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EFFECT OF WATER STRESS AND FOLAIR SPRAY OF HUMIC ACID ON GROWTH AND ESSENTIAL OIL QUALITY OF MARJORAM (*Majorana hortensis* Moench) PLANT.

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ABSTRACT

The present work was carried out during two successive seasons of 2007 / 2008 and 2008 / 2009 to investigate the impact of the water stress treatments (50, 75 and 100 % field capacity FC) and foliar spray of Humic acid (HA) treatments (control, without HA and with 1% HA) on plant height, leaves dry weight and essential oil percentage and components of *Majorana hortensis* plants.

The results can be summarized as follows: -

1- Irrigation plants with 100 % FC (control) were effective in raising the productivity of plant height, leaves dry weight and essential oil percentage and constituents.
2- Spraying plants with humic acid 1% was effective in raising the productivity of plant height, leaves dry weight and essential oil percentage and constituents.
3- The interaction between these treatments gave the best results.
4- Concerning the essential oil constituents, terpinene-4-ol was the major compound (68.24 %) resulted from 100 % FC + 1 % humic acid.

INTRODUCTION

Nowadays, the government of Arab Republic of Egypt, encourages the cultivation of medicinal and aromatic plants because they are potentially exportable crops and therefore become a source of national income, beside their importance in pharmaceutical industries, which grow enormously in Egypt.

Sweet marjoram (*Majorana hortensis* Moench), Fam. Lamiaceae (Labiatea) which was chosen for carrying out this investigation is very important member of this family. In Egypt, it is considered as the most favour economic agriculture export crop. Marjoram indigenous to the Mediterranean region. The plant is cultivated as an annual herb in Germany, Hungary, France, Tunisia, especially in Bulgaria, and small crops are planted in the United State. The true marjoram oil is constituted by several terpenes (40 %) mainly terpinene, cineole, terpineol- 4- ol, pulgone and small amount of esters (Panda, 2000). The dried leaves of marjoram are used as condiment and its volatile oil is employed medicinally due to its strong, highly aromatic spicy, its odour-like, and flavour (Lagouri *et al*., 1993). Essential oil of marjoram herb is used as stimulant, antimicrobial, digestive, carminative, diuretic, antispasmodic, better tonic, antidiabetic, antioxidant, antiasthmatic, antiparalytic drug and regulates menstruation (Yadava and Khare, 1995).

Plant reactions are affected by the amount of soil water directly or indirectly. Drought stress limits the production of 25 % of the world land (Delfine *et al*., 2005). Water stress in plant influences many metabolic

processes and the extent of its effects depend on drought severirty. The optimization of irrigation for the production of fresh herbs and essential oil is important since water is amajor component of the fresh produce and affects both weight and quality (Jones and Tardien, 1998). Water deficit in plant may lead physiological disorders, such as a reduction in photosynthesis and transpiration and in the case of aromatic plant may cause changes in the yield and composition of their essential oils (Sarker *et al*., 2005). Water deficit decrease the oil yield of rosemary (*Rosemarinus officinalis*) (Singh and Ramesh, 2000) and anise (*Pimpinella anisum*) (Zehtab- Salmasi *et al*., 2001). By contrast, Aziz *et al*. (2008) mentioned that water stress caused an increase in essential oil production of thyme (*Thymus vulg*aris) and citronella grass (*Cymbopogen winterianus* Jowitt.). The severity of the water stress response varied with cultivar and plant density (Fatima *et al*., 2000). The improvement of plant nutrition can contribute to increased resistance and production when the crop is submitted to water stress.

Humic substances are organic compounds that result from the decomposition of plant and animals materials. Humic acid and their salts which derived from coal and other sources may provide a viable alternative to liming, to ameliorate soil acidity and improve soil structurel stability. It is the humic fractions (humic acid, fulvic acid and humin) of the soil organic matter that are responsible for the generic improvement of soil fertility and improved productivity (Kononova, 1966 and Fortun *et al*., 1989). The same authors added that humic acids are known to posses many beneficial agricultural properties, they participate actively in the decomposition of organic matter, rocks and mineral, improve soil structure and change physical properties of soil, promote the chelation of many elemants and make these available to plants, aidin in correcting plant chlorisis, enhancement of photosynthesis density and plant root respiration has resulted in greater plant growth with humate application (Smidova, 1960 and Chen & Avid, 1990). Increase the permeability of plant membranes due to humate application resulted in improve growth of various groups beneficial microorganisms, accelerate cell divition, increased root growth and all plant organs for a number of horticultural crops and turfggrasses, as well as, the growth of some trees (Russo & Berlyn,1990, Sanders *et al*., 1990 and Poincelot, 1993).

The aim of research is to study the effect of water stress and humic acid foliar spray on vegetative growth, essential oil quality and chemical composition of marjoram (*Majorana hortensis* Moench) plants.

MATERIALS AND METHODS

The present work was carried out during the two successive seasons of 2007 / 2008 and 2008 / 2009 at the Experimental Station of Ornamental Plants, Fac. Agric., Mansoura University.

Plant material:

Cuttings of marjoram plants were taken from symmetry mother plants grown in the Medicinal and Aromatic Plants Farm in the Agriculture Research Center, in Giza.The cutting planted in nursery under shaded conditions for

rooting on Oct.15th 2007 and 2008 seasons. After four months, on Feb.15th the rooted cuttings were individually transplanted in pots of 30x30 cm; each pot was filled with 6.320 kg of air dried soil, which it was 2 : 1 clay : sand by volume, each pot had one plant sample (about 10 cm in height). Plants were held under natural conditions and irrigated with tap water for 8 weeks from transplanting date (2 month) until the plant samples were treated.

Experimental design and treatment:

The used experimental design was split design with three replicates.The main plots were water stress treatment (Field capacity FC %) which included three types i.e.

1. 100 % Field capacity (FC) 1701 ml / pot.
2. 75 % Field capacity (FC) 1276 ml / pot.
3. 50 % Field capacity (FC) 851 ml / pot.

Sub plots were Humic acid foliar spray treatment i.e.

1. Without Humic acid (without HA).
2. With 1 % Humic acid (with HA).

Harvesting:

The plants were harvested three times in both seasons by cutting the aerial parts of each plant (10 cm) above the soil surface. The three cuts were at the 15th of June, August and October (two month intervals).

Data recorded:

A- Vegetative growth:

Three plants were chosen randomly from each treatment, each replicates and the following characters were recorded:

1. **Plant height (cm):**
2. **Leaves dry weight (g / pot):** plants were dried at room temperature until constant weight and separated from drying plants and weighted.

B. Essential oil determination:

1. **Oil percentage (%):** was determined in the air-dried herb according to Egyptian Pharmacopeia (1984) by distilling 50 gm of dried herb for 2.50 - 3.00 hours in order to extract the essential oil. The essential oil percentage was calculated.
2. **Essential oil constituents:** Essential oil constituents were analyzed using Gas Liquid Chromatography analysis (GLC). The dehydrated oil was separately subjected to GLC analysis at the Central Laboratory of Cairo University. The constituents of the essential oil were identified by matching their retention time (RT) with those of authentic samples under the same conditions, according to Guenther and Joseph (1978).

Statistical analysis:

The data was statistically analyzed using analysis of Variance (ANOVA) technique according to Duncans(1955).

RESULTS AND DISCUSSION

1. Plant height (cm): -

1.1. Effect of water stress (field capacity FC %):-

Data in Table (1) clearly showed that, plant height as a growth character was found to be influenced by field capacity at different rates i.e.;

field capacity at the rates of 75 and 100 % stimulated the growth of marjoram plants, as the plants were elongated in comparison with field capacity at the rates of 50 %. The most effective concentration in this respect was field capacity at the rate of 100 %. Also, the application of field capacity at 100 % gave significant differences over the field capacity at 50 or 75 % in the three cuts of the two seasons. These findings met those of Khater *et al.* (1995) on *Mentha piperita* plant, Reafaat and saleh (1997) on *Ocimum basilicum* plant and Soleimanzadeh *et al*. (2010) on *Helianthus annuus* plant.

1.2. Effect of humic acid: -

Data in Table (1) was observed that, foliar spray with humic acid gave a stimulation effect in plant height habit of marjoram plants. The values of plant height were (32.47, 34.80 and 25.79 cm), and (33.06, 36.67 and 27.49 cm) in the 1st, 2nd and 3rd cuts of the both seasons, respectively. These values were found to be significantly differed from the values of without humic acid. However, the results agree with those reported by Norman *et al.* (2003) they found that tomato plant height increased considerably in plants grown in pots containing humic acids.

1.3. Effect of the interactions between water stress and humic acid:-

The results in Table (1) indicated that, plant height was increased by the interaction treatments between field capacity and humic acid foliar spray. The results also pointed out that, increasing the rate of field capacity combined with humic acid resulted in an increase in the plant height. The increments were found to be significant in all cases in the three cuts during both seasons. These results agree with the results of Said *et al*. (2009) on oregano plants.

Table (1): Plant height (cm) of marjoram plants affected by water stress and foliar spray of humic acid and their interactions of the two seasons (2007 / 2008) and (2008 / 2009).

Treatments		Plant height (cm)					
		1st seasons			2nd seasons		
		1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
Water stress (FC %)							
100 % FC		34.58[a]	36.77[a]	27.90[a]	35.15[a]	38.61[a]	29.53[a]
75 % FC		31.06[b]	33.71[b]	24.09[b]	31.57[b]	35.67[b]	25.79[b]
50 % FC		28.97[c]	30.12[c]	21.06[c]	29.57[c]	32.03[c]	22.75[c]
Humic acid							
without		30.60[b]	32.26[b]	22.91[b]	31.13[b]	34.19[b]	24.56[b]
with		32.47[a]	34.80[a]	25.79[a]	33.06[a]	36.67[a]	27.49[a]
The interactions							
100 % FC	**without**	33.98[b]	35.62[b]	26.64[b]	34.54[b]	37.53[b]	28.22[b]
	with	35.18[a]	37.92[a]	29.17[a]	35.75[a]	39.68[a]	30.85[a]
75 % FC	**without**	30.02[d]	32.89[d]	22.82[d]	30.47[d]	34.87[d]	24.52[d]
	with	32.10[c]	34.52[c]	25.36[c]	32.66[c]	36.47[c]	27.07[c]
50 % FC	**without**	27.81[e]	28.28[f]	19.27[e]	28.38[e]	30.19[f]	20.96[e]
	with	30.13[d]	31.97[e]	22.84[d]	30.76[d]	33.87[e]	24.54[d]

2. Leaves dry weight (g / pot): -

2.1. Effect of water stress (field capacity FC %):-

The results of leaves dry weight of marjoram plants as influenced by different level of field capacity were presented in Table (2). The results indicated that there was an increase in the leaves dry weight of marjoram plants by increasing the field capacity level from 50 to 100 %. Plants irrigated by the third level of field capacity (100 %) produced the heaviest dry leaves (5.12, 9.67 and 6.17 g / pot) and (5.35, 9.99 and 6.36 g / pot) at the three cuts in the two seasons, respectively. On the other hand, the lightest dry leaves weight resulted in the field capacity at the level of 50 % were (2.97, 5.16 and 3.07 g / pot) and (3.19, 5.35 and 3.28 g / pot) at the three cuts in the two seasons, respectively. Bishr (1972) on Majorana hortensis moench Osman (1989) on Cariandrum sativum L. plant Khater *et al* (1995) on Mentha piperita L and Abbas (2007) on Solanum melogena L.

2.2 Effect of humic acid: -

Data in Tables (2) showed that humic acid foliar spray have an effect to increase dry weight of leaves, due to the effect of humic acid foliar spray, the leaves dry weight (per pot) increased from 3.57 to 4.27, 6.69 to 8.12 and 4.10 to 4.98 g / pot in the three cuts, in the first season, respectively. In the second season, results of dry weight of leaves / plant (pot) were in the same trend as in the first one Norman *et al*. (2003) on tomatos, Tan and Nopamornbod (2005) on corn plants (*Zea mays* L.) and Katkat *et al*. (2009) on wheat plants.

Table (2): Leaves dry weight (g / pot) of marjoram plants affected by water stress, folier spray of humic acid and their interactions in the two seasons of (2007 / 2008) and (2008 / 2009).

Treatments		Leaves dry weight (g / pot)					
		1st seasons			2nd seasons		
		1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
Water stress (FC %)							
100 % FC		5.12[a]	9.76[a]	6.17[a]	5.35[a]	9.99[a]	6.39[a]
75 % FC		3.67[b]	7.31[b]	4.38[b]	3.89[b]	7.55[b]	4.61[b]
50 % FC		2.97[c]	5.16[c]	3.07[c]	3.19[c]	5.37[c]	3.28[c]
Humic acid							
without		3.57[b]	6.69[b]	4.10[b]	3.76[b]	6.94[b]	4.31[b]
with		4.27[a]	8.12[a]	4.98[a]	4.52[a]	8.34[a]	5.21[a]
The interactions							
100% FC	**without**	4.73[b]	9.22[b]	5.67[b]	4.95[b]	9.47[b]	5.89[b]
	with	5.51[a]	10.29[a]	6.66[a]	5.74[a]	10.51[a]	6.88[a]
75 % FC	**without**	3.37[d]	6.59[d]	4.06[d]	3.56[d]	6.87[d]	4.27[d]
	with	3.97[c]	8.02[c]	4.69[c]	4.22[c]	8.23[c]	4.94[c]
50 % FC	**without**	2.59[e]	4.27[f]	2.55[f]	2.78[e]	4.47[f]	2.76[f]
	with	3.35[d]	6.05[e]	3.59[e]	3.61[d]	6.27[e]	3.81[e]

2.3. Effect of the interactions between water stress (field capacity FC %) and humic acid:-

Data in Tables (2) showed that field capacity and humic acid foliar spray have an effect to increase dry weight of leaved in most cases. The highest values were (5.51, 10.29, 6.66, 5.74, 10.51 and 6.88 g/ pot) in the 1st, 2nd and 3rd cuts in the two seasons respectively, obtained by application of field capacity (100 %) with used of humic acid treatment. On the other hand, the fewest weights were resulted from plants treated by 50 % field capacity and without humic acid. These results were found to be in harmony with those obtained by Said *et al*. (2009) on oregano plants.

3. Essential oil (%):-

3.1. Effect of water stress (field capacity FC %):-

The data presented in Table (3) showed that essential oil percentage was greatly influenced by levels 75 and 100 % of field capacity as compared to control (50 %). Hence, in the two seasons, growing plants of marjoram at 100 % FC had enhancing effect on oil formation of herb (1.71, 1.91, 1.82, 1.73, 1.99 and 1.85 %) at the three cuts in the both seasons, respectively. A similar finding was stated by Bishr (1972) on *Majorana hortensis*h, Singh *et al.* (1997) on Lemon grass, Fatima *et al*. (2000) on *Citronella java*., Kandeal (2001) on *Rosmarinus officinalis*, Omidbaigi *et al*. (2003) on *Ocimum basilicum*, Bettaieb *et al*. (2009) on *Salvia officinalis* and Said *et al*. (2009) on oregano plants.

Table (3): Essential oil (%) of marjoram plants affected by water stress, folier humic acid and their interactions in the two seasons of (2007 / 2008) and (2008 / 2009).

Treatments		Essential oil (%)					
		1st seasons			2nd seasons		
		1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
Irrigation (%)							
100 % FC		1.71	1.91	1.82	1.73	1.99	1.85
75 % FC		1.40	1.64	1.56	1.42	1.67	1.59
50 % FC		1.09	1.29	1.25	1.13	1.35	1.29
Humic acid							
without		1.30	1.52	1.46	1.33	1.57	1.50
with		1.49	1.70	1.62	1.52	1.78	1.66
The interactions							
100 % FC	**without**	1.62	1.82	1.75	1.63	1.86	1.79
	with	1.79	1.99	1.89	1.82	2.13	1.91
75 % FC	**without**	1.31	1.56	1.48	1.34	1.58	1.49
	with	1.48	1.71	1.63	1.50	1.76	1.69
50 % FC	**without**	0.98	1.19	1.15	1.02	1.26	1.20
	with	1.20	1.40	1.34	1.25	1.44	1.38

3.2. Effect of humic acid: -

Data presented in Tables (3) revealed that, spraying the plants with humic acid gave the highest percentage of essential oil content (1.49, 1.70, 1.62, 1.52, 1.78 and 1.66 %) over than without humic acid (1.30, 1.52, 1.46, 1.33, 1.57 and 1.50 %) at the three cuts in the two seasons, respectively.

These results were coincided with those obtained by Said *et al*. (2009) on oregano plants and Zaghloul *et al.* (2009) on *Thuja orientalis*.

3.3. Effect of the interactions between water stress and humic acid:-

The data (3) showed that essential oil percentage (%) was greatly influenced by levels of field capacity with humic acid as compared to treatments by levels of field capacity without humic acid. Hence, at the three cuts in the two seasons, treating the plants of marjoram with humic acid at the level of 100 % FC had enhancing effect on oil formation of herb. This treatment produced the highest essential oil percentage. These results were in accordance with those reported by Said *et al*. (2009) on oregano plants.

4. Essential oil components (%):-

The results in Table (4) and illustrated in Figures (1 and 2) showed the different components separated and identified from marjoram oil herb samples produced from the plants treated with interaction between field capacity (water stress) and humic acid. The obtained chromatograms revealed and identified the presence of 10 components. The identified components in marjoram oil herb are α- pinene, β- pinene, myrcene, limonene, p- cymene, 1.8- cineol, terpinene-4-ol, α- terpinol, linalyl acetate and β- caryophyllene.

Table (4): Essential oil components (%) of marjoram plants affected by water stress (field capacity FC %) in the second season of (2007 / 2008).

components	Essential oil components (%) Irrigation 100 % FC	
	Without Humic acid	With Humic acid
α- pinene	0.47	0.33
β- pinene	5.25	2.88
myrcene	0.97	----
limonene	1.43	1.69
P- cymene	1.29	1.46
1,8 cineol	----	1.27
terpinene-4-ol	63.18	68.24
α- terpinol	8.87	11.89
linalyl acetate	1.08	0.85
β- caryophyllene	0.47	0.47
Known	83.01	89.08

The relative percentage areas indicated the effect of the different interaction between field capacity, humic acid on the composition of the oil samples. The highest increament was produced by 100 % FC with humic acid (89.08 %) while, treated plants with 100 % FC only was 83.01 %.

The major constituent (terpinene- 4- ol) was 68.24 % compared with 100 % FC only (without humic acid) 63.18 %. The rest of volatile oil components limonene, α- terpinol and p- cymene showed highest increaments by 100 % FC with humic acid. Similar results were reported by Omer *et al*. (1994) on marjoram and Picacagli and Marotii (1993) on several aromatic plants, Omer (1998) on *Origamum syriacum* and Said *et al*. (2009)

on oregano. It could be recommendation that to obtained the best vegetative growth (plant height and leaves dry weight, essential oil percentage and components of all studied plants they might be supplied with 100 % FC and sprayed with humic acid 1%.

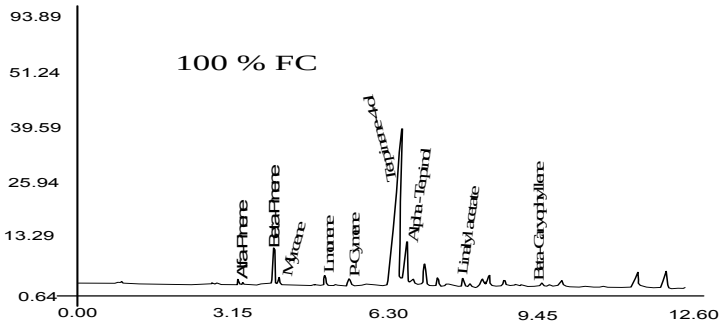

Figure (1): GLC analysis of marjoram oil extracted from 100 % FC without humic acid

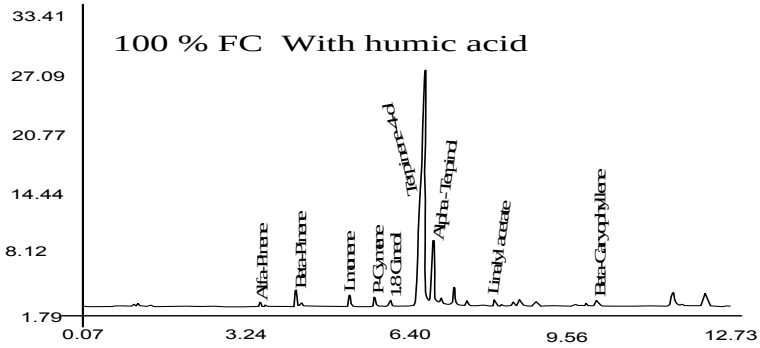

Figure (2): GLC analysis of marjoram oil extracted from 100 % FC with humic acid 1%

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تاثير الإجهاد المائي والرش الورقى بحمض الهيوميك على النمو والزيت الطيار لنبات البردقوش

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أجريت تجربتان حقليتان على نبات البردقوش فى مشتل كلية الزراعة جامعة المنصورة خلال موسمى ٢٠٠٧/٢٠٠٨ و ٢٠٠٨/٢٠٠٩ وذلك بهدف دراسة تاثيرالاجهاد المائى بثلاثة مستويات (١٠٠ % و٧٥ % و٥٠ % من السعة الحقلية) كما تم استخدام حمض الهيوميك بالرش على النباتات بمستويين (بدون رش هيوميك والرش بالهيوميك بتركيز١%) عن طريق رش النباتات مرتين بفاصل زمنى١٥ يوم بينهما وقد تم تكرار الرش عقب كل حشة ٠ بالاضافة الى تاثير التداخل بين معاملات الرى ومعاملات الهيوميك على نمو نباتات البردقوش وانتاجها من الزيت الطيار و مكوناته الفعالة٠

ويمكن تلخيص النتائج المتحصل عليها في التالي : -

١- رى النباتات بمعدل ١٠٠% من السعة الحقلية لة فاعلية فى زيادة كل من طول النبات و الوزن الجاف للاوراق و النسبة المئوية للزيت العطرى ومكوناتة ٠

٢- رش النباتات بحمض الهيوميك ١% لة فاعلية فى زيادة كل من طول النبات و الوزن الجاف للاوراق و النسبة المئوية للزيت العطرى ومكوناتة ٠

٣- التفاعل بين هذه المعاملات اعطى افضل النتائج ٠

٤- بالنسبه لمكونات الزيت العطرى كان المركب الرئيسى التربينين فوراول الناتج من التفاعل بين رى النباتات بمعدل ١٠٠% من السعة الحقلية من المعاملة ورش النباتات بحمض الهيوميك ١% وكانت نسبته ٦٨.٢٤%

قام بتحكيم البحث

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