Egyptian J. Desert Res., **64**, 153-166 (2014)

EFFECT OF ORGANIC FERTILIZATION AND ACTIVE DRY YEAST ON PRODUCTIVITY OF THREE-LOBED SAGE (*SALVIA FRUTICOSA* MILL.) PLANTS UNDER SIWA OASIS CONDITIONS

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A field experiment with split-plot design was laid out to investigate the effect of organic fertilization, foliar spray of yeast and their interaction on productivity and active constituents of three-lobed sage plants under Siwa Oasis conditions. The main plots included two compost manure levels of 10 and 20 m^3 per feddan. The sub-plots included foliar spray of active dry yeast concentrations of 0, 5 and 10 g /liter. The interaction effect proved that the highest compost manure level at 20 m^3 per feddan combined with foliar spray of the highest yeast concentration at 10 g/liter gave significantly maximum increments in vegetative growth parameters, yields of herb and essential oil as well as a higher 1,8-cineole constituent content in the volatile oil over control treatment, which recorded the lowest parameters.

Keywords: Siwa Oasis, three-lobed sage, compost, yeast, essential oil

Three-lobed sage (*Salvia fruticosa* Mill.; Family Lamiaceae) is a valuable medicinal and aromatic plant occuring in the Mediterranean regions. This species has been used in medicine as an antispasmodic, astringent, haemostatic and diuretic, but also as a culinary herb and in perfumery. The leaves of the plant are intensely aromatic and upon rubbing, clearly reminiscent of eucalyptus oil due its high cineole content. Also, antimicrobial and antiviral actions have been described for its essential oil (Wichtl, 2004; Seidemann, 2005 and Rhind, 2012).

In Egypt, cultivation of three-lobed sage plants have been extended recently to Siwa Oasis region as a promising newly reclaimed land for agriculture of medicinal and aromatic plants. So far, very little knowledge is known about farming practices of *Salvia fruticosa* as newly introduced plant under Siwa Oasis conditions. In this way, the point of the present paper is to examine the impact of applied organic fertilization, foliar spray of yeast

concentrations and their interaction on drug yield and essential oil productivity of *Salvia fruticosa* under Siwa Oasis conditions.

MATERIALS AND METHODS

This investigation was conducted during the two successive seasons of 2012/2013 and 2013/2014 at the Agricultural Experimental Station of the Desert Research Center at Khamisa Village (29.21º N and 25.40º E), Siwa Oasis, Egypt.

Seedlings of *Salvia fruticosa* were transplanted in the field on the 18th of April 2012 and 2013 for the first and second seasons, consecutively in rows; 75 cm apart and 50 cm between plants within hills. The experiment was laid out in a split plot design with four replicates. The main plots consisted of two application levels of organic fertilization of 10 and 20 m^3 compost manure per feddan. The sub-plots contained within foliar spray of active dry yeast at three concentrations of 0, 5 and 10 g dry yeast/liter of water. Compost manure was added before planting date throughout soil preparation. Half dose of the recommended chemical fertilizers was applied for plants according to Abd El-Azim (2003). Active dry yeast (*Saccharomyces cerevisiae*) was dissolved in water followed by adding sugar at ratio of 1:1 and kept overnight for activation and reproduction of yeast (El-Tohamy *et al*., 2008) and plants were sprayed with yeast culture after 45 and 90 days of transplanting, then spraying was repeated after each cut. The herb was harvested two times on the 21st of November and the 1st of March in both seasons of the investigation. L.S.D. test at 0.05 was used to compare the average means of treatments according to Snedecor and Cochran (1982). The following data were recorded:

1. Vegetative Growth and Yield Characters

a- Plant height (cm).
b- Fresh weight of herb /plant (g).
c- Dry weight of herb /plant (g).
d- Dry weight of herb /feddan (kg).

2. Chemical Analyses

2.1. Essential oil percentage

Essential oil percentage was determined in the air dried herb by hydrodistillation (British Pharmacopoeia, 1963).

2.2. Essential oil yield per plant (ml)

It was calculated as follows:

oil percentage × herb dry weight /100

2.3. Essential oil yield per feddan (l)

It was calculated as follows: essential oil yield per plant × number of plants/feddan (11200 plants /feddan).

2.4. Essential oil chemical constituents

Volatile oils of the second cut in the second season were analyzed by using Gas Chromatography-Mass Spectrometry instrument (GC-MS analysis) at the Laboratory of Medicinal and Aromatic Plants, National Research Center, Egypt.

The soil, water and compost manure samples were analyzed at the laboratories of Desert Research Center and Soils, Water and Environment Research Institute, as shown in tables (1, 2, 3 and 4). The analyses were carried out as described by Jackson (1976) and A.O.A.C. (2002).

Table (1). The mechanical analysis of soil of the experimental station at Khemisa, Siwa Oasis.

Sand (%)	Silt (%)	Clay (%)	Soil texture
92.91	5.21	1.88	Sandy

Table (2). The chemical analysis of soil of the experimental station at Khemisa, Siwa Oasis.

pH	E.C.	O.M.	Soluble anions (meq/l)				Soluble cations (meq/l)			
	(dS/m)	(%)	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
7.5	4.1	0.5	-	3.6	31.3	6.1	8.6	7.5	0.2	24.7

Table (3). The chemical analysis of irrigation water used in the experiment.

pH	E.C.	Soluble anions (meq/l)				Soluble cations (meq/l)			
	ppm	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
7.41	2155.00	-	3.63	16.87	14.43	7.27	4.50	22.12	1.04

Table (4). The chemical analysis of the used compost manure.

pH	EC (dS/m)	O.M. (%)	C/N ratio (%)	N (%)	P (%)	K (%)	Fe (%)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)
8.8	4.6	20.5	11.85	1.03	0.22	2.04	3.43	606.8	85.65	43.60

RESULTS AND DISCUSSION

1. Effect of Organic Fertilization

The effect of applying compost manure levels on productivity and active constituents of *Salvia fruticosa* plants are given in tables (5, 6, 7, 8, 9, 10 and 11). The results showed that the differences between the two applied

compost manure levels were insignificant in the first cut in most of the cases for the numerous parameters of vegetative growth, yield and essential oil productivity; whereas these variations were significant in the second cut of the experiment. These results are in agreement with those stated by Abd El-Latif (2006) and Khalail et al. (2008) on *Salvia officinalis*, Singh et al. (2008) on *Salvia sclarea* and Kocabas et al. (2010) on *Salvia fruticosa* plants.

Table (5). Effect of organic fertilization rates, foliar spray of yeast concentrations and their interaction on plant height (cm) of *Salvia fruticosa* in the two cuts of both seasons (2012/2013 and 2013/2014).

Yeast / Organic fertilization	1st cut				2nd cut			
	Without	Yeast at 5 g/liter	Yeast at 10 g/liter	Mean	Without	Yeast at 5 g/liter	Yeast at 10 g/liter	Mean
Compost at 10 m^3/feddan	20.33	21.33	27.65	23.10	34.56	40.33	40.33	38.41
Compost at 20 m^3 ton/feddan	26.67	28.00	28.67	27.78	36.70	43.50	48.33	42.84
Mean	23.50	24.67	28.16		35.63	41.92	44.33	
LSD 0.05								
Organic fertilization		NS				2.52		
Yeast		3.65				1.96		
Organic fertilization x Yeast		5.16				2.77		

* Values are means of two seasons.

Table (6). Effect of organic fertilization rates, foliar spray of yeast concentrations and their interaction on fresh weight of herb/plant (g) of *Salvia fruticosa* in the two cuts of both seasons (2012/2013 and 2013/2014).

Yeast / Organic fertilization	1st cut				2nd cut			
	Without	Yeast at 5 g/liter	Yeast at 10 g/liter	Mean	Without	Yeast at 5 g/liter	Yeast at 10 g/liter	Mean
Compost at 10 m^3/feddan	66.80	71.90	76.43	71.71	95.48	98.90	150.30	114.89
Compost at 20 m^3 ton/feddan	85.37	96.98	105.97	96.11	107.13	127.33	160.93	131.80
Mean	76.09	84.44	91.20		101.31	113.12	155.62	
LSD 0.05								
Organic fertilization		18.71				5.86		
Yeast		12.29				28.23		
Organic fertilization x Yeast		17.39				39.93		

* Values are means of two seasons.

Table (7). Effect of organic fertilization rates, foliar spray of yeast concentrations and their interaction on dry weight of herb/plant (g) of *Salvia fruticosa* in the two cuts of both seasons (2012/2013 and 2013/2014).

Yeast / Organic fertilization	1st cut				2nd cut			
	Without	Yeast at 5 g/liter	Yeast at 10 g/liter	Mean	Without	Yeast at 5 g/liter	Yeast at 10 g/liter	Mean
Compost at 10 m^3/feddan	16.22	17.48	18.57	17.42	24.98	25.97	39.28	30.08
Compost at 20 m^3 ton/feddan	19.39	23.52	28.86	23.92	28.71	36.92	44.68	36.77
Mean	17.81	20.50	23.72		26.85	31.45	41.98	
LSD 0.05								
Organic fertilization		NS				3.36		
Yeast		3.63				8.53		
Organic fertilization x Yeast		5.13				12.07		

* Values are means of two seasons.

Table (8). Effect of organic fertilization rates, foliar spray of yeast concentrations and their interaction on dry weight of herb/feddan (kg) of *Salvia fruticosa* in the two cuts of both seasons (2012/2013 and 2013/2014).

Yeast / Organic fertilization	1st cut				2nd cut			
	Without	Yeast at 5 g/liter	Yeast at 10 g/liter	Mean	Without	Yeast at 5 g/liter	Yeast at 10 g/liter	Mean
Compost at 10 m^3/feddan	181.66	195.78	207.98	195.14	279.78	290.86	439.94	336.86
Compost at 20 m^3 ton/feddan	217.17	263.42	323.23	267.94	321.55	413.50	500.42	411.82
Mean	199.42	229.60	265.61		300.67	352.18	470.18	
LSD 0.05								
Organic fertilization		NS				37.64		
Yeast		40.60				95.58		
Organic fertilization x Yeast		57.42				135.17		

* Values are means of two seasons.

Table (9). Effect of organic fertilization rates, foliar spray of yeast concentrations and their interaction on essential oil percentage of *Salvia fruticosa* in the two cuts of both seasons (2012/2013 and 2013/2014).

Yeast / Organic fertilization	1st cut				2nd cut			
	Without	Yeast at 5 g/liter	Yeast at 10 g/liter	Mean	Without	Yeast at 5 g/liter	Yeast at 10 g/liter	Mean
Compost at 10 m^3/feddan	2.03	2.13	2.28	2.15	1.27	1.31	1.48	1.35
Compost at 20 m^3 ton/feddan	2.17	2.13	2.40	2.23	1.39	1.44	1.58	1.47
Mean	2.10	2.13	2.34		1.33	1.38	1.53	
LSD 0.05								
Organic fertilization		NS				NS		
Yeast		NS				0.17		
Organic fertilization x Yeast		0.13				0.24		

* Values are means of two seasons.

Table (10). Effect of organic fertilization rates, foliar spray of yeast concentrations and their interaction on essential oil yield per plant (ml) of *Salvia fruticosa* in the two cuts of both seasons (2012/2013 and 2013/2014).

Yeast / Organic fertilization	1st cut				2nd cut			
	Without	Yeast at 5 g/liter	Yeast at 10 g/liter	Mean	Without	Yeast at 5 g/liter	Yeast at 10 g/liter	Mean
Compost at 10 m^3/feddan	0.33	0.37	0.42	0.37	0.32	0.34	0.58	0.41
Compost at 20 m^3 ton/feddan	0.42	0.50	0.69	0.54	0.40	0.53	0.71	0.55
Mean	0.38	0.44	0.56		0.36	0.44	0.65	
LSD 0.05								
Organic fertilization		NS				0.11		
Yeast		NS				0.15		
Organic fertilization x Yeast		0.12				0.21		

* Values are means of two seasons.

Table (11). Effect of organic fertilization rates, foliar spray of yeast concentrations and their interaction on essential oil yield (l/ feddan) of *Salvia fruticosa* in the two cuts of both seasons (2012/2013 and 2013/2014).

Yeast / Organic fertilization	1st cut				2nd cut			
	Without	Yeast at 5 g/liter	Yeast at 10 g/liter	Mean	Without	Yeast at 5 g/liter	Yeast at 10 g/liter	Mean
Compost at 10 m^3/feddan	3.70	4.14	4.70	4.18	3.58	3.81	6.50	4.63
Compost at 20 m^3 ton/feddan	4.70	5.60	7.73	6.01	4.48	5.94	7.95	6.12
Mean	4.20	4.87	6.22		4.03	4.88	7.23	
LSD 0.05								
Organic fertilization		NS				1.21		
Yeast		0.94				1.65		
Organic fertilization x Yeast		1.33				2.34		

* Values are means of two seasons.

2. Effect of Active Dry Yeast

According to the results presented in tables (5, 6, 7, 8, 9, 10 and 11) foliar application of active dry yeast enhanced all growth and yield characters in both seasons. The highest concentration of 10 g/liter significantly increased plant height, fresh and dry yield of herb per plant and per feddan besides oil yield per feddan over control treatment (without foliar yeast application). These findings are in harmony with those obtained by Abd El-Latif (2006) on *Salvia officinalis*, who reported that using active dry yeast significantly increased vegetative growth, oil production as compared to the control plants. Also, these results are in harmony with other investigators, who described the enhancement role of active dry yeast on medicinal and aromatic plants; i.e. El-Sayed et al. (2002) on coriander; Naguib and Khalil (2002) on *Nigella sativa*, Heikal (2005) on *Thymus vulgaris* and Ezz El-Din and Hendawy (2010) on *Borago officinalis*.

3. Effect of the Interaction

Data presented in tables (5, 6, 7, 8, 9, 10, 11 and 12) show the effect of interaction among treatments on growth, yield and active constituents. The significantly superior treatment was the application of 20 m^3 compost manure per feddan combined with foliar spray of active dry yeast at concentration of 10 g/liter, which resulted in the significantly highest plant height, fresh and dry yield of herb per plant and per feddan. Moreover, it increased volatile oil percentage, oil yield per plant and per feddan over control treatment.

The GC-MS analyses of volatile oils proved the presence of 1,8-cineole as a main compound in the volatile oil. Furthermore, it was found that all the applied fertilization treatments enhanced the accumulation of the

main component in essential oil (45.80 – 53.87% of 1,8-cineole content) in comparison to control treatment, which offered the lowest value (39.42%) (Fig. 1 and 2).

Table (12). Effect of the interaction between treatments on chemical constituents (%) of *Salvia fruticosa* essential oil.

No	R.T.	Constituents	Compost at 10 m^3/feddan without foliar spray of yeast	Compost at 10 m^3/feddan + foliar spray of yeast at 5 g/liter	Compost at 10 m^3/feddan + foliar spray of yeast at 10 g/liter	Compost at 20 m^3/feddan without foliar spray of yeast	Compost at 20 m^3/feddan + foliar spray of yeast at 5 g/liter	Compost at 20 m^3/feddan + foliar spray of yeast at 10 g/liter
1	4.57	2-thujene	0.63	0.57	0.65	0.53	0.47	0.55
2	4.76	α-pinene	5.45	4.10	5.77	4.66	4.04	3.90
3	5.21	Camphene	6.51	0.13	2.18	3.00	0.70	2.63
4	5.80	Sabinene	0.22	0.42	0.23	0.28	0.35	0.64
5	5.96	β-pinene	8.30	17.65	15.67	11.69	14.28	9.21
6	6.21	α-myrcene	3.21	4.37	6.53	6.35	4.64	5.24
7	6.81	L-phellandrene	-	-	0.08	-	0.04	-
8	7.12	α-terpinene	0.22	0.32	0.52	0.29	0.30	0.30
9	7.44	p-cymene	0.11	0.21	0.25	0.09	0.16	0.16
10	7.53	D-limonene	1.67	0.80	1.48	0.85	0.70	0.71
11	**7.67**	**1,8-cineole**	**39.42**	**45.80**	**49.47**	**47.61**	**53.87**	**46.28**
12	8.53	ç-terpinene	0.45	1.16	1.13	0.67	0.92	0.79
13	9.04	4-thujanol	0.46	0.56	0.33	0.46	0.54	0.62
14	9.50	α-terpinolene	0.13	0.09	0.16	0.10	0.12	0.11
15	10.13	L-linalool	0.15	-	-	-	-	-
16	10.21	Terpineol,cis-á-	0.24	0.30	0.22	0.18	0.18	0.29
17	10.47	β-thujone	1.02	0.37	0.48	0.74	0.83	1.71
18	10.92	α-thujone	0.78	0.23	0.54	0.34	0.59	1.16
19	11.81	Pinocarveol	-	-	-	-	-	-
20	12.18	Camphor	11.85	0.07	4.10	4.93	2.63	5.60
21	12.68	Pinocamphone	0.55	-	0.48	0.50	0.96	0.92
22	13.14	Borneol	3.42	0.61	1.12	1.40	1.03	1.42
23	13.36	3-pinanone,cis	0.22	-	0.16	0.19	0.39	0.42
24	13.45	4-terpineol	0.53	0.17	0.40	0.26	0.31	0.31
25	14.13	p-menth-1-en-8-ol	2.39	1.18	1.44	1.42	1.99	1.78
26	16.19	Linalyl acetate	0.35	-	-	0.07	-	0.12
27	17.73	L-bornyl acetate	1.79	-	0.21	0.36	0.07	0.30
28	20.07	Exo-2-hydroxycineole acetate	-	-	0.07	-	-	-

Table 12. Cont.

29	20.14	α-cubebene	-	0.11	-	-	-	-
30	20.38	α-terpinenyl acetae	0.87	0.19	0.39	0.33	0.22	0.34
31	21.10	Isoledene	-	-	-	0.07	0.05	-
32	22.61	α-gurjunene	-	0.15	-	0.08	-	0.11
33	22.74	(-)-Aristolene	0.09	-	-	-	0.04	-
34	23.18	Caryophyllene	3.93	15.43	2.37	7.46	5.56	7.41
35	23.51	1,1,3a-trimethyl-7-methylenedeca Hydro-1H-cyclopropa[a] naphthalene	-	-	0.08	0.11	0.09	0.11
36	23.76	Selina-3,7(11)-diene	-	-	0.09	0.12	0.10	0.09
37	23.95	Aromadendrene	0.44	0.14	0.75	1.09	0.90	0.91
38	24.30	ç-gurjunene	-	-	0.07	0.09	0.07	-
39	24.69	α-humulene	2.14	0.99	0.68	2.60	1.02	1.77
40	24.84	α-cyclo-homogeraniol	0.09	0.06	-	-	-	-
41	25.52	ç-muurolene	-	0.14	-	-	-	-
42	26.11	Ledene	0.17	0.07	0.26	0.36	0.22	0.30
43	26.34	Bicyclogermacrene	0.07	-	-	0.09	0.18	0.20
44	26.39	α-selinene	-	0.07	-	-	-	-
45	26.52	α-muurolene	-	0.07	-	-	-	0.10
46	27.26	ë-cadinene	0.15	0.70	0.11	0.09	-	0.26
47	29.66	(-)-spathulenol	0.16	0.15	0.15	0.14	0.24	0.22
48	29.82	(-)-caryophyllene oxide	0.31	0.42	0.09	0.18	0.19	0.20
49	30.32	Veridiflorol	0.94	-	0.90	-	0.90	2.10
50	30.48	Ledene oxide(I)	-	0.07	-	-	-	-
51	30.93	Humulene oxide	0.23	-	-	0.08	0.05	-
52	32.29	Humulane1,6dien3ol	0.07	0.23	0.09	-	-	0.14
53	32.71	(-)-caryophyllene oxide	-	-	-	-	-	-
54	32.79	α-cadinol	-	0.08	-	-	-	-
55	34.10	6-epi-shyobunol	0.27	1.82	-	-	-	-
56	34.14	Carotol	-	-	0.22	0.14	-	0.57
57	46.04	13-epimanool	-	-	0.08	-	0.06	-

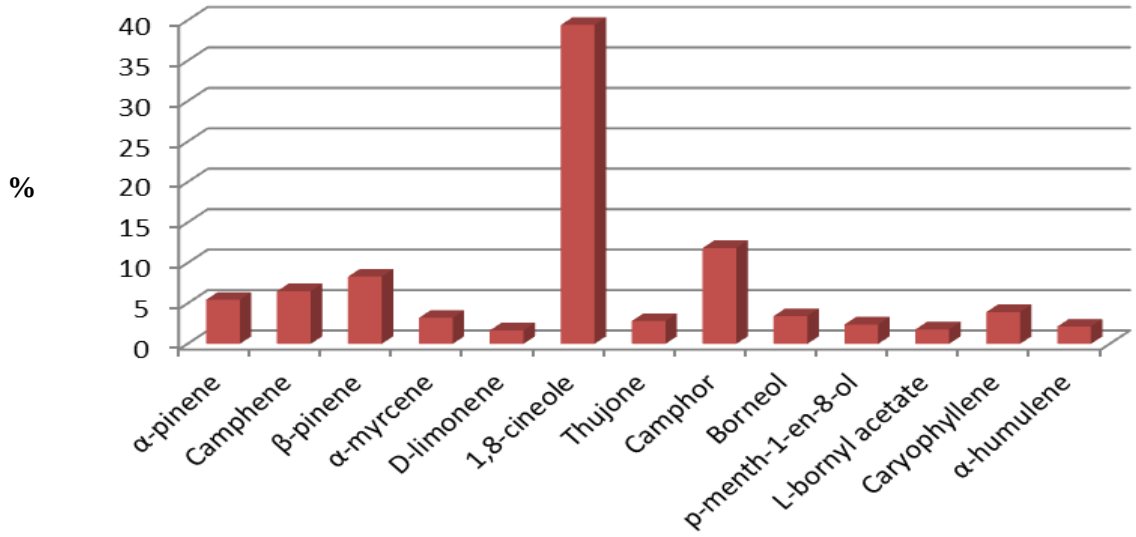

Fig. (1). Chemical constituents of volatile oil for the treatment of compost at 10 m^3/feddan without foliar spray of yeast.

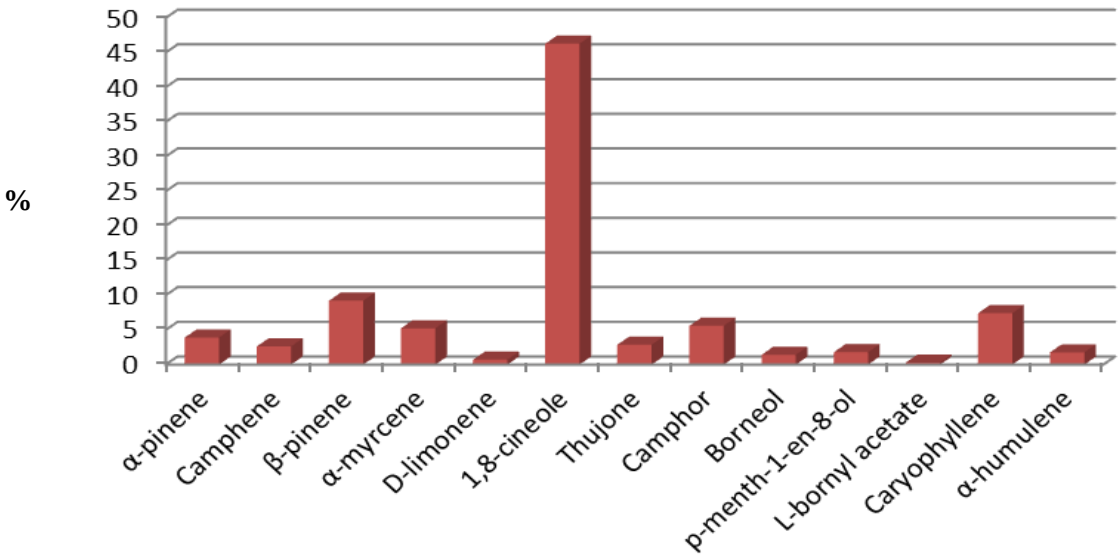

Fig. (2). Chemical constituents of volatile oil for the treatment of compost at 20 m^3/feddan with foliar spray of yeast at 10 g/liter.

The aforementioned effects may be due to the role of compost manure, which 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 degrade substrates and nutrients supplied, but also provide the soil with a wide range of microorganisms (Ryckeboer et al., 2003), including harmless heterotrophy, but potentially also plant and human pathogens. Compost as an organic material influences agricultural sustainability by improving chemical, physical, biological properties of soils, the fertility and structure of the soil and the moisture holding capacity (Saha et al., 2008). Moreover, active dry yeast is a natural safety biofertilizer causing various promotive effects on plants. It is considered as a natural source of cytokinins, which simulate cell division and enlargement as well as the synthesis of protein, nucleic acids and B-vitamin. It also releases CO_2, which is reflected in improving net photosynthesis (Amer, 2004 and Kurtzman and Fell, 2005).

Parallel findings were also reported by Kocabas et al. (2010) on *Salvia fruticosa*, who studied the effects of different organic manure applications on chemical composition of essential oils of *Salvia fruticosa*. They found that the essential oil yields increased with organic manure applications. Percentage values of 1,8 cineole, the main component of the oil, ranged from 44.0 to 50.7% with the application of organic manures. As a result, the essential oil content of three-lobed sage was increased with organic manure applications.

CONCLUSION

It could be recommended to apply compost manure at the rate of 20 m^3 per feddan before cultivation and plants should be foliar sprayed with active dry yeast, at concentration of 10 g/liter, after 45 and 90 days of transplanting, then repeated after each cut to obtain the highest herb and oil yields per feddan under Siwa Oasis conditions.

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تأثير التسميد العضوي والخميرة الجافة على إنتاجية نباتات المريمية ثلاثية الورقة تحت ظروف واحة سيوة

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أجريت هذه التجربة الحقلية بتصميم القطع المنشقة، لدراسة تأثير التسميد العضوى والرش الورقي بالخميرة وتفاعلهم على الإنتاجية والمكونات الفعالة لنباتات المريمية ثلاثية الورقة تحت ظروف واحة سيوة. إشتملت القطع الرئيسية على مستويين من سماد الكمبوست ١٠ و٢٠ م^{٣} للفدان وإشتملت القطع الفرعية على الرش الورقي بالخميرة الجافة بتركيزات صفر، ٥، ١٠ جم/ اللتر. أثبت تأثير التفاعل أن أعلى مستوى من سماد الكمبوست (٢٠ م^{٣} للفدان) مع الرش الورقي بالخميرة بأعلى تركيز (١٠ جم/اللتر) أعطى أعلى زيادات معنوية في النمو الخضري ومحصول العشب والزيت الطيار بالإضافة إلى محتوى أعلى من مركب ١,٨ سنيول في الزيت الطيار، مقارنة بالمعاملة الكنترول التى سجلت أقل القياسات.