

CULTIVATION OF *MELISSA OFFICINALIS* ,L IN THE NORTH MIDDLE NILE DELTA REGION:

B. EFFECT OF PLANTING SPACES AND ACTIVE DRY YEAST

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ABSTRACT

Two field experiments was established at Sakha Agricultural Research Station during the two seasons of 2008/2009 and 2009/2010 to evaluate the effect of planting spaces and active dry yeast on the growth ,yield and oil components of *Melissa officinalis* ,L . Three planting spaces (30, 40 and 50 cm) and four applications of active dry yeast (control, 5, 10 and 15gm / l) were used in the two seasons. The obtained results showed that wider plant spacing showed the greatest effect on growth parameters and chemical constituents. Similarly, active dry yeast levels had a promoting influence on most of the vegetative growth parameters and accelerated essential oil accumulation and chemical constituents including chlorophyll a ,b and total content and N,P and K%. The wider space between plants (40 or 50 cm) combined with maximum rate of dry yeast (10 or 15g/ l) had a favorable effect on the most growth characters and chemical constituents. The main constituent of the essential oil was, geranial (citral A) followed by camphene. Generally, it can be concluded that there is no significant difference between wider distance (50 cm) and medium one (40 cm) and rate of dry yeast (10 and15g/ l).So, it can be recommended to cultivate at distance of (40 cm) combined with applying (10 g/ l) of active dry yeast.

Keywords: *Melissa officinalis*,L , planting date , harvesting date, essential oil, oil yield.

INTRODUCTION

Lemon balm, *Melissa officinalis* L., is a perennial herb native to southern climates of Europe and North America, is presently found in both wild and cultivated states. Several other species of *Melissa* have been reported from the Mediterranean and central Asian areas, but only *Melissa officinalis* L. is cultivated. The plant grows erect and reaches a height of 0.5 to 1 m. The highest levels of essential oil have been extracted in late summer from the lower parts of the plant (Kennedy *et al.*, 2006). Lemon balm has been traditionally used for different medical purposes as tonic, antispasmodic, carminative, diaphoretic, surgical dressing for wounds, sedative-hypnotic strengthening the memory, and relief of stress induced headache as a mild sedative-hypnotic, and as an antiviral to improve healing of herpes simplex cold sores (Blumenthal *et al.*, 2000).

In suitable plant spaces, plants completely use environmental conditions (water, air, light and soil) and inter specific or intra specific competition is minimum. The effect of planting spaces on the growth, yield and active ingredients of medicinal and aromatic plants was studied by many investigators: Wahba and Ezz El-Din (2002) on *Chrysanthemum coronarium*

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,L , (Hussein *et al*,2006) on dragonhead , Khazaie *et al.* (2008) on thyme and hyssop and Berimavandi *et al*(2011) on *Calendula officinalis* .

Bio-stimulants can be generally defined as some plant growth regulators or promoting substances such as vitamins, microelements, organic acids as well as preparations containing live cells (like bread yeast or candida), which were microorganisms with the objective of increasing their number and of accelerating certain microbial process to increase the availability of nutrient elements in form which can be easily taken by plants. Thus greater attention has been directed on the use of microorganisms as bio-stimulants to provide nutrients for higher plants without any pollution to the environment (Khalil and Ismael,2010). Khedr and Farid(2000) demonstrated that the effect of dry yeast is due to its capability in induction of endogenous hormones like GA3 and IAA. The effect of dry yeast on the growth, yield and active ingredients of medicinal and aromatic plants was studied by many investigators: (Ahmed *et al*, 1998) on Roselle (*Hibiscus sabdariffa*) , Naguib and Khalil (2002)on black cumin , Wahba(2002)on oenothera and Heikal (2005) on thyme plant . Recently, dry yeast is used as an alternative source of growth substances in bio/organic fertilization system.

Continuous usage of inorganic fertilizer affects soil structure. Hence, application of dry yeast can serve as alternative to mineral fertilizers for improving soil structure and microbial biomass. This may help in increasing crop productivity. Dry yeast in comparison of the chemical fertilizer has lower nutrients content and is slow release but it is an effective as chemical fertilizer over longer periods of use. Plant spaces is dependent on the plant characters, growth period, time and method of cultivation, soil conditions, and plant length, sun light and weeds. For that the objective of this search is to study the effect of planting spaces and active dry yeast on the growth, yield and oil components of *Melissa officinalis* ,L

MATERIALS AND METHODS

This investigation was conducted at the Experimental Farm of Sakha Agricultural Research Station, Kafr El-Sheikh Governorate. The site is located at 30 56 N latitude, 31 05 E longitude with an elevation of about 6 meters above mean sea level. This location is a representative of conditions in the middle Northern Part of Nile Delta region during the two successive seasons of 2008/2009 and 2009/2010 to study the effect of planting spaces and active dry yeast on the growth, yield and oil components of *Melissa officinalis* ,L. Melissa plantlets ,originated from stem cuttings were obtained from Medicinal and Aromatic plants Department, ARC, Egypt and were transplanted in spring (11th March) in the two seasons(This time according to data in the work of A. Effect of planting and harvesting dates). The stem cuttings were cultivated in hills at three planting spaces (30 ,40 and 50 cm distances) on rows 60 cm apart in plots of 6m² (2 x 3) at the area of each planting spaces treatment. The physical and chemical properties of the experimental soil were determined before cultivation, according to Jackson (1967), as showed in Table (A)

Table (A): Physical and chemical analysis of experimental soil before cultivation

Physical analysis			Soil texture	Chemical analysis													
Sand %	Silt%	Clay%		E.c (m mhos/cm)	pH	Soluble cations (meq/ l)				Soluble anions (meq/ l)				Available N(ppm)	Available P(ppm)	Available K(ppm)	Organic matter%
			Na+			K+	Ca++	Mg++	Co ₃ -	HCo ₃ -	Cl-	So ₄ ++					
19.31	30.42	50.27	clay	1.74	7.95	13.94	1.45	5.33	1.45	-	6.96	2.35	12.86	39.40	8.37	209.3	1.6

Design of the experiments:

This experiment included 12 treatments which were the combination between three planting spaces (30 ,40 and 50 cm distances) and four yeast applications (0, 5,10 and 15g/l). Treatments were arranged in a split plot design with three replicates, different planting spaces were assigned at random in the main plots, while sub-plots were devoted to the different yeast applications.

N.P.K: the recommended dose was added for all treatment at the rate of 150 kg ammonium sulphate (20.5% N) and 50kg potassium sulphate (48% K₂O) per feddan (4200m²) was applied in two equal doses with the first and second irrigation and repeat after harvest , 200kg calcium super phosphate (15.5% P₂O₅) in one dose during soil preparation.

Yeast application:

Yeast solution was prepared according to the method described by Skoog and Miller (1957) and were applied as addition to the soil four times ,the first one after 15 days from planting and replicate after 21 days at three times interval and the same after harvesting of the 1st cut . The composition of yeast solution employed in the experiment was described in Table (B) as found by Nagodawithana (1991).

Table (B): The composition of active yeast.

Protein		47%	
Carbohydrates		33%	
Minerals		8%	
Nucleic acids		8%	
Lipids		4%	
The composition of minerals			
Na	0.12 mg/g f.w	Cu	8.00 µ/g
Ca	0.75 mg/g f.w	Se	0.10 µ/g
Fe	0.02 mg/g f.w	Mn	0.02 µ/g
Mg	1.65mg/g f.w	Cr	2.20 µ/g
K	21.0 mg/g f.w	Ni	3.00 µ/g
P	13.5 mg/g f.w	Va	0.04 µ/g
S	13.5 mg/g f.w	Mo	0.40 µ/g
Zn	0.17 mg/g f.w	Sn	3.00 µ/g
Si	0.03 mg/g f.w	Li	0.17 µ/g
The composition of Vitamins			
Thiamine		60 - 100 µ/g	
Riboflavin		35 - 50 µ/g	
Niacin		300 - 500 µ/g	
Pyridoxine		28 µ/g	
Pantorhenate		70 µ/g	
Biotin		1.3 µ/g	
Cholin		40 µ/g	
Folic acid Vit. B12		5 - 13 µ/g	
Vit.B12		0.001µ/g	

Harvesting: plants were harvested at flowering stage twice each season. The first harvest was done in the middle July and the second in the third week of October in the two seasons.

The following data were recorded per plant:

A. Vegetative growth characters:

1. Plant height (cm).
2. Number of branches/plant.
3. Leaf area (cm²)/leaf.
4. Fresh and dry weights of herb (g/plant).
5. Dry yield per fed (ton).

B. Chemical analysis:

1. Chlorophyll a, b and total mg/g f.w : were determined by using the method described by Moran (1982).

2. N, P and K%:

Nitrogen percentage was determined according to microkjeldhal method (Hach *et al*, 1985), phosphorus percentage was estimated according to A.O.A.C., (1970) and potassium percentage was determined by flame photometer(Brown and Lilleland, 1946).

3. Essential oil determination

3.1. Essential oil %

Essential oil percentage was estimated according to British Pharmacopoeia (1963).

3.2. Essential oil yield

(a): Oil yield / plant (ml)

(b): Oil yield/fed (l)

3.3. Essential oil components:

GC/Mass analysis of volatile oil during the second cut of the second season was performed with specification of the apparatus used according to Robert, (1995).

Data of both seasons were tabulated and statistically analyzed according to procedure described by Steel and Torrie (1980) and differences between the means were compared by Duncan's Multiple Range Test (Snedecor and Cochran, 1980) using COSTAT computer program.

RESULTS AND DISCUSSION

A. Vegetative growth characters:

The response of vegetative growth of *Melissa officinalis* ,L. to planting spaces and /or application of dry yeast is shown in Tables (1&2) during 2008/2009 and 2009/2010 seasons.

(1) Plant height, number of branches/plant and leaf area:

Effect of planting space: Data recorded in Table (1) indicated that raising plant spaces from 30 to 50 cm significantly increased plant height, number of branches/plant and leaf area this was true in both cuts for both seasons without significant differences between 40 and 50 cm in plant height These results may be attributed to the favorable conditions of growth such suitable amount of light and nutrients which may be more available in wide space.

Table (1): Effect of planting spaces and active dry yeast on plant height (cm), number of branches/plant and leaf area (cm²)/leaf of *Melissa officinalis* ,L herb during two seasons (2008/2009 and 2009/2010).

Treatment	Plant height (cm)				Number of branches/plant				Leaf area (cm ²)/leaf				
	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	
	1 st Season		2 nd Season		1 st Season		2 nd Season		1 st Season		2 nd Season		
	1 st Season		2 nd Season		1 st Season		2 nd Season		1 st Season		2 nd Season		
Spacing (cm)	30	60.75b	47.25b	50.75b	39.58c	13.08b	9.58c	29.67c	21.83c	8.68b	7.70a	10.19c	8.01b
	40	69.42a	53.08a	61.83a	46.42b	18.08a	12.50b	34.92b	24.83b	8.34c	7.02b	11.69b	8.84a
	50	69.75a	54.83a	60.42a	47.67a	18.50 a	13.92a	41.42a	31.17a	10.23a	7.87a	12.43a	9.57a
Yeast fertilizer	Control	63.22b	49.78b	57.44ab	43.56b	14.22c	9.89c	37.56b	23.89b	7.23d	5.61d	8.32d	6.35c
	5g/l	69.00a	50.89b	56.22b	44.00b	17.66b	12.56b	31.67c	23.33b	8.97c	6.81c	11.10c	9.22b
	10g/l	67.33a	49.56b	57.44ab	41.78c	20.22a	14.67a	41.56a	28.67a	9.58b	8.18b	12.92b	9.55ab
	15g/l	67.00a	56.67a	59.56a	48.89a	14.11c	10.89c	30.56c	27.89a	10.55a	9.53a	13.40a	10.12a
Spaces x yeast fertilizer	30 x cont	54.33f	43.67f	41.00g	38.00e	6.33g	4.67f	21.00g	17.00f	8.09d	4.82g	6.41f	5.47e
	30 x 5g/l	71.00bc	48.00e	49.33f	44.00d	22.00b	16.67ab	30.66ef	19.00f	7.38e	7.81d	10.57d	9.36b
	30 x 10g/l	63.33de	45.00f	54.00e	33.67f	16.33de	12.00de	41.33cd	31.33ab	9.21c	8.75b	12.77c	7.87c
	30 x 15g/l	54.33f	52.33cd	58.67d	42.67d	7.66g	5.00f	25.67fg	20.00ef	10.03b	9.44a	10.99d	9.34b
	40 x cont	66.67cd	55.67b	68.00a	47.33bc	16.00e	10.00e	29.33ef	20.33ef	7.18e	5.29f	7.73e	7.17cd
	40 x 5g/l	75.00ab	44.67f	60.33cd	38.00e	10.33f	6.33f	32.67e	26.67cd	6.78ef	5.58f	12.45c	7.37cd
	40 x 10g/l	68.67c	53.67bc	66.67ab	48.66b	27.00a	18.00a	30.33ef	19.00f	10.29b	7.58d	13.78b	10.74a
	40 x 15g/l	67.33cd	58.67a	52.33ef	51.67a	19.00cd	15.67bc	47.33b	33.33ab	9.10c	9.64a	12.79c	10.10ab
	50 x cont	68.67c	50.00de	63.33bc	45.33cd	20.33bc	15.00bc	44.67bc	34.33ab	6.41f	6.72e	10.84d	6.42 de
	50 x 5g/l	61.00e	60.00a	59.00d	50.00ab	20.67bc	14.67bc	28.33ef	24.33de	12.76a	7.05e	10.27d	10.92a
50 x 10g/l	70.00c	50.33de	51.67ef	43.00d	17.33de	14.00cd	53.00a	35.67a	9.25c	8.22c	12.20c	10.03ab	
50 x 15g/l	79.33a	59.00a	67.67a	52.33a	15.66e	12.00de	39.67d	30.33bc	12.51e	9.52a	16.43a	10.91a	

Means having the same letter in the column are not significantly different at 5% level according to Duncan's Multiple Range Test.

These results are similar to those obtained by HeidariZolleh *et al* (2009) on cumin and Moosavi,*et al*(2012) on *Plantago ovata* who indicated that with the increase in plant spaces from 16.6 to 33.3 plants/m², plant height was increased significantly but leaf number per plant, spike length and tiller number per plant were decreased.

Effect of dry yeast: Adding dry yeast at the rates of 5, 10 and 15g/l increased plant height, number of branches/plant and leaf area in both cuts for the two seasons of lemon balm plants compared with control treatment. Increasing the dose of dry yeast from 5 to 15g/l showed significant differences in the mean values and beneficial effect on plant height and leaf area while increasing it to 10 g/ l increased number of branches/plant in both cuts for the two seasons. Stimulating vegetative growth by using dry yeast may be due to its influence on the nutritional signal transduction producing growth regulators and suppressing pathogens (El-Ghadban,2003). It is also a natural source of cytokinins that stimulate cell proliferation and differentiation, controlling shoot and root morphogenesis and chloroplast maturation (Amer, 2004). El-Tohamy and El-Greadly , 2007 revealed that the dry yeast treatments (5 and 10 g/ l.) gave an improving in pods quality of snap beans plants (*Phaseouls vulgaris*).

Interaction effect: Taking into consideration the effect of both studied factors, results showed that the interaction between plant spaces and application of dry yeast augmented growth parameters as compared with the control. The maximum mean values of plant height and leaf area were recorded with applying 15g/ l of dry yeast at 50cm distance for both cuts in both seasons. The same trend was observed when applying the 10g/ l of dry yeast and 40cm distance in the first season and 10g/ l of dry yeast at 50cm distance in the second season for number of branches. These results are in agreement with those obtained by Yuonis *et al* (2004) on *Ammi visnaga* , L who showed that using 25m³/fed FYM with increasing planting spaces up to 30 cm apart significantly increased vegetative growth and Azizi and Kahrizi (2008)obtained a similar results on cumin .

2. Fresh and dry weights/plant and dry yield per fed:

Effect of planting space : According to Table (2) plants with spacing of 50cm had the highest fresh and dry weights (g/plant) and dry yield /fed (ton) without significant differences among 40cm distance in most cases in the two cuts of both seasons. The maximum dry yield /fed reached to 2.73 ton/fed under 40 cm distances in the first cut in the first season. The promotive effect of the widest distance on fresh and dry weights (g/plant) and dry yield /fed may be due to increment in the amount of nutrients uptake or/and getting more quantity from solar energy for plant. Similarly, different authors found the promoting effect of wider plant spacing on vegetative growth characters such as Ghaly *et al* (2003) on *Carum carvi*, L , Sadeghi *et al* (2009) on black cumin and Mohammad and Mohammad (2012) on Iranian borago (*Echium amoenum*_Fisch and Mey):

Effect of dry yeast: Table (2) clearly showed that dry yeast treatments significantly increased fresh and dry weights (g/plant) and dry yield /fed of lemon balm comparing with the control .The addition of 10 g/l gave the highest value of these parameters in the two cuts of both seasons.

Table (2): Effect of planting spaces and active dry yeast on fresh and dry weights (g/plant) and dry yield /fed (ton) of *Melissa officinalis* ,L herb during two seasons (2008/2009 and 2009/2010).

Treatment		Fresh weight (g/plant)				Dry weight (g/plant)				Dry yield /fed(ton)			
		1 st cut		2 nd cut		1 st cut		2 nd cut		1 st cut		2 nd cut	
		1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season
Spacing (cm)	30	282.69b	206.15b	483.75b	371.73b	95.14b	105.03c	105.03c	78.64c	2.11a	2.33a	1.31c	1.75a
	40	567.02a	383.24a	638.97a	442.63a	163.81a	136.61b	136.61b	96.65b	2.73a	2.28a	2.04a	1.62b
	50	548.48a	398.87a	713.01a	467.30a	184.65a	156.19a	156.19a	111.23a	2.46a	2.08b	1.74b	1.48b
Yeast fertilizer	Control	409.24c	309.83b	526.48c	390.77c	144.46ab	133.75a	133.75b	99.44b	2.27b	1.90c	1.56b	1.39d
	5g/l	492.81c	368.66a	662.38b	438.23b	137.98b	125.49b	125.49b	90.85c	2.51ab	2.08c	1.48b	1.53c
	10g/l	548.64a	377.15a	752.72a	522.80a	160.84a	158.92a	158.92a	110.12a	2.72a	2.69a	1.90a	1.85a
	15g/l	413.57c	262.05c	506.07c	357.07c	148.18ab	112.28c	125.50c	81.61d	2.25b	2.26b	1.84a	1.69b
Spacing x yeast fertilizer	30 xcont	174.08g	115.76f	382.22d	356.13de	63.80e	39.57f	86.58e	71.25cd	1.36e	1.97def	0.86h	1.44e
	30 x 5g/l	406.6ef	277.27de	585.10bc	343.86e	146.81c	76.54de	102.70de	82.36cd	2.41cd	2.28cd	1.27fg	1.83cd
	30 x 10g/l	330.18f	275.23de	591.64bc	435.79bc	61.42e	61.95e	142.21b	95.99c	3.26a	3.16a	1.70cde	2.13ab
	30 x 15g/l	219.9g	156.35f	376.05d	351.12e	108.55d	56.93ef	88.83e	64.98e	1.42e	1.92ef	1.38efg	1.58e
	40 xcont	482.37de	357.95c	604.00bc	467.62b	198.99b	106.07bc	201.61a	137.25ab	2.36cd	2.03def	1.77cd	1.58e
	40 x 5g/l	379.72f	503.49b	746.21b	501.26b	126.87cd	105.46bc	89.86e	64.35e	3.13ab	1.50g	2.01bc	1.07f
	40 x 10g/l	725.92a	301.18de	618.72bc	426.80bcd	141.77c	156.56a	133.46bc	90.44c	2.11d	2.22cde	1.76cd	1.51e
	40 x 15g/l	680.06ab	370.34c	586.95bc	374.83cde	167.60b	120.73b	121.52bcd	94.58c	3.32a	3.36a	2.61a	2.29a
	50 x cont	571.27cd	455.77b	593.23bc	348.55e	151.17c	151.75a	113.06cd	89.82c	3.07ab	1.69fg	2.02bc	1.20f
	50 x 5g/l	692.09a	325.22cd	655.83bc	469.57b	208.87ab	167.52a	183.91a	125.84b	1.98d	2.45bc	1.17gh	1.68de
	50 x 10g/l	589.81bc	555.04a	1047.78a	705.82a	230.17a	114.91b	201.31a	143.91a	2.78bc	2.68b	2.23b	1.92bc
50 x 15g/l	340.76f	259.45e	555.20c	345.25e	148.40c	87.82cd	126.48bc	85.28cd	2.02d	1.51g	1.53def	1.14f	

Means having the same letter in the column are not significantly different at 5% level according to Duncan's Multiple Range Test.

The maximum dry yield /fed reached to 2.72 ton /fed under 10 g/ l in the first cut in the first season. Similarly, Ezz El-Din and Hendawy (2010) on *Borago officinalis* plant reported that adding dry yeast at the rate of 6g/ l. was the most effective on growth parameters.

Interaction effect: Regarding the data of interaction between different planting distance and different dry yeast application, the obtained data illustrated that the maximum fresh and dry weight (g/plant) were obtained when plants were grown under the widest distance of 50cm and treated with 10g/ l dry yeast in most cases in the two cuts of both seasons. Meanwhile, the highest dry yield /fed was recorded from increasing the dose of active dry yeast to 15g/ l and 40cm distance between plants in the two cuts in both seasons, which was progressively increased to 3.36ton /fed in the second cut of the first season. The beneficial effect of widest distance and yeast application on herb dry matter may be due to both supply nutrients and microbial functions (as useful microorganisms increase the time of stomata stay open, then reducing loss from the leaf surface). It can provide chelated microelements and make them easier for plants to absorb and increasing soil aeration and acidity. These results were coincided with those of Hussein *et al* (2006) on dragonhead as it can be deduced that the maximum rate of compost (39.6 t/ha) combined with wider distance between plants (40 cm) had a favorable effect on most of growth characters.

B. Chemical analysis:

1. Chlorophyll a, b and total :

Effect of plant spacing: Data recorded in Table (3) show that various in planting spacing significantly affected on chlorophyll (a) ,(b) and total (mg/g f.w) in both cuts for each season . The spacing of 40cm gave the highest mean values of photosynthetic pigments chlorophyll(a) ,(b) and total (mg/g f.w)in both cuts for the two seasons. The promotive effect of wider distance on photosynthetic pigments was reported by El-Sherbeny *et al.* (2005) on *Sideritis montana* and Hussein *et al* (2006)on dragonhead plant .

Effect of dry yeast: Results presented in Tables (3) revealed that application of active dry yeast significantly improved chlorophyll a ,b and total(mg/g f.w) compared with control treatment in both cuts for the two seasons. Increasing applying of dry yeast to 10 g/ l gave the maximum values of chlorophyll a, b and total in both cuts for each season. The enhancing effect of dry yeast may be due to that yeast is a source of cytokinins , delaying the degradation of chlorophyll via the inhibition of chlorophyllase and enhancing the synthesis of protein and RNA that are closely related with delaying the aging of leaves . Our results were fortified by those of Seleim (2005) on spearmint and sage plants who found that all used dry yeast treatments significantly increased leaf chlorophyll a and b of the two plants . Similar results were obtained by Khalil and Ismael(2010) on *Lupinus termis* and Shahram *et al* (2011) on lemon balm

Interaction effect: The combination effect between plant spacing and different concentrations of dry yeast fertilizer is presented in Table (3) which show that plant spacing at different distances and fertilized by dry yeast at various levels had a promotive effect on photosynthetic pigments compared with those unfertilized in both cuts for both seasons. However, the highest

mean values of chlorophyll a, b and total were recorded with wider plant spacing 40 and/or 50 cm and increasing dry yeast level to 10g/ l . Applied 10g / l dry yeast and 40cm recorded the highest chl a in the two cuts in the second season and chl b in the two cuts in the two seasons. Meanwhile, applied 10gm/ l dry yeast and 50cm recorded the highest chl a in the two cuts in the first season and total chl in the two cuts for the two seasons. This might be reasonable since the improvement of growth characters is mainly a result of stimulation of photosynthetic apparatus that leads to more photosynthesis and food reserve. The beneficial effects of dry yeast on the accumulation of photosynthetic pigments were previously observed by El-Sherbeny et al. (2005) on *Sideritis Montana* plants and Hussein et al , 2006 on *Dracocephalum moldavica* plants .

Table (3): Effect of planting spaces and active dry yeast on chlorophyll (a, b and total) mg/g fresh weight of *Melissa officinalis* , L herb during the two seasons (2008/2009 and 2009/2010).

Treatment		Chlorophyll (a) mg/g f.w				Chlorophyll (b) mg/g f.w				Total chlorophyll mg/g f.w			
		1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut
		1 st Season		2 nd Season		1 st Season		2 nd Season		1 st Season		2 nd Season	
Spacing (cm)	30	3.36b	3.18b	2.84b	2.57b	1.97b	1.40c	1.07b	0.49b	7.44b	6.68b	5.70c	5.02b
	40	3.59a	3.24a	3.01a	2.59a	2.11a	1.50b	1.25a	0.57a	7.56a	6.84a	6.08a	5.15a
	50	3.33c	3.18b	2.44c	2.56c	1.92c	1.60a	0.91c	0.45b	7.28c	6.57c	5.75b	5.02b
yeast fertilizer	Cont	3.57b	3.35b	2.68b	2.58b	1.55d	1.39c	0.88c	0.47b	7.19c	6.74b	5.94b	5.07a
	5g/ l	3.29c	2.96d	2.62c	2.58b	1.72c	1.83a	0.69d	0.53a	7.01d	6.41c	5.31c	5.10a
	10g/ l	3.63a	3.47a	2.70b	2.60a	2.55a	1.45b	1.50a	0.52a	8.12a	7.30a	6.18a	5.10a
	15g/ l	3.21d	3.02c	3.05a	2.53c	2.18b	1.32d	1.24b	0.48b	7.39b	6.34d	5.95b	5.02b
Spacing x yeast fertilizer	30 x cont	3.03i	3.65b	3.02d	2.58b	0.86j	1.84c	0.97f	0.60c	6.60g	5.76j	6.57c	5.18c
	30 x 5g/ l	3.42h	2.91i	2.61h	2.57bc	2.29e	1.99b	0.71h	0.40fg	7.06f	7.19e	5.46h	4.95f
	30 x 10g/ l	3.52d	3.61c	2.80f	2.58b	2.25e	0.78j	1.02e	0.38fg	7.14f	7.57b	5.58g	4.98d
	30 x 15g/ l	3.46g	2.52j	2.92e	2.52e	2.49d	0.99i	1.59b	0.57cd	8.31c	6.19i	5.17j	5.09d
	40 x cont	3.84b	3.42e	2.92e	2.59b	2.71c	0.77j	1.47c	0.46e	6.39h	6.97f	6.10d	5.09d
	40 x 5g/ l	3.48f	3.05f	2.70g	2.59b	1.24h	1.80d	0.91g	0.65b	7.77e	6.34h	5.40i	5.28a
	40 x 10g/ l	3.52d	3.03g	3.29a	2.63a	3.30a	2.27a	1.48c	0.70a	8.08d	6.73g	5.94e	5.23b
	40 x 15g/ l	3.51e	3.47d	3.14b	2.53de	1.21h	1.14h	1.16d	0.48e	8.00d	7.32d	6.88b	5.01d
	50 x cont	3.83c	2.99h	2.10j	2.57bc	1.08i	1.55f	0.22j	0.36g	8.57b	7.49c	5.14j	4.95ef
	50 x 5g/ l	2.98j	2.92i	2.55i	2.59b	1.63g	1.70e	0.45i	0.54d	6.22i	5.69k	5.06k	5.07d
	50 x 10g/ l	3.86a	3.77a	2.01k	2.59b	2.11f	1.29g	2.01a	0.49e	9.14a	7.60a	7.03a	5.11d
	50 x 15g/ l	2.65k	3.05f	2.10c	2.55cd	2.85b	1.85c	0.99ef	0.41f	5.85j	5.51l	5.78f	4.96ef

Means having the same letter in the column are not significantly different at 5% level according to Duncan's Multiple Range Test.

2. N, P and K %:

Effect of plant spacing: Data in Table (4) revealed that nitrogen, phosphorus and potassium % of lemon balm herb were significantly increased by increasing plant spacing so as to reach their maximum records under the highest of 50cm distance in the two cuts for both seasons . The increase in N,P and K % may attributed to the less competition between plants for light ,minerals ,aeration and water which reflected on more accumulation of minerals (N,P and K)in widest spacing .These results were similarly documented by many résearches done in this field e.g Yuonis et al (2004) on *Ammi visnaga* ,L and Nejad (2011) on cummin.

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Table (4): Effect of planting spaces and active dry yeast on N, P and K% of *Melissa officinalis*, L herb during the two seasons (2008/2009 and 2009/2010).

Treatment		N%				P%				K%			
		1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut
		1 st Season		2 nd Season		1 st Season		2 nd Season		1 st Season		2 nd Season	
Spacing (cm)	30	2.47b	2.62c	1.84b	2.38c	0.35b	0.60c	0.30a	0.50b	1.63b	1.89b	1.37b	1.56b
	40	2.53b	2.67b	1.86b	2.47b	0.36a	0.64b	0.28b	0.49c	1.67a	1.58c	1.22c	1.37c
	50	2.64a	2.87a	2.29a	2.57a	0.36a	0.74a	0.31a	0.62a	1.63b	1.96a	1.44a	1.59a
Yeast fertilizer	Cont	2.47b	2.50c	2.10b	2.15c	0.36b	0.50d	0.30a	0.42d	1.57c	1.50d	1.30b	1.21d
	5g/l	2.57a	2.72b	2.08b	2.50b	0.34c	0.59c	0.29b	0.57b	1.61b	1.73c	1.35a	1.46c
	10g/l	2.59a	2.96a	1.69c	2.76a	0.36b	0.72b	0.30a	0.63a	1.70a	1.83b	1.34ab	1.56b
	15g/l	2.56a	2.70b	2.14a	2.47b	0.38a	0.77a	0.285c	0.52c	1.68a	2.19a	1.37a	1.80a
Spaces x yeast fertilizer	30 x cont	2.47cde	2.30i	2.00e	2.00f	0.356bc	0.50f	0.296cd	0.44e	1.56f	1.41g	1.48b	1.12h
	30 x 5g/l	2.51c	2.89d	2.06de	2.70b	0.32e	0.51e	0.30cd	0.50d	1.55fg	1.54e	1.37c	1.44e
	30 x 10g/l	2.38de	2.52h	1.49g	2.30e	0.346cd	0.62d	0.316b	0.55d	1.81c	1.68d	1.20d	2.00b
	30 x 15g/l	2.49c	2.79e	1.82f	2.51c	0.386a	0.64c	0.29d	0.50d	1.58f	2.94a	1.41bc	1.68c
	40 x cont	2.48cd	2.23j	2.06de	2.00f	0.38a	0.50f	0.30cd	0.40g	1.65e	1.54e	1.07e	1.40g
	40 x 5g/l	2.63b	2.75e	2.06de	2.52c	0.36b	0.64c	0.28e	0.50d	1.69d	1.68d	1.46b	1.55d
	40 x 10g/l	2.64b	3.08b	1.08h	2.97a	0.35bcd	0.64c	0.26e	0.50 d	1.87b	1.42g	1.08e	1.12h
	40 x 15g/l	2.37e	2.61g	2.26c	2.40d	0.356bc	0.77b	0.27e	0.55c	1.45h	1.68d	1.25d	1.42f
	50 x cont	2.45cde	2.98c	2.23c	2.45d	0.35bcd	0.50f	0.31bc	0.42f	1.51g	1.54e	1.33c	1.12h
	50 x 5g/l	2.55bc	2.52h	2.11d	2.30e	0.34d	0.64c	0.303bcd	0.71b	1.58f	1.96c	1.23d	1.40g
50 x 10g/l	2.74a	3.27a	2.48a	3.00a	0.38a	0.91a	0.33a	0.83a	1.43h	2.38a	1.74a	1.55d	
50 x 15g/l	2.81a	2.70f	2.33b	2.51c	0.38a	0.91a	0.30cd	0.50d	2.00a	1.96c	1.46b	2.30a	

Means having the same letter in the column are not significantly different at 5% level according to Duncan's Multiple Range Test.

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Effect of dry yeast: Application of active dry yeast in different levels resulted in significant increase in nitrogen, phosphorus and potassium % as compared with control treatment. The maximum values were obtained from plants treated with 10 and 15 g/ l in most cases. Applying 10 g/ l gave the highest value of N% in the two cuts for both seasons and the highest P% in the two cuts for the second season. Meanwhile, 15g/ l gave the maximum value of P% in the two cuts for the first season and the highest value of K% in the two cuts for both seasons compared with control treatment. These results may be due to that yeast applications stimulate the synthesis of protein (Stino, 2009).

Interaction Effect: The interaction between different plant spaces level and different rates of active dry yeast had significant effect on nitrogen, phosphorus and potassium %. The widest plant spaces (50cm) combined with (10g/ l) yeast without significant difference with 50cm and 15g/ l yeast in some cases gave the highest significant value of N,P and K% in the two cuts for both seasons. These results may be due to the positive effect of yeast treatment under widest spaces conditions that yeast provided plants with essential nutrients elements required for protein formation. Similar results obtained by Yuonis *et al* (2004) indicated that nitrogen, phosphorus and potassium content of the aerial parts of plant significantly increased by increasing the rate of organic manure and planting spaces.

3. Oil %, Oil yield/plant and /fed

Effect of plant spacing: The results in Table (5) showed that oil %, oil yield/plant and /fed were highly significant increased with increasing plant spacing. The highest means were observed under 50cm distance in the two cuts for both seasons. Wider spaces offer ample quantity of nutrients, light and other factors which in turn reflected on the biomass formation, plant height and essential oil content. On the other hand, when calculating the yield in a unit area, the narrower spaces will contain high number of plants and consequently more yields will be obtained. Thus, the heaviest plants obtained in the wide spaces in the same unit area may reach the yield obtained in the narrower distance noticed. In the present study a positive relationship between increasing plant spacing and oil percentage and oil yield / fed was found; similar findings were reported by Shalaby *et al* (1993) on lemon balm, Azizi and kahrizi (2008) on cumin, Berimavandi *et al* (2011) on *Calendula officinalis* L.

Effect of dry yeast: The different rates of active dry yeast induced significant effect on oil %, oil yield/plant and /fed as compared with the control. Also, data indicated that there was a gradual decrease in oil %, oil yield/plant and /fed with decreasing rates of active dry yeast applied. However, application of 10 and 15 g/ l yeast recorded the highest significant values in oil %, oil yield/plant and /fed in most cases in both cuts for both seasons. These results were in harmony with that reported by El-Ghadban *et al* (2008) on lavender

Interaction effect: As for the effect of combined treatments of planting distance and adding dry yeast, the maximum mean value of essential oil % was observed as a result of the interaction between widest space (50cm) and 15g/ l yeast in the two cuts during the two seasons.

Table (5): Effect of planting spaces and active dry yeast on essential oil %, oil yield/plant and /fed of *Melissa officinalis* ,L herb during two seasons (2008/2009 and 2009/2010).

Treatment		Oil %				Oil yield/plant (ml)				Oil yield/fed (l)			
		1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut
		1 st Season		2 nd Season		1 st Season		2 nd Season		1 st Season		2 nd Season	
		1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut
Spacing (cm)	30	0.45c	0.22c	0.42b	0.30c	0.48b	0.23c	0.53b	0.24c	10.76a	5.05c	5.41c	5.26a
	40	0.51b	0.26b	0.42b	0.33b	0.81a	0.34b	0.24c	0.32b	10.90a	5.73b	7.15b	5.30a
	50	0.52a	0.37a	0.48a	0.36a	0.84a	0.58a	0.59a	0.40a	14.01a	7.70a	9.88a	5.35a
Yeast fertilizer	cont	0.41d	0.20d	0.40d	0.29d	0.60b	0.26d	0.40b	0.28c	9.43b	4.40d	6.23b	4.81c
	5g/l	0.46c	0.23c	0.41c	0.31c	0.73a	0.30c	0.48a	0.28c	12.52a	4.95c	7.82a	4.72c
	10g/l	0.53b	0.31b	0.44b	0.34b	0.74a	0.52a	0.50a	0.38a	12.48a	8.36a	8.33a	6.30a
	15g/l	0.55a	0.37a	0.49a	0.39a	0.77a	0.44b	0.44a	0.33b	13.14a	6.94b	7.55a	5.39c
Spacing x yeast fertilizer	30 x cont	0.42h	0.20g	0.40f	0.30e	0.24f	0.17f	0.60c	0.21ef	5.46g	3.85ef	3.52i	4.75de
	30 x 5g/l	0.43g	0.24e	0.40f	0.31d	0.67cd	0.25e	0.67bc	0.26de	15.01bc	5.48d	6.80defg	5.74bc
	30 x 10g/l	0.52d	0.22f	0.42e	0.30e	0.40e	0.31d	0.48d	0.29d	9.07f	6.94cd	5.51gh	6.40b
	30 x 15g/l	0.42h	0.20g	0.44d	0.29f	0.60d	0.18f	0.38ef	0.19f	13.51bcd	3.95e	5.82fgh	4.14ef
	40 x cont	0.42h	0.18h	0.40f	0.27g	0.96b	0.36d	0.15h	0.36c	12.89cd	6.05cd	8.09cd	6.08b
	40 x 5g/l	0.50e	0.20g	0.40f	0.28f	0.89b	0.18f	0.30fg	0.18f	11.98de	2.99f	8.93c	3.04g
	40 x 10g/l	0.50e	0.26d	0.40f	0.22d	0.63d	0.35d	0.24g	0.29d	8.46f	5.78d	6.44efgh	4.83de
	40 x 15g/l	0.60b	0.40c	0.46c	0.46a	0.77c	0.49c	0.26g	0.43b	10.29ef	8.10b	5.15h	7.25a
	50 x cont	0.40i	0.22f	0.40f	0.30e	0.59d	0.25e	0.42de	0.27d	9.93ef	3.32ef	7.07def	3.59fg
	50 x 5g/l	0.46f	0.26d	0.44d	0.32d	0.63d	0.48c	0.46de	0.40bc	10.57ef	6.38cd	7.74cde	5.37cd
	50 x 10g/l	0.56 c	0.46b	0.50b	0.40c	0.12a	0.93a	0.78a	0.57a	19.90a	12.34a	13.04a	7.68a
50 x 15g/l	0.64a	0.52a	0.58a	0.42b	0.93b	0.66b	0.70ab	0.36c	15.63b	8.77b	11.67b	4.77de	

Means having the same letter in the column are not significantly different at 5% level according to Duncan's Multiple Range Test.

However, oil yield/plant and /fed reached maximum values as a result of the combination treatment between the wider space (50 cm) and 10g/ l yeast in the two cuts during the two seasons. Moreover, it can be observed that oil yield/fed reached to 19.90 l/fed. The increment of essential oil yield may be due to the enhancement of mass production or/and essential oil (%).

4. Essential oil constituents:

The relative percentage of the main constituents of the essential oil of different treatments during the second cut and the second season are shown in (Table 6). Chemical composition of lemon balm oil is strongly affected by planting distance and active dry yeast. Eight compounds were identified in lemon balm oil as geranial (citral A), neral(citral B), camphene,β-Pinene ,myrcene ,linalool ,geranyl acetate and β-caryophyllene .For the combination between the narrow (30 cm) or medium distance (40 cm) treating with 10 or 15 g/ l dry yeast, geranial (citral A) was found to be the major compound followed by camphene as comparing with the control , β-caryophyllene was the major compound followed by geranyl acetate. On the other hand, the combination between the wider distance (50 cm) and treating with 10 or 15 g/ l dry yeast, neral(citral B) was found to be the major compound followed by geranial (citral A). In this connection, Shahram *et al* (2011) reported that the essential oil content of lemon balm was geraniol , citronellol ,citronellal ,linalol, eugenol acetate and nerol. Generally, it could be observed that there was a positive correlation between total hydrocarbon compounds percentage and planting distance. The inconsistent trend for the effect of various dry yeast levels and different plants distances on essential oil constituents of lemon balm plants was also reported by Hussein *et al* , 2006 on *Dracocephalu. moldavica* plants .

Table (6): Effect of planting spaces and active dry yeast on essential oil constituents of *Melissa officinalis* ,L herb during the second cut of the second season.

Components	30cm			40cm			50cm		
	Control	10g/ l	15g/ l	Control	10g/ l	15g/ l	Control	10g/ l	15g/ l
Camphene	19.90	21.77	28.145	1.007	24.68	28.778	2.165	9.57	5.98
β-Pinene	-	3.16	0.202	0.866	2.97	4.611	25.739	1.92	0.69
Myrcene	1.57	-	0.242	3.499	0.51	0.636	57.392	-	1.79
Linalool	-	-	3.440	0.589	1.97	0.243	0.456	1.04	1.13
Neral(citral B)	2.09	8.52	0.253	31.736	7.49	0.007	2.396	51.86	53.95
Geranial (citral A)	7.45	57.98	7.148	53.190	41.19	51.764	0.497	29.73	32.24
Geranyl acetate	27.72	5.72	0.170	3.646	3.08	0.187	6.939	4.01	3.19
β-caryophyllene	37.29	0.69	0.422	2.489	0.91	8.241	-	0.75	-
Total identified	96.02	97.84	90.022	97.022	82.80	94.467	95.584	98.88	98.97
Unidentified compound	3.98	2.16	9.978	2.978	17.20	5.533	4.416	1.12	1.03

CONCLUSION

In conclusion, it could be concluded that under Kafr El-sheikh Governorate environmental condition, the application of dry yeast fertilizer at level of 10 g/ l to *Melissa officinalis* ,L cultivated at 40 cm distance between plants is recommended for good plant growth with highest chemical

constituents as well as more essential oil content, which lead to improving of the productivity of this plants.

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زراعة المليسا في منطقة شمال وسط الدلتا:
(ب) تأثير مسافة الزراعة والخميرة

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أجريت تجربتان حقليتان في مزرعة محطة البحوث الزراعية بسخا خلال موسمي النمو ٢٠٠٨/٢٠٠٩ ، ٢٠٠٩ / ٢٠١٠ للدراسة تأثير مسافات الزراعة والخميرة على النمو والمحصول ومحتوى الزيت لنبات المليسا. كانت مسافات الزراعة (٣٠ و ٤٠ و ٥٠ سم) وكانت معاملات الخميرة (الكنترول و ١٠ و ١٥ جم/لتر) إضافة الى التربة. وقد أوضحت النتائج أن الزراعة على مسافة واسعة كانت الأفضل تأثيرا على قياسات النمو والمكونات الكيميائية . وقد شجعت معدلات الخميرة معظم قياسات النمو الخضري وزيادة نسبة الزيت والمكونات الكيميائية مثل مكونات النبات من كلوروفيل أ وب والكلوروفيل الكلي ونسبة النيتروجين والفسفور والبوتاسيوم. وكانت الزراعة على مسافة واسعة ٤٠ او ٥٠ سم بين النباتات مع إضافة ١٠ او ١٥ جم /لتر خميرة افضل تأثيرا على معظم صفات النمو والمكونات الكيميائية . كان المكون الرئيسي للزيت هو الجيرانيال (السترال) يلية الكامفن . عامة يتضح انه لا يوجد فرق معنوي بين الزراعة على مسافة واسعة ٥٠ و متوسطة ٤٠ سم وبين إضافة ١٠ و ١٥ جم /لتر خميرة لذلك يمكن ان نوصي بالزراعة على مسافة متوسطة ٤٠ سم مع إضافة ١٠ جم /لتر خميرة في منطقة شمال وسط الدلتا .

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