

EFFECT OF BIO- FERTILIZATERS AND PLANT EXTRACTS ON GROWTH, ESSENTIAL OIL AND CHEMICAL CONSTITUENTS OF SAGE (*Salvia officinalis* L.) PLANT, UNDER WATER STRESS CONDITIONS.

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ABSTRACT

The present work was carried out at a private Farm in Sammenoud area, Gharbieh Governorate during the two successive seasons of 2011-2012 and 2012-2013. The present study aimed to investigate the effect of bio fertilizers (VAM fungi and phosphorine) and foliar spray of plant extracts (garlic and aloe) on some vegetative growth (plant height, fresh and dry weight) essential oil percentage and chemical constituents of sage (*Salvia officinalis* L.) plant under different water stress conditions (plants irrigated every 15, 30 and 45 days).

The results showed that irrigation of plants every 15 and 30 days were effective in increasing the productivity of vegetative growth, essential oil percentage and uptake of N,P and K % in sage. Spraying plants with garlic extract was more effective than aloe extract at in improving the productivity of vegetative growth, essential oil percentage and uptake of N, P and K content. Plants treated with VAM fungi were effective in increasing the productivity of vegetative growth, essential oil percentage and uptake of N, P and K %.

INTRODUCTION

Sage (*Salvia officinalis* L.) plant, Fam. Lamiaceae is a perennial herb indigenous in Southern Europe, cultivated in central Europe and found now in temperature climate regions. It is now almost entirely utilized in the food industry as a flavoring and seasoning herb and condiment. Large quantities have been used in the commercial meat packing and processing. Sage is also a most important ingredient of mixed herb as well as being used in herbal teas for medicinal purposes, (Haridi, 1987).

Water stress in plant influences many metabolic processes and the extent of its effects depend on drought severity. The optimization of irrigation for the production of fresh herbs is important since water is a major component of the fresh produce and affects both weight and quality, (Jones and Tardien, 1998). Drought stress limits the production of 25 % of the world land, (Delfine *et al.*, 2005). 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, (Sarker *et al.*, 2005).

The importance of using biofertilizers is to reduce the amount and cost of chemical fertilizers and to eliminate environmental pollution, (Abd EL-Fattah, 1998). Biofertilizers are generally capable of inducing beneficial effect

on a compatible host based on altering the rhizosphere flora, by seed or soil inoculation with certain organisms. Biofertilizers mainly comprise phosphate solubilizers or Vesicular Arbuscular Mycorrhizae (VAM). These organisms may affect their host plant by one or more mechanisms such as nitrogen fixation, production of growth promoting substances or organic acids, enhancing nutrient uptake or protection against pathogens. The significant effect of biofertilizers may be due to the effect of different strain groups and nutrients mobilizing microorganisms which increased levels of extractable minerals (EL-Kramany *et al.*, 2000). Khater (2001) found that caraway plants receiving Phosphorine inoculation were significantly taller, had more fresh and dry weights of leaves, stems and whole plant in addition to higher N, P and K % in herb per plants over than uninoculated control plants. Shalan *et al.* (2001) observed that Phosphorine biofertilizer resulted in the highest values of vegetative growth of *Matricaria chamomilla* plant when compared with control plants. Abo EL-Ala (2002) showed that roots colonization by N-fixers and P-dissolving bacteria significantly improved marjoram and basil plant growth as compared with control. Eisa (2004) found that all biofertilizer treatments increased the vegetative growth and N, P and K % in *Salvia officinalis* L. plants. Abbaspour *et al.* (2012) indicated that when the arbuscular mycorrhiza (AM) were inoculated to pistachio (*Pistacia vera* L.), plant growth was higher for well-watered than for water-stressed plants, and that P, and K contents in AM treated shoots were greater than those in non-AM shoots under well-watered conditions and drought stress, while N content was higher under drought stress conditions. Gholamhosini *et al.* (2013) mentioned that irrespective of the mycorrhizal species and the drought stress intensity, sunflower inoculated plants produced more dry matter and were highest in P and N % in both leaves and seeds.

Different plant extracts also affect plant growth. Helmy (1992) remarked that soil side dressing of garlic extract at 250 mg DW / plant gave the best results in increasing the number of flowers of summer squash plants. Lindsey *et al.* (2002) showed that aloe extract could be used to improve the germination, vegetative growth and flowering of plants. EL- Shayeb (2009) reported that spraying aloe extract at 75 % significantly increased potassium content the next value was with garlic extract at 75 %. of *Oenothera biennis* plant. El-Shayeb (2009) declared that the highest concentration of aloe extract increased fresh and dry weights of *Oenothera biennis* flowers. Mady (2009) showed that treating *Majorana hortensis* and *Salvia officinalis* plants with garlic extract concentration at 50 or 100 % stimulated fresh and dry weights in the two cuts. Ahmed *et al.* (2014) indicated that, most treatments of some medicinal plant extracts (garlic at 50 % and *Aloe vera* with four concentrations 25, 50, 75 and 100 %), significantly increases plant height, number of branches, dry weight of herb and essential oil yield compared with control on basil (*Ocimum basilicum*) plant. Helmy (2003) found that the highest fixed oil percentages were obtained from the treatment irrigated every 3 week and fertilized with half doses of NPK - recommended rate combined with both biofertilizers (biogen + phosphorin) in both seasons of roselle plants.

The major objective of the present study was to investigate the effect of some sources of biofertilizers and some plant extracts and their combinations on the growth, essential oil percentage and chemical content of sage (*Salvia officinalis* L.) plant under different water stress conditions.

MATERIALS AND METHODS

The field experiment was conducted during the two successive seasons of 2011/2012 and 2012/2013 at a private Farm in Sammanoud, Gharbieh Governorate, Egypt. Seeds of sage were obtained from Arish, North Sinai, Egypt. Seeds were sown in prepared nursery beds on October 15th in both seasons. The growing seedlings were transplanted after 80 days from sowing at 20 cm apart on the eastern side of rows in an irrigated soil. The experimental area was divided into 1.5 x 3 m plots, each containing five ridges 1.5 m at 60 cm distance. Each row contained 5 plants 30 cm apart (25 plants per plot). Soil samples were obtained at 30 cm depth from soil surface and were analyzed at laboratories of Agriculture Research Center, Ministry of Agriculture. The soil physical and chemical properties are presented in Table (A).

Table (A): Some physical and chemical characteristics of the experimental soil before the two seasons.

The analysis	1 st season	2 nd season
Physical		
Sand %	27.35	27.79
Silt %	30.89	31.74
Clay %	41.57	41.13
Soil texture	clay	clay
Chemical		
pH	7.9	8.1
Organic matter %	1.85	1.93
Available nutrients		
N (mg / kg)	263	290
P (mg / kg)	25.7	27.3
K (mg / kg)	539	527
Fe (mg / kg)	5.29	5.05
Mn (mg / kg)	3.53	3.89

The experimental design was split– split plot design with 3 replicates. Main plots were assigned for water stress of 15, 30 and 45 days (I₁, I₂ and I₃ respectively) . Sub-plot were assigned for biofertilizer treatments control (C), Phosphorine (P) and Vesicular Arbuscular ycorrhizae VAM (M). The Sub-sub plots were foliar spray with plant extracts; Aloa extract (A) and Garlic extract (G), were distributed randomly. Three water stress treatments combined with two application rates of plant extracts and three applications of bio fertilizers formed 18 interaction treatments.

Preparation of inoculums:

Sage seedlings were inoculated before transplanting with dissolved bacteria (1 kg/ fed) in 5-liter water and 100 g arabic gum was applied, and the root of sage seedlings were dipped in this suspension for 10 minutes after one month from transplanting. The soil inoculation was repeated by bacteria fertilizers at 4 kg/fed, mixed with wet soft soil (1:10 ratio) into the root absorption zone of the plant then covered with the fine soil and irrigated immediately during the both seasons. Bacteria fertilizer (Phosphorine) was prepared as described by (El-Zeiny et al., 2001). The soil of the used pots was mixed with VAM spores as described by Musandu and Giller (1994). The spores count was found to be about 132 spores / 1 g soil. This soil which contained mixture of VAM spores, mycelia and chopped roots was applied at about 50g /plant, and was incorporated into the soil before irrigation of the soil and transplanting.

Preparation of the plant extract:

Aloe extract was prepared as described by Wilfred et al. (1990). Garlic extract was prepared as described by El- Desouky et al. (1998). Foliar spray of aloe and garlic extracts at 75 % for each one sprayed twice every 21 days in the both seasons.

Harvesting:

Sage plants were harvested twice yearly by cutting the aerial parts of each plant (10-cm) above the soil surface. The first cut was at the 15th May (at commencement of the flowering), while the second one was done four months after the first cut.

Data recorded

A random sample of four plants from each experimental unit were taken at the harvesting stage at two cuts in both seasons for determination of vegetative growth (plant height, fresh and dry weight).

Essential oil percentage was determined in the dried leaves samples (100g) by subjecting to hydro distillation using modified Clevenger traps in British Pharmacopeia (2000).

Plant samples were analyzed at the laboratory of the Medicinal and Aromatic Plants Dept., Hort. Institute, Agric. Res. Center. At cutting date herb was dried in an electric oven at 70C for 24 hr. according to A.O.A.C. (1970), then finely ground for chemical determination of NPK. The total nitrogen was determined according to the method of Jackson (1967) by a modified micro-Kjildahle apparatus. But, Phosphorus was determined calorimetrically according to the method of Murphy and Reily (1962). While, Potassium was measured using Flame photometer as described by Wilde et al. (1985).

Statistical analysis:

All the obtained data were statistically analyzed of variance (ANOVA) in split-split plot design. The treatment means were compared using the least significant difference (L.S.D.) test at 5% as described by Gomez and Gomez (1984),

RESULTS AND DISCUSSION

Vegetative growth characters:

Effect of water stress:

Data in Table (1) showed that vegetative growth (plant height fresh and dry weight) of sage plants was influenced by water stress. Water intervals of 30 and 15 days stimulated plant growth and elongation as compared with water stress at 45 days interval. The most effective irrigation treatment was 30 days irrigation intervals. It has been shown that several biochemical parameters could be affected by moderate water stress due to changes in hormone and enzyme activities. Abscisic acid is a plant hormone that is produced in the roots in drying soils and it is transported by water flow in xylem to the shoot for regulating the shoot physiology and limits stomata conductance (Kang and Zhang, 2004). It is well known that water is lost through transpiration, and CO₂ is absorbed for photosynthesis through stomata. Therefore, any variation in stomata opening will affect stomatal conductance and photosynthesis rate. Reduced stomatal conductance in early stages of water stress inhibits transpiration rate more than it reduces the intercellular CO₂ concentration, which is the driving factor for photosynthesis. The advantage of water stress irrigation at 30 days intervals is that moderate deficit irrigation would maintain a favorable plant water status, while the roots in the dry side promote the increase in abscisic acid production and decrease the stomatal conductance (Saeed *et al.*, 2000). However, at severe water stress, the leaf water potential in mesophyll cells decreases and stomata will close to a greater extent that inhibits the photosynthesis rate, which is known as hydraulic signaling (Taiz and Zeiger, 2002).

These results are in agreement with those obtained by Nour Eldeen (2010) on majoram, Cerekovic *et al.* (2013) on *Ribes nigrum* L. plant, and Stagnari *et al.* (2014) on citrus.

Effect of biofertilizers:

It is also clear from data in Table (1) that biofertilizers (VAM fungi and Phosphorine) enhanced vegetative growth (plant height fresh and dry weights) of *Salvia officinalis* plants at the two cuts in both seasons, and VAM treatment was the superior in this respect. VAM fungi gave a significant superiority in vegetative growth characters at the first cut in the two seasons in comparison to treated plants with Phosphorine biofertilizer. The promoting effect of VAM fungi on vegetative growth could be due to the increase in both number and length of stem internodes. It is clearly known that VAM fungi play an important role in enhancing plant growth and metabolism. Such results may be attributed to the main role of VAM fungi to achieve maximum growth by increasing uptake of soil phosphate more than other nutrients and its translocation to the host root through a specific efficient active mechanism (Cooper and Tinker, 1978). The positive effect of VAM may be due to its improving effect on photosynthesis and respiration in addition to its roles in cell division and development of meristematic tissues (Mengel and Kirkby, 1982). The increment in phosphorus uptake could accelerate cell division and

growth, energy transfer, signal transduction, biosynthesis of macromolecules, photosynthesis and respiration (Plaxton and Carswell, 1999). In addition, VAM inoculation could be associated with substantial improve in phosphorus uptake and increasing phosphorus concentration in plant tissues (Mohamed and Saad, 2004).

These results are in harmony with the findings of Nasseem et al. (2000) on sodan-grass, Shalan (2001) on chamomile, Helmy (2003) on roselle, Kandeel and Sharaf (2003) on marjoram and Eisa (2004) on sage.

Effect of plant extracts:

Data dealing with the effect of plant extracts on the vegetative growth (plant height, fresh and dry weights of sage plants) during the two cuts in the both growing seasons were presented in Table (1). Spraying sage plants with garlic extract produced the highest values of growth parameters when compared with the treatment of aloe extract, which gave the lowest values of these characters in the two cuts of both seasons. The superiority of plant growth with spraying of garlic extract might be attributed to the fact that garlic extract contains considerable amounts of plant nutrients, especially sulfur, in addition to the protective effect of garlic extract against most plant pathogen infections, (Lampkin, 1994). Regarding the growth enhancing potential of garlic extract might be attributed to being contain natural sources of many growth promoting substances (macro and micronutrients, GA₃). The fresh extracts of *Allium sativum* can be used to improve the vegetative growth of many plants, (El-Desouky et al., 1998).

These results are in agreement with those obtained by Youssef (1997) on *Delphinium ajacis* L., *Antirrhinum majus* L. and *Callistephus chinensis* plants, Tartoura et al. (2013) on squash.

Effect of the interaction treatments :

Data presented in Table (2) disclosed that plant height fresh and dry weights per plant significantly increased due to the interaction effect among irrigation stress, bio-fertilizers and plant extracts. Irrigating plants every 30 days with VAM fungi inoculation and spraying by garlic extract produced the highest values of vegetative growth characters at the two cuts in both seasons. Whereas, the lowest values were obtained from the plants irrigated every 45 days without bio-fertilizers and sprayed with aloe extract. Regarding interactions between foliar application and biofertilizer treatments, data in the same table showed that, fertilized plants with VAM fungi inoculation and sprayed with garlic extract had better vegetative growth parameters. These increases may be attributed to the phosphate solubilizing bacteria of Phosphorine inoculums, which may have played a great role in contributing growth hormones, such as auxins, gibberellins or cytokinins, which could stimulate plant growth (EL-Sheekh, 1997). Sheng Wu et al. (2013) on citrus using AMF under water stress, indicated that AM colonization produced a positive effect on plant growth and photosynthesis, even under drought stress. This review provides an overview of possible mechanisms involved in DS tolerance through improved water and nutrient uptake (especially P, Mg, K and Ca nutrition); effective spatial configuration of root system, carbohydrates.

Essential oil percentage:

Effect of water stress:

The essential oil percentage in the dried herb of sage plants varied by water stress (all 15, 30 and 45 days) treatments (Table, 3). Irrigated plants all 15 or 30 days increased the essential oil percentages in sage herb when compared with irrigated plants at 45 days. The highest oil percentage obtained from the irrigated plants all 30 days were 1.02 and 0.97% respectively, in the first and second cuts of first growing seasons, and these values were significant as compared with irrigated plants at 15 or 45 days in the two cuts during first season. While the least oil percentages were 0.77 and 0.88 % produced from irrigated plants all 45 days. The same trend was observed in the second season as in the first one. Shoala (1992) indicated that irrigation had a significant effect on the oil production of lemongrass (*Cymbopogon citratus* L.) plant. The highest oil % was produced in most cases with 30 days irrigation intervals.

These results are in agreement with those obtained by Nour Eldeen(2010) on *majorum*.

Effect of biofertilizers :

Treatments with vesicular arbuscular Mycorrhizae (VAM) and Phosphorine, as shown in Table (3), clearly showed that inoculation with VAM fungi produced the highest values of the essential oil percentage, were 1.00 and 0.93 % when compared with phosphorine biofertilizer 0.91 and 0.86 % respectively, at both cuts through first season,. The differences between inoculated sage plants with VAM fungi and phosphorine biofertilizer were significant increases the essential oil percentage in the dried herb at two cuts, of first season., the same trend was observed as in the first one in the second season. Khater (2001) noticed that caraway essential oil % in fruits was significantly increased with treating plants by Phosphorine inoculation when compared to untreated plants. Kandeel and Shaeaf (2003) showed that essential oil percentage of marjoram herb was considerably influenced by inoculation with Vesicular Arbuscular Mycorrhizae fungi plus application of half or full doses of NPK compared with control plants (full dose NPK).

These results are in agreement with those obtained by Eisa (2004) on sage.

Effect of plant extracts:

Data illustrated the effect of plant extracts on the essential oil percentage in the dried herb of plants are presented in Table (3). It revealed that during first season, application of garlic extract caused an increase in the essential oil percentage (0.93 and 0.88 % in two cuts, respectively). The increment in the second cut during the first season was significant if compared to sprayed plants with aloe extracts. Also, in the second season the essential oil percentage increased in sage herb when sprayed with garlic extract and this increment was significant if compared with aloe extracts at the both cuts.

These results are in agreement with those obtained by Mady (2009) on *Majorana hortensis* and *Salvia officinalis* plants and Ahmed *et al.* (2014) on basil

Table (3): Effect of irrigation, bio fertilizers and plant extracts on essential oil % of *Salvia officinalis* L. plant at the two cuts during 2011/2012 and 2012/2013 seasons.

Treatments	Essential oil percentage / plant			
	1 st seasons		2 nd seasons	
	1 st cut	2 nd cut	1 st cut	2 nd cut
Irrigation				
I ₁	0.91	0.85	0.92	0.85
I ₂	1.02	0.97	1.04	0.97
I ₃	0.83	0.77	0.86	0.75
L.S.D at 0.05	0.010	0.018	0.006	0.005
Plant Extracts				
A	0.91	0.85	0.93	0.85
G	0.93	0.88	0.95	0.86
L.S.D at 0.05	0.006	0.008	0.010	0.010
Bio fertilizers				
C	0.85	0.80	0.88	0.78
P	0.91	0.86	0.86	0.87
M	1.00	0.93	1.02	0.95
L.S.D at 0.05	0.007	0.001	0.004	0.005

I = irrigation G = Garlic extract A = Aloa extract P = Phosphorine M= Mycorrhizae

Effect of the interaction treatments :

It is clear from the data in Table (4) that there was significant effect of interaction between water stress, biofertilizers plus plant extracts on the essential oil percentage of herb compared with plants uninoculated with mycorrhizae or phosphorine biofertilizer.

Table (4): Effect of the interaction treatments on essential oil (%) of *Salvia officinalis* L. plant at the two cuts during 2011/2012 and 2012/2013 seasons.

Treatments			Essential oil (%)			
			1 st seasons		2 nd seasons	
			1 st cut	2 nd cut	1 st cut	2 nd cut
I ₁	C	A	0.84	0.75	0.90	0.78
		G	0.84	0.80	0.87	0.77
	P	A	0.92	0.87	0.95	0.82
		G	0.92	0.87	0.95	0.83
	M	A	0.95	0.92	0.97	0.95
		G	0.98	0.92	0.97	0.96
I ₂	C	A	0.92	0.87	0.97	0.84
		G	0.93	0.88	0.97	0.85
	P	A	0.98	0.97	0.98	0.98
		G	1.00	0.98	1.00	0.98
	M	A	1.11	1.00	1.11	1.09
		G	1.20	1.10	1.20	1.10
I ₃	C	A	0.78	0.75	0.77	0.71
		G	0.79	0.76	0.81	0.72
	P	A	0.81	0.72	0.84	0.72
		G	0.81	0.73	0.85	0.73
	M	A	0.87	0.82	0.93	0.82
		G	0.90	0.85	0.94	0.87

L.S.D at 0.05	0.019	0.020	0.31	0.030
I = irrigation	G = Garlic extract	A = Aloa extract	P = Phosphorine	M= Mycorrhizae

In the first season, the maximum essential oil percentage were produced from irrigated plants all 30 days, inoculated with mycorrhizae and sprayed with garlic extract, were 1.00 and 0.98 % at two cuts, respectively. The percentage for increasing are over irrigated plants all 45 days (0.84 and 0.75 %) by 13.7 and 23.7 % in two cuts, respectively. Similar effect was obtained of the second season. The data recorded in the season confirmed those of the first one. Helmy (2003) found that the highest fixed oil percentages were obtained from the treatment irrigated every 3 week and fertilized with half does of NPK - recommended rate combined with both biofertilizers (biogen + phosphorin) in both seasons of roselle plants. These results are in agreement with those obtained by Ahmed et al. (2014) on basil plant.

Nitrogen, phosphorus and potassium uptakes:

Effect of water stress:

Data in Table (5) clearly showed that nitrogen percentage in herb tissues was found to be influenced by water stress at different rates. The moderate water stress (at 30 days irrigation intervals) stimulates the uptake of nutrients in both seasons as compared with 15 and 45 days irrigation intervals. Skinner et al. (1999) indicated that moderate water stress successfully increased N uptake and reduced the potential for NO₃⁻ leaching under environmental conditions, which allowed adequate root development. This was reflected positively on increasing N concentration in cabbage plants. On the other hand, water stress treatments were associated with a decrease in P and K concentration in plant (Kang and Zhang 2004). It is obvious that soil nutrients availability is a function of soil chemistry and regulated by the dynamic changes of soil moisture. For the nutrient transport from the soil to the root surface, mass flow and diffusion are two different mechanisms. Water stress treatments reduce both mass of flow and diffusion rates and the release of slowly released nutrient into available form. To explain these results, we should mention that diffusion is the main mechanism for the movement of phosphorus and potassium to the root surface and it contributes with more than 90 % for P and 80 for K from the whole P and K uptake, (Marschner 1995). Helmy (2003) indicated that irrigated roselle plant every 1 week and fertilized with 50 % NPK recommended rate + biogen + phosphorin produced the highest anthocyanin content as well as N, P and K contents in both seasons.

Effect of biofertilizers:

According to the data in Table (5) colonization of plant roots by arbuscular mycorrhizal fungi can greatly increase the plant uptake of nitrogen phosphorus and potassium. The most prominent contribution of arbuscular mycorrhizal fungi to plant growth is due to uptake of nutrients by extra radical mycorrhizal hyphae. The utilization of soil nutrients may depend more on efficient uptake of phosphate, nitrate, ammonium and potassium from the soil solution even at stress conditions (e.g. water stress) than on mobilization

processes in the rhizosphere (George et al., 1995). These results are in agreement with those obtained by Eisa (2004) on sage.

Effect of plant extracts:

The data of N, P and K (%) in herb of plants were shown in Table (5). The results clearly demonstrated that, garlic extract caused a slight increase in the percentage of N, P and in herb during both cuts in the two cuts during both seasons. The differences between spraying with garlic and aloe extracts were significant in the all cut in the two seasons. Concerning the effect of plant extracts spraying, it is clear that plant spraying with garlic extract was the superior treatment on enhancing nutrients uptake by plants. This could be attributed to the protective effect of garlic extracts against plant pathogens, which improved plant vitality, growth and the efficiency of nutrients uptake. These results are in agreement with those obtained by Tartoura et al. (2013) on squash.

Effect of the interactions treatments :

Data presented in Table (6) disclosed that the nitrogen, phosphorus and potassium concentrations significantly increased due to the interaction effect among irrigation intervals, biofertilizers and plant extract treatments. Irrigation the plants every 30 days with inoculum plants with VAM fungi and spraying by Garlic extract produced the highest values of nutrients concentrations of N% in plant tissues, and Irrigation the plants every 15 days with inoculums plants with VAM fungi and spraying by Garlic extract produced the highest values of nutrients concentrations of P and K % in plant tissues. Omirou et al. (2013) on watermelon plants using water stress (W) and no water stress (NW)) and using AM fungi (non mycorrhiza (NM) and with mycorrhiza (M) mentioned significant reduction of root -N and -P content. Inoculation of plants grown under water stress resulted in a significant increase of water use efficiency. Heidari and Karami (2014) on sunflower using water stress treatment (W1=90,W2 =70 and W3=50 % of field capacity) and two different mycorrhiza species, mentioned that by increasing water stress from control (W1) to (W3) treatment, the content of potassium in seeds significantly decreased due to water stress but water stress up to W2 treatment increased the content of phosphorus, nitrogen and oil content of seeds. These results are in agreement with those obtained by Eisa (2004) on sage.

It could be recommended that irrigating plants every 30 days, inoculated with VAM fungi and sprayed with garlic extract gave the highest values of vegetative growth characters as well as essential oil percentage and N %.

REFERENCES

- A.O.A.C. (1970). Official Methods of Analysis of the Association of Official Agricultural Chemists (A.O.A.C.) Twelfth Edition, Washington. D.C.
- Abbaspour, H.; S. Saeidi-Sarb; H. Afshari and M.A. Abdel-Wahhab (2012). Tolerance of Mycorrhiza infected Pistachio (*Pistacia vera* L.) seedling to drought stress under glasshouse conditions. J.Plant Physiology 169: 704– 709.
- Abd El-Fattah, A.E. (1998). Effect of bio and mineral phosphate fertilization on growth and productivity of globe artichoke (*Cynara scolymus*) under newly reclaimed calcareous soil conditions. Assiut J. Agric. Sci., 29(3): 227-240.
- Abdel-Fattah, G. M. and Y. Shabana (2003). The use of *Glomus fasciculatum*, Mycorrhizal fungi to overcome the effect of the industrial wastes on the growth of cowpea. J. Agric. Sci. Mansoura Univ., 28 (7): 5387-5403.
- Abo EL-Ala (2002). Biofertilization techniques used for improving production of some medicinal plants in desert soil . Ph.D. Thesis, Inst. Environ. Studies and Res. Ain shams Univ., Cairo Egypt.
- Ali, A.F., Eatemed Osman and M.R. khater (2001). Effect of Phosphorine and potassium sulphate on guar, *Cyamopsis tetragonoloba* L. Taub. Egypt.
- Ahmed, K.Sh.; Kh. Hammam and A. Amer (2014): Effect of bio fertilization and some medicinal plant extraction on the growth, yield and chemical constituents of basil plant. J. Plant Production Mansoura Univ. 5(2): 193-210.
- British Pharmacopoeia (2000): The Pharmaceutical Press, 17. Bloomsbury Square, London W.C.L.
- Cooper, K.M. and P.B. Tinker (1978). Lipid physiology of vesicular-arbuscular mycorrhizas. I. composition of lipids in root of onion, clover and ryegrass with *Glomus mosseae* New, Phytol. 88: 327.
- Cerekovic, N.; M. Pagter; H. Pedersen; R. Brennan and K. Petersen (2013): Effects of drought stress during flowering of two pot-grown blackcurrant (*Ribes nigrum* L.) cultivars. Scientia Horticulturae. 162: 365–373.
- Delfine, S., F. Loreto, P. Pinell, R. Tognetti and A. Alvino (2005). Isoprenoids content and photosynthetic limitations in rosemary and spearmint plants under water stress. Agric.Ecosystems and Envi. 106: 243-252.
- Eisa Abo El-Ala. (2004). Effect of some biofertilizers on salvia plant. Ph. D. Thesis, Fac. Agric., Mansoura Univ.
- EL-Shayeb, N. S. A. (2009). Physiological Studies on *Oenothera biennis* (Bio-fertilizer & plant extracts) (Evening primrose). Ph. D. Thesis, Fac. Agric., Moshtohor, Benha Univ., Egypt.
- El-Desouky, S. A., A. Waneis and Z. Khedr (1998). Utilization of some natural plant extracts (garlic and yeast) as seed – soaked materials to squash (*Cucurbita pepo* L.) I- Effect on growth, sex – expression and fruit yield and quality. Ann. Agric. Sci., Moshtohor, 36 (2) 839 – 854.
- El-Kramany, M.F.; Ahmed, M.K.; Bahr, A.A. and Kabesh, M.O. (2000). Utilization of bio-fertilizers in field crop production. Egypt. J. Appl. Sci., 15(11): 137.

- EL-Sheekh, H.M. (1997). Effect of bio and mineral phosphate fertilizers on growth, yield quality and storability of onion . Egypt, J. Appl. Sci., 12 (12): 213-231.
- EL-Zeiny, O.A., U.A. EL-Behariy and M.. Zaky (2001). Influence of biofertilizer on growth, yield and quality of tomato grown under plastic house. J. Agric. Sci. Mansoura Univ., 26 (3): 1749-1763.
- George, E., H. Marschner and I. Jakobsen (1995). Role of Arbuscular Mycorrhizal Fungi in Uptake of Phosphorus and Nitrogen from Soil. 15:257-270.
- Gholamhosini, M., A. Ghalavand, A. Dolatabadian, E. Jamshidi and A. Khodaei-Joghan (2013). Effects of arbuscular mycorrhizal inoculation on growth, yield, nutrient uptake and irrigation water productivity of sunflowers grown under drought stress. Agricultural Water Management. 117: 106–114.
- Gomez, K.H. and A.A. Gomez (1984). Statistical Procedures for Agriculture Research. John Willy and Sons, Inc., New York.
- Haridi, S.M.M. (1987). Biochemical studies on sage plant (*Salvia officinales* L.) Ph. D. Thesis, Fac. Agric. Ain Shams Univ.
- Helmy, E.M.S. (1992). Response to summer squash application methods of fresh garlic extracted by different solvents. Alexandria J. Agric. Res., 37: 125-142.
- Helmy, Laila M. (2003). Studies on the effect of irrigation intervals, bio and chemical fertilization on roselle plant productivity. J. Agric. Sci. Mansoura Univ., 28 (5): 3927-3945.
- Heidari, M. and V. Karami (2014): Effects of different mycorrhiza species on grain yield nutrient uptake and oil content of sunflower under water stress. J. Saudi Society Agric. Sci.13: 9-13.
- Jackson, M.L. (1967). Soil Chemical Analysis. Printic Hall of India, New Delhi, pp. 144-197.
- Jones, H.G. and F. Tardien (1998). Modelling water relations of horticultural crops: a review. Sci. Hort., 74,21-46.
- Kandeel, A.M. and M.S. Sharaf (2003). Productivity of *Majorana hortensis*, L. plants as influenced by the interaction between mineral and biological fertilization. J. Agric. Sci. Mansoura Univ., 28 (2): 1373-1389.
- Kang, S. Z. and J. H. Zhang (2004). Controlled alternate partial root-zone irrigation: its physiological consequences and impact on water use efficiency. J. Exp. Bot. 55:2437-2446.
- Khater, Rania M.R. (2001). Effect of some fertilizers treatments on the growth and volatile oil yield on *Carum carvi* plants. M. Sci. Thesis, Fac. of Agric. Zagazig Univ.
- Lampkin, N. (1994). Organic farming. Published by Farming Press Books and Videos Wharfedale Road, Ipswich Ip 14 LG, UK., pp. 13-85.
- Lindsey, K. L., A. K. Jager and A. M. Viljoen (2002). Cyclooxygenase inhibitory activity of aloe species. South African. J of Bpt., 68,(1), 47-50.
- Mady, A. (2009). Effect of certain medicinal plant extracts on growth, yield and metabolism of some medicinal aromatic and plants. M. Sc., Al-Azher Univ.

- Marschner H. (1995). Mineral Nutrition of Higher Plants (2nd Edition). ACADEMIC PRESS Harcourt Brace & Company, Publishers London San Diego New York Boston Sydney Tokyo Toronto.
- Mengel, K. and E.A. Kirkby (1982). Principles Plant Nutrition, International Potash Institute, Bern, Switzerland.
- Mohamed, M. and O. Saad (2004): Effect of VA-Mycorrhizae and Azotobacter on growth and oil production of *Achillea millefolium* L. plant under different water regime. J. Agric. Sci. Mansoura Univ., 29 (1): 391-407.
- Murphy, J. and J.P. Reily (1962). A modified single method for determination of phosphorus in natural water. Anal. Chemi. Acta., 27: 31-36.
- Musandu, A.A.O. and K.B. Giller (1994). Effect of Vesicular-Arbuscular Mycorrhiza on kudzu (*Pueraria phaseoloides*) growth in phosphate fixing Kenya soil. African crop Sci. J. 2, 97.
- Nasseem, M.G.; F.I. Radwan; M.A. Hussin and A.M. Omran (2000). Effect of VA-Mycorrhizae, sulphur and phosphorus on growth and nutrient contents of millet and sudan-grass crops. Adv. Agric Res. Alexandria Univ., Vol. 5 No 1: 1173-1185.
- Nour Eldeen, El-S. A. El-S (2010): Effect of water stress on marjoram (*Majorana hortensis* L.). M. Sc. Thesis Fac. Agric. Mansoura Univ.
- Omirou, M.; L. M. Loannides and C. Ehalotis (2013): Mycorrhizal inoculation affects arbuscular mycorrhizal diversity in watermelon roots, but leads to improved colonization and plant response under water stress. Applied Soil Ecology . 63: 112-119.
- Plaxton, W.C. and M.C. Carswell (1999): Metabolic aspects of the phosphate starvation response in plants. In Responses to Environmental Stresses: From phytohormones to Genome Reorganization. Edited by Lerner HR. New York: Dekker; 1999: 349-374.
- Saeed, T. W.; V. Nouman; E. EL-Sayed and S. Sari EL-Deen (2000). Effect of Mycorrhizae inoculation and Phosphorine fertilization on growth patterns and leaf mineral content in transplants of two almond cultivars. Zagazig J. Agric. Res. 27(2): 397-410.
- Sarker, B., M. Hara and M. Uemura (2005). Proline synthesis, Physiological responses and biomass, yield of eggplants during and after repetitive soil moisture stress. Sci. Hort., 103, 387-402.
- Shalan, M.N., E. EL-Ghawwas, M. Dessouky and S. Soliman (2001). Effect of sources and levels of phosphorus fertilization on polish chamomile (*Matricaria chamomilla* L.). J. Agric. Sci. Mansoura Univ., 26(4): 2215-2233.
- Skinner, R.H., J.D. Hanson and J.G. Benjamin (1999). Nitrogen uptakes and partitioning under alternate- and every-furrow irrigation. Plant Soil 210, 11–20.
- Stagnari, F.; A. Galieni; S. Speca and M. Pisanta (2014): Water stress effects on growth, yield and quality traits of red beet. Scientia Horticulturae. 165: 13–22.
- Sheng Wu, Q.; A.K. Srivastava and Ying-Ning Zoua (2013): AMF-induced tolerance to drought stress in citrus: A review. Scientia Horticulturae. 164: 77–87.

- Shoala, A. W. (1992): Effect of irrigation and chemical fertilization treatments on lemongrass (*Cymbopogon citratus* L.) plants. M.Sc. Thesis, Fac. Agric., Cairo Univ.
- Taiz, L. and E. Zeiger (2002). Plant physiology, 3rd edn., Sinauer Associates, Sunderland, Mass.
- Tartoura, E. A. A. ; E. E. El-Gamily.; Y. B. A. El-Warakly and A. A. M. Fayed (2013) : The impact of organic and mineral fertilizations, plant spacing and foliar application of yeast and garlic extract on seed production of squash. *J. Plant Production Mansoura Univ.* 4(11): 1691-1705.
- Wilde S.A., R.B. Corey, J.G. Lyer and G.K. Voigt (1985). "Soil and Plant Analysis for Tree Culture" pp. 93-106, 3rd Ed. Oxford and IBM. Publishing Co., New Delhi.
- Wilfred, T. MaBusela; Alistair; M. Stephen Marthinus; and C. Botha (1990). Carbohydrate polymers from *Aloe ferox* leaves. *Photochemistry*, 29(11): 3555-3558.
- Youssef, H. M. A. (1997). Physiological studies on some annual plants. M. Sc. Thesis, Fac. of Agric., Moshtohor, Zagazig Univ.

تأثير التسميد الحيوي و المستخلصات النباتية على النمو والمكونات الكيماوية في نبات المريميه تحت ظروف الاجهاد المائى.

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اجريت هذه الدراسة في مزرعة خاصه بمدينة سمنود محافظه الغربيه خلال الموسمين المتتاليين ٢٠١١-٢٠١٢ ، ٢٠١٢-٢٠١٣ لدراسة تأثير استخدام الاسمدة الحيوية (فوسفورين و ميكروهيذا) والرش بالمستخلصات النباتيه (الثوم والصبار) على النمو الخضرى (طول النبات و الوزن الطازج و الجاف للعشب)، والنسبة المئوية للزيت الطيار والمكونات الكيماوية في نبات المريميه النامى تحت ظروف الاجهاد المائى (نباتات تروى كل ١٥ و ٣٠ و ٤٥ يوم) .
أظهرت النتائج أن رى النباتات كل ١٥ و ٣٠ يوم أدى الى زيادة مؤثرة فى كل النمو الخضرى والنسبة المئوية للزيت الطيار والنيتروجين والفوسفور والبوتاسيوم. وأيضاً أدى تلقيح النباتات بفطر الميكوهيذا الى تحسين صفات كل من النمو الخضرى والنسبة المئوية للزيت الطيار وامتصاص النيتروجين والفوسفور والبوتاسيوم وكذلك مستخلص الثوم كان افضل من مستخلص الصبار فى تحسين النمو الخضرى،
ولذلك نوصى برى نباتات المريميه كل ٣٠ يوم ورشها بمستخلص الثوم بالاضافة الى تلقيح النباتات بفطر الميكوهيذا للحصول على اعلى القيم من حيث صفات النمو الخضرى والنسبة المئوية للزيت الطيار و النيتروجين.

Table (1): Effect of irrigation, biofertilizers and plant extracts treatments on plant height, plant fresh and dry weight of *Salvia officinalis* L. plant.

Treatments	Plant height (cm)				Fresh weight (g / plant)				Dry weight (g / plant)			
	1 st seasons		2 nd seasons		1 st seasons		2 nd seasons		1 st seasons		2 nd seasons	
	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut
	Irrigation											
I ₁	66.34	37.45	68.21	39.52	542.58	537.56	541.21	534.10	151.64	155.31	146.19	154.49
I ₂	75.84	43.46	77.30	45.90	655.10	652.39	647.70	652.41	194.65	208.22	187.40	202.75
I ₃	55.55	32.06	57.64	35.78	426.57	416.38	445.57	429.77	112.80	110.84	107.99	112.79
L.S.D at 0.05	2.82	1.56	1.36	0.76	2.52	1.55	1.29	0.66	1.59	0.65	1.47	0.13
	Plant Extracts											
A	64.84	37.21	66.96	39.75	537.03	526.55	539.75	534.70	151.07	154.47	144.63	154.70
G	66.98	38.10	68.47	41.05	545.80	544.33	549.90	542.82	154.99	161.78	149.76	158.65
L.S.D at 0.05	0.98	0.90	0.63	0.57	1.23	1.32	1.14	1.44	0.96	0.07	0.41	0.15
	Bio fertilizers											
C	60.20	34.30	61.90	36.68	476.60	473.47	496.20	484.09	128.45	132.78	126.83	133.33
P	66.74	37.55	68.59	39.76	532.19	524.15	537.73	524.24	150.11	155.13	145.22	153.67
M	70.78	41.13	72.65	44.76	615.46	608.71	600.55	607.96	180.52	186.46	169.53	183.03
L.S.D at 0.05	2.19	1.10	0.82	0.91	1.348	1.32	1.73	1.61	1.00	0.16	0.61	0.35

I = irrigation G = Garlic extract A = Aloa extract P = Phosphorine M = Mycorrhizae

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	Plant height (cm)	Fresh weight (g/plant)	Dry weight (g/plant)
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Table (2): Effect of the interaction between irrigation, biofertilizers and plant extracts treatments on plant height, plant fresh and dry weight of *Salvia officinalis* L. plant

			1 st seasons		2 nd seasons		1 st seasons		2 nd seasons		1 st seasons		2 nd seasons	
			1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut
I ₁	C	A	55.78	33.00	57.40	36.03	427.9	435.12	468.15	456.47	113.79	114.34	114.25	118.69
		G	59.83	33.23	61.23	36.33	474.73	470.75	474.7	465.35	120.66	128.89	116.12	123.74
	P	A	68.75	36.00	70.13	38.20	513.15	505.75	488.55	478.5	141.9	142.79	132.73	141.94
		G	70.41	38.16	72.13	38.13	513.25	509.95	518.12	483.6	145.30	147.16	145.38	142.08
	M	A	69.56	42.16	73.53	43.30	661.9	642.41	648.85	651.3	191.79	192.19	183.61	197.51
		G	73.73	42.16	74.83	45.13	664.6	661.4	648.9	669.4	196.43	206.53	185.10	203.01
I ₂	C	A	71.16	40.06	72.40	41.20	592.75	573.4	597.3	584.95	168.73	174.18	166.44	175.53
		G	71.73	40.30	73.33	42.60	592.8	618.0	606.5	589.03	174.24	188.78	173.33	177.06
	P	A	75.10	43.83	76.90	45.00	675.9	665.55	668.73	674.25	200.36	215.61	193.33	210.04
		G	77.50	44.13	78.16	47.06	679.19	680.4	668.85	685.3	205.25	220.96	194.8	215.65
	M	A	78.76	45.43	80.83	49.00	690.12	686.1	671.25	688.41	208.53	223.16	196.9	217.77
		G	80.80	47.00	82.16	50.56	699.86	690.9	673.6	692.55	210.81	226.66	199.63	220.45
I ₃	C	A	49.50	29.06	52.50	31.50	384.0	368.8	408.93	398.73	94.54	93.45	92.17	100.22
		G	53.23	30.13	54.56	32.46	387.45	374.79	421.66	410	98.79	97.09	98.68	104.75
	B	A	54.16	31.16	56.83	34.16	400.5	383.15	426.05	411.21	103.22	101.15	100.09	105.58
		G	54.53	32.00	57.40	36.00	411.19	400.1	456.08	412.6	104.67	103.15	105.04	106.75
	M	A	60.80	34.23	62.16	39.40	487.12	478.7	480.02	468.5	136.82	133.36	122.16	125.1
		G	61.06	35.80	62.40	41.16	489.17	492.75	480.72	477.6	138.79	136.86	129.83	134.39
L.S.D at 5%			2.932	2.698	1.881	1.696	3.675	3.963	3.372	4.328	2.878	0.221	1.22	0.461

Table (5): Effect of irrigation, bio fertilizers and plant extracts treatments on N, P and K concentration of *Salvia officinalis* L. plant.

Treatments	Nitrogen concentration (%)				Phosphorus concentration (%)				Potassium concentration (%)			
	1 st seasons		2 nd seasons		1 st seasons		2 nd seasons		1 st seasons		2 nd seasons	
	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut
	Irrigation											
I ₁	1.586	1.576	1.633	1.636	0.373	0.383	0.389	0.383	3.854	3.891	3.926	3.953
I ₂	1.766	1.770	1.776	1.772	0.316	0.338	0.339	0.348	3.611	3.642	3.623	3.706
I ₃	1.480	1.438	1.513	1.493	0.240	0.279	0.265	0.287	3.109	3.258	3.241	3.336
L.S.D at 0.05	0.0002	0.0021	0.0047	0.0047	0.0047	0.0013	0.0001	0.0038	0.0292	0.0786	0.0475	0.0006
	Plant Extracts											
A	1.606	1.586	1.634	1.625	0.305	0.329	0.326	0.335	3.481	3.576	3.565	3.657
G	1.616	1.603	1.647	1.642	0.314	0.338	0.337	0.343	3.568	3.618	3.628	3.674
L.S.D at 0.05	0.011	0.0115	0.0105	0.0114	0.0114	0.0112	0.0118	0.0094	0.1159	0.1166	0.1084	0.1124
	Bio fertilizers											
C	1.550	1.524	1.572	1.556	0.274	0.303	0.296	0.308	3.28	3.41	3.41	3.43
P	1.592	1.573	1.626	1.634	0.315	0.336	0.331	0.344	3.50	3.55	3.55	3.67

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Treatments	N (%)				P (%)				K (%)			
	1 st seasons		2 nd seasons		1 st seasons		2 nd seasons		1 st seasons		2 nd seasons	
	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut

M	1.690	1.687	1.724	1.711	0.340	0.362	0.367	0.365	3.78	3.82	3.81	3.88
L.S.D at 0.05	0.0001	0.0022	0.0037	0.0037	0.0037	0.0001	0.0001	0.0030	0.0508	0.0484	0.0383	0.0014

I = irrigation G = Garlic extract A = Aloa extract P = Phosphorine M= Mycorrhizae

I ₁	C	A	1.495	1.435	1.520	1.513	0.351	0.358	0.366	0.363	3.711	3.888	3.868	3.9
		G	1.497	1.470	1.533	1.513	0.358	0.361	0.371	0.363	3.723	3.865	3.88	3.903
	P	A	1.502	1.496	1.591	1.615	0.376	0.387	0.393	0.386	3.91	3.917	3.942	3.97
		G	1.522	1.524	1.595	1.636	0.38	0.393	0.396	0.389	3.919	3.769	3.95	3.975
	M	A	1.749	1.755	1.778	1.769	0.383	0.398	0.402	0.396	3.927	3.948	3.957	3.984
		G	1.756	1.779	1.784	1.770	0.39	0.405	0.409	0.402	3.936	3.959	3.963	3.99
I ₂	C	A	1.683	1.713	1.704	1.680	0.247	0.298	0.278	0.299	3.153	3.192	3.191	3.267
		G	1.708	1.718	1.707	1.725	0.252	0.308	0.3	0.31	3.448	3.517	3.549	3.28
	P	A	1.783	1.779	1.787	1.785	0.338	0.347	0.348	0.361	3.598	3.655	3.568	3.874
		G	1.785	1.795	1.797	1.807	0.341	0.352	0.353	0.364	3.7	3.674	3.575	3.884
	M	A	1.812	1.805	1.825	1.815	0.359	0.362	0.376	0.377	3.882	3.898	3.915	3.963
		G	1.827	1.815	1.836	1.820	0.364	0.366	0.382	0.378	3.885	3.916	3.941	3.969
I ₃	C	A	1.459	1.392	1.480	1.453	0.215	0.241	0.231	0.255	2.75	2.941	2.96	3.111
		G	1.461	1.416	1.489	1.457	0.221	0.253	0.234	0.261	2.904	3.084	3.067	3.144
	P	A	1.474	1.420	1.492	1.467	0.225	0.262	0.234	0.271	2.928	3.141	3.126	3.151
		G	1.489	1.429	1.499	1.494	0.234	0.279	0.263	0.298	2.976	3.146	3.172	3.214
	M	A	1.497	1.486	1.537	1.530	0.259	0.315	0.308	0.314	3.474	3.605	3.562	3.694
		G	1.5	1.486	1.584	1.562	0.286	0.328	0.325	0.327	3.624	3.636	3.563	3.707
L.S.D at 5%			0.0355	0.0347	0.0316	0.0343	0.0342	0.0338	0.0354	0.0283	0.3478	0.3499	0.3252	0.3373

Table (6): Effect of the interaction treatments on N, P and K % of *Salvia officinalis* L. plant

