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# BIO-PRODUCTION OF *ORIGANUM VULGARE* L. PLANT UNDER EGYPTIAN SANDY SOIL CONDITION

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**ABSTRACT:** *The bio-production of oregano was investigated under Egyptian sandy soil condition in response to compost and biofertilizers. Field experiments were carried out at the Experimental Station of Medicinal and Aromatic Plants of Sekem Company, EL Sharkiya, Egypt during the two successive seasons of 2013 & 2014. The plants were fertilized with compost at 5, 10 and 15 $m^3$ individually or in combination with biofertilizers mixture of nitrogen fixing bacteria (Azotobacter chroococcum, Azospirillum lipoferum), phosphate solubilizing bacteria (Bacillus polymixa, Bacillus megatherium var phosphaticum and Pseudomonas fluorescence) at concentration of $1x10^8$ $ml^{-1}$ dilution by 1:20 L of water and added by 50 ml per as a soil drench. All growth and yield traits were significantly enhanced due to combining biofertilizers and 15 $m^3$ compost. Volatile oil % was maximized when plants were fertilized with only compost at 5 $m^3$, meanwhile, the overall higher oil yields were obtained from plants treated by combining biofertilizer with 15 $m^3$ compost. Combining bio fertilizers with all compost treatments significantly enhanced nutrition element as well as carbohydrate contents. Overall, combining biofertilizers with compost at 15 $m^3$ compost gave the best results for maximizing growth and yield of oregano herb. The results of our study gave also the chance of shifting toward clean agriculture to reduce the contamination impact of chemical fertilizers.*

***Key words**: Oregano, Origanum vulgare, biofertilizer, compost, yield, volatile oil.*

## INTRODUCTION

*Origanum vulgare* ssp. *hirtum*, is a perennial herbs endemic to the Mediterranean area (Skoula and Harborne, 2002). The plant is also adapted to wide range of conditions including dry, rocky calcareous soils in the mountainous area of southern Europe and Southwest Asia. Oregano is known widely in the world of herbs and spices for its volatile oils that are rich in the phenolic monoterpenoids, mainly carvacrol, occasionally thymol (Kintzios, 2002). Also the oil is has a great potential of antimicrobial activity against several species of bacteria, and fungi and therefore can be used as a natural preservative ingredient in food and/or pharmaceutical industry (Sahin *et al,* 2004).

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 their levels (Hellal *et al,* 2011). Many ways had been used to substitute chemical fertilizers including organic fertilizers mainly from animal product or bio-fertilizers.

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 microorganism. Azospirillum, *Azotobacter chroococcum*, *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). Chemicals free or bio production of many medicinal plant crops using this plant growth promoting rhizobacteria has been successfully used in different trials; Gomaa and Abo Aly (2001) on anise plant, Kandeel *et al* (2001), Mahfouz and Sharaf-Eldin (2007) on *Foeniculum vulgare*, Nofal *et al* (2001) on *Ammi visnaga* and Shaalan (2005) on *Nigella sativa*, L.

Bio fertilizers are not usually used solitary to stimulate growth since they needs organic matter to stimulate activity (Garcia *et al,* 1994 and Pascual *et al,* 1997). 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 materials influences agricultural sustainability by improving chemical, physical, biological properties of soils, the fertility and structure of the soil and the moisture holding capacity (Follet *et al*, 1981; Frederickson *et al,* 1997 and 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 bio-fertilizers and compost on growth, yield and essential oil of *Origanum vulgare* ssp. *hirtum*.

## MATERIALS AND METHODS

### 1. Plant material

Two field experiments were carried out at the Experimental Station of Medicinal and Aromatic Plants of Sekem Company, EL Sharkiya, Egypt during the two successive seasons of 2013 & 2014 in order to study the possibility of bio production of oregano herb under sandy soil conditions of Belbis desert, AL Sharkia Governorate, Egypt.

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. Seedlings were ready for transplant when they were 7-10 cm long. The physical and chemical properties of the experimental soil are presented in Table (1):

**Table1. Physical and chemical properties of the experimental soil**

| Soil type | Ph | E.C. (ds/cm) | O.M. (%) | Cations (meq/l) | | | | Anions (meq/l) | | | TDS (mg/l) | N (mg/l) | P (mg/l) | K (mg/l) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $Ca^{++}$ | $Mg^{++}$ | $Na^{+}$ | K+ | $HCO_3^-$ | $SO4^{--}$ | $Cl^-$ | | | | |
| 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 |

## 2. Soil preparation

Before planting, rock phosphate was added at 150 kg/fed for all experimental plots. Land 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.

## 3. Seedling plantation

The Seedlings were transferred from nursery to the experimental site on 18$^{th}$ March for the first season and on 20$^{th}$ March for the second season. Uniform seedlings free form any kind of damage or root disease with average of 7-10 cm long with seven pairs of leaves were selected. Seedlings were mulched out from nursery and moved directly for planting under dripper's position and irrigation was done immediately after transplanting for five hours until soil saturation. One week after transplanting all the dead seedlings were replaced with fresh-mulched ones at the same growth stage. During the experiment all the agricultural practices were done as usual.

## 4. Compost fertilizer

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 (2)

## 5. Biofertilizer

A mixture of equal percentages of five strains of bacteria namely, *Azotobacter chroococcum*, *Azospirillum lipoferum* (as nitrogen fixing bacteria), *Bacillus polymixa*, *Bacillus megatherium* var phosphaticum and *Pseudomonas fluorescence* (as phosphate solubilizing bacteria) was used as a source of biofertilizers. The biofertilizer mixture was provided by Sekem Co., Egypt from the company lab at concentration of $1x10^8$ $ml^{-1}$.

The biofertilizer treatments were prepared by dilution 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 done after transplanting with 30 and 45 days, and after the first cut with 15 and 30 days, immediately after biofertilizer application, irrigation was done.

## 6. Production evaluation

Harvesting oregano fresh herb was done two times per seasons at July 1$^{st}$ and November 1$^{st}$ and the data was the mean of the two cuts. Harvest was done early as possible and plant height (cm) was recorded before harvest using measuring tape. After harvest, plants were removed to sorting and drying station for sampling and fresh herb measurements i.e. herb fresh weigh/plant (g) and calculated herb fresh weight yield/fed (kg). Samples were air dried under shade to estimate herb dry weigh/plant (g) and herb dry weight yield/fed (kg). After drying leaves were detached from stems and weighed separately to estimate dry leaves/plant (g) and dry leaves yield /fed (kg).

**Table 2. Chemical analysis of the used compost.**

| PH | E.C (mhos) | Soluble cations (meq/l). | | | | Soluble anions (meq/l). | | | Ash% | O.M% | N% | P% | K% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $Ca^{++}$ | $Mg^{++}$ | $Na^{+}$ | $K^{+}$ | $HCO3^{-}$ | $Cl^{-}$ | $SO4^{--}$ | | | | | |
| 8.10 | 3.10 | 15.00 | 45.00 | 90.00 | 30.00 | 45.00 | 10.00 | 140.50 | 9.00 | 65.00 | 2.00 | 1.50 | 1.00 |

## 7. Volatile oil productivity

Oregano volatile oil % was determined by hydro distillation according to the method described in British Pharmacoepea (1963), samples of 100 g of air dried leaves moved into distillation flask and the distillation period extended to three hours. Volatile oil percentage was calculated using the following equation: Volatile oil percentage = (oil volume in the graduated tube/fresh weight of samples) x 100. Volatile oil percentage was used to calculate volatile oil yield/plant as well as volatile oil yield/fed was calculated.

## 8. GC/ Mass analysis of volatile oil

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.

## 9. Chemical analysis

Chlorophyll a, b and total chlorophyll were determined in leaf fresh samples (mg/g f.w.) as described by Saric *et al* (1967). N, P and K percentages as well as total carbohydrates in the leaves were determined. Nitrogen was determined by modified micro Kjeldahle method as described by A.O.A.C. (1970). Phosphorus was colorimetrically determind using the method described by Murphy and Riley (1962) using spectrophotometer at 882 µv. As for potassium % it was estimated using flame photometry according to Cottenie *et al* (1982). Total carbohydrates were determined according to Chaplin and Kennedy (1994).

## 10. Experimental design and statistical analysis

The experiment was designed as randomized complete block design with three replicates for each treatment. 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

## 1. Plant height

It is evident from data presented in Table (1) that, plant height of oregano was positively enhanced by combining biofertilizer with compost treatments during both successive seasons. Compost treatment at the low dose ($5m^3$) recorded the shortest plants compared to other treatment combinations. The tallest plants for both seasons were obtained by combining bio fertilizer and compost at either $10m^3$ or $15m^3$ without significant differences in between.

## 2. Plant fresh weight and yield/fed

Oregano plant fresh weight and total yield /fed were significantly increased due to compost treatments and combinations with biofertilizer as shown in Table (1). Combining bio fertilizer and compost at either 10 $m^3$ or 15 $m^3$ recorded the highest fresh weight /plant as well as fresh weight yield /fed in both seasons. The highest fresh weight/plant (91.55 and 87.67 g) was recorded by biofertilizer combined with 15

$m^3$ of compost in both seasons, respectively.

**Table (1). Vegetative growth characters and yield components of oregano treated with biofertilizers and compost treatments in two seasons.**

| Treatments | Plant height (cm) | Fresh weight (g/plant) | Fresh weight yield (kg/fed) | Dry weight (g/plant) | Dry weight yield (kg/fed) | Dry leaves (g/plant) | Dry leaves yield (kg/fed) | Leaf/ stalk ratio |
|---|---|---|---|---|---|---|---|---|
| First season | | | | | | | | |
| Compost ($5m^3$) | 21.28 | 59.61 | 1311 | 19.75 | 434.5 | 9.725 | 213.9 | 49.16 |
| Compost ($10m^3$) | 22.22 | 64.39 | 1417 | 21.80 | 479.7 | 10.86 | 239.1 | 49.89 |
| Compost ($15m^3$) | 26.56 | 73.11 | 1608 | 24.40 | 536.9 | 13.19 | 290.2 | 54.07 |
| Biofert* + Compost ($5m^3$) | 25.84 | 68.33 | 1503 | 23.72 | 521.8 | 12.67 | 278.7 | 53.56 |
| Biofert + Compost ($10m^3$) | 28.00 | 80.26 | 1766 | 28.68 | 631.0 | 17.47 | 384.3 | 60.73 |
| Biofert + Compost ($15m^3$) | 28.67 | 91.55 | 2014 | 32.16 | 707.5 | 20.16 | 443.5 | 62.56 |
| LSD0.05% | 6.77 | 13.55 | 298.2 | 2.87 | 63.14 | 2.15 | 47.31 | 4.38 |
| Second season | | | | | | | | |
| Compost ($5m^3$) | 22.77 | 59.22 | 303 | 19.11 | 420.5 | 9.335 | 205.4 | 49.29 |
| Compost ($10m^3$) | 23.17 | 60.61 | 1333 | 20.98 | 461.6 | 10.47 | 230.3 | 49.98 |
| Compost ($15m^3$) | 25.83 | 68.44 | 1506 | 23.33 | 513.3 | 12.41 | 273.0 | 53.62 |
| Biofert + Compost ($5m^3$) | 26.39 | 70.83 | 1558 | 22.83 | 502.3 | 12.51 | 275.3 | 54.86 |
| Biofert + Compost ($10m^3$) | 27.11 | 84.22 | 1853 | 28.00 | 616.0 | 17.01 | 374.3 | 60.83 |
| Biofert + Compost ($15m^3$) | 27.06 | 87.67 | 1929 | 30.71 | 675.7 | 19.42 | 427.3 | 63.17 |
| LSD0.05% | 4.25 | 7.76 | 170.7 | 2.72 | 59.74 | 1.44 | 31.62 | 4.80 |

**Biofert*** means biofertilizers mixture of nitrogen fixing bacteria (*Azotobacter chroococcum*, *Azospirillum lipoferum)* and phosphours solubilizing bacteria *(Bacillus polymixa*, *Bacillus megatherium* var phosphaticum and *Pseudomonas fluorescence*).

## 3. Plant dry weight and yield/fed

Plant dry weight and total dry yield /fed followed the same trend as in the case of fresh weight and fresh yield/fed as shown in Table (1). Combining bio fertilizer and compost at $15m^3$ recorded the highest dry weight /plant as well as dry yield /fed in the first season. However there were non significant differences between combining bio fertilizer and compost at either 10 $m^3$ or $15m^3$ in the second season. The highest dry weight/plant (32.16 and 30.71 g) was recorded for biofertilizer combined with 15 $m^3$ in both season as yielded in the highest yield/fed (707.5 and 675.7 kg) for both seasons, respectively.

## 4. Dry weight of leaves/plant and yield /fed

Data presented in Table (1) indicate that leaves dry weight/plant and leaves dry weight yield /fed were significantly increased due to combinations between compost and biofertilizer in most cases compared to compost treatment only. Combining bio fertilizer and compost at $15m^3$ recorded the highest dry leaves weight /plant as well as dry leaves yield /fed in both seasons (20.16 and 19.42 g respectively). Similar results were obtained for leaves dry weight yield /fed by combining bio fertilizer and compost at $15m^3$ recorded 443.5 and 427.3 kg/fed respectively for both seasons.

## 5. Leaf/stalk ratio

Leaf ratio was significantly increased due to compost treatments and combinations with biofertilizer as shown in Table (1). Leaf/stalk ratio was positively enhanced by combining biofertilizer with compost at $15m^3$ treatment during both successive seasons. Compost treatment at the low dose ($5m^3$) recorded the lowest leaf/stalk ratio compared to other combinations treatments (49.16 and 49.29 %). The highest ratios for both seasons were obtained by combining bio fertilizer and compost at either 10 or $15m^3$ without significant difference in between.

## 6. Volatile oil percentage

Oregano volatile oil content recorded a reverse direction in response to compost and biofertilizer treatments. Increasing fertilizer levels recorded almost a gradual decrease in volatile oil % in both experimental seasons. Fertilizing oregano with only 5 $m^3$ recorded the highest values for volatile oil % (1.2 and 1.23 %) for both seasons, respectively as shown in Table (2). Meantime, combining bio fertilizer and compost at $15m^3$ recorded the lowest values in this concern.

## 7. Volatile oil composition

The results of GC-MS analysis of volatile oil showed that the main components found in descending order according to their value were p-cymene, carvacrol and ç-Terpinene (Table, 3). The identified volatile oil components were positively affected by compost and their combination with biofertilizers. An increase in p-cymene and carvacrol were recorded as a result of all combinations treatments, while an obvious decrease in ç-Terpinene content was also recorded.

## 8. Volatile oil yield

Although, volatile oil % recorded a reverse direction in response to compost and biofertilizer treatments, total oil yield/fed recorded different results as shown in Table (2), there was no constant trend for the obtained results in this respect. The highest oil yield /fed (5.82 and 5.60 l) was observed after treating oregano plants with biofertilizer combined with compost at $15m^3$ during both successive seasons.

## 9. Chlorophyll content

Chlorophyll b showed non significant differences between the different treatments, while chlorophyll a and total chlorophyll were significantly increased due to individual compost and combinations with biofertilizer treatments as shown in Table (2). Combining bio fertilizer and compost at $15m^3$ recorded the highest values of chlorophyll a in both seasons (0.506 and 0.509 mg/g f.w.) without significant difference compared to combining biofertilizer and compost at $10m^3$. The same trend was observed regarding total chlorophyll.

## 10. Nutrient elements (%)

Data presented in Table (2) emphathized that combining bio fertilizer and compost treatments increased N, P and K percentages in oregano leaves in comparison with individual compost treatments. The highest N percentage was obtained by combining bio fertilizer and compost at $15m^3$ in both seasons (1.74 and 1.74 %, respectively). Regarding P percentage, applying bio fertilizer in combination with compost treatment recorded significant difference in most cases in comparison with compost treatment only. Concerning K percentage combining bio fertilizer with $15m^3$ compost treatment recorded the highest values

(2.64 and 2.63% in both seasons, respectively).

## 11. Total carbohydrates (%)

Total carbohydrates percentage was significantly increased due to combining compost treatments with biofertilizer as shown in Table (2). Compost treatment at the lower dose ($5m^3$) recorded the lowest carbohydrates percentage (11.88 and 12.27 %) compared to other combinations treatments. The highest percentages for both seasons was obtained by combining bio fertilizer and compost at either 10 or $15m^3$ without significant differences in between.

**Table (2). Chemical constituents of oregano plants treated by biofertilizers and compost.**

| Treatment | Volatile oil (%) | Volatile oil yield (l/fed) | Chloro-phyll a ($mgg^{-1}$) | Chloro-phyll b ($mgg^{-1}$) | Total chloro-phyll ($mgg^{-1}$) | N% | P% | K% | Total carbo-hydrates (%) |
|---|---|---|---|---|---|---|---|---|---|
| First Season | | | | | | | | | |
| Compost ($5m^3$ ) | 1.20 | 5.16 | 0.315 | 0.121 | 0.436 | 1.14 | 0.62 | 1.87 | 11.88 |
| Compost ($10m^3$ ) | 1.08 | 5.12 | 0.339 | 0.127 | 0.466 | 1.17 | 0.67 | 2.05 | 12.55 |
| Compost ($15m^3$ ) | 0.99 | 5.37 | 0.359 | 0.124 | 0.483 | 1.31 | 0.74 | 2.15 | 13.52 |
| Biofert* + Compost ($5m^3$ ) | 0.91 | 4.77 | 0.417 | 0.143 | 0.559 | 1.49 | 0.80 | 2.49 | 14.44 |
| Biofert + Compost ($10m^3$ ) | 0.91 | 5.75 | 0.451 | 0.150 | 0.601 | 1.44 | 0.83 | 2.53 | 18.41 |
| Biofert + Compost ($15m^3$ ) | 0.84 | 5.82 | 0.506 | 0.128 | 0.634 | 1.74 | 0.84 | 2.64 | 19.86 |
| LSD0.05% | 0.08 | 0.64 | 0.075 | 0.038 | 0.08 | 0.10 | 0.09 | 0.11 | 2.6 |
| Second season | | | | | | | | | |
| Compost ($5m^3$ ) | 1.23 | 5.14 | 0.307 | 0.125 | 0.432 | 1.16 | 0.75 | 2.01 | 12.27 |
| Compost ($10m^3$ ) | 1.09 | 4.97 | 0.315 | 0.129 | 0.444 | 1.25 | 0.80 | 1.89 | 12.93 |
| Compost ($15m^3$ ) | 1.01 | 5.16 | 0.368 | 0.131 | 0.498 | 1.39 | 0.86 | 2.02 | 13.20 |
| Biofert + Compost ($5m^3$ ) | 0.92 | 4.63 | 0.403 | 0.136 | 0.539 | 1.48 | 0.88 | 2.25 | 16.95 |
| Biofert + Compost ($10m^3$ ) | 0.90 | 5.41 | 0.464 | 0.141 | 0.605 | 1.51 | 0.92 | 2.39 | 18.74 |
| Biofert + Compost ($15m^3$ ) | 0.84 | 5.60 | 0.509 | 0.149 | 0.657 | 1.74 | 0.93 | 2.63 | 19.64 |
| LSD0.05% | 0.08 | 0.65 | 0.11 | 0.04 | 0.11 | 0.15 | 0.04 | 0.39 | 1.55 |

**Biofert*** means biofertilizers mixture of nitrogen fixing bacteria (*Azotobacter chroococcum*, *Azospirillum lipoferum)* and phosphate solubilizing bacteria *(Bacillus polymixa*, *Bacillus megatherium* var phosphaticum and *Pseudomonas fluorescence*.

**Table (3). The main components of oregano volatile oil treated by compost and combination with biofertilizers treatments of the second season.**

| Components | RT | Compost ($5m^3$) | Biofert + compost ($5m^3$) | Biofert + compost ($10m^3$) | Biofert + compost ($15m^3$) |
|---|---|---|---|---|---|
| | | Volatile oil components (%) | | | |
| α-Thujene | 8.82 | 8.08 | 6.03 | 4.9 | 5.31 |
| α-Pinene | 10.39 | 0.38 | | | |
| Sabinene | 10.45 | 0.5 | | | |
| 1-Octen-3-Ol | 10.94 | | | 0.94 | |
| α-Myrcene | 11.15 | 3.85 | 2.01 | | 1.95 |
| α-Terpinene | 11.9 | 0.66 | | | 0.74 |
| o-Cymene | 12.4 | 5.2 | 19.52 | | |
| p-Cymene | 12.7 | 27.9 | 35.12 | 56.3 | 42.9 |
| cis-Ocimene | 12.97 | | | | 1.95 |
| ç-Terpinene | 14.08 | 28.2 | 11.36 | 8.27 | 10.6 |
| Borneol | 17.45 | 0.43 | | 1.28 | 0.56 |
| Carvacrol methyl ether | 20.21 | 0.63 | 1.48 | 1.49 | 2.09 |
| Carvacrol | 23.67 | 23 | 23.8 | 25.7 | 33.3 |
| Caryophyllene | 26.03 | 0.42 | 0.67 | 0.66 | 0.61 |
| Germacrene-D | 27.86 | 0.38 | | | |
| α-Bisabolene | 28.73 | 0.31 | | | |
| Caryophyllene oxide | 30.87 | | | 0.42 | |
| Total | | 99.94 | 99.99 | 99.96 | 99.99 |

**Biofert*** means biofertilizers mixture of nitrogen fixing bacteria (*Azotobacter chroococcum*, *Azospirillum lipoferum)* and phosphate solubilizing bacteria *(Bacillus polymixa*, *Bacillus megatherium* var phosphaticum and *Pseudomonas fluorescence*).

## Discussion:

It is evident from our obtained results that the combined treatment of compost and biofertilizers significantly enhanced growth characters and yield compared to individual compost treatment. Generally, the combination between $15m^3$ compost with biofertilization resulted in the maximum measurements followed by $10m^3$ compost with biofertilization.

These results may be due to the combined promotive effects of each fertilizer source and consequently recorded better results compared to compost treatment alone. Compost as a form of organic fertilizer can lead to enhance growth in many direct and indirect ways i.e. through improving physical, chemical, and biological properties of soil. The enhancing effect includes increasing soil organic matter, cation exchange capacity, and availability of mineral nutrients (Cacciari *et al,* 1989), decreasing bulk density, increasing water holding capacity, preventing nutrient leaches and providing the plant with nutrient requirements (Reddy *et al*, 2005 and Gichangi *et al,* 2009) and increasing exchangeable potassium in soil (Ohallorans *et al,* 1993).

Enhancing growth and yield of oregano was significant due to combining compost with biofertilizers. Plant growth promoting rhizobacteria (PGPRs) can influence plant growth through nitrogen fixation and production of bio-control agents against soil-borne phytopathogens (Glick, 2003). Also biofertilizers affect reducing membrane potential of the root, synthesis of some enzymes (such as ACC deaminase) that modulate the level of plant hormones as well as the solubilization of inorganic phosphate and mineralization of organic phosphate, which make phosphorus the essential component of the energy compounds (ATP and ADP) and phosphoproteins (Marschner, 1995) available to the plants (Rodriguez and Fraga, 1999).

Furthermore, this stimulative effect may be related to the good equilibrium of nutrients and water in the root medium (Abdelaziz *et al*, 2007 a) or to the beneficial effects of bacteria on vital enzymes and hormonal, stimulative effects on plant growth (Bashan *et al,* 1989). The non-symbiotic N2-fixing bacteria produce adequate amounts of IAA and cytokinins with increasing the surface area per unit root length and enhanced the root hair branching with an eventual increase in the uptake of nutrients from the soil (Jagnow *et al,* 1991 and Rodriguez and Fraga, 1999). Several studies proved similar results by many others investigators as Edris *et al.* (2003) on *Origanum majorana*, Kandeel and Sharaf (2003) and Mahfouz (2003) on *Marjorana hortensis*, Hamed (2004) on *Salvia officinalis* and Al-Fraihat *et al* (2011) on marjoram.

On the other hand, the highest essential oil % was recorded for the lower dose of compost at 5 $m^3$. Meanwhile the highest essential oil yields were obtained from plants treated by combing biofertilizer and 15 $m^3$ compost. In this respect, it can be suggested that the simulative effect of the mixture of compost and microorganisms on increasing essential oil yield might be attributed to their enhancing effect on vegetative growth characteristics and plant chemical composition. These results are in accordance with the findings of Mahfouz (2003) on *Majorana hortensis*, Hamed (2004) on *Salvia officinalis* plants. Toaima (2005) on *Achillea millefolium* L. Abdelaziz *et al.* (2007 b) on *Rosmarinus officinalis* and Khalil *et al* (2008) on sage.

Meanwhile there was a remarkable increase in chlorophyll content since chlorophyll synthesis has been related to phosphate fixing bacteria. Phosphobacteria stimulate chlorophyll synthesis through encouraging pyridoxal enzymes formation, that play an important role in α-amino levulinic acid synthetase as a primary compound in chlorophyll synthesis (Ramadan *et al*, 2003). Increasing chlorophyll content the main source for biosynthesis in the plant leads to increasing carbohydrate content in the leaves. Our–results agreed with those stated by Khalil and El-Sherbeny (2003) on three *Mentha* species, Hussein *et al* (2006) on *Dracocephalum moldavica* L. family Lamiaceae. Corrêa *et al* (2009) on oregano and El-Kallaf (2011) on *Mentha spicata*, L.

The combined promotion effects of each fertilizer source also enhanced the percentages of N, P and K elements in oregano herb. Phosphate solubilizing bacteria release organic and inorganic acids which decrease soil pH leading to change of phosphorus and other nutrients to available forms ready for uptake by plants (Singh and Kapoor, 1999). Enhanced root growth with an eventual increase on the uptake of nutrients from the soil in addition of fixation of nitrogen and phosphorus eventual increase increased the percentages of N, P and K elements in the herb and this increment led to promote the growth and yield of oregano plants. Similar reults were obtained by Mahfouz (2003) on Majorana, Hamed (2004) on *Salvia officinalis*, Abdelaziz *et al* (2007 b) on *Rosmarinus officinalis*, Ali (2008) on *Origanum syriacum* L. Al-Fraihat *et al* (2011) on marjoram and Abou Seedo *et al* (2014) on *salvia officinalis*.

## REFERENCES

A.O.A.C. (1970). Official Methods of Analysis of Association of Official Agriculture Chemists. Washington, D .C., 10 th ed.

Abdel Wahab, M. M. and A. Z. A. Hassan. (2013). Response of fennel plants to organic biofertilizer in replacement of chemical fertilization. Top Class Journal of Agric. Res. 1(3):29-35.

Abdelaziz, M. E., A. H. Hanafy Ahmed, M. M. Shaaban and R. Pokluda (2007a). Fresh weight and yield of lettuce as affected by organic manure and bio-fertilizers. Proc. Conference of Organic Farming, Czech Univ. Agric ,.Czech Republic: 212-214.

Abdelaziz, M., R. Pokluda and M. Abdelwahab (2007b). Influence of compost, microorganisms and NPK fertilizer upon growth, chemical composition and essential oil production of *Rosmarinus officinalis* L. Not. Bot. Hort. Agrobot. Cluj, 35, Issu 1.

Abou Seedo, K. F., A. A. Taha and A. A. Salih (2014). Effect of three different growth media on yield constituents of sage (*Salvia officinalis*) under protected agriculture conditions. Sci. Res. Essays, 2(10):478-485.

Al-Fraihat, A.H., S. Y. A. Al-dalain, Z. B. Al-Rawashdeh, M. S. Abu-Darwish and J. A. Al-Tabbal (2011). Effect of organic and biofertilizers on growth, herb yield and volatile oil of marjoram plants grown in Ajloun region, Jordan. J. Med. Plants Res., 5(13):2822-2833.

Ali, M. Y. M. (2008). Effect of fertilization, irrigation and planting distances on the growth and production of *Origanum syriacum* L. plant under Sinai conditions. Ph.D. Thesis, Fac. Agric., Cairo Univ.,Egypt.

Antunes, M. D. C and A. M. Cavaco (2010). The use of essential oils for postharvest decay control. A review. Flavour Fragr J., 25:351-366.

Bashan, Y., Y. Ream, H. Levanovy and A. Sade (1989). Nonspecific responses in plant growth, yield and root colonization or non cereal crop plant to inoculation with *Azospirillum brasilense*, Publication: Can. J. Bot., 67:1317-1324.

British Pharmacoepea. (1963). Determination of Volatile Oil in Drugs. Pharmaceutical Press, London.W.C.I.

Cacciari, D. L., T. Pietrosanti and W. Pietrosanti (1989). Phytohormones–like substances produced by single and mixed diazotrophic cultures of Azospirillum and Arthrobacter. Plant Soil, 115:151-153.

Chaphlin, M. F. and J. F. Kennedy (1994). Carbohydrate Analysis, 2nd ed. Oxford University Press, New York.

Corrêa, R. M., J. E. B. P. Pinto, É. S. Reis, C. De Oliveira, E. M. De Castro and R. D. Brant (2009). Leaf anatomical traits of oregano (*Origanum vulgare* L.) plants, subjected to different sources and levels of organic fertilization. Acta Sci. Agron., 31(3):439-444.

Cottenie, A., M. Verloo, M. Velghe and R. Camerlynck (1982). Chemical Analysis of Plant and Soil. Laboratory of Analytical and Agrochemistry. State Univ. Ghent, Belgium.

Edris, A. E., A. Shalaby and H. M. Fadel (2003). Effect of organic, agriculture practices on the volatile aroma components of some essential oil plants growing in Egypt. II: Sweet marjoram (*Origanum majorana* L.) essential oil. Flavour Fragr J., 18(4): 345-351.

El-Kallaf, H. H. A. (2011). Influence of organic, inorganic fertilization and bio fertilization on growth, chemical composition and essential oil content of spearment (*Mentha spicata,* L.). Ph.D. Thesis, Fac. Agric (Saba Basha), Alexandria Univ., Egypt.

Follet, R., R. Donahue and L. Murphy (1981). Soil and Soil Amendments. Prentice. Hall, Inc., New Jersey.

Frederickson, J., K. R. Butt, M. R. Morris and C. Daniel (1997). Combining vermiculture with green waste composting system. Soil. Biol. Biochem., 29 (3/4), 725.730.

Garcia, C., T. Hernandez, F. Costa and B. Ceccanti (1994). Biochemical parameters in soil regenerated by addition of organic wastes. Waste. Manag. Res, 12: 457-466.

Gichangi, E. M., P. N. Mnkeni and P. C. Brookes (2009). Effects of goat manure and inorganic phosphate addition on soil inorganic and microbial biomass phosphorus fractions under laboratory incubation conditions. J. Plant Nutr. Soil Sci., 55: 764-771.

Glick, B. R. (2003). Plant growth promoting bacteria. In: Glick, B.R. and J.J. Pasternak (eds.), Molecular Biology-Principles and Applications of Recombinant DNA, p: 436–54. ASM Press, Washington DC, USA.

Gomaa, A. O. and H. E. Abo-Aly (2001). Efficiency of biofertilization in the presence of inorganic and organic fertilizers on growth, yield and chemical constituents of anise plant (*Pimpinella anisum*,L.). Proc. 5th Arabian Hort. Conf., Ismailia, Egypt, March. 24-28: 73-80.

Hamed, E. S. (2004). Studies on planting of some medicinal plants in the desert. M.Sc. Thesis, Fac. Agric., Kafr El-Sheikh, Tanta Univ., Egypt.

Hellal, S. A. Mahfouz and F. A. S. Hassan (2011). Partial substitution of mineral nitrogen fertilizer by bio-fertilizer on *Anethum graveolens* L. plant. Agric. Biol. J. N. Am., 2(4):652-660.

Hussein, S. E. El-Sherbeny, M. Y. Khalil, N. Y. Naguib and S. M. Aly (2006). Growth characters and chemical constituents of *Dracocephalum moldavica* L. plants in relation to compost fertilizer and planting distance. Sci Hortic, 108:322–331.

Jagnow, G., G. Hoflich and K. H. Hoffman (1991). Inoculation of non-symbiotic rhizosphere bacteria. Possibilities of increasing and stabilizing yield. Angew Botanik, 65: 97-126.

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: 1373-1389.

Kandeel, Y. R., E. S. Nofal, F. A. Menesi, K. A. Reda, M. N. Taher and Z. T. Zaki (2001). Effect of some cultural practices on growth and chemical composition of *Foeniculum vulgare*, Mill. Proc. 5th Arabian Hort. Conf., Ismailia, Egypt, March. 24-28: 61-72.

Khalil, M. Y. and S. E. El-Sherbeny (2003). Improving the productivity of three *Mentha* species recently cultivated under Egyptian conditions. Egypt. J.Appl. Sci., 18 (1):285–300.

Khalil, M. Y., M. A. M. Kandil and M. F. Swaefy Hend (2008). Effect of three different compost levels on fennel and Salvia growth characters and their essential oils. Res. J. Agric. & Biol. Sci, 4(1):34-39.

Kintzios, S. E. (2002). Profile of the multifaceted prince of the herbs. In: Kintzios SE (Ed.) Oregano: The genera *Origanum* and *Lippia*. Medicinal and Aromatic Plants Industrial Profiles 25. Taylor and Francis/ CRC Press USA, 3–8, 236–242.

Mahfouz, S. A. and M. A. Sharaf-Eldin (2007). Effect of mineral vs. biofertilizer on growth, yield and essential oil content of fennel (*Foeniculum vulgare*.Mill). Int. Agrophys, 21:361-366.

Mahfouz, S. A. S. (2003). Effect of biofertilization on growth and oil production of marjoram (*Majorana hortensis* Moench.) plant. Ph.D. Thesis, Fac. Agric., Cairo Univ., Egypt.

Marschner, H. (1995). Mineral Nutrition in Higher Plants. (2nd ed), Academic Press, London, New York., 861 PP.

Murphy, J. and J. H. Riley (1962). A modified single solution for the determination of phosphate in natural waters. Annal. Chem. Acta, 27:31-36.

Nofal, E. S., Y. R. Kandeel, F. A. Menesi, K. A. Reda, M. Taher and Z .T. Zaki (2001). Effect of some cultural practices on growth and chemical composition of *Ammi visnaga*,L. Proc. 5th Arabian Hort. Conf., Ismailia, Egypt, March. p: 51-60.

Ohallorans, J. M., M. A. Munoz and O. A. Colberg (1993). Effect of chicken manure on chemical properties of mollisil and tomato production. J. of Agric. of the Univ. of Puerto Rico., 77(3): 181-191.

Pascual, J. A., T. Hernandez, M. Ayuso and C. Garcia (1997). Changes in the microbial activity of arid soils amended with urban organic wastes. Biol Fert Soils, 24: 429-434.

Ramadan, B. S., H. R. Hassan and F. A. Abdo (2003). Effect of minerals and biofertilizers on photosynthetic pigments, root quality, yield components and anatomical structure of sugar beet (*Beta vulgaris*, L.) plants grown under reclaimed soil. J. Agric. Sci. Mansoura Univ., 28(7): 5139-5160.

Reddy, D. D., S. A. Rao. and M. Singh (2005). Changes in P fractions and sorption in an Alfisol following crop residue application. JPNSS, 168: 241-247 .

Rivero, C., T. Chirenje, L. Q. Ma and G. Martinez (2004). Influence of compost on soil organic matter quality under tropical conditions. Geoderma., 123: 355-361.

Rodriguez, H. and R. Fraga (1999). Phosphate solubilizing bacteria and their role in plant growth promotion .Biotech. Advan., 17: 319-339.

Ryckeboer, J., J. Mergaert, K. Vaes, S. Klammer, D. De Clercq, J. Coosemans, H. Insam and J. Swings (٢٠٠٣). A survey of bacteria and fungi occurring during composting and self-heating processes. Ann Microbiol, 53: 349-410.

Sadhana, B. (2014). “*Arbuscular Mycorrhizal* Fungi (AMF) as a biofertilizer”. IJCMAS . 3(4):384-400.

Saha, S., B. L. Mina, K. L. Gopinath, S. Kundu and H. S. Gupta (2008). Relative changes in phosphatase activities as influenced by source and application rate of organic composts in field crops. Biores Technol., 99:1750–1757.

Sahin, F., M. Güllüce, D. Daferera, A. Sökmen, M. Sökmen, M. Polissiou, G. Agar and H. özer (2004). Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp.*vulgare* in the Eastern Anatolia region of Turkey. Food Control, 15: 549–557.

Saric, M., R. Kastrori, R. Curic, T. Cupina and I. Gric (1967). Chlorophyll determination. University of “Noveon Sadu Praktikum iz Fiziologize Biljaka”, Belgrade ,Serbia.

Shaalan, M. N. (2005). Influence of biofertilization and chicken manure on growth, yield and seeds quality of *Nigella sativa*, L. plants. Egypt, J. Agric. Res., 83(2): 811-828.

Singh, S. and K. K. Kapoor (1999). Inoculation with phosphate-solubilizing microorganisms and vesicular arbuscular mycorrhizal fungus improves dry matter yield and nutrients uptake by wheat grown in sandy soil. Biol. Ferti. Soils, 28: 139-144.

Skoula, M. and J. B. Harborne (2002). The taxonomy and chemistry of *Origanum.* In: Kintzios, S.E. (Ed.), Oregano: The genera *Origanum* and *Lippia*. Taylor and Francis, London, p: 67–108.

Snedecor, G. W. and W. G. Cochran (1982). Statistical Methods. The Iowa State Univ., Press., Ames., Iowa, U.S.A., 507 pp.

Toaima, I. M. W. (2005). Production of yarrow (*Achillea millefolium* L) under Sinai conditions. M. Sc. Thesis, Fac. Agric., AL-Azhar Univ.

## الإنتاج الحيوى لنبات الأوريجانو تحت ظروف التربة الرملية المصرية

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### الملخص العربى

أجريت هذه الدراسة بغرض دراسة الإنتاج الحيوى للأوريجانو تحت ظروف التربة الرملية المصرية باستخدام الكومبوست والتسميد الحيوى. تم اجراء الدراسة بمحطة تجارب النباتات الطبية والعطرية بشركة سيكم، محافظة الشرقية، مصر. لتحقيق الغرض من الدراسة تم تسميد النباتات باستخدام الكومبوست منفرداً بمعدل ٥م$^{٣}$، ١٠م$^{٣}$ و ١٥م$^{٣}$ للفدان أو بالتفاعل مع التسميد الحيوى كخليط متساوى من خمس سلالات بكتيرية مثبتة للنتروجين ومذيبة للفوسفور يتم تخفيفه بنسبة ١:٢٠ واضافته للتربة بمعدل ٥٠ مل/نبات. أظهرت النتائج زيادة معنوية فى كافة صفات النمو الخضرى وإنتاجية النبات خاصة عند وجود التفاعل بين التسميد الحيوى و١٥م$^{٣}$ كمبوست. وبينما كانت هناك زيادة معنوية فى صفات النمو والإنتاجية لوحظ إنخفاض محتوى أوراق النبات من الزيت الطيار إلا أن الزيادة الكلية للمحصول نتيجة التسميد الحيوى بالتداخل مع الكمبوست ١٥م$^{٣}$ أدت إلى زيادة محصول الفدان من إنتاجية الزيت الطيار. وبصفة عامة تشير نتائج البحث إلى إمكانية الانتاج الحيوى لنبات الأوريجانو سواء لإنتاج العشب الطازج أو الجاف المطلوب للتصدير أو لإنتاج الزيت الطيار.