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EFFECT OF VA-MYCORRHIZAE AND AZOTOBACTER ON GROWTH AND OIL PRODUCTION OF *Achillea milefolium* L. PLANT UNDER DIFFERENT WATER REGIME

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ABSTRACT

Nowadays competition for the available water supply, for irrigation, is increasing. This study which carried out for two seasons aimed to investigate the effect of vesicular-arbuscular mycorrhizae (VAM) and *Azotobacter* inoculation on growth and essential oil production of the aromatic plant *Achillea millefolium,* which has different usage as a medicinal aromatic plant. Plants were transplanted in sandy soil, which maintained at 50, 75 or 100% of field capacity. In addition to the control treatment, plants were inoculated with *A. chroococcum* and/or VAM. Field capacity significantly affected plant growth measured parameters; plant height, herb fresh and dry weight, flower fresh and dry weight as well as volatile oil yield. Microbial inoculation, significantly improved plant growth as well as its volatile oil yield compared with the control plants. The highest essential oil yield of flower tops (0.185 ml/plant) has been estimated when soil inoculated with both of the microorganism and maintained in 50% FC. *Azotobacter* inoculation significantly increased N% but not P% of yarrow herb however, VAM significantly increased P% of the herb. Inoculation with both of microorganisms had a beneficial effect in plant growth especially under lower levels of FC. Moreover inoculation with both of microorganisms had more pronounced effect than single inoculation.

INTRODUCTION

Achillea millefolium L, plant which known as yarrow is a perennial plant belongs to family *Asteraceae*. Armitage (1992) emphasis the importance of *A. millefolium* as an ornamental plant, cut flower as well as a dried material. Essential oils of yarrow have several physiological active substances, mainly the sesquiterpens guaiazulen. Yarrow has been known for a long time as a medicinal plant and used in the folk medicine. It has a beneficial remedy in diseases of the mucous surfaces, relieving irritation and profuse secretion. It smoothes intestinal irritation and overcomes mild forms of diarrhea. It is of benefit in improving the tone of the urinary apparatus, relieving irritation, overcoming suppression of the urine. The drug stimulates the flow of gasteric secretions; it has relieved effect in the obstructed perspiration and commencement of fevers (Stary and Jirasck, 1975).

Competition for the limited water supply, which is available for irrigation, is increasing. An adequate supply of water is just essential to the successful growth of plants, photosynthesis and other biochemical processes. The unpredictable nature of drought and depletion of underground water supply sources increase the uncertainty about the feature of irrigation and water supply. Water stress, in general, reduces nutrient uptake by roots and transport from roots to shoots (Goicoechea *et al.*, 1997). Different

agricultural treatment has been used to overcome water shortage by enhancing drought resistance mechanisms, water-use efficiency and plant growth. Nitrogen fertilization, which might be as a result of *Azotobacter* inoculation, has been found to improve the growth of different plant species under water stress (Shangguan, 1997 and Shangguan *et al.*, 2000). VAM presumably increases the efficiency of water and mineral absorption, chiefly because the hyphae extend out into the soil and increase the absorption surface. VAM maintains an active water absorption of older roots after they have become suberized (Bowen, 1973). The enhancement of mineral uptake by VAM colonized plants has been extensively reviewed (Smith and Read, 1997). VAM fungi colonize plant roots and often enhance host plant growth and mineral nutrient acquisition, particularly for plants grown under infertile soil conditions. These influences in plant water status could be due to increasing the absorption surface, hydrolyzing certain nutrients in the soil such as organic and inorganic phosphate (Faber *et al.*, 1991). Therefore, the present investigation aimed to find-out the potentiality of VAM and *A. chroococcum* on growth of yarrow plants under water regime conditions.

MATERIAL AND METHODS

This experiment had been carried out in 2001/2002 and repeated in 2002/2003 at the experimental nursery, Hort. Dept., Fac. of Agric., Minia Univ. Stock plants were divided into single uniform plants in 1st Oct. then, plants were transplanted in plastic pots each filled with 7 kg of air-dried sandy soil. Filed capacity (FC) of container was estimated before transplanting as described by Tuomela (1997). After one month of transplanting, uniform plants were arranged in 3 x 4 factorial experiment in a complete randomized block arrangement design. So, the treatment was 3 water treatments (100, 75 and 50% of FC every week) and 4 soil inoculations (control, *A. chroococcum*, VAM and *A. chroococcum* + VAM). Therefore for each treatment there was 20 plants divided into 4 replicates.

One-week-irrigation cycle had been applied throughout the experiment commencement after a month of transplanting. Loosing water was calculated every week by weighting 5-randomized-selected pots from the control (100% FC) plants. Loosing water was compensated to 100, 75 or 50% of FC. This was expected to result in different levels of water stress in plant in the 3 watering treatments.

Water treatment was commenced after a week of inoculation. Plants were fertilized with 4 g/plant ammonium sulphate (20.5% N), 2 g/plant calcium superphosphate (15.5% P_2O_5) and 1 g/plant potassium sulphate (48% K_2O). Phosphorus fertilizer was added as a one dose during the transplanting while the nitrogen and potassium fertilizers were divided into 3 doses. First dose had been added after 45 days from transplanting and the other 2 doses after 1 and 2 months of the first one.

In addition to the 4 replicates 30 plants were treated as the control. These plants had been used to calculate the required amount of water, needed for irrigation. To eliminate the plant weight, which could, affect the

calculated required amount of water to achieve the FC., five randomly selected plants were used to calculate the required amount of water at 1 st of each month during the experiment period. The amount of lossing water was calculated as (container weight at 100 % FC – container weight excluding the plant weight).

Flowering tops were collected 4 times every 2 weeks during the flowering season. Fresh and air-dried yield of the 4-cuttings flowering tops of individual plants was estimated. Air-dried flower tops of the 5 plants in each replicate was carefully hand crushed to measure the percentage of EO content (Guenther, 1961). At the end of growth seson, plant heights were measured, also herb of individual plants were cut above the soil surface and weighted thereafter the air-dried weights were estimated. Then herb of the 5 plants in each replicate was hand crushed to measure the % of EO. Nitrogen content *of A. millefolium* plants was determined using microkjeldahl method (Eastin, 1978). Phosphorus contents had been colorimetrically determined according to Wilde *et al.*, (1979).

Preparation of microorganism inocula

A. chroococcum from the stock culture collection, department of Agric. Microbiology, Fac., Agric., Minia University, Egypt was used. Two VAM species, (*Glomus fasiculatum* and *G. mosseas*) were kindly supplied by Botany Dept., Fac. of Agric., Kafer-El-Sheikh, Tanta University, Egypt.

A. chroococcum was grown for 7 days at 30 °C in 250-ml Erlenmeyer flask containing 100 ml of modified liquid Ashby's medium (Abdel Malek and Ishac, 1968). The suspension that had 19-23 x 10^8 cell/ml viable cell had been used as inocula. For preparing VAM inoculum, fired clay pots of 30 cm in diameter were filled with autoclaved sandy loam soil. Soil in each pot was inoculated with the two species of these endomycorrhizal fungi. Five onion seedlings were transplanted in each pot as a host plant. At the end of the growth stage roots of onion plants were mixed together and VAM spores were counted in 135-152 g soil as described by Musandu and Giller (1994). The mixture of VAM spores, mycelia and chopped roots were used as VAM inoculum.

Determinations of microbial density

Samples from rhizosphere were taken at 15 days interval up to 120 days of_inoculation to follow counts of *Azotobacter*. The rhizosphere was collected by mechanical removal of the tightly adhering soil left after shaking the roots together. The basic dilution was made by adding 10 g of the rhizosphere soil to 90 ml of sterilized water and stirring for 5 min. Serial dilutions for rhizosphere samples were prepared and number of *Azotobacter* was determined by the dilution frequency method in Ashby's liquid medium using most-probable number tables (Cochran, 1950). Percentage of mycorrhizal root colonization was assessed microscopically by the slide method (Mosse and Giovanetti, 1980).

Results of plant growth characters, EO, N and P content were submitted to an analysis of variance (ANOVA). Means were compared using

LSD test ($p<0.05$) between any pair of data (Clewer, and Scarisbrick, 2001). The analysis was performed using MSTATC for DOS.

RESULTS AND DISCUSSIONS

Number of *Azotobacter*

Table (1) showd higher cell numbers of *Azotobacter* in the rhizosphere of inoculated yarrow than the uninoculated plants after 15 days of inoculation with *Azotobacter*. The highest difference in cell number between the rhizosphere of inoculated plants and those of uninoculated were recorded after 90 days of inoculation. Results showed a decline in rhizosphere surviving cell number of plants inoculated with *Azotobacter* at 120 days of inoculation in all treatments. These results may be explained by the stimulation effect of plant roots and their exudates until the age of 90 days of inoculation. These exudates make rhizosphere zone a relatively nutrient rich environment, in which inoculant can grow and multiply (Breland and Bakken, 1991 and Attia and Saad, 2001). In addition, the results showed that the number of *Azotobacter* depended on the FC at the same time after inoculation. The highest cell number has been obtained when soil was maintained at 75% FC and the lowest being when FC was 100%. For example in the first season, after 90 dayes of inoculation, number of *Azotobacter* cells were 91, 94 and 88 $\times 10^5$/g of dry soil under 50, 75 and 100% of FC respectively (Table 1). Moreover, the results showed that inoculation with VAM plus *Azotobacter* led to an increase in the *Azotobacter* cell number compared with inoculation with mycorrhizae alone or uninoculated ones. This may be due to the positive interaction between *Azotobacter* and VAM.

VAM colonization

The colonization of yarrow roots by endo VA-Mycorrhizal fungi was assessed microscopically after 120 days of inoculation. VAM root colonization in both *Azotobacter*, inoculated and uninoculated plants were recorded in Table (2). The highest number of VAM root colonization was observed when plants inoculated with VAM plus *Azotobacter*. These results are in agreement with those of Khalifa and Badr (1992); Saad (1995); Saad and Ahmed (2002). This result may indicate that the presence of *Azotobacter* may facilitate the mycorrhizal infection percentage. VAM root colonization was decreased with increased FC% (Table 2). In addition, in absence of VAM inoculum, low ratios of VAM root colonization were detected in roots of *A. millefolium* plant inoculated with *Azotobacter*. This may indicate the presence of low number of native mycorrhizal fungi in the experimental soil location.

Table 1: Cell number of *Azotobacter* (10^5) in rhizosphere soil inoculated and uninoculated *A. millefolium* L. plant. under different soil field capacity at different growth stages during two seasons

Treatments	% of soil field capacity																	
	100						75						50					
	Days after inoculation						Days after inoculation						Days after inoculation					
	0	15	30	60	90	120	0	15	30	60	90	120	0	15	30	60	90	120
First season 2001																		
Unino.	12.3	23.4	30.8	41.5	56.3	42.8	12.3	27.2	38.6	50.1	62.2	48.6	12.3	25.8	35.4	46.3	60.5	45.7
VAM	12.3	22.8	31.2	40.9	57.2	43.6	12.3	27.8	39.2	51.6	63.2	49.7	12.3	26.7	36.8	48.1	61.5	44.9
Azot.	12.3	53.4	66.8	77.4	88.6	72.9	12.3	61.7	73.4	85.3	94.8	79.6	12.3	59.2	70.8	81.6	91.3	76.3
V+A	12.3	43.8	50.8	55.6	70.1	54.8	12.3	48.8	55.2	62.8	74.3	59.8	12.3	46.3	52.6	58.4	72.6	56.3
Second season 2002																		
Unino.	11.7	21.2	31.2	40.8	55.6	43.2	11.7	26.8	39.2	52.4	61.9	46.3	11.7	24.5	36.2	47.7	58.3	44.2
VAM	11.7	22.1	32.8	41.2	56.7	44.8	11.7	28.3	42.4	54.1	63.5	48.2	11.7	25.9	39.4	50.2	60.1	46.3
Azot	11.7	54.6	65.2	76.9	87.8	70.3	11.7	60.6	75.8	86.7	93.9	77.8	11.7	58.8	71.2	83.7	92.3	75.8
V +A	11.7	42.6	51.3	56.8	68.2	53.1	11.7	45.8	55.2	63.4	76.2	60.6	11.7	43.8	53.6	59.2	70.8	55.8

Unino.= uninocualated, VAM .= Myco, Azot= *Azotobacter* and V+A= VAM+*Azotobacter*

Table 2:Percentage of mycorrhizae colonization in *A. millefolium* L. plant under different field capacity during two seasons

Treatments	% of soil field capacity					
	100	75	50	100	75	50
	1 st season 2001			2nd season 2002		
Uninoculated	4	6	65	5	8	7
VAM (V)	62	74	73	61	76	75
Azotobacter (A)	7	10	9	8	11	10
V+ A	65	78	76	64	77	68

Plant growth and oil production

Plant height

As shown in (Table 3) plant height of yarrow plants, in the first season, decreased from 69.7 to 67.0 and 60.0 cm when soil FC maintained at 100, 75 and 50 % respectively. There was a significant difference in plant height only between plants grown in soil at 100 and 50% FC ($p< 0.5$). Microbial inoculation significantly increased plant height over the control plants (52.5 cm). The highest increment in plant height over the control plants; 48% was estimated when soil inoculated with VAM + *A. chroococcum*. However, the difference between plants grown in soil was inoculated with VAM and *A. chroococcum* (65.4 and 66.7 cm respectively) was not significant. In addetion the interaction between the inoculation and water treatment was not significant. Overall, control plant which has been grown in soil at 50 % FC had the lowest plant height 58.7 cm whereas, the highest plant height 81.3 cm was estimated when grown in soil at 100 % FC and inoculated with VAM + *A. chroococcum*. Similar results have been documented in the second season.

Herb fresh and dry weight

Both of water treatment as well as microbial inoculation had a significant effect on herb fresh and dry weight (FW and DW, respectively) of yarrow plants in both seasons. In the first season, FW of plants grown in soil at 100, 75 and 50 % of FC were 299.9, 314.5 and 252.9 g/plant, respectively. However, there was only significant difference between the third value and any of the other two values. Microbial inoculation significantly increased herb FW over the control plants (212.2 g/plant). This increment was 36, 44 and 59% when soil inoculated with VAM, *A. chroococcum* or VAM + *A. chroococcum,* respectively. Control plants which grown in soil at 100% FC had FW higher than those of plants grown in soil with 75% FC. However, plants inoculated with *A. chroococcum*, VAM or *A. chroococcum* + VAM and grown in soil with 75% FC had FW higher than those of inoculated plants grown in soil at 100% FC. Results showed that the interaction between the studied factors was not significant. Overall, control plants grown in soil at 50 % FC had the minimum FW 167 g/plant. Whereas plants grown in soil at the

same water content but inoculated with *A. chroococcum* + VAM had a FW 312 g/plant.

Table (3) :Effect of soil FC, VAM and *Azotobacter* inoculation on plant height and herb fresh and dry weight of *A. millefolium* in two seasons

Treatments	% of soil field capacity (A)							
	1 st season				2 nd season			
	100	75	50	Mean (B)	100	75	50	Mean (B)
	Plant height (cm)							
Control	58.7	52.62	46.3	52.5	58.9	56.2	42.9	52.7
VAM	67.1	68.4	60.7	65.4	69.6	73.1	64.1	68.9
Azot	71.8	69.1	59.2	66.7	73.4	68.3	57.6	66.4
V+A	81.3	78.0	75.0	78.1	81.4	80.4	73.8	78.6
Mean A	69.7	67.0	60.3		70.8	69.5	59.6	
LSD 5%	A 5.7	B.6.5	AB ns		A 7.8	B 9.0	AB ns	
	Herb fresh weight (g/plant)							
Control	225.9	243.7	167.0	212.2	242.3	231.7	179.1	217.7
VAM	313.9	300.1	252.3	288.7	304.4	310.3	256.8	290.5
Azot	333.7	303.4	279.5	305.5	317.9	335.0	291.7	314.9
V+A	352.2	384.3	312.7	349.7	402.8	353.6	322.6	359.7
Mean A	306.4	307.8	252.9		316.8	307.7	262.5	
LSD 5%	A 28.15	B 32.72	AB ns		A 35.6	B 41.1	AB ns	
	Herb dry weight (g/plant)							
Control	71.2	64.0	60.7	65.3	68.4	64.7	62.7	65.3
VAM	84.8	89.5	76.0	83.4	88.0	91.9	77.3	85.8
Azot	91.4	84.3	73.2	83.0	93.0	86.4	73.8	84.4
V+A	107.5	93.9	74.7	92.0	109.7	93.8	82.8	95.4
Mean A	88.7	82.9	71.1		89.8	84.2	74.1	
LSD 5%	A 7.2	B 8.3	AB 14.35		A 8.7	B 10.0	AB ns	

V+A =VAM + *Azotobazter*, ns= not signficant

In the first season, plant DW decreased with decreasing soil FC. The obtained DW values were 88.7, 82.9 and 71.1 g/plant when soil had 100, 75 and 50 % FC, respectively. However, there was no significant differences ($P>0.05$) between plants grown in soil at 100 % FC and 75% FC. Soil

inoculation significantly increased herb DW over the control plants. Recorded values were 65.3 g/plant and 83 g/plant for the control and inoculated plants, respectively. There was no significant difference in herb DW between plants inoculated with VAM or *A. chroococcum* since, both prodced similar herb DW 83 g/plant (Table 3). This value was significantly higher than that of uninoculated plants. It should be mentioned that plants inoculated with VAM + *A. chroococcum* had significantly the highest herb DW 92 g/plant.

The interaction between soil FC and plant inoculation was significant. Herb DW under any water regime gradually increased in the following inoculated order *A. chroococcum* > VAM > *A. chroococcum* + VAM. DW of control plants decreased gradually with decreasing FC of the soil (Table 3). However, plant DW increased when soil was inoculated with the tested microorganism and when FC was reduced from 100 to 75 %. The reduction in DW of control plants when soil had 50% FC rather than 100% was 40% (which was significant) but, when soil inoculated with *A. chroococcum*, VAM or *A. chroococcum* + VAM this reduction was 10, 13 and 20%, respectively (these reductions were significant in all cases). Herb DW of plants grown in soil at 50% FC and inoculated with any type of evaluated microorganisms was higher than those of uninoculated plants grown in soil at 100% FC. When soil was maintained at 75% FC, herb DW of plants treated with *A. chroococcum*, VAM and *A. chroococcum* + VAM increased over the control plants by 39.8, 42.8 and 67.9% respectively but when soil maintained at 50% FC these increment was 26, 21.6 and 21.6 % respectively. These results indicated that tested microorganisms were more effective in plant growth when soil had 75% FC than 100 or 50 % FC. Similar results were observed in the second season for both herb fresh and dry weights.

Flower tops fresh and dry weight

Table (4) showed that FW of yarrow flower tops insignificantly increased from 65.4 to 71.7 g/plant when soil FC decreased from 100% to 75%. Thereafter, this value significantly decreased to 54.5 g/plant when FC being 50%. Microbial inoculation significantly increased flower tops FW over the control plants, which had the minimum weight (51.7 g/plant). When soil had 100% FC, there was no significant difference between *A. chroococcum*- and VAM-inoculated plants (62.8 and 65.4 g/plant respectively). Whereas, under 50 and 75% FC the highest values were achieved in plants inoculated with *A. chroococcum* + VAM. Overall, the highest weight of flower tops (91.5 g/plant) recorded when plants were gown in soil at 75 % FC and inoculated with *A. chroococcum* + VAM. This value was 90 % higher than the weight of control plants grown in soil at 100% FC.

Flower tops DW significantly varied according to FC and inoculation treatment. Flower tops DW insignificantly increased from 23.98 to 25.22 g/plant when FC of the soil reduced to 75%. Thereafter, this value insignificantly reduced to 22.15 g/plant when FC was 50%. Both of *A. chroococcum* or VAM significantly increased the DW of the flower tops (23.28 and 24.48 g/plant respectively) compared to the control plants, which had 18.67 g/plant. But, when soil was inoculated with both of the microorganisms this value was 27.38 g/plant. There was insignificant interaction between soil

FC and soil inoculation in the first season however this interaction was significant in the second season. Under any inoculation treatment the highest value of flower tops DW was recorded in plants grown in soil at 75% FC. Moreover, under any FC level the highest values of flower tops DW were achieved when plants were inoculated with the combined *A. chroococcum* + VAM. Data of the second season was similar to those recorded in the first one as shown in (Table 4).

Table (4): Effect of soil FC, VAM and *Azotobacter* inoculation on flower tops fresh and dry weight of *A. millefolium* in two seasons

Treatments	% of soil field capacity (A)							
	1 st season				2 nd season			
	100	75	50	Mean (B)	100	75	50	Mean (B)
	Flower fresh weight (g/plant)							
Control	48.4	66.5	45.1	53.3	50.9	67.1	44.9	54.2
VAM	61.3	71.8	55.2	62.8	74.3	73.8	60.5	69.5
Azot	75.5	75.0	55.6	68.7	74.9	74.8	52.6	67.4
V+A	48.4	91.5	67.0	81.0	87.4	93.6	67.4	82.8
Mean A	67.4	76.2	55.72		71.9	77.3	56.3	
LSD 5%	A 7.8	B 9.0	AB 15.6		A 9.7	B 11.3	AB ns	
	Flower dry weight (g/plant)							
Control	17.65	21.52	19.83	19.67	18.80	21.38	21.65	20.61
VAM	22.18	25.28	22.38	23.28	23.20	26.90	22.70	24.27
Azot	22.60	25.28	23.55	23.95	22.88	27.08	25.75	25.23
V+A	25.60	26.80	25.85	26.07	25.18	29.28	25.12	26.52
Mean A	22.00	24.82	22.90		22.51	26.17	23.81	
LSD 5%	A 2.46	B 2.84	AB ns		A 2.72	B 3.15	AB 5.44	

V+A =VAM + *Azotobazter*, ns= not signficant

Obtained results showed that plant height, herb and flower tops FW and DW have been significantly affected by the level of FC. These results illustrated the requirement of an adequate FC for optimum vegetative and flowering growth of yarrow plants. Generally, vegetative growth decreased by decreasing FC to 50%. However, some growth parameters were significantly higher at 75% FC than 100%. This indicates that growth, development cell division and enlargement of different organ require different available water.

Some previous workers reviewed the reduction in plant growth due to inadequate moisture supplies, which cause alteration in different physiological process. These results which have been reported on Thymus by Letchamo *et al.*, (1995), on fennel by Mohamed and Abdou, (2003) and on wheat by Sivamani *et al.*, (2000) are similar to these results. Moreover results showed that soil inoculation with microorganism significantly stimulated yarrow plant height as well as herb and flower tops FW and DW. The positive effect of *Azotobacter* might be due to the increment in nitrogen availability (Attia and Saad 2001). Mycorrhizal fungi colonize plant roots and often enhance host plant growth particularly for plants grown under infertile soil conditions (Mathur and Vyas, 2000). Goicoechea *et al.*, (1997) found that VAM inoculation improved the growth of alfalfa under water stress condition as VAM increases water and nutrients uptake under water stress conditions.

Volatile oil percentage and yield of herbage

Percentage of essential oil (EO) of herb was significantly affected by water treatment and microorganism inoculation in the first season. Whereas, in the second season only microorganism inoculation had insignificant effect in EO%. There was a significant difference in EO% only between plants grown in soil at 100 and 50% FC (0.81 and 0.112 respectively). Percentage of EO and yield (0.081% and 0.072 ml/plant respectively) increased (0.112 % and 0.078 ml/plant respectively) by reducing soil FC from 100 to 50%. However, this increment of EO yield was not significant.

In both seasons microbial inoculation had a significant effect in EO yield but insignificant increment in EO% of the second season. Percentage and yield of EO of herb increased due to inoculation in the following order control, *A. chroococcum*, VAM and *A. chroococcum* + VAM. Control plants had the lowest EO % and yield (0.088% and 0.054 ml/plant, respectively) while plants inoculated with *A. chroococcum* and VAM had the highest EO % and yield (0.1% and 0 .086 ml/plant, respectively). There was no significant difference in EO yield among plants except when plants were inoculated with *A. chroococcum* + VAM compared with the control plants (Table 5).

Table (5): Effect of soil FC, VAM and *Azotobacter* inoculation on essential oil (EO) percentage of herb and flower tops of *A. millefolium* in two seasons

Treatments	% of soil field capacity (A)							
	1 st season				2 nd season			
	100	75	50	Mean (B)	100	75	50	Mean (B)
	EO% of herb							
Control	0.080	0.080	0.103	0.088	0.080	0.088	0.105	0.091
VAM	0.082	0.095	0.115	0.098	0.088	0.098	0.117	0.101
Azot	0.078	0.090	0.115	0.094	0.075	0.085	0.122	0.094
V+A	0.085	0.098	0.117	0.100	0.085	0.095	0.120	0.100
Mean A	0.081	0.091	0.112		0.082	0.091	0.116	
LSD 5%	A 0.030	B 0.035	AB 0.061		A 0.030	B ns	AB ns	
	EO% of flower tops							
Control	0.459	0.494	0.626	0.526	0.462	0.508	0.622	0.531
VAM	0.457	0.528	0.613	0.532	0.451	0.517	0.611	0.527
Azot	0.481	0.535	0.648	0.555	0.480	0.538	0.646	0.555
V+A	0.437	0.562	0.715	0.571	0.454	0.565	0.711	0.577
Mean A	0.459	0.530	0.651		0.462	0.532	0.648	
LSD 5%	A 0.053	B ns	AB ns		A 0.051	B ns	AB ns	

V+A =VAM + *Azotobazter*, ns= not signficant

Overall, in the first season the lowest EO yield were estimated when control plants were grown in soil with 100 % FC, whereas, the highest values were in soil with 100% FC inoculated with *A. chroococcum* +VAM. Results in Table (5) showed that under any water treatment, any type of inoculation increased EO % and yield of yarrow over the control treatment. Control plants had the highest EO yield when grown in soil at 100 % FC. Whereas plants inoculated with *A. chroococcum* had the highest EO yield when grown in soil with 50% of FC. On the other hand, plants inoculated with VAM had the highest EO yield when grown in soil with 75% FC.

Volatile oil percentage and yield of flower tops

In both seasons, percentage of EO of flower top has been significantly affected with FC but not with microbial inoculation. While EO yield was significantly affected with both of treatments. However, there was insignificant interaction between both of the studied factor (Table 6). In the first season, percentage of EO significantly increased (0.459, 0.530 and 0.651%) by reducing FC of the soil from 100 to 75 and 50%, respectively. There was no significant difference in EO yield between plants grown in soil with (100 and 75%) or (75 and 50%) FC. But plants grown in soil with 50% FC had EO yield of 0.145 ml/plant which was significantly higher than these of plants grown in soil with 100% FC.

Table(6): Effect of soil FC, VAM and *Azotobacter* inoculation on essential oil (EO) yield of herb and flower tops of *A. millefolium* in two seasons

Treatments	% of soil field capacity (A)							
	1 st season				2 nd season			
	100	75	50	Mean (B)	100	75	50	Mean (B)
	EO yield of herb (ml/plant)							
Control	0.057	0.051	0.055	0.054	.....	0.190	0.066	0.152
VAM	0.073	0.089	0.086	0.083	0.077	0.090	0.091	0.086
Azot	0.071	0.076	0.084	0.077	0.070	0.07	0.089	0.076
V+A	0.089	0.084	0.086	0.086	0.093	0.089	0.100	0.094
Mean A	0.072	0.075	0.078		0.110	0.110	0.086	
LSD 5%	A ns	B 0.035	AB ns		A ns	B 0.035	AB ns	
	EO yield of flower tops (ml/plant)							
Control	0.081	0.108	0.124	0.108	0.087	0.108	0.136	0.110
VAM	0.101	0.134	0.137	0.124	0.104	0.140	0.145	0.130
Azot	0.109	0.130	0.152	0.130	0.109	0.145	0.173	0.142
V+A	0.119	0.150	0.185	0.151	0.115	0.171	0.153	0.146
Mean A	0.105	0.130	0.151		0.104	0.141	0.152	
LSD 5%	A 0.030	B 0.035	AB ns		A 0.030	B 0.035	AB ns	

V+A =VAM + *Azotobazter*, ns= not signficant

Microbial inoculation had no significant effect in EO percentage which ranged from 0.526%, of control plants to, 0.571% of plants inoculated with *A. chroococcum* + VAM. While EO yield had been significantly affected due to microbial inoculation. All types of microbial inoculation increased EO yield over the control plants that had the lowest EO yield 0.098 ml/plant. There was no significant difference in EO yield between plants inoculated with *A. chroococcum* and VAM or between (VAM and VAM + *A. chroococcum*). Plants inoculated with *A. chroococcum* + VAM had the highest EO yield 0.159 ml/plant. Results showed that there was no significant interaction between water treatment and inoculation. When soil had 100% FC there was no significant difference among control plants and inoculated plants. Overall, control plants grown in soil with 75% FC had the lowest EO yield 0.081 ml/plant whereas plants grown in soil with 50% FC inoculated with *A. chroococcum* + VAM had the highest EO yield (0.185 ml/plant) which is 128% higher than the previous value. EO of control plants increased 37 % when FC reduced from 100 to 50% whereas these increments were 35, 39 and 55 % when plants were inoculated with *A. chroococcum*, VAM and *A. chroococcum* + VAM respectively.

Results indicated that yarrow herbage and flower tops had a higher percentage of volatile oil under low soil moisture content. However, unlike EO yield of flower tops, EO yield of herbage did not affect due to water treatment. The highest flower tops volatile oil yield has been obtained when plants were grown in soil with 50% FC. The relationship between water stress and percentage as well as EO yield of aromatic plants might be due to the reduction of plant growth with no effect in EO biosynthesis. Reduction in biomass (plant growth) will increase percentage of EO due to increase intensity of oil gland (Charles *et al*, 1990) The variation in EO by the alteration of water supply in different aromatic plants had been observed by Fatima *et al.*, (2002) in Cymbopogon, Mohamed *et al.*, (2000) in Tagetes. Plant inoculation increased volatile oil percentage as well as yield of both of herbage and flower tops especially under water stress condition. That could be as a result of water uptake improvement as well as plant nutrition state.

Nitrogen and phosphorus percentage

Results showed that both of water treatment and microorganism inoculation significantly affect N% of yarrow plants in both season. In the first season, there was no significant difference in N% between plants grown in soil at 100 and 75% (4.131and 4.558% respectively). However, plants grown in soil at 50% FC had N% (3.374%) significantly lower than the other two values. There was no significant difference in N% of yarrow plants between control and VAM-inoculated plants (3.158 and 3.488 % respectively) or between *A zotobacter* and *A zotobacter* + VAM inoculated plants (4.459 and 4.977 % respectively) Table (7).

There was a significant interaction between the two studied treatments. When plants, were grown in soil at 50% FC there was no significant difference in N% between the control and any inoculation treatment. All plants grown in soil at 75% FC had N% higher than those grown in soil at 100 or 50% FC. Similar results were recorded in the second

season. The lowest N% (2.721%) was estimated in uninoculated plants grown in soil with 100% FC whereas, the highest N % (5.840%) was in plants grown in soil with 75% FC and inocualted with *Azotobacter* + VAM. Nitrogen % of plants inoculated with any microorganism treatment decreased with reduction in FC%.

Table (7): Effect of soil FC, VAM and *Azotobacter* inoculation on percentage of N and P of *A. millefolium* in two seasons

Treatments	% of soil field capacity (A)							
	1 st season				2 nd season			
	100	75	50	Mean (B)	100	75	50	Mean (B)
	Nitrogen %							
Control	2.721	3.593	3.162	3.158	3.204	2.707	3.281	3.064
VAM	3.489	3.656	3.321	3.488	3.643	3.221	3.345	3.404
Azot	4.740	5.143	3.496	4.459	5.523	4.69	3.407	4.540
V+A	5.574	5.840	3.517	4.977	5.830	5.510	3.608	4.983
Mean A	4.131	4.558	3.374		4.550	4.033	3.410	
LSD 5%	A 0.588	B 0.679	AB 1.176		A 0.409	B 0.472	AB 0.817	
	P %							
Control	0.284	0.230	0.191	0.235	0.285	0.232	0.208	0.242
VAM	0.351	0.557	0.416	0.441	0.367	0.572	0.432	0.457
Azot	0.252	0.274	0.266	0.264	0.248	0.280	0.264	0.264
V+A	0.373	0.622	0.569	0.521	0.369	0.640	0.561	0.523
Mean A	0.315	0.421	0.360		0.310	0.431	0.366	
LSD 5%	A 0.030	B 0.035	AB 0.060		A 0.030	B 0.031	AB 0.040	

V+A =VAM + *Azotobazter*, ns= not signficant

In the first season, P% in yarrow plants significantly increased from 0.360 to 0.421% when soil FC increased from 50 to 75%, respectively. Thereafter P% significantly decreased to 0.315% when soil FC achieved at 100%. *Azotobacter* inoculation had no significant effect on P% as compared to the control plants. Whereas, VAM inoculation significantly increased P % (0.441%) compared to control or *Azotobacter* treatment (0.264%). Inoculation

with both microorganisms had the highest significant effect in P% over the other treatments. There was a significant interaction between the two studied factors. Plants grown in soil at 75% FC and inoculated with both microorganism had the highest P% (0.622%). VAM or VAM + *Azotobacter*-inoculated plants had significantly higher P percentage when soil FC decreased to 75%. Similar results were recorded in the second season.

The reduction in N and P % of yarrow plants grown in soil with low water content is similar to these results obtained by Mohamed and Abdou, (2003), in fennel. Low moisture content results in a decrease in the diffusion rate of nutrients particularly P from the soil matrix to the absorbing root surface (Goicoechea *et al.*, 1997). *Azotobacter* inoculation increased N% of yarrow plants whereas, VAM inoculation increased P% of plants these results are in agreement with Saad and Hammad, (1998).

So it is recommended to maintain soil FC of yarrow plants grown under theses condition at 50% with *Azotobacter* and VAM inoculation to obtain the highest EO yield using the lowest quantity of water. Soil inoculation with *Azotobacter* and/or VAM had a pronounced effect in yarrow growth and EO yield especially under water shortage condition.

REFRENCES

Abdel-Malek, Y. and Y.Z Ishac. (1968). Evaluation of methods used in counting *Aztobacter. J. Appl. Bact.*, 31: 269-275.

Armitage, A.M. (1992) Field Studies of *Achillea* as a cut flower: longevity, spacing, and cultivar response. *J. Amer. Soc. Hort. Sci.*, 117: 65-67.

Attia, F.A. and O.A.O. Saad (2001). Biofertilizers as partial alternative of chemical fertilizer for *Catharanthus roseus* G. Don. *J. Agric. Sci. Mansoura Univ.*, 26 (11): 7193-72080.

Bowen, G.D. (1973) Mineral nutrition of ectomycorrhizae. In Ectomycorrhizae (Marks G.C. and Kozlowski T.T ed.) pp. 313-319, Acadimic Press, London.

Breland, T.A. and L R. Bakken (1991). Microbial growth and nitrogen immobilization in the root zone of barley (*Hordeum vugar* L.), Italian regress (*Lolium multiforum Lam.*), and White clover (*Trifolium repens* L.). *Biol. Fertil. Soils*, 12, 154.

Charles, D.J.; R.J. Joly and J.E. Smith (1990) Effect of osmotic stress on essential oil content and composition of peppermint. *Phytochem.*, 2: 2837- 2840.

Clewer, A.G and D.H. Scarisbrick (2001) Practical Statistics and Experimental Design for Plant and Crop Science, John Wiley and Sons, Ltd., Chicheter, New York, Weinheim, Brisbane, Singapore and Toronto.

Cochran, W.G. (1950). Estimation of bacterial densities by mean of the most probable number. *Biometrics*. 6: 105.

Eastin, E.F. (1978). Total nitrogen determination for plant nutrient. *Analytical Biochem.*, 85, 591.

Faber, B.A.; R.J. Zasoski; D.N. Munns and K. Shackel (1991) A method for measuring hyphal nutrient and water uptake in mycorrhizal plant. *Can. J. Bot.*, 69: 87-94.

Fatima, S.; F. A. Abad and S. Sharma (2002) Physiological and m etabolic response of different genotype of *Cymbopogon martinii* and *C. winterianus* to water stress. *Plant Growth Regu.*, 37: 143-149.

Goicoechea, N.; M.C. Antolin and M. Sanchez-Diaz (1997). Influence of arbuscular mycorrhizal and rhizobium on nutrient content and water relations in drought stressed alfalfa. *Plant and soil*, 192: 261:268.

Guenther, E. (1961) The essential oils, Vol. 1 D. Von Nostrand Co., New York, 236.

Khalifa, M.R. and M.M. Bader (1992). Interaction between *Fusarium* root rot disese of faba bean and inoculation with mycorrhizal fungi and/or *Rhizobium leguminosarum* under different levels of NPK fertilization. *Egypt. J. appl. Sci.*, 7 (12): 851-861.

Letchamo, W.; X.U. Hi and A. Gosselin (1995) Photosynthetic potential of *Thymus vulgaris* selection under 2 light regimes and 3 soil-water levels. *Scientia Hort.*, 62:89-101.

Mathur, N and A. Vyas (2000) Influence of arbuscular mycorrhizae on biomass production, nutrient uptake and physiological changes in *Ziziphus mauritianalam.* under water stress. *J of Ari. Envi.*, 45: 191–195

Mohamed, M.A.H. and M Abdou. (2003) Growth and oil production of *Foeniclum vulgare* Mill 1. Effect of number of irrigation and rates of potassium fertilization. *Egyptian J. of Agric. Res.*, NRC- 1: 227-291.

Mohamed, M.A.H.; P.J.C. Harris and J. Henderson (2000) *In vitro* selection and characterisation of a drought tolerant clone of *Tagetes minuta. Plant Sci.*, 159, 213-222.

Mosse, B. and M. Giovanetti (1980). An evaluation of techniques for measuring vesicular arbusculr mycorrhizal infection in roots. *New Phytol.*, 84, 489-500.

Musandu, A.A.O. and K.E. Giller (1994). Effect of vesicular-arbusculr mycorrhiza on Kudzu Pueraria phaseoloides growth in phosphate fixing kenya soil. *Afri. Crop Sci. J.*, 2: 285-290.

Saad, O.A.O. (1995). Effect of plant residues, farm yard manure and endomycorrhizal fungi on nodulation growth and yield of rhizobia-inoculated faba bean. *Minia J. Agric. Res & Dev.*, 17: 375-388.

Saad, O.A.O. and E. T. Ahmed (2002). Response of *Leucaena leucocephala* seedlings to inoculation with Rhizobia, VA-mycorrhizal fungi, chemical ammendments and organic fertilization. Proc. Minia 1st Conf. for Agric. & Environ. Sci., Minia, Egypt, March 25-28, 2002. pp. 2017-2032.

Saad, O.A.O. and A.M.M. Hammad (1998). Fertilizing wheat plants with rock phosphate combined with phosphate dissolving bacteria and VA-mycorrhizae as alternative for Ca superphosphate. *Ann. Agric. Sci. Ain Shams Univ*, 43: 445-460.

Shangguan, Z.P., (1997) Regulation of nitrogen nutrition on photosynthetic characteristics of winter wheat in dryland. *Plant Nutr. Fert. Sci.*, 3: 105–110.

Shangguan, Z. P.; P. Shao and J. Dyckmans (2000) Nitrogen nutrition and water stress effects on leaf photosynthetic gas exchange and water use efficiency in winter wheat. *Envi. and Exp. Botany,* 44: 141–149

Sivamani, E.; A. Bahieldinb J.Wraith; T.Al-Niemi; W. E Dyer.; H. T.David and R. Qu. (2000) Improved biomass productivity and water use efficiency under water deficit conditions in transgenic wheat constitutively expressing the barley *HVA*1 gene. *Plant Sci.,* 155: 1–9

Smith, S. E. and D.J.Read (1997). *Mycorrhizal Symbosis*. San Diego, Academic Press.

Stary, F. and V. Jirasck (1975) A Concise Guide in Colour Herbs. Hamlyn, London, NewYork, Sydney and Tornto.

Tuomela, K. (1997) Leaf water relations in six provenances of *Eucalyptus microtheca*: A greenhouse experiment. *Forest Eco. and Manag.*, 92: 1-10.

Wilde, S.A.; R.B.Corey; J.G.Lyer and G.K. Voigt (1979). Soil and Analysis for Tree Culture. Ist Ed, p.98, Oxford and IBH Publishing Co., New Delhi.

تأثير الميكورهيزا والأزتوباكتر على نمو و إنتاج الزيت لنبات الأشيليا ميليفوليـــام تحت مستويات مختلفة من الماء

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لقد أزدادت المنافسة على المياه المتاحة للرى ، لذلك أجريت هذه الدراسة فى موســمين لدراسة تأثير التلقيح بالميكورهيزا و الأزتوباكتر على النمو و إنتاج الزيوت الطيارة لنباتات الاشيليا ميليفوليام .ولهذا النبات استخدامات مختلفة طبية و عطرية . وتم الزراعة فى تربــة رمليــة مــع استخدام ثلاث مستويات من الرى و هى ٥٠ ، ٧٥ ، ١٠٠ % من السعة الحقلية بالإضــافة إلــى الكنترول ، وتم تلقيح النباتات بكل من الميكورهيزا أو الأزتوباكتر أو خليط منهما . وقد أوضــحت النتائج أن للسعة الحقلية تأثير معنوى على الصفات المقدرة مثل طول النبات و وزن العشب طازج و جاف و وزن الأزهار طازج و جاف و كذلك محصول الزيوت الطيارة بالمقارنة بــالكنترول ، وقد أدى التلقيح الميكروبى إلى تحسين معنوى فى نمو النبات وفى محصــول الزيــوت الطيــارة بالمقارنة بالكنترول .وكان أعلى محصول للزيت فى القمم الزهرية ١٨٥، مل لكــل نبــات و قــد تحصل عليها فى النباتات الملقحة بخليط من الميكورهيزا و الازتوباكتر و النامية فــى ٥٠% مــن السعه الحقلية . وكان للتلقيح بالازتوباكتر زيادة معنوية فى النسبة المئويــة للنيتــروجين و غيــر معنوية فى الفسفور فى العشب بينما كان للميكورهيزا زيادة معنوية فى النسبة المئوية للفسفور فى العشب . وأيضا كان للتلقيح بخليط الميكروبات تأثير مفيد في نمو النبات خصوصا تحت المستوى المنخفض من السعة الحقلية. بالاضافة إلى أنه كان للتلقيح بخليط الميكروبات تأثير مفيد عنه فــي حالة التلقيح بأحد الميكروبات منفردا .