COMBINED EFFECT OF ORGANIC AND BIOFERTILIZER ON HERB YIELD AND ESSENTIAL OIL PRODUCTION OF *ORIGANUM VULGARE* L. PLANTS UNDER SANDY SOIL CONDITIONS

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ABSTRACT

This study was carried out on *Origanum vulgare* L. plant during the two successive seasons of 2013 and 2014, at the Experimental Station of Medicinal and Aromatic Plants of Sekem Company, Belbis desert- EL Sharkiya, Egypt. The aim of this research was to study the effect of the combination between compost, biofertilizer and algae extract on the growth, productivity and essential oil production of *Origanum vulgare* L. plants. The organic fertilization of Sekem compost was added at rates of 5, 10 and15m^3/ fed. A mixture of equal percentages of five strains of bacteria namely, *Azotobacter chroococcum*, *Azospirillum lipoferum* (as nitrogen fixing bacteria), *Bacillus polymixa*, *Bacillus megatherium* and *Pseudomonas fluorescence* (as phosphate solubilizing bacteria) was used as a source of biofertlizer. The biofertilizer a mixture at concentration of 1x10^8 m^l was obtained from Sekem Co, Egypt. Algae extract was obtained from Algae Production Unit at the National Research Centre. It was an extract of the algae *Spirulina platensis*. The results showed that, the highest significant increment for growth and yield characters was resulted from the treatment of algae extract + biofertilizer + 15m^3 compost this combination treatment resulted in the highest mean value of oil yield (ml/ plant). As general the main chemical constituents of oregano essential oil were carvacrol, p-cymene, ç-terpinene, α-thujene, α-myrcene, α-terpinene, o-cymene, carvacrol methyl ether and caryophyllene.

Keywords: Oregano, Origanum vulgare, algae extract, compost, yield, biofertilization, volatile oil.

INTRODUCTION

It is of interest to increase the production of medicinal and aromatic plants to avoid chemical therapy side effects on human health, as it has a major role in the treatment of human and animal diseases. Chemical fertilizers and pesticides used in agriculture for increasing yield and controlling pests can contaminate the water, air, food, lowed soil fertility as well as have bad influence on growth of soil microorganisms and human health **(Abdel-Ghany, 2007 and Hamed, 2011).**

Most widely used is the genus Origanum (family Lamiaceae) (from the Greek words oros– mountain and hill and ganos– ornament). Oregano is the most valued spice and the common name for a general aroma and flavor primarily derived from more than 60 plant species used worldwide as a seasoning. *Origanum* spp. (Lamiaceae) includes more than 70 species, subspecies, varieties, and hybrids, and most of the species are shrubs **(Kintzios, 2002).** One of the most important commercially grown species is *Origanum vulgare* subsp. *hirtum*, which is endemic to the Mediterranean area **(Skoula and Harborne, 2002)**.

Oregano is found in many areas and is a perennial shrub native to the dry, rocky limestone soils in the mountainous area of southern Europe and southwest Asia. It is also cultivated because of its uses as a herb and its healing properties,, which have been known since Antiquity **(Bariceric and Bartal, 2002).**In addition, the significance of herbs such as oregano has increased in recent years, especially with the interest in growing alternative crops and in nutraceutical and functional foods. Sixty percent of all are recorded to grow in Turkey, indicating this country as the gene center of *Origanum* **(Kintzios, 2002).** *Origanum* is known widely in the world of herbs and spices for its volatile oils. Oregano is the commercial name of those species that are rich in the phenolic monoterpenoids, mainly carvacrol, occasionally thymol, while marjoram is the commercial name of those that are rich in bicyclic monoterpenoids cis- and trans-sabinene hydrate **(Kintzios, 2002)**.

The essential oil of *Origanum vulgare* subsp. *vulgare* has a great potential of antimicrobial activity against all 10 bacteria, and 15 fungi and yeast species tested. The result may suggest that the essential oil *O. vulgare* subsp. *vulgare* possesses compounds with antimicrobial properties as well as antioxidant activity, and therefore can be used as a natural preservative ingredient in food and/or pharmaceutical industry **(Sahin *et al.,* 2004).** *Origanum vulgare* subsp. *hirtum* showed antioxidant activity **(Milos *et al*., 2000)**. *Origanum vulgare* ethanolic extract and essential oil revealed antibacterial properties and antioxidant activity **(Bárbara *et al.,* 2013).**

As a valuable medicinal plant, chemical free production and food safety is one of the major issues related to fresh products **(Antunes and Cavaco, 2010)**. Recently, the production of chemical-free medicinal and aromatic plants has been the main goal of many researchers and producers in order to ensure the high quality and safety of the product. Therefore, it would be beneficial to use alternatives to chemical fertilizers or at least to minimize the levels of these chemical fertilizers **(Hellal *et al.,* 2011).**

Bio-fertilizers are microbial preparations containing living cells of different organisms (bacteria, fungi, cyanobacteria, etc.), which have the ability to mobilize plant nutrients in soil from unusable to usable form through biological processes. These fertilizers are not harmful to crops or other plants like the chemical fertilizers. They let the plants grow in a healthy environment. Use of bio fertilizers in the soil, makes the plants healthy as well as protect them from getting many diseases. They are also environment friendly and do not cause the pollution of any sort **(Sadhana, 2014).**

In the last two decades, bio fertilizers have been increasingly used in modern agriculture due to the extensive knowledge in rhizospheric biology and the discovery of the promotive microorganisms. Azospirillum, *Azotobacter chroococcum* and *Azospirillum lipoferum* (confirmed as nitrogen fixing bacteria), *Bacillus polymixa*, *Bacillus megatherium* var phosphaticum and *Pseudomonas fluorescence* (confirmed as phosphate solubilizing bacteria) are known as plant growth promoting rhizobacteria (**Abdel Wahab and Hassan, 2013).**

Moreover, it is known that compost is required to improve the quality of soil organic matter **(Rivero *et al,* 2004)** by various ways. When composts are applied to soil, not only degradable substrates and nutrients are supplied, but also a wide range of microorganisms (**Ryckeboer *et al,* 2003)**, including harmless heterotrophy but potentially also plant and human pathogens. Compost as an organic material influences agricultural sustainability by improving chemical, physical, biological properties of soils, the fertility and structure of the soil and the moisture holding capacity (**Saha *et al*, 2008).**

Considering the importance of organic and biofertilizers for sustainable agriculture and the necessity to reduce chemical fertilizers application in agricultural, this study was conducted to evaluate the effect of organic and biofertilization on growth, production and chemical constituents of *Origanum vulgare* L. subsp *hirtum* plants.

MATERIALS AND METHODS

The present study was carried out during the two successive seasons of 2013 & 2014 at the Experimental Station of Medicinal and Aromatic Plants of Sekem Company, Belbis , EL-Sharkiya Governorate, Egypt (30° 22'47.59" N 31°39'40.63" E).The objective of this work was to study the effect of organic and biofertilization on growth, productivity and chemical constituents of *Origanum vulgare* subsp. *hirtum* L. plants.

Oregano seeds were imported from Germany by Pharmasaat Co, (straβe am west bahnh of D-06556 Artem), Tel/Fax: ++49(0)3466/324599, by Sekem Co. Seeds were sown in the nursery on the first week of November in both seasons. The Seedlings were transplanted from nursery to the experimental site on 18 th March for the first season and on 20th March for the second one .

All soil and water samples were analyzed in the Desert Research Center laboratories according to **Rainwater and Thatcher (1960**) are presented in Tables (A&B)

Table (A): The physical and chemical properties of the experimental soil

Soil type	pH	E.C. (dS m^{-1})	O. M. (%)	Cations (meq/l)				Anions (meq/l)			TDS (mg/l)	N (mg/l)	P (mg/l)	K (mg/l)
				Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^-	SO_4^{--}	Cl^-				
Fine Sandy	7.5	0.93	1.9	2.98	1.05	4.70	0.59	2.45	3.36	3.55	584.9	71.2	5.12	65.4

Table (B): Water analysis of the irrigation water

TDS mg/l	pH	EC (dSm^{-1})	Soluble cations (mg/l.)				Soluble anions (mg/l.)			
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^-	SO_4^{--}	CL^-
233.6	7.2	397	33.2	11.0	33.0	5.0	21.0	112.9	44.5	29.6

Before planting, rock phosphate was added at 100 kg/fed for all experimental plots. The soil was divided into four plots each plot was provided with compost at three levels. After soil preparation, drip irrigation system was installed at 60 cm between rows and 30 cm between plants within the row. Drippers were set up at 2.0 liter/hour/plant every day.

Compost fertilizer was supplied by Sekem Co., Egypt from the company own compost production facility. Plants were treated with compost fertilization at 5, 10 and 15 m^3/fed. Compost was added during soil preparation one day before seedling transplanting. After adding compost, soil was irrigated until saturation. Analysis of the added compost is presented in Table (C).

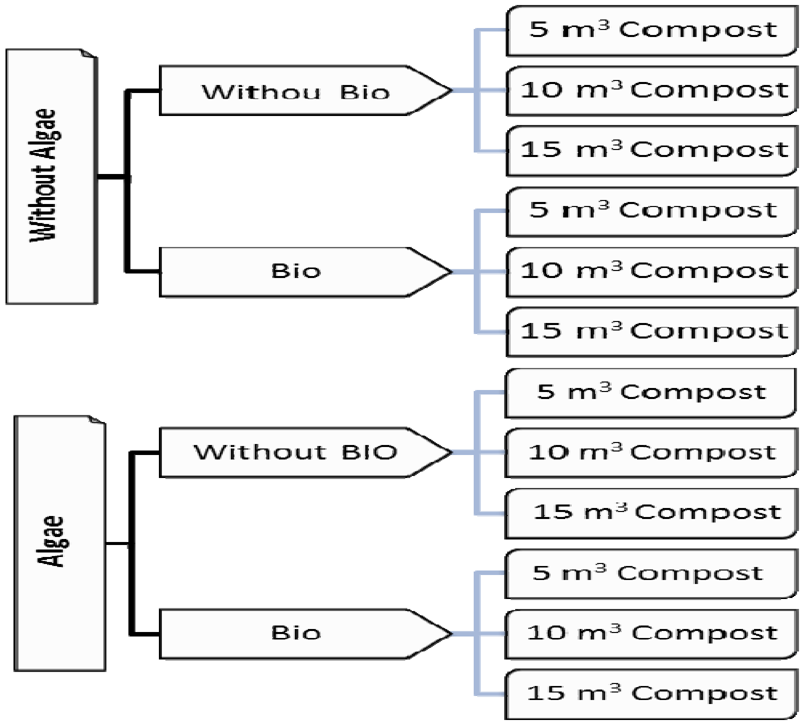
Table (C): Chemical analysis of the used compost

pH	E.C (dSm^{-1})	Soluble cations (meq/l).				Soluble anions (meq/l).			Ash	O.M%	N%	P%	K%
		Ca^{++}	Mg^{+}	Na^{+}	K^{+}	HCO_3^-	Cl^-	SO_4^{--}					
8.10	3.10	15.00	45.0	90.0	30.0	45.00	10.0	140.5	9.00	65.00	2.0	1.5	1.00

Biofertilizer was added as a mixture of equal percentages of five strains of bacteria namely: *Azotobacter chroococcum*, *Azospirillum lipoferum*, *Bacillus polymixa*, *B.megatherium* and *Pseudomonas fluorescence* ($1x10^8$/ ml) which were obtained from Sekem Co, Egypt. The biofertilizer treatment was prepared by dilution of 1 L mixture with 20 l of tap water and added as a soil drench at 50 ml per plant four times per season. Application of biofertilizer was after transplanting at 30 and 45 days, and after the first cut at 15 and 30 days, immediately after biofertilizer application, irrigation was done.

Algae extract 1% was obtained from Algae Production Unit at the National Research Center. It was an extract of the algae *Spirulina platensis*. The Algae extract treatment was prepared by dilution of 1 L mixture with 50 l of tap water and added as a spray on plant every 30 days per season. Application was after transplanting with 30 days.

The treatments were conducted as follows:



Harvesting of oregano fresh herb was done twice per season on July 1st and November 1st and data were the mean of the two cuts.

The following parameters were recorded at harvesting time:

1- Plant vegetative growth

- Herb fresh weight (g/plant and kg/fed).
- Herb dry weight (g/plant and kg/fed).
- Yield of dry leaves (g/plant and kg/fed).

2- Determination of essential oil:

- Essential oil percentage (%).
- Essential oil yield per plant (ml).
- Essential oil constituents by GC-MS analysis.

Oregano volatile oil % was determined by hydro distillation according to the method described in **British Pharmacopoeia (1963)**.

The GC-MS analysis for oil samples from the second season was carried out at the Central Laboratory of National Research Center, Giza. Essential oil GC/ Mass analysis was performed using a Hewlett-Packard 5890 A series 11 instrument equipped with flame ionization detector (FID) and a carbon wax fused silica column (50 m x 0.25 mm. i. d., film thickness 0.32 µm). Initial column temperature was 50 Cº and held for 3 minutes, then raised to 60 cº by rate 3.0 Cº per minute and raised to 260 Cº by rate 3.0 Cº per minute and hold at 260 Cº for 5 minutes. The volatile oil components were identified by comparing their retention times and mass spectrum with those of standards, NIST library of the GC-MS system and literature data.

Identifications were made by library searches **(Adams, 2007)** combining MS and retention data of authentic compounds by comparison of their GC retention indices (RI) with those of the literature or with those of standards available in our laboratories **(Adams, 2007)**.

The layout of the experiment was a split-split plot design with three replicates using algae extract as the main plot, biofertilizer as sub plot and compost in the sub sub plot. The results were statistically analyzed using MSTAT program, USA. Means were compared using LSD test at 0.05 level according to **Sendecor and Cochran, (1982).**

RESULTS AND DISCUSSION

A- Vegetative growth:

A-1-Fresh weight (g/plant and kg/fed):

As presented in table (1) for the effect of the interaction between algae, biofertilization and compost on fresh weight/plant and yield fresh weight per fed, in both seasons, the significantly heaviest plants resulted from the treatment of algae + biofertilization + 15 m^3 compost/fed. However the lightest plants, in both seasons, resulted from the treatment of without (algae and biofertilization) + 5 m^3 compost/fed. Also the highest yield fresh weight per fed. was resulted from the treatment of algae + biofertilization + 15 m^3 compost/fed.

These results were in parallel line with those achieved by **Kandeel and Sharaf (2003)** who indicated that, *Azotobacter chroococcum* showed an increment in fresh weight of *Marjorana hortensis* L. plants. On mint plants (*Mentha arvensis* L.), **Rajamanickam *et al*. (2011)** revealed that, application of 100% NPK + vermicompost at 5 t ha^{-1}+ consortium of biofertilizers significantly increased the fresh herbage yield. **Edris *et al*. (2003)** on *Origanum majorana* and **El-Sherbeny *et al.* (2007)** on *Ruta graveolens* L. reported that compost significantly improved most vegetative growth characters as fresh weight of leaves and stems. **Shariatmadari *et al.* (2011)** on tomato, cucumber and squash showed that addition of all algae extract can enhance fresh weight of leaf and stem and **Bindhu (2013)** on *Pisum sativum*, found that 20% concentration of aqueous extract of Azolla promoted the fresh weight of plant.

Table (1): Effect of interaction between algae extract, biofertilizer and compost on herb fresh weight (g/plant) and yield fresh weight (kg/fed) of oregano plant during 2013 and 2014 seasons

Algae x Bio x Compost			Fresh weight (g/plant)						Yield fresh weight (kg/fed)					
			2013			2014			2013			2014		
Algae	Bio	Comp	1^{st} cut	2^{nd} cut	Total yield	1^{st} cut	2^{nd} cut	Total yield	1^{st} cut	2^{nd} cut	Total yield	1^{st} cut	2^{nd} cut	Total yield
Without	Without	5 m^3	59.78	60.44	120.22	60.11	58.33	118.44	1395	1410	2805	1403	1361	2764
		10 m^3	59.89	68.89	128.78	56.44	64.78	121.22	1397	1607	3004	1317	1512	2829
		15 m^3	70.56	75.67	146.23	66.45	70.44	136.89	1646	1766	3412	1551	1644	3195
	With	5 m^3	58.11	78.56	136.67	69.56	72.11	141.67	1356	1833	3189	1623	1683	3306
		10 m^3	74.63	85.89	160.52	77.67	90.78	168.45	1741	2004	3745	1812	2118	3930
		15 m^3	84.43	98.67	183.10	80.78	94.56	175.34	1970	2302	4272	1885	2206	4091
With	Without	5 m^3	80.22	87.78	168.00	83.11	89.89	173.00	1872	2048	3920	1939	2097	4036
		10 m^3	95.97	95.00	190.97	97.00	100.00	197.00	2239	2217	4456	2263	2333	4596
		15 m^3	99.78	113.80	213.58	102.90	108.60	211.50	2328	2655	4983	2401	2534	4935
	With	5 m^3	134.60	146.50	281.10	129.20	143.40	272.60	3141	3418	6559	3015	3346	6361
		10 m^3	137.00	149.30	286.30	131.2	143.40	274.60	3197	3484	6681	3061	3346	6407
		15 m^3	150.30	157.4	307.70	149.10	153.30	302.40	3507	3673	7180	3479	3577	7056
$LSD_{0.05}$			21.93	26.93		18.62	14.15		482.5	592.4		409.7	311.3	

A-2-Dry weight (g/plant and kg/fed):

The effect of interactions between algae, biofertilizer and compost treatments on plant dry weight and dry yield /fed. are shown in Table (2).

The results indicated that, the maximum plant dry weight as well as dry yield /fed. were obtained when oregano plants were treated with algae, biofertilizer and compost at 15 m^3, these results were observed in all cuts in both seasons. With note that the three levels of compost in the first cut of the first season, compost at 10 and 15 m^3 in the second cut in the same season and the first cut in the second season there were non significant differences among them. These results were coincided with those obtained by **Al-Fraihat *et al*. (2011)** showed that, marjoram treated with halex-2 biofertilizers gave the highest values of herb dry yield.

The stimulatory effect of the treatment of algae + biofertilization + 15 m^3 compost/fed. on both fresh and dry weight/plant may be due to the stimulatory role of algae, bio and organic fertilization on the plant physiological processes (**Toaima, 2005; Abdel-Moneim and Abd-Allah, 2008 and Saha *et al*, 2008;)**.

Table (2): Effect of the interaction between algae extract, biofertilizer and compost on herb dry weight (g/plant) and (kg/fed) of oregano during 2013 and 2014 seasons

Algae x Bio x Compost			**Dry weight (g/plant)**						**Dry weight (kg/fed)**					
			2013			**2014**			**2013**			**2014**		
Algae	Bio	Comp	**1st cut**	**2nd cut**	**Total yield**	**1st cut**	**2nd cut**	**Total yield**	**1st cut**	**2nd cut**	**Total yield**	**1st cut**	**2nd cut**	**Total yield**
Without	Without	5 m^3	19.07	20.67	39.74	19.59	18.67	38.26	444.9	482.3	927.2	457.1	435.6	892.7
		10 m^3	21.08	22.53	43.61	20.00	21.97	41.97	491.9	525.7	1017.6	466.7	512.6	979.3
		15 m^3	24.92	23.89	48.81	22.56	24.10	46.66	581.5	557.4	1138.9	526.4	562.3	1088.7
	With	5 m^3	23.93	23.51	47.44	23.00	22.67	45.67	558.4	548.6	1107.0	536.7	529.0	1065.7
		10 m^3	28.40	28.97	57.37	25.47	30.53	56.00	662.7	676.0	1338.7	594.3	712.4	1306.7
		15 m^3	30.09	34.22	64.31	29.33	32.09	61.42	702.1	798.5	1500.6	684.4	748.8	1433.2
With	Without	5 m^3	25.84	31.21	57.05	27.57	31.85	59.42	602.9	728.2	1331.1	643.3	743.2	1386.5
		10 m^3	30.15	31.67	61.82	33.00	33.33	66.33	703.5	739.0	1442.5	770.0	777.7	1547.7
		15 m^3	36.62	36.78	73.40	34.11	35.22	69.33	854.5	858.2	1712.7	795.9	821.8	1617.7
	With	5 m^3	43.67	42.87	86.54	40.43	39.54	79.97	1019.0	1000.3	2019.3	943.4	922.6	1866.0
		10 m^3	43.74	49.00	92.74	42.33	43.87	86.20	1019.0	1143.3	2162.3	987.7	1023.6	2011.3
		15 m^3	50.56	52.22	102.78	46.56	50.50	97.06	1179.7	1218.4	2397.8	1086.4	1178.3	2264.7
$LSD_{0.05}$			10.57	8.03		5.71	5.91		232.6	176.6		125.6	130.0	

A-3-Leaves dry weight (g/plant) and (kg/fed.):

The effects of interactions between algae, biofertilizer and compost treatments on leaves dry weight (g/plant) and yield of leaves dry weight (kg/fed.) are shown in Table (3). The results indicated that, the maximum values of leaves dry weight/plant as well as yield of leaves dry weight/fed. were obtained from treating oregano plants with algae, biofertilizer and compost at 15 m^3, these results were observed in all cuts in both seasons although there were non-significant differences among compost at 5, 10, 15 m^3 in the first cut of the first season and compost at 10 and 15 m^3 in second cut of the first season and both cuts in the second season.

Table (3): Effect of the interaction between algae extract, biofertilizer and compost on leaves dry weight (g) / plant and (kg) / fed of oregano during 2013 and 2014 seasons

Algae x Bio x Compost			Leaves d.w./plant						Leaves d.w./fed					
			2013			2014			2013			2014		
Algae	Bio	Comp	1st cut	2nd cut	Total yield	1st cut	2nd cut	Total yield	1st cut	2nd cut	Total yield	1st cut	2nd cut	Total yield
Without	Without	5 m^3	9.22	10.27	19.49	9.39	9.28	18.67	215.1	239.6	454.7	220.0	216.5	436.5
		10 m^3	10.62	11.11	21.73	9.31	11.63	20.94	247.8	259.2	507.0	217.2	271.4	488.6
		15 m^3	13.26	13.12	26.38	12.38	12.44	24.82	309.4	306.1	615.5	288.9	290.3	579.2
	With	5 m^3	12.43	12.91	25.34	12.62	12.40	25.02	290.0	301.2	591.2	294.5	289.3	583.8
		10 m^3	17.21	17.73	34.94	15.43	18.60	34.03	401.6	413.7	815.3	360.0	434.0	794.0
		15 m^3	19.05	21.27	40.32	17.91	20.93	38.84	444.5	496.3	940.8	417.9	488.4	906.3
With	Without	5 m^3	12.77	15.75	28.52	13.11	15.80	28.91	298.0	367.5	665.5	305.9	368.7	674.6
		10 m^3	15.04	17.24	32.28	17.00	18.84	35.84	350.9	402.3	753.2	396.7	439.6	836.3
		15 m^3	19.48	19.69	39.17	20.03	21.01	41.04	454.5	459.4	913.9	467.4	490.2	957.6
	With	5 m^3	26.49	26.33	52.82	25.67	26.84	52.51	618.1	614.4	1232.5	599.0	626.3	1225.3
		10 m^3	27.48	31.33	58.81	28.07	30.07	58.14	641.2	731.0	1372.2	655.0	701.6	1356.6
		15 m^3	32.39	33.62	66.01	31.69	32.74	64.43	755.8	784.5	1540.3	739.4	763.9	1503.3
$LSD_{0.05}$			5.98	5.09		4.55	2.79		131.5	111.9		100.2	61.35	

These results were in line with the results of herb dry weight/plant and herb dry yield /fed. and were in agreement with those obtained by **El-Sherbeny *et al.* (2007)** on *Ruta graveolens* L. who recorded that compost significantly improved dry weight of leaves and **Shariatmadari *et al.* (2011)** on tomato, cucucmber and squash who showed that addition of all algal extract can enhance dry weight of leaf.

B- Oil percentage and oil yield per plant:

The effect of interactions of algae, biofertilizer and compost treatments are shown in Table (4). The results showed significant differences between most treatments. The highest values of oil percentage resulted from treated plants without algae, without biofertilizer and 5m^3 compost. While in the first cut in first season the highest values resulted from treatment of algae, with biofertilizer and compost at 15 m^3 with non significant differences between compost levels. In the first cut of the second season the highest values resulted from treatment algae, with biofertilizer and compost at any levels.

Table (4): Effect of the interaction between algae extract, biofertilizer and compost on volatile oil percentage and oil yield (ml/plant) of oregano plant during 2013 and 2014 seasons

Algae x bio x compost			Oil (%)				Oil per plant (ml)			
			2013		2014		2013		2014	
Alge	Bio	Comp	1st cut	2 nd cut	1st cut	2 nd cut	1st cut	2 nd cut	1st cut	2 nd cut
Without	Without	5 m^3	1.52	0.89	1.53	0.92	0.25	0.18	0.30	0.17
		10 m^3	1.36	0.79	1.38	0.80	0.29	0.18	0.28	0.18
		15 m^3	1.23	0.76	1.25	0.77	0.31	0.19	0.28	0.18
	With	5 m^3	1.17	0.65	1.17	0.66	0.28	0.16	0.27	0.15
		10 m^3	1.19	0.63	1.73	0.63	0.34	0.18	0.30	0.19
		15 m^3	1.04	0.63	1.05	0.62	0.31	0.21	0.31	0.20
With	Without	5 m^3	1.16	0.50	1.12	0.51	0.30	0.16	0.31	0.16
		10 m^3	0.94	0.40	0.98	0.39	0.28	0.13	0.33	0.13
		15 m^3	0.73	0.37	0.72	0.38	0.27	0.14	0.24	0.13
	With	5 m^3	1.16	0.35	1.15	0.36	0.51	0.15	0.47	0.14
		10 m^3	1.04	0.33	1.10	0.34	0.45	0.16	0.47	0.15
		15 m^3	1.07	0.33	1.00	0.34	0.54A	0.17	0.47	0.17
$LSD_{0.05}$			0.19	0.08	0.11	0.08	0.09	0.054	0.077	0.002

Similar results were obtained by **Mahfouz (2003)** who showed that biofertilization of *Majorana hortensis* increased essential oil percentage and oil yield per plant. **Toaima (2005)** on *Achillea millefolium* L. recorded that, in the presence of second chemical fertilizers level of NPK (300 kg ammonium sulfate + 200 kg calcium super phosphate + 50 kg potassium sulfate/fed.) plus organic manure (15 m^3/fed sheep manure) + biofetilizer (a mixture of *Azotobacter chroococcum*, *Azospirillum lipoferum* and *Bacillus megatherium*) one addition per month enhanced essential oil percentage and oil yield per plant. **Al-Fraihat *et al*. (2011)** showed that treating marjoram plants with halex-2 biofertilizers gave the highest significant values of volatile oil percentage and oil yield/plant and **Khalil *et al*. (2008) and EL-Leithy *et al*. (2013)** found that all levels of compost significantly increased oil (%) and oil yield (ml/plant) of sage.

3- Oil yield (l/ fed.):

The effect of interactions of algae, biofertilizer and compost treatments on oil yield per feddan are shown in Table (5). The highest oil yield per feddan was obtained from the treatment of algae, with bio fertilizer and compost at 15m^3 while in the second season resulted from treatment of algae, with bio fertilizer and compost at 10 m^3 as these results were obtained from treatment of algae, with bio fertilizer and compost at $15m^3$.

Table (5): Effect of the interaction between algae extract, biofertilizer and compost on oil yield (l/fed) of oregano during 2013 and 2014 seasons

Algae x bio x compost			Oil yield (l/fed.)					
			2013			2014		
Algae	**Bio**	**Comp**	**1st cut**	**2 nd cut**	**Total yield**	**1st cut**	**2 nd cut**	**Total yield**
With out	**Without**	**5 m^3**	5.83	4.20	10.03	7.00	3.97	10.97
		10 m^3	6.77	4.20	10.97	6.53	4.20	10.73
		15 m^3	7.23	4.43	11.66	6.53	4.20	10.73
	With	**5 m^3**	6.53	3.73	10.26	6.30	3.50	9.80
		10 m^3	7.93	4.20	12.13	7.00	4.43	11.43
		15 m^3	7.23	4.90	12.13	7.23	4.67	11.90
With	**Without**	**5 m^3**	7.00	3.73	10.73	7.23	3.73	10.96
		10 m^3	6.53	3.03	9.56	7.70	3.03	10.73
		15 m^3	6.30	3.27	9.57	5.60	3.03	8.63
	With	**5 m^3**	11.90	3.50	15.40	10.97	3.27	14.24
		10 m^3	10.50	3.73	14.23	10.97	3.50	14.47
		15 m^3	12.60	3.97	16.57	10.97	3.97	14.94
$LSD_{0.05}$			2.16	0.97		1.60	0.73	

These findings were in agreement with these reported by **Mahfouz (2003)** who showed that biofertilization on *Majorana hortensis* increased oil yield per feddan, **Abdelaziz *et al*. (2007)** on *Rosmarinus officinalis* L. found that the treated plants by a mixture of compost and microorganisms showed a significant increment in essential oil production.

4- Essential oil constituents:

Data of the effect of organic and biofertilization tretments on oil constituents of *Origanum vulgare* L. subsp *hirtum* in two cuts during the two seaseons are presented in Table (6).

As general the main chemical constituents of oregano essential oil were carvacrol, p-cymene, ç-terpinene, α-thujene, α-myrcene, α-terpinene, o-cymene, carvacrol methyl ether and caryophyllene).

Carvacrol was the most dominant component in oregano essential oil composition for the different treatments. Carvacrol is a monoterpenoid phenol, it has a characteristic pungent, warm odor. The quality of oregano oil is determined by the carvacrol percentage in the oil. **Skoula and Harborne (2002)** reported that carvacrol is the dominant component of its essential oil. **Andreas and Dimitra (2013)** found that carvacrol is the dominant component in the essential oil of *O.vulgare* subsp. *hirtum*. **Lagouri *et al*. (1993), Aeschbach *et al*. (1994) and Yanishlieva *et al*. (1999)** revealed that antioxidant effect of this plant is as a result of carvacrol. **Mastelic *et al*. (2008)** reported its antimicrobial and antioxidant properties and in addition, revealed carvacrol's antiproliferative activity on tumor cells of Hela.

Table (6): The main components (%) of oregano volatile oil treated by compost and their combination with biofertilizers treatments of the second season

Components	RT	C1	B+C1	B+C2	B+C3	A+C1	A+C2	A + C3	A+B+ C1	A+B+ C2	A+B+ C3
α-Thujene	8.82	8.08	6.03	4.9	5.31	9.83	8.04	6.52	4.44	11.01	6.81
α-Pinene	10.39	0.38									
Sabinene	10.45	0.5							1.73		
1-Octen-3-Ol	10.94			0.94							
α-Myrcene	11.15	3.85	2.01		1.95	3.97	3.18	0.59	1.92		3.13
α-Terpinene	11.9	0.66			0.74				1.00		
o-Cymene	12.4	5.2	19.52			15.49	16.94	19.38	34.61		15.73
p-Cymene	12.7	27.9	35.12	56.3	42.9	12.7	25.55	28.52	10.58	27.15	21.92
cis-Ocimene	12.97				1.95		1.66				1.06
ç-Terpinene	14.08	28.2	11.36	8.27	10.6	20.72	19.79	15.96	10.17	22.25	21.43
Borneol	17.45	0.43		1.28	0.56	0.78		0.46	0.62	1.62	0.46
Carvacrol methyl ether	20.21	0.63	1.48	1.49	2.09	0.79	2.68	1.49	1.47	4.26	1.00
Carvacrol	23.67	23	23.8	25.7	33.3	34.34	22.16	26.55	32.45	33.72	27.67
Caryophyllene	26.03	0.42	0.67	0.66	0.61	1.02		0.53	0.51		0.53
à-Humulene	27.04					0.35					
Germacrene-D	27.86	0.38							0.27		0.27
α-Bisabolene	28.73	0.31									
Caryophyllene oxide	30.87			0.42					0.23		
Total		99.94	99.99	99.96	99.99	99.99	100	100	100	100	100

A* means algae extract. B* means biofertilizers mixture.C1* means compost at 5 m^3.
C2* means compost at 10 m^3. C3* means compost at 15 m^3.

It was obvious from previous data that the different fertilization treatments have a remarkable influence on carvacrol percentage in the oil. The highest carvacrol percentage was obtained from the treatments:- algae + 5 m^3 compost/fed, algae +biofertilization + 10 m^3 compost and biofertilization + 15 m^3 compost while the lowest carvacrol percentage in the oil was obtained from the control treatment:- algae + 10m^3 compost.

These results were in agreement with those obtained by **El Leithy *et al*. (2006)** on rosemary who found that the highest oil content and essential oil constituents (alpha-pinene, β-pinene, limonene, 1-8-cineole, linalool, camphor, β-terpineol, borneol, terpinen 4- ol, carvone, thymol, carvacrol, linalylacetate, geranylacetate, β-caryophyllene, caryophyllene oxide) were highly significant increased under biofertilizer (*Azotobacter vinelandii*) treatments**. Hendawy *et al.* (2010)** on *Thymus vulgaris* L. reported that 20m^3 compost fed^{-1} combined with 10 l fed^{-1} of tea compost and /or feldspar, rock phosphate at the level of 150 kg fed^{-1} were superior in most cases of growth characters, yield and oil percentage of *Thymus vulgaris*.

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الملخص العربى

التأثير المشترك للسماد العضوى والحيوى على محصول العشب وإنتاجية الزيت الطيار لنباتات الأوريجانو تحت ظروف الأراضى الرملية

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والإنتاجية وإنتاج الزيت الطيار لنباتات الأوريجانو. تم إضافة التسميد العضوى بكمبوست سيكم بمعدل 5 ، 10 ، 15 م^3/فدان ، تم استخدام كمصدر للسماد الحيوى خليط من نسب متساوية من خمس سلالات من البكتريا وهى *Azotobacter chroococcum*, *Azospirillum lipoferum* (بكتريا مثبتة للنيتروجين) و *Bacillus polymixa*, *Bacillus megatherium* *Pseudomonas fluorescence* (بكتريا ميسرة للفوسفات) وقد تم الحصول على خليط السماد الحيوى بتركيز 1×10^8 مل$^{-1}$ من شركة سيكم ، مصر ، تم الحصول على مستخلص الطحالب من وحدة إنتاج الطحالب بالمركز القومي للبحوث وهو عبارة عن مستخلص طحلب *Spirulina platensis* . أوضحت النتائج أنة قد نتجت أعلى زيادة معنوية فى صفات النمو والمحصول وأعلى متوسط قيمة لمحصول الزيت (مل/نبات) من المعاملة بمستخلص الطحالب + السماد الحيوى + 15 م^3 كمبوست/فدان وبصفة عامة كانت المكونات الكيميائية الرئيسية للزيت الطيار للأوريجانو هى

carvacrol, p-Cymene, ç-terpinene, α-thujene, α-myrcene, α-terpinene, o-cymene, carvacrol methyl ether and caryophyllene.