Effect of Organic and Biofertilizers on Growth, Oil Yield and Chemical Composition of the Essential Oil of *Ocimum basillicum* L. Plants

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ABSTRACT

This work was carried out during two successive seasons of 2012 and 2013 at Agric. Research Station, Alex. Univ. Egypt, to investigate the effect of different rates of organic fertilizers (cattle manure(CM) 0, 40, 60 and 80 m^3/fed.) and four concentrations of biofertilizer (active dry yeast (ADY) 0.0, 2.0, 4.0 and 6.0 g/l) on the vegetative growth, oil yield, and the chemical composition of essential oil of *Ocimum basilicum* L. The experimental design was a split-plot in three replicates. Each replicate contained 16 treatments (4CM × 4Bio. Fer.), the main plot represented the organic fertilizer (CM) treatments while, the sub-plot was the biofertilizer concentration (ADY). The obtained results can be summarized as follows; using the different rates of organic manure separately or in combination with the different concentrations of biofertilizer (ADY) stimulated the vegetative growth and oil yield compared, to the control. The highest values of vegetative growth (plant height, number of branches, leaves numbers, leaf area and leaves dry weight), total chlorophyll content and oil percentage were obtained by adding CM at 80 m^3/fed. combined with 6.0 g/l biofertilizer (ADY). Moreover. The highest significant increases in the main chemical components of leaves essential oil (estragole, eucalyptol, linalool, and trans-4-methoxycinnamaldehyde) were obtained by using organic fertilizer (CM) at 60 m^3/fed. combined with 2.0 and/or 4.0 g/l of biofertilizer (ADY).

Key words: *Ocimum basilicum,* Vegetative growth, Organic fertilizer, Active dry yeast, essential oil.

INTRODUCTION

Medicinal and aromatic plants contain substances that can be used for therapeutic purposes, it is known in the modern and ancient civilization for their healing properties. These compounds called "active ingredient" and the plant is considered as a source for this compounds.

Sweet basil (*Ocimum basilicum* L.) comprises several aromatic and medicinal lamiaceae herbs belong to different species. *Ocimum sp.* contains between 50 to 150 species of herbs and shrubs. Basil is native to the tropical regions of Asia, Africa and Central and South America (Gill and Randhawa, 1997). These are a great variability of both morphology and chemotypes of basil; it is an annual herb with 50-60 cm plant height with leaves of color from green to purple and with small white or pink flowers (Sharafzadeh and Alizadeh, 2011).

Traditionally, sweet basil has been used as a medicinal plant for various ailments, such as headaches, coughs, diarrhea, constipation, warts, worms and kidney malfunction, antispasmodic, stomachicum, carminative, expectorant, antimalarial, febrifuge and stimulant (Simon et al., 1999). Fresh and dry leaves of plant are used in food and spice industries, perfumery, dental and oral products (Vieira and Simon, 2000). *O. basilicum* is a popular culinary herb and a source of essential oils extracted by steam distillation from the leaves and the flowering tops which are used to flavor foods, in dental and oral products, and in fragrances (Akgul, 1989).

Basil has shown antioxidant, antimicrobial and antiumer activities due to its phenolic acids and aromatic compounds (Gutierrez et al., 2008). The high economic value of basil oil is due to the presence of phenyl propanoids, like eugenol, chavicol and their derivatives or terpenoids like monoterpen alchole linalool, methyl cinnamate, and limonene (Marotti et al., 1996).

Organic fertilizers have been known to improve the biodiversity (Enwall et al., 2005; Birkhofer et al., 2008) and may prove a large depository for excess carbon dioxide (Lal, 2004). Organic fertilizers in comparison of the chemical fertilizers have lower nutrient content and are slow release but they are as effective as chemical fertilizers over longer periods of use (Naguib, 2011). Organic fertilizers are obtained from animal sources such as animal manure or plant sources like green manure. Continuous usage of inorganic fertilizer affects soil structure. Hence, organic manures can serve as alternative to mineral fertilizers for improving soil structure (Dauda et al., 2008) and microbial biomass (Suresh et al., 2004).

Organic manures and biofertilization are very important for medicinal and aromatic plants to produce the best product in both quantity and quality and it is safe for human, animal and the environment. Dewidar (2007) found that the combination between compost and biofertilizers increased plant fresh and dry weights in the fourth cut compared to the other treatments of marjoram plants. Abo Elazm (2008) found that the highest values of the herb fresh and dry weight, and yield of oil/plant of marjoram were obtained with using poultry manure combined with phosphorein or yeast biofertilizers at 25 m^3/ ha.

Active dry yeast, a natural biofertilizer, is safety and causes various promoted effects on plants and is a natural source of cytokinins which simulates cell division and enlargement as well as the synthesis of protein, nucleic acid and B-vitamin (Ezz El-Din and Hendawy, 2010). Also, active dry yeast releases CO_2 which reflected in improving net photosynthesis (Kurtzman and Fell, 2005).

The objective of the present study was to study the effect of organic and bio-fertilizers (active dry yeast) on vegetative growth, oil yield, and the chemical composition of essential oil of *Ocimum basilicum* L.

MATERIALS AND METHODS

The present work was carried out during 2012 and 2013 seasons at Agriculture Research Station, Faculty of Agriculture, Alexandria University, to study the effect of organic and active dry yeast (biofertilizer) on vegetative growth and oil yield of *Ocimum basilicum* L.

Preparation of *Ocimum basilicum* Plants

Seeds were sown on the 12th March 2012 and 2013 in the first and second seasons, respectively, in sandy clay soil in seed pans. After one month from sowing, when the seedling reached 8-10 cm height with 6:8 leaves and 4 branches, they were transplanted to 30 cm clay pots using sandy clay soil. Herb harvest was took place twice, in the first cut when inflorescence shoots occurred (50% flowering) in July and after two months the second cut harvest was done (in September) in both seasons (Taie *et al.,* 2010). Each time all the plant parts were cut at the height of 6–7 cm above the ground (Biesiada and Kuś, 2010).

Soil analysis:

The physical analysis of the used soil revealed that it was sandy clay loam soil wich contained 25, 13 and 62% clay, silt and sand, respectively. The chemical analysis cleared that, it contained the available N, P and K values at 427, 46.6 and 610 ppm respectively. The electric conductivity (EC) was 9.6 (dsm^{-1}) with pH of 7.82.

Experimental treatments:

The fertilizers used:

The cattle manure (CM) was used as an organic fertilizer and it brought from the Animal Production Farm, Faculty of Agriculture, Alexandria University. The amount of the used fertilizer (0, 40, 60 and 80 m^3/fed.) was added during soil preparation at one dose.

Biofertilizer treatments (Active dry yeast) were applied as a foliar spray (0, 2, 4 and 6 g/l) at three times, the first one after one month from transplanting, the second after two weeks from the first one and the third one after one month from the first cut.

Experimental design:

The experimental layout was split plot design for arrangement of pots with three replicates. Each replicate contained 16 treatments (4CM × 4Bio. Fer.) and five plants were used as an experimental unit (Snedecor and Cochran, 1974). Organic fertilizer treatments were arranged in the main treatment (main-plot), while, the biofertilizer levels were randomly distributed in the sub-treatment (sub-plot).

Growth characteristics:

The vegetative growth parameters were included: (I) plant height (cm), (II) number of branches, (III) leaves numbers, (IV) leaf area (cm^2), (V) leaves dry weight (g).

Chemical composition:

1- Total chlorophyll content of the fresh leaves at harvesting time was measured according to method reported by Yadava (1986) using Minolta SPAD Chlorophyll Meter model-502.
2- The essential oil was extracted by water distillation method according to Novak et al. (2002). The amount of obtained oil from five plants was measured and oil percentage (%) was calculated according to Charles and Simon (1990).
3- Chemical constituents of the essential oil of the leaves were analyzed using the Trace GC Ultra/Mass Spectrophotometer ISQ (Thermo Scientific) (GC/MS) apparatus to determine their main constituents at Institute of Graduate Studies and Researches- Alexandria University, Alexandria, Egypt. The main chemical components of the oil are (I) Estragole (II) Linalool (III) Eucalyptol (IV) Trans-4-methoxycinnamaldehyde.

RESULTS AND DISCUSSION

Vegetative growth

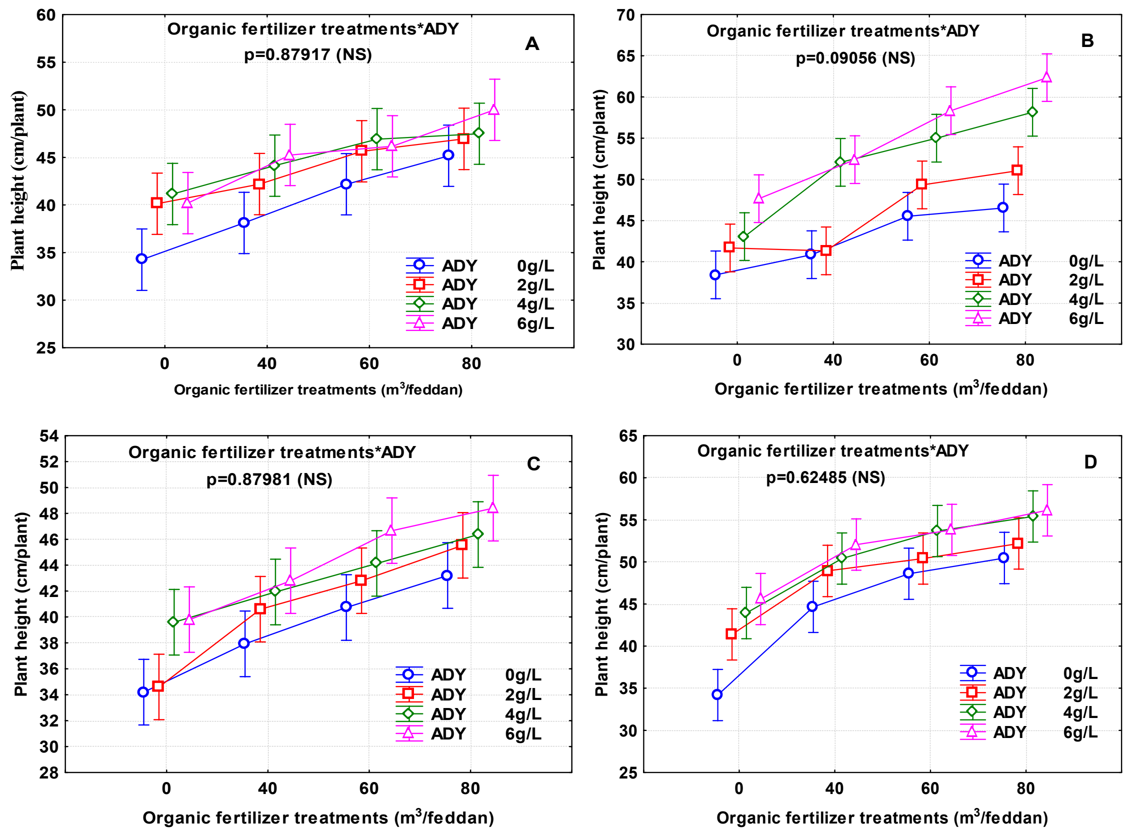
1. Plant height (cm):

Data in (Table 1 and Fig. 1) showed that there were significant effects of all levels of cattle manure (CM) as an organic fertilizer on the height of basil plants compared with the control in the two cuts of the two seasons.

Table 1: Means of plant height (cm) of *O. basilicum* plants as influenced by organic (CM) and active dry yeast (ADY) fertilizers and their combinations in the two seasons of 2012 and 2013.

CM (A) m^3/fed.	First cut					Second cut				
First season (2012)										
	Active Dry Yeast (B) g/l									
	0	2	4	6	mean	0	2	4	6	mean
0	34.25	40.13	41.15	40.20	38.93	38.41	41.68	43.06	47.66	42.71
40	38.11	42.20	44.13	45.25	42.42	40.86	41.33	52.06	52.40	46.66
60	42.17	45.64	46.91	46.16	45.22	45.53	49.33	55.00	58.33	52.05
80	45.16	46.95	47.48	50.00	47.40	46.53	51.06	58.15	62.35	54.52
mean	39.93	43.73	44.92	45.40		42.84	45.85	52.07	55.18	
$LSD_{0.05}$	A=3.09	B= 2.13	A×B= NS			A= 1.77	B= 2.21	A×B= NS		
Second season (2013)										
	Active Dry Yeast (B) g/l									
	0	2	4	6	mean	0	2	4	6	mean
0	34.20	34.60	39.60	39.80	37.05	34.20	41.40	43.93	45.60	41.28
40	37.93	40.60	41.93	42.80	40.82	44.66	48.93	50.40	52.06	49.02
60	40.73	42.80	44.13	46.66	43.58	48.60	50.40	53.66	53.80	51.62
80	43.20	45.53	46.36	48.40	45.87	50.46	52.20	55.40	56.13	53.55
mean	39.02	40.88	43.01	44.41		44.48	48.23	50.85	51.90	
$LSD_{0.05}$	A=1.95	B=1.83	A×B= NS			A=2.91	B= 2.02	A×B= NS		

NS= Not significant at the level of probability of 0.05.



A: first season first cut; B: first season second cut; C: Second season first cut, D: Second season second cut

Figure 1: Effect of organic fertilizer (CM) and active dry yeast (ADY) treatments on the height (cm) of *O. basilicum* plants.

The highest values of plant height were obtained with treating plants with 80 m^3/fed in the first season with 47.40 cm and 54.52 cm and in the second season with 45.87 cm and 53.55 cm for first and second cuts, respectively. There was no significant effect between the levels of 60 and 80 m^3/fed in the second cut of the second season. For the effect of ADY, there was a significant effect on basil height compared with control but in the first cut of the first season there was no significant effect between all the treatments of ADY. In the second cut there was a significant effect between ADY levels and the best result (55.18 cm) was obtained by supplying plants with the ADY rate at the level of 6 g/l. Furthermore, in the second season there was a significant effect of ADY treatments on plant height but there is no significant effect between the ADY levels of 4 and 6 g/l in both cuts. Moreover, there was no significant effect of the interaction among the treatments of CM and ADY on basil height. The elongation and improving of plant height may be due to the role of CM and ADY fertilizers by enhancing the cell division rate and cell enlargement (Shalaby and El-Nady, 2008). Additionally, organic fertilization is a very important method of providing plants with their nutritional requirement without having an undesirable impact on the environment (El-Sayed et al., 2002).

Subsequently, increasing in plant height might be attributed to the effect of organic fertilizer that improves physical, chemical, and biological properties of soil; that is, increasing soil organic matter, cation exchange capacity, and water holding capacity and availability of mineral nutrients and, this in turn, increases plant height (Al-Fraihat, 2011).

These results are in agreement with Jacoub (1999) on *Ocimum basilicum* and *Thymus vulgaris*, Abd EL-Gawad (2001) on *Coriandrum sativum* and Hussain (2002) on *Majorana hortensis*.

2. Number of branches/plant:

From the results observed in (Table 2) it is clear that by increasing the CM fertilization levels, number of branches per plant in both cuts of the both seasons was significantly increased. The superior treatment 80 m^3/fed gave the following values 58.57, 96.88, 71.60 and 148.28 branch/plant, respectively, in both cuts of the both seasons.

Moreover, the treated plants with ADY gave significant effect on number of branches in both cut of the both seasons. The maximum mean values of number of branches during the both harvest in the two seasons were obtained by spraying the plants with 6 g/l ADY (53.738, 83.18, 63.83 and 115.36 branch/plant), respectively. The interaction in the first cut of the both seasons was not significant but it was significant in the second cut of the both seasons. The maximum mean values was obtained with using the treatment of 4 g/l or 6 g/l ADY with 80 m^3/fed.

Table 2: Means of the number of branches/plant of *O. basilicum* plants as influenced by organic (CM) and active dry yeast (ADY) fertilizers and their combinations in the two seasons of 2012 and 2013.

First season (2012)										
	First cut					**Second cut**				
CM (A) m^3/fed.	**Active Dry Yeast (B) g/l**									
	0	**2**	**4**	**6**	**mean**	**0**	**2**	**4**	**6**	**mean**
0	28.00	33.53	36.68	45.23	35.86	37.76	39.60	50.46	56.80	46.15
40	36.86	49.13	49.40	47.00	45.60	59.20	65.06	67.60	68.46	65.08
60	45.42	53.08	52.58	56.77	51.96	63.26	72.20	82.46	90.40	77.08
80	53.33	56.88	58.13	65.94	58.57	68.13	89.31	113.01	117.08	96.88
mean	40.90	48.15	49.20	53.738		57.09	66.54	78.38	83.18	
$LSD_{0.05}$	A= 2.79	B= 3.09	A×B= NS			A=4.51	B= 4.37	A×B= 8.46		
Second season (2013)										
	First cut					**Second cut**				
CM (A) m^3/fed.	**Active Dry Yeast (B) g/l**									
	0	**2**	**4**	**6**	**mean**	**0**	**2**	**4**	**6**	**mean**
0	44.20	43.26	43.93	47.00	44.60	48.00	54.00	58.80	62.80	55.90
40	45.06	50.00	52.13	58.46	51.41	70.73	81.93	83.06	82.26	79.50
60	56.86	56.26	64.93	71.73	62.45	79.46	86.06	109.20	131.40	101.53
80	61.73	68.26	78.28	78.13	71.60	104.93	131.33	171.86	185.00	148.28
mean	51.96	54.45	59.82	63.83		75.78	88.33	105.73	115.36	
$LSD_{0.05}$	A= 4.69	B=3.72	A×B= NS			A= 11.2	B=6.55	A×B=14.94		

NS= Not significant at the level of probability of 0.05.

Muller and Leopold (1966) demonstrated that the enhancing effects of yeast application might be due to yeast cytokinins that improving the accumulation of soluble metabolites. Also, cytokinins stimulate cell proliferation and differentiation, controlling shoot and root morphogenesis and chloroplast maturation (Amer 2004). These results were in agreement with these was obtained by Jacoub (1999) on *Ocimum basilicum* and *Thymus vulgaris*, Naga (2004) on Fennel plants and Abou-Dahab et al. (2009) on Lovage plants.

3. Number of leaves/plant:

From the data in (Table 3) it is clear that there was significant increment in number of leaves per plant of basil. In the first season, the minimum number of leaves (368.49 and 428.18 leaf/pant) was obtained by control treatment and the maximum (679.15 and 1065.10 leaf/plant) produced by the treatment of 80 m^3/fed during the first and second cut, respectively. In the second season, the minimum number of leaves was 314.84 and 457.66 leaf/plant produced by control treatment and the maximum was 820.66 and 862.39 leaf/plant under the treatment of 80 m^3/fed during the first and second cut, respectively.

Moreover, there was significant effect of ADY treatment on number of basil leaves in both harvesting of the two seasons. The best result was recorded when treated plants with 6g/l ADY (647.49, 870.25, 671.48 and 751.56 leaf/plant, respectively). Concerning to the interaction among the treatments of CM and ADY, in the first season there was no significant effect in the first cut but there was significant effect in the second cut and the most effective treatment was 4g/l or 6 g/l ADY with CM at the level of 80 m^3/fed (1324.20 and 1348.93 leaf/plant, respectively). In the second season there was significant effect in the first cut and the best result was obtained with using 6 g/l ADY with 80 m^3/fed (1074.11 leaf/plant) and there was no significant effect in the second cut.

This increase in the number of basil leaves may be due to cell multiplication, cell enlargement and cell differentiation which have resulted in increasing of number of leaves. Also, these results may be attributed to the effect of yeast extract in increasing levels of endogenous hormones in treated plants which could be interpreted by cell division and cell elongation (Khedr and Farid, 2000). These results were in harmony with Khalid et al. (2006) on *Ocimum basilicum*, Moghadam et al. (2012) on *Lilium asiatic* and Khaled et al. (2014) on majoram plant.

4. Leaf area (cm^2)/plant:

Data in (Table 4 and Fig. 2) showed that there was significant effect of CM treatments on basil leaf area in both harvesting in the two seasons compared with untreated plants. The most effective treatment was 80 m^3/fed with the following values in both cuts in the two seasons; 1612.67, 2897.7, 2314.59 and 2433.49 cm^2/plant, respectively, compared with the control plants (833.97, 1349.4, 866.63 and 1370.56 cm^2/plant, respectively).

Table 3: Means of number of leaves /plant of *O. basilicum* plants as influenced by organic (CM) and active dry yeast (ADY) fertilizers and their combinations in the two seasons of 2012 and 2013.

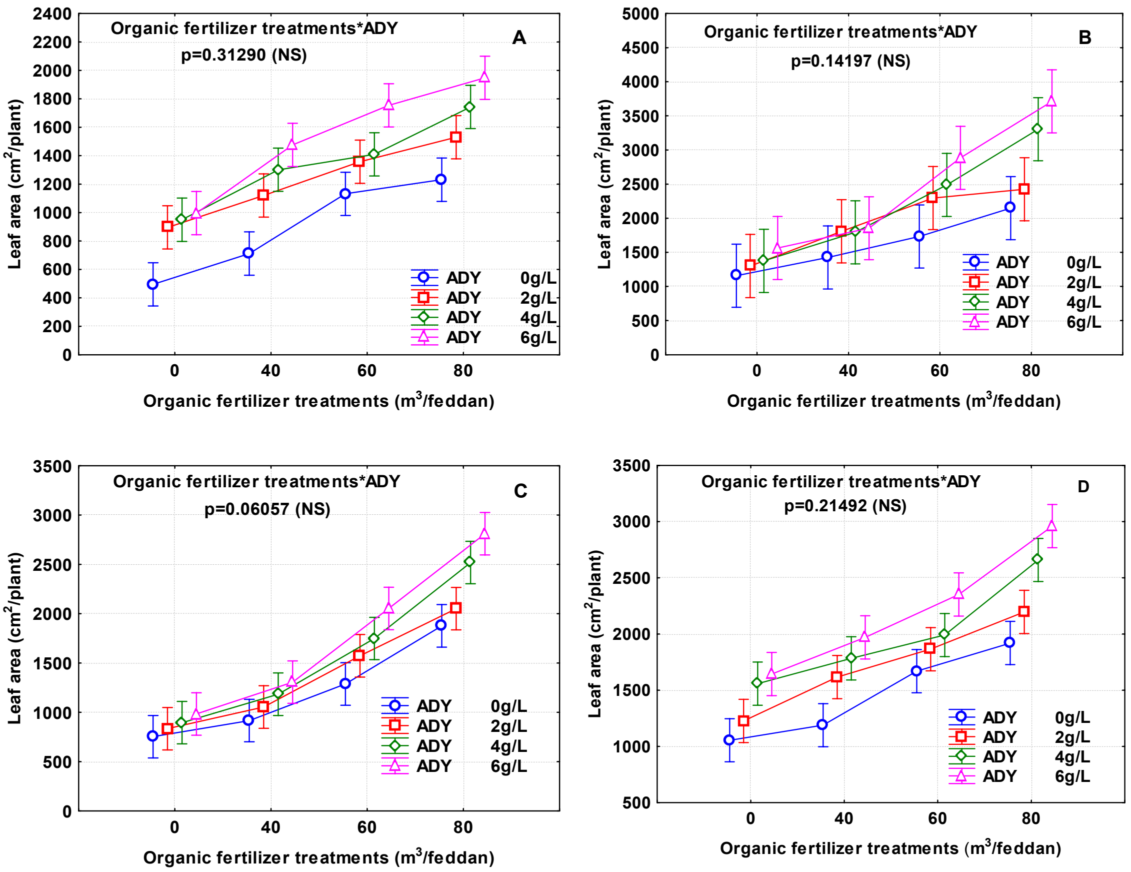
CM (A) m^3/fed.	First season (2012)									
	First cut					Second cut				
	Active Dry Yeast (B) g/l									
	0	2	4	6	mean	0	2	4	6	mean
0	245.20	314.90	471.13	442.72	368.49	369.32	438.74	429.63	475.02	428.18
40	389.50	436.46	456.86	655.61	484.61	446.38	519.46	629.06	706.69	575.40
60	466.05	526.40	540.87	685.54	554.72	569.70	663.86	675.87	950.37	714.95
80	510.47	670.82	729.25	806.06	679.15	667.03	920.24	1324.20	1348.93	1065.10
mean	402.81	487.15	549.53	647.49		513.11	635.58	764.69	870.25	
$LSD_{0.05}$	A=117.67	B=75.95	A×B= NS			A=159.13	B=101.98	A×B=223.93		
	Second season (2013)									
	First cut					Second cut				
CM (A) m^3/fed.	Active Dry Yeast (B) g/l									
	0	2	4	6	mean	0	2	4	6	mean
0	275.46	306.36	346.39	331.15	314.84	333.79	409.43	506.58	580.80	457.66
40	328.21	401.32	442.25	531.08	425.72	420.81	540.62	538.56	607.26	526.81
60	450.92	512.72	674.21	749.57	596.86	575.48	580.42	665.47	797.08	654.62
80	613.75	724.64	870.12	1074.11	820.66	683.69	852.95	891.84	1021.09	862.39
mean	417.09	486.26	583.25	671.48		503.44	595.86	650.61	751.56	
$LSD_{0.05}$	A=43.89	B= 48.17	A×B= 91.02			A=74.41	B= 40.62	A×B= NS		

NS= Not significant at the level of probability of 0.05.

Table 4: Means of the leaf area (cm^2)/plant of *O. basilicum* plants as influenced by organic (CM) and active dry yeast (ADY) fertilizers and their combinations in the two seasons of 2012 and 2013.

First season (2012)										
	First cut					**Second cut**				
CM (A)	**Active Dry Yeast (B) g/l**									
m^3/fed.	**0**	**2**	**4**	**6**	**mean**	**0**	**2**	**4**	**6**	**mean**
0	494.86	895.41	949.65	995.94	833.97	1158.20	1300.34	1375.59	1563.47	1349.4
40	711.70	1120.32	1300.83	1476.12	1152.25	1426.21	1808.90	1794.37	1853.76	1720.8
60	1131.81	1357.27	1409.80	1753.74	1413.16	1732.49	2295.89	2487.78	2884.68	2350.2
80	1230.99	1529.86	1742.63	1947.18	1612.67	2148.02	2424.49	3305.23	3713.10	2897.7
mean	892.34	1225.72	1350.73	1543.25		1616.20	1957.40	2240.70	2503.80	
LSD$_{0.05}$	A= 117.52		B=110.18		A×B= NS	A= 435.05		B=309.53		A×B= NS
Second season (2013)										
	First cut					**Second cut**				
CM (A)	**Active Dry Yeast (B) g/l**									
m^3/fed.	**0**	**2**	**4**	**6**	**mean**	**0**	**2**	**4**	**6**	**mean**
0	753.45	834.40	895.03	983.64	866.63	1054.13	1226.16	1558.26	1643.69	1370.56
40	916.95	1054.26	1184.12	1306.12	1115.36	1188.83	1616.12	1783.07	1969.50	1639.38
60	1287.58	1573.43	1748.81	2053.79	1665.91	1669.08	1865.02	1990.46	2351.39	1968.99
80	1877.12	2051.58	2518.74	2810.90	2314.59	1919.35	2196.62	2658.58	2959.40	2433.49
mean	1208.78	1378.42	1586.68	1788.62		1457.85	1725.98	1997.60	2231.00	
LSD$_{0.05}$	A=188.46		B=148.97		A×B= NS	A=188.95		B= 125.38		A×B= NS

NS= Not significant at the level of probability of 0.05.



A: first season first cut; B: first season second cut; C: Second season first cut, D: Second season second cut

Figure 2: Effect of organic fertilizer (CM) and active dry yeast (ADY) treatments on the leaf area (cm^2) per plant of *O. basilicum*.

Furthermore, with increasing the concentration of ADY till 6 g/l, the leaf area/plant was increased in the two cuts of the two seasons (1543.25, 2503.80, 1788.62 and 2231.00 cm^2/plant, respectively). The interaction among the treatments of ADY and CM had no significant effect on the leaf area in the both seasons. The increment of basil leaf area may be caused by the increasing of cell enlargement, cell number, number of leaves or all of them.

These results may be attributed to the effect of yeast extract in increasing levels of endogenous hormones in treated plants which could be interpreted by cell division and cell elongation (Khedr and Farid, 2000). In addition, these results may be due to the physiological roles of vitamins and amino acids in the yeast extract which increased the metabolic processes role and levels of indogenous hormones, i.e., IAA and GA_3 (Chaliakhyan, 1957).

The present investigated results were in accordance to those found by Jacoub (1999) on *Ocimum basilicum* and *Thymus vulgaris*, Abd El-latif (2006) on *Salvia officinalis* and Costa et al. (2008) on *Ocimum selloi*.

5. Leaves dry weight g/plant:

Data in (Table 5 and Fig. 3) showed that there was significant effect of all levels of CM on leaves dry weight of basil plants in the both harvest of the two seasons compared with untreated plants. The best result was obtained with using 80 m^3/fed. Moreover, there was significant effect of all levels of ADY on leaves dry weight of basil plants in the both harvest of the two seasons compared with control. Leaves dry weight per plant was increased with increase the level of ADY till 6 g/l.

According to the interaction among the CM and ADY treatments in the first season there was no significant effect in the first cut but there was a significant effect in the second cut and the best treatment was 6 g/L (ADY) with 80 m^3/fed (CM). In the second season there was significant effect in the first cut and the best result was obtained with treating plants with 80 m^3/fed (CM) with 6g/l (ADY) but there was no significant effect in the second cut.

This increase in leaves dry weight may be due to increment in number of leaves and leaf area. These results were in harmony with EL-Gendy et al. (2001), Alves et al. (2005) on *Coriandrum sativum* Carmen et al. (2006) and Salman (2006) on *Ocimum basilicum*.

Chemical composition

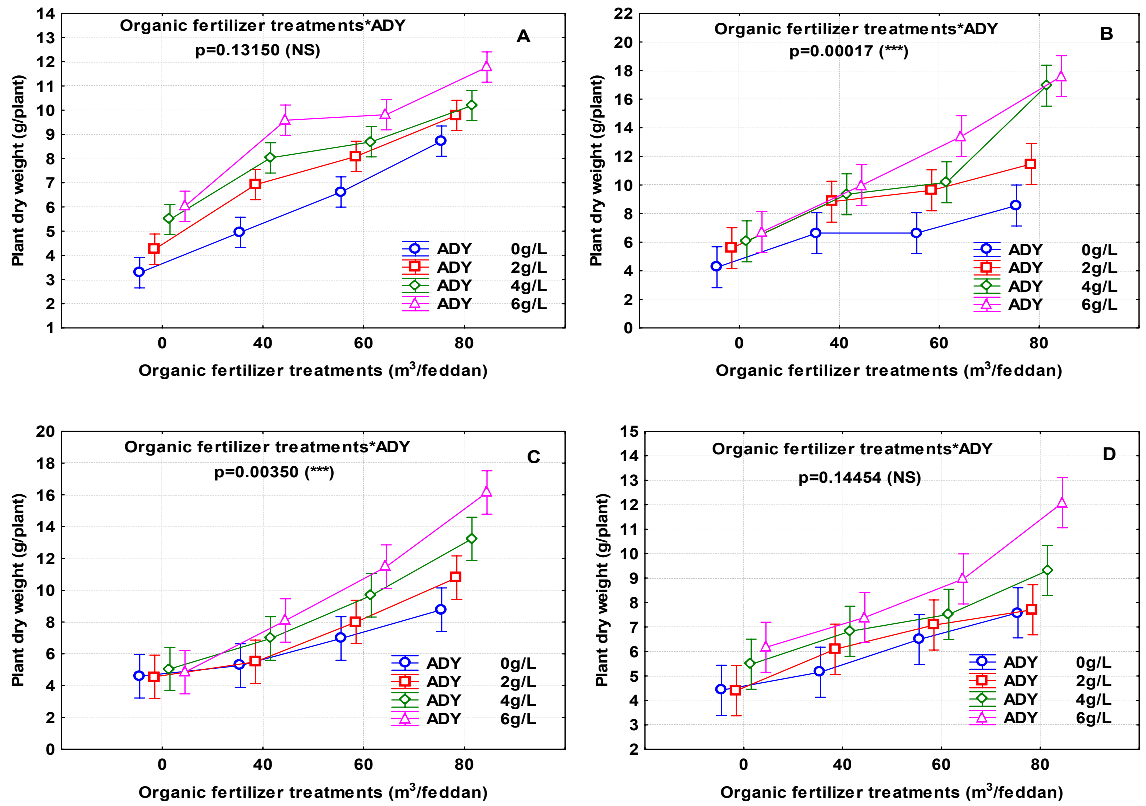
1. Total chlorophyll content

Data obtained in (Table 6) showed that there was significant effect of CM treatments on total chlorophyll content of *Ocimum basilicum* plants compared with control treatment. In the first season, the highest value was obtained with supplying plants with 80 m^3/fed in the two cuts (43.89 and 47.61, respectively). Followed by 60 m^3/fed (42.055 and 45.703 respectively). In the second season there were significant effects of the treatments in comparing with the control and there was no significant effect between all levels of CM, the highest value was recorded with using 80 m^3/fed in the two cuts (42.025 and 41.74, respectively).

Table 5: Means of leaves dry weight (g/plant) of *O. basilicum* plants as influenced by organic (CM) and active dry yeast (ADY) fertilizers and their combinations in the two seasons of 2012 and 2013.

First season (2012)										
	First cut					Second cut				
CM (A) m^3/fed.	Active Dry Yeast (B) g/l									
	0	2	4	6	mean	0	2	4	6	mean
0	3.28	4.25	5.48	6.03	4.76	4.24	5.58	6.06	6.72	5.65
40	4.95	6.92	8.02	9.58	7.37	6.64	8.83	9.35	9.98	8.70
60	6.61	8.09	8.69	9.81	8.304	6.64	9.62	10.18	13.41	9.96
80	8.72	9.78	10.19	11.78	10.12	8.56	11.46	16.94	17.60	13.64
mean	5.89	7.26	8.099	9.302		6.52	8.87	10.63	11.92	
$LSD_{0.05}$	A=0.63	B=0.40	A×B= NS			A= 0.84	B=1.10	A×B= 2.03		
Second season (2013)										
	First cut					Second cut				
CM (A) m^3/fed.	Active Dry Yeast (B) g/l									
	0	2	4	6	mean	0	2	4	6	mean
0	4.586	4.56	5.05	4.85	4.763	4.41	4.39	5.47	6.17	5.114
40	5.27	5.49	6.97	8.10	6.459	5.15	6.09	6.82	7.39	6.365
60	6.96	8.00	9.68	11.48	9.035	6.49	7.08	7.52	8.96	7.515
80	8.78	10.79	13.23	16.15	12.24	7.58	7.70	9.30	12.08	9.168
mean	6.401	7.215	8.734	10.147		5.908	6.319	7.28	8.65	
$LSD_{0.05}$	A=1.48	B=0.83	A×B= 1.93			A=0.96	B=0.69	A×B= NS		

NS= Not significant at the level of probability of 0.05.



A: first season first cut; B: first season second cut; C: Second season first cut, D: Second season second cut

Figure 3: Effect of organic fertilizer (CM) and active dry yeast (ADY) treatments on the leaves dry weight (g/plant) of *O. basilicum*.

Table 6: Means of total chlorophyll content (SPAD unit) of *O. basilicum* plants as influenced by organic (CM) and active dry yeast fertilizers and their combinations in the two seasons of 2012 and 2013.

					First season (2012)					
CM (A) m^3/fed.	**First cut**					**Second cut**				
	Active Dry Yeast (ADY) (B) g/l									
	0	**2**	**4**	**6**	**mean**	**0**	**2**	**4**	**6**	**mean**
0	35.60	36.68	39.45	40.09	37.95	31.37	37.70	41.38	45.14	38.89
40	39.04	40.00	40.72	42.79	40.64	38.26	44.30	41.98	45.27	42.45
60	40.34	41.14	43.06	43.66	42.055	38.64	48.63	46.76	48.77	45.70
80	40.37	44.81	44.86	45.53	43.89	40.71	48.65	50.53	50.55	47.61
mean	38.84	40.66	42.02	43.02		37.24	44.82	45.16	47.43	
$LSD_{0.05}$	A= 1.26		B=1.42	A×B= NS		A=1.79	B=1.62		A×B=3.197	
					Second season (2013)					
CM (A) m^3/fed.	**First cut**					**Second cut**				
	Active Dry Yeast (ADY) (B) g/l									
	0	**2**	**4**	**6**	**mean**	**0**	**2**	**4**	**6**	**mean**
0	28.53	32.02	39.50	33.12	33.293	29.31	32.50	37.02	36.96	33.95
40	36.66	39.10	42.03	44.29	40.523	35.78	38.83	40.47	40.25	38.83
60	36.71	38.83	42.19	46.31	41.012	39.43	39.77	40.73	42.23	40.54
80	36.18	40.49	43.29	48.13	42.025	41.34	39.78	41.10	44.74	41.74
mean	34.52	37.61	41.75	42.96		36.46	37.72	39.83	41.05	
$LSD_{0.05}$	A= 3.014		B= 3.06	A×B= NS		A=3.088	B=2.35		A×B= NS	

NS= Not significant at the level of probability of 0.05.

Moreover, all concentrations of ADY showed significant effect on total chlorophyll content of basil compared with control treatment and the best value was obtained with using 6 g/l ADY in the two harvests of the two seasons (43.02, 47.43, 42.96 and 41.05, respectively).

Furthermore, the interaction among the treatments of CM and ADY was significant in the second cut of the first season and the best value of chlorophyll content was recorded with using 80 m^3/fed CM with 4 or 6 g/l ADY (50.53 and 50.55) followed by 60 m^3/fed CM with 6 g/l ADY (48.77), 80 m^3/fed CM with 2 g/l ADY (48.65) and 60 m^3/fed CM with 2 g/l ADY (48.63). Moreover, in the second season there was no significant effect in the both cuts.

Such increase in photosynthetic pigments formation could be attributed to the role of yeast cytokinins which delaying the aging of leaves by reducing the degradation of chlorophyll and enhancing the protein and RNA synthesis (Castelfranco and Beale, 1983).

These results were in agreement with Costa et al. (2008) on *Ocimum selloi*, Abdou et al. (2011) on clove basil plant, Taie et al. (2010) on *Ocimum basilicum* plant, and Abdou et al. (2012) on fennel plants.

2. Oil percentage (%)

From the data in (Table7 and Fig. 4), it is clear that there were significant effects of all levels of CM fertilizers on oil percentage of *Ocimum basilicum* in the both harvests in the two seasons, compared with the control treatment. Generally, the highest values were obtained with using 80m^3/ fed CM with values of 0.54, 0.547, 0.431 and 0.419% in the two cuts of the two seasons, respectively. Moreover, there was no significant effect between the levels of 40 and 60 m^3/fed CM and between 60 and 80 m^3/fed. in the second cut of the first and the second seasons respectively.

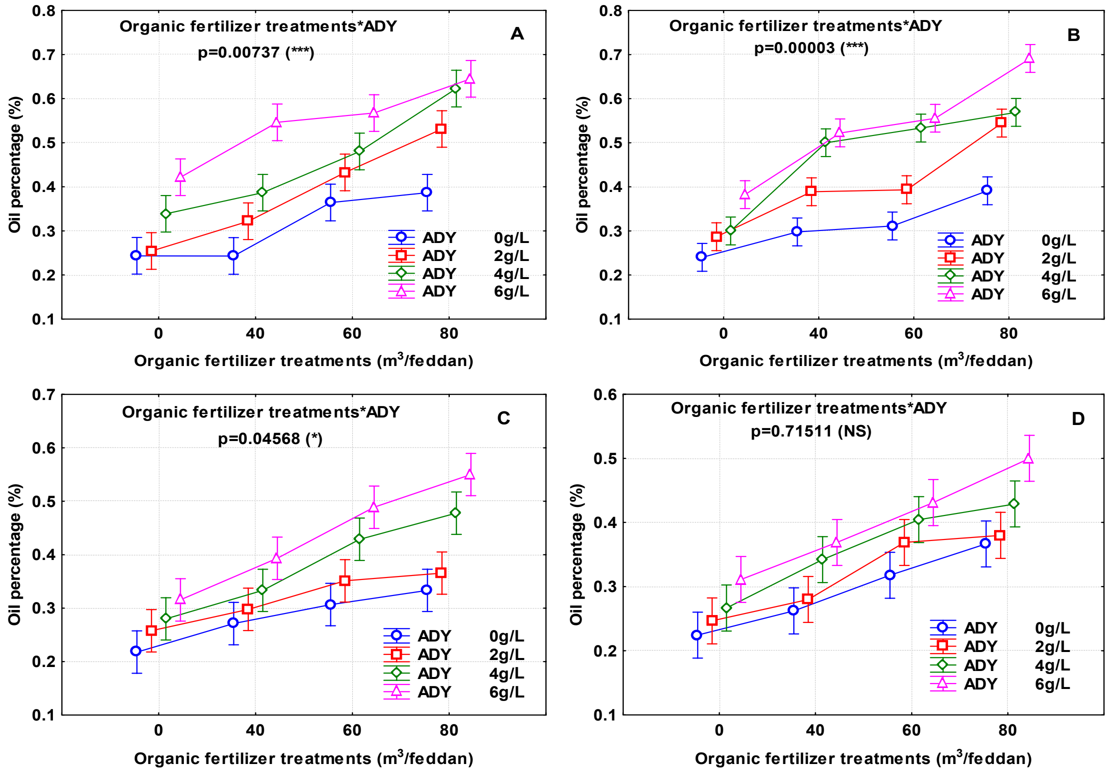
Additionally, in the second season the highest values of oil percentage (0.431% and 0.419%) was obtained by using 80 m^3/ fed CM in the two cuts respectively. Concerning to ADY, there were significant differences of all concentrations of ADY in the two cuts of the two seasons compared with control. The highest values were obtained with spraying plants with 6 g/l ADY (0.54, 0.537, 0.435 and 0.402 % respectively).

Furthermore, the interaction among the treatments of CM and ADY was significantly affected the oil percentage of *Ocimum basilicum* plants in the two cuts of the two seasons except of the second cut of the second season. The best result was recorded with using 80 m^3/fed CM with 6 g/l ADY (0.64, 0.69 and 0.550%, respectively). This increasing in oil percentage may be due to increasing the number of oil glands or enlargement in oil glands or both of them.

Table 7: Means of oil percentage (%) of *O. basilicum* plants as influenced by organic (CM) and active dry yeast fertilizers and their combinations in the two seasons of 2012 and 2013.

CM (A) m^3/fed.	First season (2012)									
	First cut					Second cut				
	Active Dry Yeast (ADY) (B) g/l									
	0	2	4	6	mean	0	2	4	6	mean
0	0.24	0.25	0.33	0.42	0.31	0.24	0.28	0.30	0.38	0.302
40	0.24	0.32	0.38	0.54	0.37	0.29	0.39	0.50	0.52	0.426
60	0.36	0.43	0.48	0.57	0.46	0.31	0.39	0.53	0.55	0.448
80	0.38	0.53	0.62	0.64	0.54	0.39	0.54	0.56	0.69	0.547
mean	0.31	0.38	0.45	0.54		0.309	0.403	0.475	0.537	
$LSD_{0.05}$	A= 0.028	B=0.03	A×B= 0.058			A= 0.032	B= 0.018	A×B= 0.044		
	Second season (2013)									
CM (A) m^3/fed.	First cut					Second cut				
	Active Dry Yeast (ADY) (B) g/l									
	0	2	4	6	mean	0	2	4	6	mean
0	0.216	0.256	0.280	0.313	0.266	0.223	0.246	0.266	0.310	0.261
40	0.270	0.296	0.333	0.393	0.323	0.263	0.280	0.343	0.370	0.314
60	0.306	0.350	0.426	0.486	0.392	0.316	0.370	0.403	0.430	0.38
80	0.333	0.366	0.476	0.550	0.431	0.366	0.380	0.430	0.500	0.419
mean	0.281	0.317	0.379	0.435		0.292	0.319	0.360	0.402	
$LSD_{0.05}$	A=0.036	B= 0.026	A×B= 0.056			A= 0.043	B=0.019	A×B= NS		

NS= Not significant at the level of probability of 0.05.



A: first season first cut; B: first season second cut; C: Second season first cut, D: Second season second cut

Fig. 4: Effect of organic fertilizer (CM) and active dry yeast (ADY) treatments on the oil percentage (%) of *O. basilicum.*

These results can be explained in the light of facts that, using organic manure, led to increase organic matter, availability of nutrients, nitrogen fixation and rizosphere microorganisms that release phytohormones, and substances which lead to increased growth and dry matter accumulation which in turn increases the concentration of oil (Edris et al., 2003; Jung et al. 2004).

These results were agreement with those obtained by Naga (2004) on *Foeniculum vulgare* Mill, and *Carium carvi* L. plants, Costa et al. (2008) on *Ocimum basilicum* plants, Louise et al. (2009) on *Plectranthus neochilus* plants, and Daneshian et al. (2011) on *Ocimum basilicum* plants.

3. The main chemical components of the essential oil *Ocimum basillicum* leaves.

The results in (Tables 8 - 11 and Figures 5 and 6) showed that there were no significant effects of CM levels on the percentages of the main 4 compounds in both cuts. On the other hand, the percentages of estragole, eucalyptol, linalool, and trans-4-methoxycinnamaldehyde (first cut) were significantly affected by using ADY treatments. Moreover, the interactions among the CM and ADY treatments gave significant effects on all the four chemical components in both cuts.

a. Methyl chavicol (estragole) (Table 8) ranged from (51.64-75.11%) in the first cut and the highest percentage was obtained by treated plants with 60 m^3/fed CM without ADY (75.11%) followed by the treatment of 2 g/l ADY (71.45%) without CM. In the second cut, the average values ranged from 55.2-73.15% and the highest percentage was obtained by the treated *Ocimum basilicum* plants with 2 g/l ADY without CM (73.145%) followed by the treatment of 60 m^3/fed (72.06%).

(Kandil et al., 2009) found that the main essential oil constituents were only slightly affected by fertilization with different ratios of organic fertilizers.

b. Linalool (Table 9) ranged from 1.59- 17.28% in the first cut and the highest percentage was obtained by treated plants with 60 m^3/fed CM with 2 g/l ADY (17.280%) followed by 4 g/l ADY without CM (16.06%). In the second cut, linalool was ranged from 2.96- 21.31% and the highest percentage was obtained by the treated plants with 60 m^3/fed CM combined with 6 g/l ADY (21.31%) followed by 60 m^3/fed CM with 2 g/l ADY (18.355%).

Table 8: Means of estragole content (%) of *O. basilicum* plants as influenced by organic (CM) and active dry yeast (ADY) fertilizers and their combinations in season of 2012.

CM (A) m^3/fed.	First cut					Second cut				
	Active Dry Yeast (B) g/l									
	0	2	4	6	mean	0	2	4	6	mean
0	64.760	71.450	58.695	69.595	66.125	63.700	73.145	62.115	68.195	66.789
40	62.466	63.150	63.125	61.085	62.457	71.455	57.625	67.480	71.685	67.061
60	75.110	51.640	57.760	52.350	59.215	72.060	59.245	70.480	55.195	64.245
80	55.970	60.310	62.930	55.410	58.655	62.080	68.045	67.915	69.550	66.898
mean	64.577	61.638	60.628	59.610		67.323	64.515	66.997	66.156	
$LSD_{0.05}$	A= NS	B=3.157	A×B=9.494			A= NS	B=1.677	A×B=9.121		

NS= Not significant at the level of probability of 0.05.

Table 9: Means of Linalool content (%) of *O. basilicum* plants as influenced by organic (CM) and active dry yeast (ADY) fertilizers and their combinations in season of 2012.

CM (A) m^3/fed.	First cut					Second cut				
	Active Dry Yeast (ADY) (B) g/L									
	0	2	4	6	mean	0	2	4	6	mean
0	12.365	2.855	16.060	4.360	8.91	12.050	2.955	13.520	3.580	8.026
40	6.400	10.860	12.695	3.535	8.372	7.810	16.570	8.535	4.580	9.374
60	1.585	17.280	4.240	14.850	9.489	5.070	18.355	7.465	21.310	13.050
80	14.890	6.610	4.240	7.530	8.318	15.785	9.335	8.270	5.590	9.745
mean	8.810	9.401	9.308	7.568		10.178	11.803	9.447	8.765	
$LSD_{0.05}$	A= NS	B=1.152	A×B =4.172			A= NS	B=0.408	A×B =3.218		

NS= Not significant at the level of probability of 0.05.

c. Eucalyptol (1-8, cineol) (Table 10) was ranged from 1.51- 4.886% in the first cut and the highest percentage was obtained with 60 m^3/fed CM combined with 4g/l ADY (4.886%) followed by 40 m^3/fed CM with 6 g/l ADY (4.035%). In the second cut, the values ranged between 1.61-4.27% and the highest percentage was obtained with 60 m^3/fed CM combined with 4 g/l ADY (4.270%) followed by the treatment of 40 m^3/fed CM with 2 g/l ADY (4.025%).

d. Trans-4-methoxycinnamaldehyde values (Table 11) ranged from 1.65-7.865% in the first cut; where the highest value was obtained with treated plants with 60 m^3 CM/fed combined with 4g/l ADY (7.865%) followed by 80 m^3 CM/fed with 6 g/l ADY (7.030%). As for in the second cut, the values ranged from 2.306-4.635% and the highest value was obtained with treated plants with 6 g/l ADY only (4.635%) followed by 4 g/l ADY (3.720%) without CM (0 m^3 CM/fed).

Table 10: Means of Eucalyptol content (%) of *O. basilicum* plants as influenced by organic (CM) and active dry yeast (ADY) fertilizers and their combinations in season of 2012.

CM (A) m^3/fed.	First cut					Second cut				
	Active Dry Yeast (ADY) (B) g/L									
	0	2	4	6	mean	0	2	4	6	mean
0	2.800	2.385	3.635	3.600	3.105	3.425	2.870	2.710	3.040	3.011
40	3.155	3.975	2.895	4.035	3.515	1.750	4.025	2.870	2.680	2.831
60	2.365	3.925	4.886	2.975	3.537	3.115	3.515	4.270	3.510	3.603
80	2.135	1.510	2.260	3.860	2.441	2.430	3.130	2.765	1.610	2.484
mean	2.613	2.948	3.419	3.617		2.680	3.385	3.153	2.710	
$LSD_{0.05}$	A= NS	B=0.587	A×B =2.217			A= NS	B=0.347	A×B = NS		

NS= Not significant at the level of probability of 0.05.

Table 11: Means of Trans-4-methoxycinnamaldehyde content (%) of *O. basilicum* plants as influenced by organic (CM) and active dry yeast (ADY) fertilizers and their combinations in season of 2012.

CM (A) m^3/fed.	First cut					Second cut				
	Active Dry Yeast (ADY) (B) g/L									
	0	2	4	6	mean	0	2	4	6	mean
0	3.045	3.975	3.825	5.035	3.970	2.325	3.210	3.720	4.635	3.473
40	1.653	3.885	3.075	6.275	3.722	2.445	2.535	2.995	3.540	2.879
60	3.655	4.990	7.865	5.665	5.544	3.695	2.550	3.385	2.465	3.024
80	5.295	6.585	6.515	7.030	6.356	3.045	2.825	2.740	2.306	2.729
mean	3.412	4.858	5.320	6.001		2.877	3.210	2.780	3.236	
$LSD_{0.05}$	A= NS	B=1.269	A×B =3.010			A= NS	B= NS	A×B =3.601		

NS= Not significant at the level of probability of 0.05.

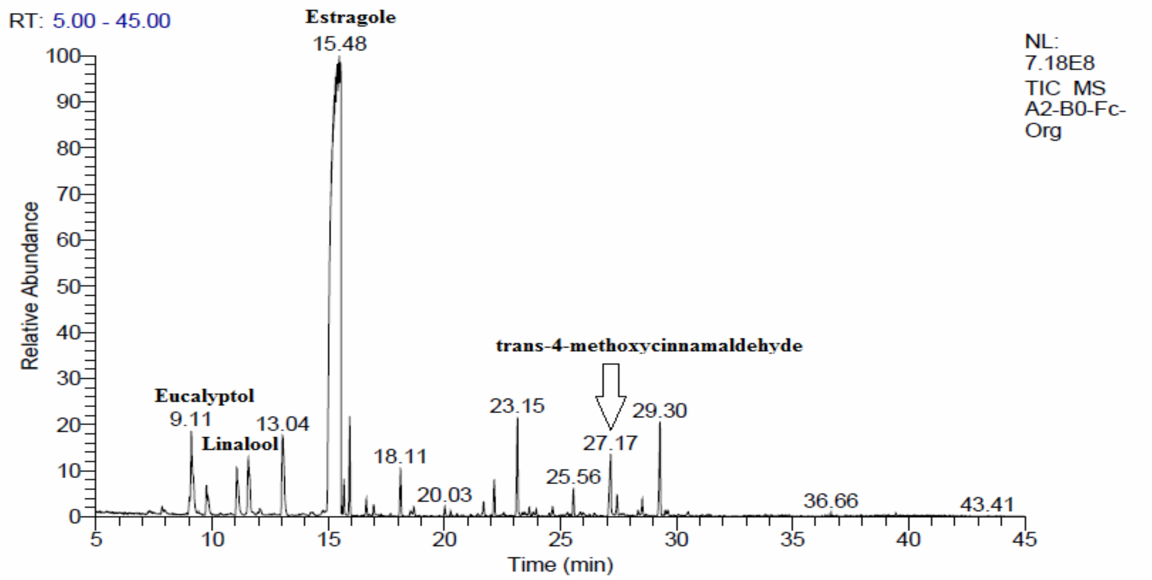


Figure 5: GC/MS chromatogram for the effect of CM and ADY treatments on the essential oil composition of *O. basilicum* plants in the first cut of the first season.

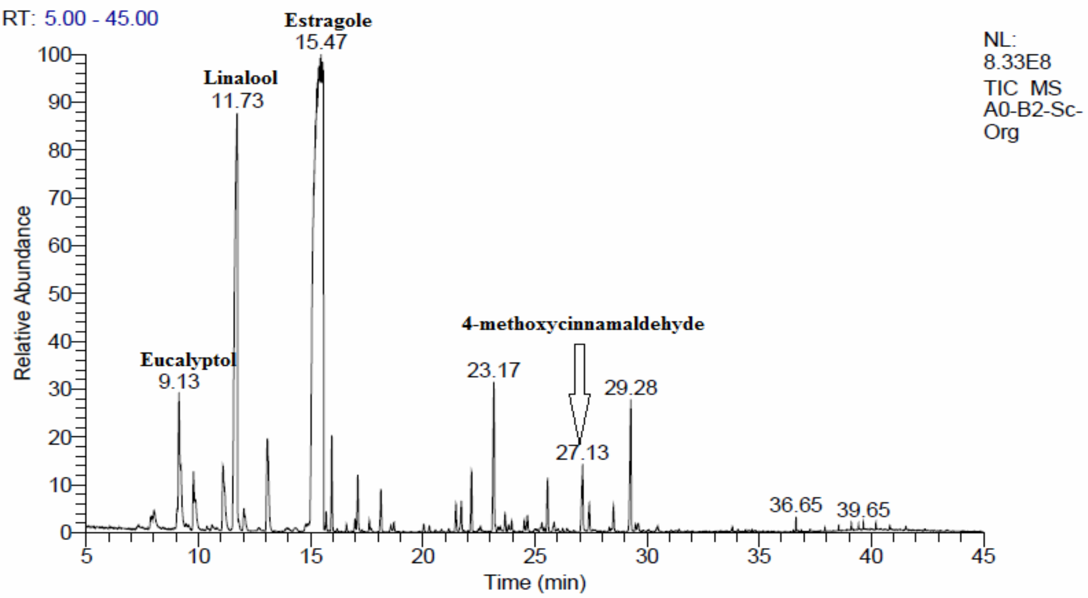


Figure 6: GC/MS chromatogram for the effect of CM and ADY treatments on the essential oil composition of *O. basilicum* plants in the second cut of the first season.

As was stated in other studies on the chemical constituents of sweet basil essential oil; methyl chavicol (estragole), β-(*E*)-ocimene, linalool, eugenol, 1,8-cineole (eucalyptol) and α-(*E*)-bergamotene were found to be the dominant constituents in *O. basilicum* plants as affected also by organic fertilizer as combined with ADY treatments (Simon et al., 1990; Khatri et al. 1995; Sajjadi, 2006; Telci et al. 2006; Chalchat and Özcan 2008; Pripdeevech et al. 2010). Additionally, the chemical composition of sweet basil essential oil did not change due to the fertilization type or level; rather the relative percentages of certain constituents were affected and these results were in agreements with Gharib *et al.* (2008) on marjoram essential oil.

It was stated that using organic and biofertilizers lead to a change in the composition of essential oil in the different plant species (Tanu *et al,* 2004; Darzi *et al.* 2009). It is demonstrated that using vermicompost and compost led to an increase in the relative content of linalool and methyl chavicol in basil (*Ocimum basilium*) (Anwar *et al.* 2005).

Accordingly, the main 4 constituents (estragole, linalool, eucalyptol and trans-4-methoxycinnamaldehyde) were subjected to statistical analysis to show the significant effects of the treatments. The results showed that there were no significant effects of CM on the percentages of the main 4 compounds in both cuts. On the other hand, the percentages of estragole, eucalyptol, linalool, and trans-4-methoxycinnamaldehyde (first cut) were significantly affected by ADY treatments. Moreover, the interactions among the CM and ADY treatments gave significant effects for all the four chemical components in both cuts. The main essential oil constituents were only slightly affected by fertilization with different ratios of organic fertilizers (Kandil et al. 2009). In the present study, the effect of different amounts of fertilizer on the essential oil composition was very slight and was not significant. But the amount of some component such as estragole and linalool was changed with using the fertilizer. These results are in agreement with Alizadeh et al. (2010) on *Satureja hortensis* L. (Lamiaceae) cultivated in Iran. Other study reported that the essential oil yield of *Origanum majorana* was significantly increased due to biofertilizer treatments relative to non-inoculated plants, without alteration of oil composition (Banchio et al., 2008).

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الملخص العربي

تأثير التسميد العضوي والحيوى على النمو وإنتاج الزيت والمحتوى الكيماوي للزيت الطيار لنباتات الريحان

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أجرى هذا البحث فى موسمين متتاليين (٢٠١٢/ ٢٠١٣) بمحطة البحوث الزراعية التابعة لكليـة الزراعـة- جامعة الإسكندرية بهدف دراسة تأثير معدلات مختلفة من السماد العضوي هى: ٠,٠، ٤٠، ٦٠ و ٨٠ كجم/ فـدان وأربع تركيزات من السماد الحيوي (الخميرة الجافة النشطة) هى: ٠,٠، ٢,٠، ٤,٠ و٦,٠ جم/ لتر علـى النمـو الخضرى وإنتاج الزيت والمحتوى الكيماوي للزيت الطيار لنباتات الريحان (*Ocimum basilicum* L.). صممت هـذه التجربة في صورة القطع المنشقة في ثلاث مكررات عشوائية تحتوي كل مكررة على ١٦ معاملة، خصصت القطع الكبيرة لمعدلات التسميد العضوي، أما القطع الصغيرة فكانت تركيزات السماد الحيـوي وقـد أوضـحت النتـائج المتحصل عليها أن معاملة النباتات بالسماد العضوي والحيوي بصورة فردية أو متداخلة قد أدت الى زيادة معنويـة في كل من قياسات النمو الخضري وإنتاج الزيت مقارنة بنباتات معاملة المقارنة (الكنترول). وتحققت أفضل النتائج لصفات النمو الخضري (إرتفاع النبات، عدد الأفرع / نبات، عدد الأوراق / نبات، المساحة الورقية /نبات والـوزن الجاف للأوراق) ومحتوى الأوراق من الكلورفيلات الكلية والنسبة المئوية للزيت عند إضافة السماد العضوي بمعدل ٨٠ كجم/فدان مع اضافة السماد الحيوي بتركيز ٦ جم/لتر، في حين أوضحت نتائج التحليل الكيماوي للزيت الطيـار أن أعلى زيادة معنوية في كل من الإسـتراجول (Estragole) والايوكليبتـول (eucalyptol) واللينـالول (Linalool) والترانس -٤- ميثوكسي سنامالدهيد (Trans-4-methoxycinnamaldehyde) عند استخدام السماد العضوي بمعدل ٦٠ كجم/فدان مع اضافة السماد الحيوي بتركيز ٢,٠ أو ٤,٠ جم/لتر وذلك فى كلا الموسمين مقارنه بمعاملة الكنترول.