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# Response of Basil Essential Oil to Cultivation Date and Organic Fertilization

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**Abstract: Purpose**: This investigation was carried out during the two successive seasons (2013 and 2014) to investigate the effect of date of cultivation and organic fertilization on the production and constituents of the essential oil of *Ocimum basilicum,* var. Genovese. The experiment was designed as a split-plot with three replications. Two sowing dates (March and April) were the main plots and the sub plots consisted of nine treatments of yeast extract and / or algae extract. From the above mentioned results the recommended treatment to obtain the best oil characteristics of *O. basilicum* is to cultivate it in April with application of 2 ml/l algae + 6 g/l yeast for essential oil %, essential oil yield ( ml/plant and L/Fed.), the major compound was identified as linalool in the essential oil of the studied treatments with relative percentage ranged from 60.23 to 66.47% from all separated compounds. The second major compound was identified as 1,8-cineol in the essential oil of all treatments and reached to 16.48%.
**Key words**: *Ocimum basilicum,* oil, yeast, algae, sowing dates.

## Introduction:

The genus *Ocimum* includes about a dozen species and subspecies native to the tropical and sub-tropical regions of the world. "Genovese" basil (*Ocimum basilicum,* var. Genovese) is a variety of sweet basil that originated in Italy. It is widely cultivated for the production of essential oils and is also marketed as a herb, either fresh, dried or frozen[1]. Basil has been utilized for its expectorant, carminative and stimulant properties in folk medicine. In addition, it was used as insecticide[2], flea and moth repellent and against to snake, scorpion and insect bite[3].

Cultivation date plays an important role in the performance, production and consequently the yield of medicinal and aromatic plants which in turn affect the farm income. Changing in planting date leads to significant changes in the weather which affects the total period of plant growth and exposure to the environment. Planting Planting date may influence the crop productivity and its inner components [4].The effect of sowing date on growth, yield and active ingredients of medicinal and aromatic plants was studied by many investigators; such as [5] on *Ruta Graveolens*[6], on *Coriandrum sativum,*[7] on *Artemisia annua* and [8] on anise plant.

Active dry yeast is a natural safety biofertilizers causes various promotion effects on plants. It is considered as a natural source of cytokinins which stimulates cell division and enlargement as well as the synthesis of protein, nucleic acid and B-vitamin[9]. It also releases $CO_2$ which reflected in improving net photosynthesis[10]. Foliar application of dry yeast enhanced growth, plant nutritional and essential oil yield of

thyme plants[11]. The effect of dry yeast is due to its capability in induction of endogenous hormones like GA3 and IAA[12].

Recently, the algal extract used as a fertilizer since it contains plant hormones, amino acids, fatty acids and trace elements responsible for controlling plant growth and development and for improving the resistance to pathogens . The positive effects of algae and algal extracts on the growth of vegetables, fruits and other crops had been reported. Algal extracts are used both: for conditioning seeds or as fertilizers for soil or foliar application during the growing season and flowering. They stimulate seed germination, growth and yield of different crops[13,14,15]. More than 200 compounds from the essential oil were identified and different chemotypes have been classified for *O. basilicum* according to the essential oil chemical composition[16,17]. *Ocimum basilicum* L, contains essential oils based primarily on monoterpene derivatives such as linalool[18].

Omer *et. al*[19] found that linalool is the most prominent component in Genovese basil grown in Egypt. [20]reported that the essential oil of basil cultivated in Egypt contained 48% linalool, 3.04% methyl chavicol and 5.9% eugenol. The essential oil of basil oil showed different biological activities i.e. antimicrobial, antioxidant[21,22], antifungal [23] and insecticidal[24,25]. Some of its components, such as 1, 8-cineole, linalool, and camphor are known to be biologically active[26].

This study was carried out to investigate the influence of planting dates and/or organic fertilizer on the essential oil of *Ocimum basilicum*, var. Genovese .

## Materials and Methods

This study was carried out in the Hawareya village, Beheira Governorate, Egypt during the two successive seasons of 2013 and 2014 in clay soil. The soil was carefully prepared and initial soil samples to 30 cm depth from experimental site were collected and analyzed for some important chemical and physical properties. The results are presented in Table (1). The chemical analysis of water irrigation is shown in Table (2).

**Table (1): Physical and chemical analysis of the tested soil**

| **Available macronutrients (ppm)** | | | | | **Available micronutrients (ppm)** | | | |
|---|---|---|---|---|---|---|---|---|
| **N** | **P** | **K** | **$Ca^{++}$ (Meq/l)** | **$Mg^{++}$ Meq/l)** | **Fe** | **Mn** | **Zn** | **Cu** |
| 20 | 5 | 382 | 2.5 | 1.8 | 3.5 | 4.5 | 0.75 | 2.7 |
| **Mechanical Characteristics** | | | **Soil particle size distribution** | | | | | |
| **EC mmohos / cm** | | **pH** | **Sand%** | **Silt %** | **Clay %** | **Soil texture** | | |
| 0.8 | | 8 | 21.31 | 20.94 | 54.20 | Clay | | |

**Table (2): Chemical composition of water used for irrigation**

| **pH** | **EC mmohos /cm** | **Soluble ions (Meq/l)** | | | | | | | **SAR** |
|---|---|---|---|---|---|---|---|---|---|
| | | **$Ca^{++}$** | **$Mg^{++}$** | **$Na^{+}$** | **$K^{+}$** | **$HCO_3^{-}$** | **$Cl^{-}$** | **$SO_4^{-}$** | |
| **6.9** | 0.81 | 3 | 2.1 | 2.74 | 0.23 | 1.6 | 1.8 | 4.7 | 2 |

**SAR: sodium adsorption ratio.**

The experimental layout was a split-plot design with three replications. The main plots contained the two sowing date treatments; while the sub plots were allocated to the nine organic and bio fertilizer treatments as follow:

**Control**

Oligo- x at 1ml /l (O1)
Oligo- x at 2ml/l (O2)
Yeast at 3 g/l (Y3)
Yeast at 6 g/l (Y6)
Oligo- x at 1ml/l + Yeast at 3 g/l (O1 + Y3)

Oligo- x at 1ml/l + Yeast at 6 g/l (O1 + Y6)
Oligo- x at 2ml/l + Yeast at 3 g/l (O2 + Y3)
Oligo- x at 2ml/l + Yeast at 6 g/l (O2 + Y6)

Seeds of basil (*Ocimum basilicum var.* Genovese) were obtained from SEKEM Company, and were sown in seed bed at two consecutive months: 1st March and 1st April during 2013 and 2014 seasons. When seedlings height reached 10 cm (after 45 days), they were transplanted in the permanent field at the distance of 30 cm between hills and intro-row spacing of 60 cm.

After 2 weeks of transplanting, some plots were sprayed with Oligo-x (as algae extraction) with different levels (1 and 2 ml/l) and one week later, some plots were sprayed with different levels of yeast (3 and 6 g/l) . These foliar application treatments were repeated after the 1st cut. So, this investigation included nine treatments as mentioned before

Algae extract formulation: Algae extract (oligo-x) was obtained from AGAS (Arabian group for agricultural service) company having the following composition: oligo saccharide (3%), algnic acid (5%), phytin (0.003%), menthol (0.001%), natural growth regulators (cytokinine 0.001 %, indol acetic acid 0.0002% and pepsin 0.02%) and minerals (potassium oxide 21% , phosphorus oxide 0.5%, N 1% , Zn 0.3% ,Fe 0.2% and Mn 0.1%).Yeast Application: Yeast solution was prepared according to method described by [27,28].The composition of yeast solution used in the experiment was described in Table (3)

**Table (3): The composition of yeast solution**

| **minerals (µ / g)** | | | | **vitamins (µ/ g)** | | **the major components (%)** | |
|---|---|---|---|---|---|---|---|
| **Na** | 0.12 | **Cu** | 8.00 | **Thiamine B1** | 60 – 100 | **Protein** | 47 |
| **Ca** | 0.75 | **Se** | 0.10 | **Riboflavin B2** | 35 – 50 | **Carbohydrates** | 33 |
| **Fe** | 0.02 | **Mn** | 0.02 | **Niacin B3** | 300 – 500 | **Nucleic acids** | 8 |
| **Mg** | 1.65 | **Cr** | 2.20 | **Pyridoxine HCL B6** | 28 | **Minerals** | 8 |
| **K** | 21.0 | **Ni** | 3.00 | **Pantorhenate B5** | 70 | **Lipids** | 4 |
| **P** | 13.5 | **Va** | 0.04 | **Biotin B7** | 1.3 | | |
| **S** | 13.5 | **Mo** | 0.40 | **Cholin B4** | 40 | | |
| **Zn** | 0.17 | **Sn** | 3.00 | **Folic acid B 9** | 5 – 13 | | |
| **Si** | 0.03 | **Li** | 0.17 | **Cobalamin B12** | 0.001 | | |

All treatments received 15 $m^3$/Fed of manure + 300 Kg/Fed superphosphate during preparing and hoeing the soil.

Two cuts (harvests) were carried out during the two successive seasons of study. The 1st cut was carried out after 2 months of transplanting (full bloom stage, which is the optimal commercial stage for oil production as mentioned by[29,30,31]. The second cut was done after two months from the 1st cut at the beginning of bloom stage.

**1**. **Essential oil percentage**: The volatile oil of air dried herb for each treatment was extracted by hydro distillation for 3 hours according to[32]. The resulted essential oil was dehydrated over anhydrous sodium sulfate and was kept at deep freezer till GC-MS analyses. The percentage of essential oil was calculated and expressed as volume (ml/weight) on the base of dry weight. Essential oil yield per plant was calculated by multiplying the oil percentage by the average plant yield of dry herb.

**2**. **GC/MS**: GC-MS analysis for the essential oil of the first cut for the second season was analyzed using gas chromatography–mass spectrometry instrument stands at the Department of Medicinal and Aromatic Plants Research, National Research Centre with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-WAX MS column (30 m x 0.25 mm i.d., 0.25 $\mu$m film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10 using the following temperature program: 40 $^{o}$C for 1 min; rising at 4.0 C/min to 160 C and held for 6 min; rising at 6 $^{o}$C/min to 210 $^{o}$C and held for 1 min. The injector and detector were held at 210 $^{o}$C. Diluted samples (1:10 hexane, v/v) of 0.2 $\mu$L of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450.

**Qualitative and quantitative analyses**: Most of the compounds were identified using mass spectra (Wiley spectral library collection and NIST library). The identification of the separated components was confirmed by the published data i.e.[33].

Data were subjected to analysis of variance using[34]. The mean values of all parameters were compared by LSD test according to[35]. Simple correlation coefficient and simple linear regression analysis were determined according to[36].

## Result and Discussion

### 1. Essential oil percentage:

### 1.1. Effect of sowing date:

The mean values indicating that planting date significantly affected the essential oil percentage (Table, 4). April planting date gave the highest oil percentages (0.630 % and 0.640 for first cut against 0.690 % and 0.660 % for 2nd cut at 1st and 2nd seasons, respectively) than that obtained in March planting date (0.560 % and 0.550 for first cut against 0.560 % and 0.580 % for 2nd cut at 1st and 2nd seasons, respectively) during 1st and 2nd seasons, respectively.

In this respect, [37] concluded that *Artemisia vulgaris* plants transplanted in the period from January to March contained moderate oil percentage (0.20 to 0.28 %) while that transplanted in June had the highest oil percentage (0.4%). [38]obtained the highest oil content (%) in bergamot mint (*Mentha citrate*) planted on 17th March as compared with former sowing date (15th December). [5]found that delaying sowing date of *Ruta graveolens* from 20th October to 20th November resulted in significant promotion for percentage and yield of essential oil of herb. [39]studied the effect of three different sowing dates on *Coriandrum sativum* and showed that sowing dates affected on essential oil %. The more delayed sowing date the more the highest oil percentage was given.

The relationship between essential oil content and sowing date has not been established. It has been hypothesized that cultivating medicinal plants for essential oil content could theoretically be later than medicinal plants for canopy because plants do not have to be harvested at maturity[40]. Early planting increases the total length of time that the plant is in the field and exposed to the environment and also is associated with increased incidences of several diseases[41].

**Table (4): The mean values of the essential oil percent of basil as affected by two sowing dates in the two seasons 2013 and 2014**

| Sowing dates | 1st season | | | | | |
|---|---|---|---|---|---|---|
| | 1st cut | | | 2nd cut | | |
| | Oil % | Oil (ml/plant) | Oil (L/Fed) | Oil % | Oil (ml/plant) | Oil (L/Fed) |
| **1st sowing date (March)** | 0.560 | 0.250 | 5.56 | 0.560 | 0.287 | 6.390 |
| **2nd sowing date (April)** | 0.630 | 0.318 | 7.06 | 0.690 | 0.379 | 8.330 |
| **LSD at 0.05** | **0.005** | **0.001** | **0.01** | **0.004** | **0.001** | **0.004** |
| | 2nd season | | | | | |
| **1st sowing date (March)** | 0.550 | 0.212 | 4.71 | 0.580 | 0.218 | 4.850 |
| **2nd sowing date (April)** | 0.640 | 0.298 | 6.62 | 0.660 | 0.297 | 6.599 |
| **LSD at 0.05** | **0.006** | **0.002** | **0.01** | **0.004** | **0.002** | **0.133** |

### 1.2. Effect of Fertilizer:

Algae extract and/or yeast applications significantly affected essential oil percentage of both cuts during both seasons (Table, 5). Untreated plants produced the lowest mean values of essential oil % during both seasons.

The two applied algae levels significantly increased essential oil percentage comparing with control for both cuts during the two seasons. Algae extract at 2ml/l resulted in the highest mean values of essential oil

percentage (0.560 % and 0.530 % for 1st cut as well as 0.580 % for 2nd cut during the 1st and 2nd seasons, respectively).

A significant effect was observed for the two applied levels of yeast (3 and 6 g/l) comparing with control. Application of yeast at 3 or 6 g/l was superior than the other two applied levels and gave the same value (0.580 %) in the 1st cut of 1stseason.The maximum mean values of essential oil % (0.650 % for 2nd cut of 1st season, 0.580% and 0.610% during the 2nd season for 1st and 2nd cut, respectively) compared with control, the two applied algae levels and the lowest level of yeast (3 g/l).

The combined treatment algae extract at 2 ml/l + yeast extract at 6 g/l (O2 + Y6) produced the highest mean value of essential oil % (0.700% and 0.770% for 1st cut as well as 0.730 % and 0.740% for 2nd cut during 1st and 2nd seasons, respectively) followed by that of algae extract at 2 ml/l + yeast at 3 g/l (O2 + Y3) during both seasons comparing with control and all other treatments. These results are in agreement with those obtained by [42]on Margoram plant, [43]on *Salvia officinalis* and [44]on *Melissa officinalis* , [45]on borage and [46]on fennel**.**

**Table (5): Essential oil percent, oil content (ml/plant) and oil yield (L/Fed.) of basil plants as affected by algae extract and/or yeast applications for two cuts in the two seasons 2013 and 2014.**

| Fertilizer | 1st season | | | | | |
|---|---|---|---|---|---|---|
| | 1st cut | | | 2nd cut | | |
| | Oil % | Oil (ml/plant) | Oil (L/Fed) | Oil % | Oil (ml/plant) | Oil (L/Fed) |
| **Control** | 0.510 | 0.200 | 4.45 | 0.520 | 0.219 | 4.87 |
| **O1(1ml / l)** | 0.520 | 0.297 | 6.59 | 0.570 | 0.324 | 7.20 |
| **O2 (2ml /l)** | 0.560 | 0.283 | 6.29 | 0.580 | 0.405 | 8.99 |
| **Y3 (3g/l)** | 0.580 | 0.313 | 6.96 | 0.590 | 0.390 | 8.67 |
| **Y6 (6g/l)** | 0.580 | 0.209 | 4.64 | 0.650 | 0.278 | 6.18 |
| **O1+Y3** | 0.600 | 0.312 | 6.93 | 0.640 | 0.255 | 5.28 |
| **O1+Y6** | 0.620 | 0.304 | 6.75 | 0.660 | 0.308 | 6.83 |
| **O2+Y3** | 0.670 | 0.312 | 6.93 | 0.670 | 0.389 | 8.64 |
| **O2+Y6** | 0.700 | 0.329 | 7.31 | 0.730 | 0.432 | 9.59 |
| **LSD at 0.05** | **0.009** | **0.003** | **0.01** | **0.014** | **0.001** | **0.01** |
| **2nd season** | | | | | | |
| **Control** | 0.480 | 0.170 | 3.77 | 0.500 | 0.145 | 3.23 |
| **O1(1ml / l)** | 0.500 | 0.232 | 5.16 | 0.570 | 0.241 | 5.33 |
| **O2 (2ml /l)** | 0.530 | 0.248 | 5.52 | 0.580 | 0.296 | 6.57 |
| **Y3 (3g/l)** | 0.570 | 0.263 | 5.85 | 0.600 | 0.278 | 6.17 |
| **Y6 (6g/l)** | 0.580 | 0.264 | 5.87 | 0.610 | 0.268 | 5.96 |
| **O1+Y3** | 0.620 | 0.252 | 5.60 | 0.640 | 0.244 | 5.42 |
| **O1+Y6** | 0.650 | 0.274 | 6.08 | 0.670 | 0.257 | 5.71 |
| **O2+Y3** | 0.710 | 0.259 | 5.76 | 0.680 | 0.254 | 5.64 |
| **O2+Y6** | 0.770 | 0.334 | 7.42 | 0.740 | 0.338 | 7.50 |
| **LSD at 0.05** | **0.007** | **0.004** | **0.01** | **0.013** | **0.003** | **0.29** |

### 1.3. Effect of the combination between sowing date and fertilizers treatments:

The combination interaction effect between 2nd sowing date and both algae extract at 2 ml + 6 g/l from yeast which recorded 0.730% and 0.840% in the 1st cut as well as 0.790% and 0.800% in the 2nd cut during 1st and 2nd seasons, respectively (Tables 6 &7). On the other hand, the lowest mean values of essential oil % (0.460 % and 0.400% for 1st cut, while 0.510% and 0.470% for 2nd cut during 1st and 2nd seasons, respectively) were obtained as a result of 1st sowing date without fertilizers treatments. Similar results were reported by [46]on fennel.

**Table (6): Essential oil percent, oil content (ml/plant) and oil yield (L/Fed.) of basil plants as affected by algae extract and/or yeast applications and date of sowing for two cuts in the first season 2013.**

| Sowing dates | Fertilizer | 1st season | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1st cut | | | 2nd cut | | |
| | | Oil % | Oil (ml/plant) | Oil (L/Fed) | Oil % | Oil (ml/plant) | Oil (L/Fed) |
| 1st sowing date (March) | Control | 0.460 | 0.178 | 3.96 | 0.510 | 0.219 | 4.87 |
| | O1(1ml / l) | 0.480 | 0.252 | 5.60 | 0.540 | 0.277 | 6.16 |
| | O2 (2ml /l) | 0.510 | 0.223 | 4.96 | 0.540 | 0.331 | 7.36 |
| | Y3 (3g/l) | 0.550 | 0.291 | 6.47 | 0.530 | 0.417 | 9.27 |
| | Y6 (6g/l) | 0.560 | 0.184 | 4.09 | 0.550 | 0.211 | 4.69 |
| | O1+Y3 | 0.570 | 0.249 | 5.53 | 0.530 | 0.201 | 4.47 |
| | O1+Y6 | 0.550 | 0.282 | 6.27 | 0.550 | 0.204 | 4.53 |
| | O2+Y3 | 0.670 | 0.313 | 6.96 | 0.590 | 0.344 | 7.64 |
| | O2+Y6 | 0.660 | 0.281 | 6.24 | 0.660 | 0.382 | 8.49 |
| 2nd sowing date (April) | Control | 0.550 | 0.222 | 4.93 | 0.530 | 0.219 | 4.87 |
| | O1(1ml / l) | 0.560 | 0.341 | 7.58 | 0.590 | 0.371 | 8.24 |
| | O2 (2ml /l) | 0.610 | 0.343 | 7.62 | 0.620 | 0.478 | 10.62 |
| | Y3 (3g/l) | 0.600 | 0.335 | 7.44 | 0.640 | 0.363 | 8.07 |
| | Y6 (6g/l) | 0.600 | 0.233 | 5.18 | 0.740 | 0.345 | 7.67 |
| | O1+Y3 | 0.620 | 0.375 | 8.33 | 0.740 | 0.308 | 6.84 |
| | O1+Y6 | 0.690 | 0.325 | 7.22 | 0.770 | 0.411 | 9.13 |
| | O2+Y3 | 0.670 | 0.310 | 6.89 | 0.750 | 0.434 | 9.64 |
| | O2+Y6 | 0.730 | 0.377 | 8.38 | 0.790 | 0.481 | 10.69 |
| | **LSD at 0.05** | **0.015** | **0.004** | **0.02** | **0.021** | **0.001** | **0.018** |

**2.Essential oil yield ( ml/plant and L/Fed.):**

**2.1. Effect of sowing date:**

Data tabulated in Table (4) indicated that the maximum mean values of essential oil yield (0.318 and 0.298 ml/plant for 1st cut as well as 0.379 and 0.297 ml/plant for the 2nd one during 1st and 2nd seasons, respectively) were obtained as a result of planting in April, comparing with March planting date which recorded 0.250 and 0.212 ml/plant in the 1st cut, while the 2nd one produced 0.287 and 0.218 ml/plant during the two successive seasons, respectively. This increment may be due to the increase in herb dry weight (g/plant) and / or essential oil%. The highest essential oil yield (L/Fed) was recorded with the second planting date (April) which produced 7.06 and 6.62 L/Fed for 1st cut as well as 8.33 and 6.599 for 2nd one at first and second seasons, respectively (Table,4). The lowest values of essential oil yield 5.56 and 4.71 L/Fed for 1st cut beside 6.39 and 4.85 L/Fed. were obtained from plants cultivated in the 1st planting date (March), during 1st and 2nd seasons, respectively.

**2.2. Effect of Fertilizer:**

Application of algae extract at 1 ml/l gave the maximum mean values of essential oil yield (0.297 ml/plant) at the 1st cut of 1st season, while algae extract at 2 ml/l gave the highest one (0.405 ml/plant) in the 2nd cut of the same season (Table, 5). In the second season, plants sprayed with algae extract at 2 ml/l contained 0.248 and 0.296 ml/plant in the 1st and 2nd cuts, respectively. Essential oil yield (L/Fed.) gave the same trend as observed with essential oil yield (ml/plant). The highest mean values of essential oil content (ml/plant) which recorded 0.329 and 0.334 ml/plant for 1st cut versus 0.432 and 0,338 for 2nd cut at 1st and 2nd seasons, respectively were produced by the combination between algae extract at 2 ml/l + yeast at 6 g/l treatments compared with control and all other fertilizers treatments.

Essential oil yield (L/Fed.) showed the same trend such as ml/plant as the result of fertilizers treatments where the combination between algae extract at 2 ml/l + yeast at 6 g/l treatments resulted in the highest oil yield comparing with control and all other fertilizers treatments. This increment may be attributed to the increase in the essential oil content (ml/plant).

### 2.3. Effect of the combination between sowing date and fertilizers treatments:

Essential oil content was significantly affected by the combination treatments for both seasons (Tables, 6 & 7). The highest values of essential oil content (0.377 and 0.378 ml/plant at 1st cut versus 0.481 and 0.373 at 2nd cut, 8.38 and 8.40 L/Fed. in the 1st cut against 10.69 and 8.29 in the 2nd cut during first and second seasons, respectively) were observed with the combination treatments between 2nd sowing date (April) with algae extract at 2 ml/l + yeast at 6 g/l during both seasons.

**Table (7): Essential oil percent, oil content (ml/plant) and oil yield (L/Fed.) of basil plants as affected by algae extract and/or yeast applications and date of sowing for two cuts in the second season 2014.**

| Sowing dates | Fertilizer | 2nd season | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1st cut | | | 2nd cut | | |
| | | Oil % | Oil (ml/plant) | Oil (L/Fed) | Oil % | Oil (ml/plant) | Oil (L/Fed) |
| 1st sowing date (March) | Control | 0.400 | 0.155 | 3.44 | 0.470 | 0.133 | 2.96 |
| | O1(1ml / l) | 0.430 | 0.142 | 3.16 | 0.530 | 0.199 | 4.42 |
| | O2 (2ml /l) | 0.460 | 0.178 | 3.96 | 0.560 | 0.234 | 5.20 |
| | Y3 (3g/l) | 0.530 | 0.201 | 4.47 | 0.550 | 0.211 | 4.69 |
| | Y6 (6g/l) | 0.550 | 0.220 | 4.89 | 0.550 | 0.215 | 4.78 |
| | O1+Y3 | 0.600 | 0.220 | 4.89 | 0.630 | 0.221 | 4.91 |
| | O1+Y6 | 0.620 | 0.245 | 5.44 | 0.640 | 0.229 | 5.09 |
| | O2+Y3 | 0.690 | 0.256 | 5.69 | 0.600 | 0.220 | 4.89 |
| | O2+Y6 | 0.690 | 0.290 | 6.44 | 0.670 | 0.302 | 6.71 |
| 2nd sowing date (April) | Control | 0.560 | 0.184 | 4.09 | 0.520 | 0.157 | 3.49 |
| | O1(1ml / l) | 0.560 | 0.322 | 7.16 | 0.600 | 0.282 | 6.27 |
| | O2 (2ml /l) | 0.600 | 0.318 | 7.07 | 0.600 | 0.357 | 7.93 |
| | Y3 (3g/l) | 0.610 | 0.325 | 7.22 | 0.650 | 0.344 | 7.64 |
| | Y6 (6g/l) | 0.600 | 0.308 | 6.84 | 0.670 | 0.321 | 7.13 |
| | O1+Y3 | 0.630 | 0.284 | 6.31 | 0.640 | 0.267 | 5.93 |
| | O1+Y6 | 0.670 | 0.302 | 6.71 | 0.690 | 0.285 | 6.33 |
| | O2+Y3 | 0.730 | 0.262 | 5.82 | 0.750 | 0.287 | 6.38 |
| | O2+Y6 | 0.840 | 0.378 | 8.40 | 0.800 | 0.373 | 8.29 |
| | **LSD at 0.05** | **0.011** | **0.006** | **0.01** | **0.020** | **0.004** | **0.45** |

### 3. The Main Constituents Of Essential Oil:

The main constituents of the essential oil of the different treatments were determined qualitatively and quantitatively with GC-MS. Thirty three compounds were identified in the essential oil of all treatments in the two cuts of the two planting dates.

The average values for components of *O.basilicum* var. Genovese essential oilin the first date of planting (March 2013) are shown in Table (8). Total identified compounds ranged from 98.42to 99.49 % from all separated compounds. The major compound was identified as linalool in the essential oil of the studied treatments with relative percentage ranged from 60.23 to 65.39% from all separated compounds. The second major compound was identified as 1,8-cineol in the essential oil of all treatments and reached to 13.44% as the highest relative percent and to 9.96% as the lowest relative percent from the separated compounds.

The total mono-terpenoids were the principal compounds since they ranged from 85.3to 90.71 % while the sesquiterpenoid compounds ranged from 8.45 to 14.19 %. The total oxygen containing compounds were the dominant since they ranged from85.38 to 89.47 %, while the hydrocarbon compounds ranged from 9.69 to 13.84%.

The major sesquiterpene was identified as α-copaene which ranged from 3.12% (control) to 4.59% (O1+Y6). The second sesquiterpene was found to be cedra-8-ene which ranged from1.23% (control) to 1.83 % (O1). Regarding the effect of the studied treatments on the major compound (linalool) of the essential oil of basil plant in the first cut of the first date of planting (March 2013), it could be observed that the highest relative percent (65.39%) was detected in the essential oil of O2+Y3 treatment, while the lowest one (60.23%) was recorded in the essential oil of O1 treatment. No considerable changes in the relative percentage of linalool due to the application of organic fertilizers or/and algae extracts were observed. The differences between the highest

relative percent of linalool (65.39% for O2+Y3) and the lowest one (60.23 for O1) reached only 8.6%. Application of O1 and O2 treatment decreased linalool (6.36%) and (0.68%) compared to the control plant, respectively. On the other hand, application of Y3 increased linalool (0.28%), whileY6 increased it with (0.61%).

**Table (8): The average values for the identified components of *O.basilicum var. Genovese* essential oil in the first date of planting (March 2013).**

| Compound | Rt | Area | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | control | O1 | O2 | Y3 | Y6 | O1+Y3 | O1+Y6 | O2+Y3 | O2+Y6 |
| α-pinene | 5.38 | 0.34 | 0.31 | 0.21 | 0.39 | 0.35 | 0.42 | 0.35 | 0.42 | 0.33 |
| sabinene | 6.51 | 0.28 | 0.28 | 0.18 | 0.23 | 0.19 | 0.26 | 0.21 | 0.26 | 0.26 |
| β-pinene | 6.7 | 0.84 | 0.73 | 0.5 | 0.81 | 0.72 | 0.88 | 0.76 | 0.93 | 0.79 |
| myrcene | 6.97 | 0.57 | 0.43 | 0.29 | 0.42 | 0.31 | 0.44 | 0.32 | 0.49 | 0.44 |
| limonene | 8.41 | 0.29 | 0.26 | 0.19 | 0.25 | 0.21 | 0.26 | 0.23 | 0.25 | 0.27 |
| 1,8-cineol | 8.58 | 12.16 | 11.58 | 9.96 | 13.07 | 11.08 | 12.85 | 12.37 | 13.44 | 12.38 |
| trans linalool oxide | 10.02 | 0.39 | 0.45 | 0.46 | 0.46 | 0.22 | 0.4 | 0.6 | 0.28 | 0.58 |
| linalool oxide | 10.69 | 0.25 | 0.3 | 0.27 | 0.28 | 0.16 | 0.25 | 0.43 | 0.16 | 0.38 |
| linalool | 11.32 | 64.32 | 60.23 | 63.88 | 64.5 | 64.71 | 63.75 | 60.94 | 65.39 | 60.6 |
| cis-epoxy-ocimene | 12.92 | 0.2 | 0.21 | 0.16 | 0.13 | 0.1 | 0.14 | 0.13 | 0.1 | 0.19 |
| camphore | 13.47 | 0.39 | 0.59 | 0.39 | 0.86 | 0.45 | 0.56 | 0.74 | 0.58 | 0.71 |
| Borneol-l | 14.5 | 0.58 | 0.61 | 0.55 | 0.52 | 0.57 | 0.72 | 0.76 | 0.46 | 0.61 |
| terpineol | 15.55 | 1.31 | 1.24 | 1.04 | 1.24 | 0.98 | 1.11 | 1.21 | 0.97 | 1.23 |
| estragole | 15.68 | 0.93 | trac | trac | 0.44 | 0.41 | trac | 1.99 | trac | 0.73 |
| n-octyl acetate | 16.06 | 0.19 | 0.18 | 0.15 | 0.14 | 0.11 | 0.17 | 0.15 | 0.12 | 0.14 |
| linalyl acetate | 19.39 | 1.76 | 1.8 | 1.34 | 1.54 | 1.7 | 1.93 | 1.92 | 1.06 | 1.71 |
| limonen oxide | 21.86 | 0.09 | 0.12 | 0.09 | 0.11 | 0.1 | 0.1 | 0.11 | 0.1 | 0.1 |
| eugenol | 22.68 | 0.78 | 0.43 | 0.67 | 0.35 | 0.65 | 0.34 | 0.53 | 0.54 | 0.75 |
| β-elemene | 23.87 | 0.71 | 1.16 | 1.2 | 0.61 | 0.79 | 0.87 | 0.75 | 0.67 | 0.82 |
| trans caryphellene | 25.22 | 0.08 | 0.15 | 0.14 | 0.1 | 0.13 | 0.12 | 0.12 | 0.11 | 0.13 |
| a-cis-Ocimene | 25.78 | 4.78 | 5.49 | 4.55 | 3.7 | 4.98 | 4.09 | 3.07 | 4.4 | 4.39 |
| α-humulene | 26.8 | 0.4 | 0.62 | 0.61 | 0.44 | 0.56 | 0.46 | 0.5 | 0.41 | 0.59 |
| germacrene-D | 27.91 | 0.95 | 1.68 | 1.74 | 0.64 | 1.35 | 0.78 | 1.04 | 0.85 | 1.39 |
| Cis-β-farnesene | 28.01 | 0.18 | trac | 1.74 | 0.18 | trac | 0.18 | trac | 0.19 | trac |
| germacrene-B | 28.52 | 0.19 | 0.32 | 0.33 | 0.21 | 0.27 | 0.19 | 0.22 | 0.21 | 0.3 |
| Farnesol | 28.75 | 0.52 | 0.91 | 0.97 | 0.45 | 0.64 | 0.57 | 0.6 | 0.54 | 0.7 |
| Neryl acetate | 29.06 | 0.26 | 0.44 | 0.42 | 0.22 | 0.31 | 0.25 | 0.7 | 0.24 | 0.35 |
| cedra-8-ene | 29.34 | 1.23 | 1.83 | 1.82 | 1.35 | 1.49 | 1.47 | 1.6 | 1.31 | 1.74 |
| globulol | 31.73 | 0.29 | 0.32 | 0.31 | 0.3 | 0.43 | 0.37 | 0.42 | 0.29 | 0.41 |
| (z.z)-α-pharmene | 32.06 | 0.28 | 0.62 | 0.49 | 0.47 | 0.4 | 0.48 | 0.55 | 0.33 | 0.54 |
| Trans-α-bergamotene | 33.6 | 0.31 | 0.51 | 0.46 | 0.4 | 0.41 | 0.48 | 0.49 | 0.35 | 0.48 |
| α-copaene | 34.77 | 3.12 | 4.44 | 4.08 | 3.79 | 3.77 | 3.85 | 4.59 | 3.23 | 4.07 |
| (+)-lavandulol | 35.27 | 0.19 | 0.33 | 0.3 | 0.21 | 0.22 | 0.25 | 0.36 | 0.18 | 0.31 |
| Total identified compound | | 99.16 | 98.57 | 99.49 | 98.81 | 98.77 | 98.99 | 98.76 | 98.86 | 98.42 |
| Total non identified compound | | 0.84 | 1.43 | 0.51 | 1.19 | 1.23 | 1.01 | 1.24 | 1.14 | 1.58 |
| Total non oxygenated constituents | | 9.69 | 13.19 | 13.84 | 10.19 | 10.82 | 11.02 | 11.61 | 9.9 | 12.02 |
| Total oxygenated constituents | | 89.47 | 85.38 | 85.65 | 88.62 | 87.95 | 87.97 | 87.15 | 88.96 | 86.4 |
| Total sesquiterpenoids | | 8.45 | 12.89 | 14.19 | 9.15 | 10.46 | 10.07 | 11.24 | 8.67 | 11.48 |
| Total monoterpenoids | | 90.71 | 85.68 | 85.3 | 89.66 | 88.31 | 88.92 | 87.52 | 90.19 | 86.94 |

The results of the second date of planting (April 2013) are shown in Table (9) in which the total identified compounds ranged from 97.98 to 99.25 % from all separated compounds. The major compound was identified as linalool in the essential oil of all studied treatments with relative percentage ranged from 62.16 to 66.47% from all separated compounds. The second major compound was identified as 1,8-cineol in the

essential oil of all treatments and reached to 16.48% as the highest relative percent and to 4.61 as the lowest relative percent from the separated compounds. The total mono-terpenoids were the principal compounds since they ranged from 82.31 to 93.01%, while the sesquiterpenoid compounds ranged from 5.87 to 15.67%. The total oxygen containing compounds were the dominant since they ranged from 84.57 to 90.09 %, while the hydrocarbon compounds ranged from 9.07 to 13.41%. The major sesquiterpene was identified as α-copaene which ranged from 2.22% (O2+Y6) to 5.58% (O1), followed by cedra-8-ene as the second main sesquiterpene which ranged from 0.82% (O2+Y6) to 1.97 (O1).

**Table (9): The average values for the identified components of *O.basilicum var. Genovese* essential oil in the second date of planting (April 2013).**

| compound | Rt | Area | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | control | O1 | O2 | Y3 | Y6 | O1+Y3 | O1+Y6 | O2+Y3 | O2+Y6 |
| α-pinene | 5.38 | 0.46 | trac | 0.47 | 0.12 | 0.36 | 0.47 | 0.23 | 0.26 | 0.66 |
| sabinene | 6.51 | 0.33 | trac | 0.33 | 0.16 | 0.3 | 0.33 | 0.21 | 0.25 | 0.52 |
| β-pinene | 6.7 | 0.95 | 0.09 | 0.97 | 0.4 | 0.81 | ..97 | 0.58 | 0.7 | 1.43 |
| myrcene | 6.97 | 0.63 | trac | 0.55 | 0.29 | 0.47 | 0.55 | 0.34 | 0.46 | 0.89 |
| limonene | 8.41 | 0.3 | trac | 0.27 | 0.19 | 0.24 | 0.27 | 0.2 | 0.26 | 0.33 |
| 1,8-cineol | 8.58 | 14.04 | 4.61 | 12.01 | 8.47 | 11.76 | 12.01 | 10.78 | 12.03 | 16.48 |
| trans linalool oxide | 10.02 | 0.42 | 0.4 | 0.32 | 0.24 | 0.39 | 0.26 | 0.52 | 0.41 | 0.28 |
| linalool oxide | 10.69 | 0.31 | 0.27 | 0.17 | Trac | 0.26 | 0.17 | 0.37 | 0.24 | 0.16 |
| linalool | 11.32 | 63.71 | 66.43 | 62.96 | 66.47 | 64.42 | 62.96 | 63.79 | 62.16 | 65.68 |
| cis-epoxy-ocimene | 12.92 | 0.21 | 0.28 | 0.22 | 0.25 | 0.14 | 0.16 | 0.16 | 0.16 | 0.15 |
| camphore | 13.47 | 0.44 | 0.54 | 0.44 | 0.4 | 0.61 | 0.44 | 0.49 | 0.41 | 0.31 |
| Borneol-l | 14.5 | 0.58 | 0.67 | 0.57 | 0.47 | 0.57 | 0.57 | 0.56 | 0.61 | 0.25 |
| terpineol | 15.55 | 1.18 | 1.09 | 1.06 | 0.98 | 1.11 | 1.06 | 1.14 | 1.08 | 1.1 |
| estragole | 15.68 | 1.31 | 0.52 | 1.11 | Trac | trac | 0.29 | trac | trac | trac |
| n-octyl acetate | 16.06 | 0.14 | 0.11 | 0.17 | 0.22 | 0.12 | 0.1 | 0.13 | 0.16 | 0.13 |
| linalyl acetate | 19.39 | 1.95 | 1.94 | 1.48 | 1.79 | 1.36 | 1.48 | 1.47 | 1.57 | 1.02 |
| limonen oxide | 21.86 | 0.1 | 0.1 | 0.09 | 0.1 | 0.11 | 0.1 | 0.13 | 0.11 | 0.07 |
| eugenol | 22.68 | 0.53 | 0.52 | 0.38 | 0.13 | 0.12 | 0.17 | 0.44 | 0.08 | trac |
| β-elemene | 23.87 | 0.61 | 1.4 | 0.58 | 1.14 | 0.91 | 0.88 | 0.99 | 0.91 | 0.52 |
| trans caryphellene | 25.22 | 0.07 | 0.18 | 0.1 | 0.11 | 0.12 | 0.1 | 0.11 | 0.09 | 0.05 |
| a-cis-Ocimene | 25.78 | 4.07 | 4.22 | 6.36 | 5.72 | 5.12 | 6.36 | 5.01 | 7.49 | 3.35 |
| α-humulene | 26.8 | 0.34 | 0.84 | 0.39 | 0.54 | 0.51 | 0.45 | 0.51 | 0.42 | 0.28 |
| germacrene-D | 27.91 | 0.81 | 2.11 | 0.7 | 1.82 | 1.54 | 1.07 | 1.45 | 1.13 | 0.58 |
| Cis-β-farnesene | 28.01 | 0.15 | trac | 0.33 | Trac | trac | 0.28 | trac | 0.34 | 0.14 |
| germacrene-B | 28.52 | 0.15 | 0.36 | 0.13 | 0.32 | 0.27 | 0.23 | 0.34 | 0.25 | 0.13 |
| Farnesol | 28.75 | 0.41 | 1.13 | 0.42 | 0.9 | 0.67 | 0.7 | 0.69 | 0.67 | 0.36 |
| Neryl acetate | 29.06 | 0.23 | 0.52 | 0.22 | 0.42 | 0.33 | 0.29 | 0.37 | 0.31 | 0.2 |
| cedra-8-ene | 29.34 | 0.99 | 1.97 | 1.45 | 1.67 | 1.44 | 1.45 | 1.67 | 1.57 | 0.82 |
| globulol | 31.73 | 0.27 | 0.71 | 0.42 | 0.26 | 0.25 | 0.2 | 0.25 | 0.14 | 0.1 |
| (z.z)-α-pharmene | 32.06 | 0.24 | 0.51 | 0.32 | 0.38 | 0.41 | 0.47 | 0.62 | 0.37 | 0.31 |
| Trans-α-bergamotene | 33.6 | 0.29 | 0.55 | 0.37 | 0.41 | 0.39 | 0.38 | 0.47 | 0.39 | 0.24 |
| α-copaene | 34.77 | 2.91 | 5.58 | 3.45 | 4.03 | 3.57 | 3.45 | 4.2 | 3.39 | 2.22 |
| (+)-lavandulol | 35.27 | 0.12 | 0.33 | 0.15 | 0.19 | 0.19 | 0.29 | 0.31 | 0.22 | 0.12 |
| Total identified compound | | 99.25 | 97.98 | 98.96 | 98.59 | 98.87 | 97.99 | 98.53 | 98.64 | 98.88 |
| Total non identified compound | | 0.75 | 2.02 | 1.04 | 1.41 | 1.13 | 2.01 | 1.47 | 1.36 | 1.12 |
| Total non oxygenated constituents | | 9.16 | 13.41 | 10.31 | 11.47 | 11.22 | 10.28 | 11.81 | 10.7 | 9.07 |
| Total oxygenated constituents | | 90.09 | 84.57 | 88.65 | 87.12 | 87.65 | 87.71 | 86.72 | 87.94 | 89.81 |
| Total sesquiterpenoids | | 7.36 | 15.67 | 8.81 | 11.77 | 10.27 | 9.95 | 11.61 | 9.89 | 5.87 |
| Total monoterpenoids | | 91.89 | 82.31 | 90.15 | 86.82 | 88.6 | 88.04 | 86.92 | 88.75 | 93.01 |

It is clear that the different treatments did not show big change in the main constituents of the essential oil, which may be attributed to that the biosyntheses of these compounds did not affect by these treatments. In other words the enzymatic systems responsible for the biosyntheses of these compounds did not affect by the applied treatment, Essential oil composition depends upon internal, environmental and agricultural practices as well as factors affecting the plant such as genetics and ecological conditions[47,48]. These results agree with the findings of [49,19] who found that linalool is the most prominent component in Genovese basil grown in Egypt and also the results of [20]who reported that the essential oil of basil cultivated in Egypt contained 48% linalool, 3.04% methyl chavicol and 5.9% eugenol. The linalool content was about 5% higher in plots fertilized with 75% of the recommended dose of NPK compared with those fertilized with 100% NPK. But 50% of the recommended NPK gave the lowest linalool content. *Ocimum basilicum* L, contains essential oils based primarily on monoterpene derivatives such as linalool[18]. [50] showed that, fifteen hydrocarbon compounds were detected in the essential oil of Genovese basil in which linalool was the main constituent. [47] reported that the oxygenated monoterpenes were the major compounds in Turkish *O. basilicum* essential. [51]also reported that *O. basilicum* essential oil from Bangladesh contains linalool as the main component. Jirovetz and Buchbauer[52] found a high level of linalool (71.4%) in *O. basilicum* essential oil from Bulgaria. Gurbuz *et al.*[53] mentioned that linalool (41.2%) was the main compound in the essential oil of *O. basilicum* extracted with hydro-distilled from Turkey. [54]identified linalool as the main component of sweet basil (*Ocimum basilicum* L.). [55]found that linalool, methyl chavicol and 1,8-cineole as the main component of three sweet basil (*Ocimum basilicum* L.) cultivars. [17]studied oil content, and oil composition of sweet basil and reported, linalool, methyl chavicol and 1,8-cineole as the main component of sweet basil.

Other reports indicated that basil (*O. basilicum* L.) essential oil contains high proportions of phenolic derivatives, such as eugenol, methyl chavicol (estragole) and methyl cinnamate, often combined with various proportions of linalool (a monoterpenol).

## Conclusion

From the above mentioned results it might be recommend the cultivation of basil (*O. basilicum*) in April with application of 2 ml/l algae + 6 g/l yeast for production of the essential oil from quantity and quality point of view.

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