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Effect of planting density and biofertilization on growth and productivity of *Cymbopogon citratus* (DC.) Stapf. (Lemongrass) plant under Siwa Oasis conditions

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Abstract
This experiment was conducted in north western desert of Egypt in Siwa Oasis region to study the effect of planting density and biofertilization treatments on productivity and quality of lemongrass (*Cymbopogon citratus*) plants in two seasons of 2013/2014 and 2014/2015. The experiment was installed in split plot design. Cultivation was done at two planting densities:- planting in rows 50 cm apart (16800 plants/feddan) and in rows 75 cm apart (11200 plants/feddan). The used biofertilizer was a mixture of *Azotobacter chroococcum*, *Bacillus megaterium* and *Saccharomyces cerevisiae*. The interaction effects showed that cultivation at wider inter-row spacing with biofertilization increased herb biomass and oil yield per plant while cultivation at narrow inter-row spacing with biofertilization increased productivity per feddan. In most cases, the quality of produced essential oil was in agreement with the minimum ISO standard of citral content. The effect of agriculture practices on citral content was more obvious in first cut of the experiment. The extracted oil possessed a strong antimicrobial activity. Biofertilizers application increased the antagonistic activity of lemongrass oil against tested pathogenic microbes.

Keywords: Lemongrass, plant density, biofertilization, essential oil, citral content

Introduction
The Egyptian government recommended increase of the production of medicinal and aromatic plants in order to face the extending demands of the local markets and exportation. Cultivation of these plants extended now to the newly reclaimed lands in the desert for increasing production.

Siwa Oasis is a natural depression located in northern part of the Western Desert of Egypt (about 50 km east of the Libyan border and 300 km south of the Mediterranean Sea) in the Sahara desert. Its average depth is around 18 meters below sea level and covers an area of about 250,000 feddans of which 15,000 feddans are currently cultivated based on groundwater available from both of dug wells and natural flowing springs giving a total discharge of about 130 million m^3 /year [1- 4]. Climate in Siwa Oasis is arid to semiarid with a negligible rainfall, the monthly mean maximum temperature range from 20 °C in January to 38 °C in July, with a yearly average of approximately 30 °C. The monthly mean minimum temperature ranges from 4 °C in January to 21 °C in July. Absolute maximum temperatures can reach 50 °C while the absolute minimum temperature measured was 4.5 °C. Mean monthly relative humidity ranges from 30 to 58% [5- 6]. The Oasis is considered a new promising region in Egypt for cultivation and production of medicinal and aromatic plants with its environmental conditions favorable for the growth of these plants [7- 10].

One of the most important medicinal and aromatic plants is West Indian lemongrass (*Cymbopogon citratus* (DC.) Stapf, Family: Poaceae), notable for its lemon flavor and fragrance. It is used in cooking as a major source of lemon flavoring. Medically, it is anti-inflammatory, antidiabetic, analgesic, anthelmintic, antibacterial, antifungal, anticancer, antioxidant, antiplatelet, hepatoprotective, sedative and vasorelaxant. Citral is the major constituent of its essential oil. The oil is carminative, depressant, analgesic, antipyretic, antibacterial and antifungal [11- 14].

Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that actively colonize plant root and increase plant growth [15].

The mechanisms by which PGPR directly contribute to the plant growth are phytohormone production, enhancing plant nutrition by solubilization of minerals such as phosphorus and iron, production of siderophores and enzymes, lowering of ethylene levels and induction of systemic resistance, PGPR indirectly benefit the plant growth by the biocontrol of deleterious microorganisms or root pathogens that inhibit plant growth, including antibiotic and hydrogen cyanide production, parasitism, competition for nutrients and niches within the rhizosphere, synthesis of extracellular enzymes to hydrolyze the fungal cell wall and decreasing pollutant toxicity [16]. Medicinal plants are known to be rich in secondary metabolites and are potentially useful to produce natural drugs. Medicinal plants support a great diversity of microflora in their rhizosphere including PGPR. Biofertilizers application in medicinal plants production in sustainable agriculture with aim of quality increasing and sustainability of yield is very important [17].

Siwa Oasis is a virgin area in Egypt thereby, using of Good Agriculture Practices (GAP) is essential for agriculture production there i.e. choosing of suitable cultivation density and suitable fertilizer kinds. Until now, very little information is available on the agronomic practices of lemongrass plants under the ecosystem conditions of Siwa Oasis; therefore, the present study was carried out to investigate the effect of planting density and biofertilization treatments on productivity and quality of *Cymbopogon citratus* plants under Siwa Oasis conditions.

Materials and Methods

This work was implemented during the two successive seasons of 2013/2014 and 2014/2015 in semiarid region of the Agricultural Experimental Station of the Desert Research Center at Khamisa Village (29.21º N and 25.40º E), Siwa Oasis, Egypt. Lemongrass plants were propagated vegetatively through slips obtained by the splitting up of individual adult clumps. The rooted slips were cultivated on 20th of April during the two successive seasons of the experiment at spacing of 50 cm between hills in rows under drip irrigation system. The experiment was laid out in a split plot design with four replications. The main plots were consisted of two levels of planting density; D_1: planting in rows 50 cm apart (50x50 cm spacing = 16800 plants/feddan) and D_2: planting in rows 75 cm apart (75x 50 cm spacing = 11200 plants/feddan). The sub-plots included biofertilization treatments as without and with, respectively. Half dose of the recommended chemical fertilizers (150 kg calcium super phosphate, 200 kg ammonium sulphate and 50 kg potassium sulphate /feddan) was applied as follows:- calcium super phosphate and compost manure at10 m^3/feddan was conducted before planting in only one dose, nitrogen and potassium fertilizers were divided into two equal doses; the first was added after 45 and 90 days of planting date and the second was added after the first cut [18- 20].

Different soil samples were collected from different sites of Siwa Oasis for isolation of Azotobacter and phosphate dissolving bacteria (PDB). Purification trials were carried out and purified isolates were maintained for further study. Obtained isolates were examined for N_2 fixation according to modified Keldahl method [21] for Azotobacter isolates and Phosphate solubilization [22] for Bacillus isolates. The selected isolates were subjected to different biochemical tests for screening their activities toward production of phytohormones by [23] and enzymes [24] then identification of highly active isolates using 16S rRNA genes sequencing according to [25] at Sigma company for scientific services. Fresh liquid mixed culture of *Azotobacter chroococcum*, *Bacillus megaterium* and yeast (*Saccharomyces cerevisiae*) strains was used for plants inoculation at the rate of 10^8 colony forming unit (cfu/ml). The biofertilizer was added as a soil drench after 45, 90 and 135 days of planting date. The addition of biofertilizer was repeated after the first cut for one time. Lemongrass plants were harvested twice per season in November 20th and February 27th by cutting the vegetative parts of plants 15 cm above the soil surface.

The following data were recorded

A- Growth and yield characters

Plant height (cm), fresh weight of herb/plant (g), fresh weight of herb/feddan (kg), dry weight of herb/plant (g) and dry weight of herb/feddan (kg).

B- Chemical analyses

1. Determination of essential oil percentage **:**Essential oil percentage was determined in the air dried herb by hydrodistillation for 3 hours using a Clevenger type apparatus [26].
2. Determination of essential oil yield per plant (ml) as follows: Oil percentage x plant dry weight / 100
3. Determination of essential oil yield per feddan (L) as follows: Oil yield per plant x number of plants/feddan.
4. Determination of essential oil components: The essential oil samples of the second season were analyzed by using gas liquid chromatography apparatus GLC at the Central Laboratory of National Research Center, Giza, Egypt.

C- Microbiological determinations

1- Total microbial counts in rhizosphere soil: Rhizosphere soil samples were collected at 1st and 2nd cuts for both seasons and analyzed for total counts of microorganisms according to [27]. For counting and growing phosphate dissolving bacteria using Pikovskaya's agar medium (PVK) [22]. For counting and growing Azotobacters, modified Ashby's media [28], Yeast counts on yeast extract malt extract agar medium according to [29] and CO_2 evolution according to [30].

2- Antimicrobial activity of lemongrass essential oil: Antimicrobial activity of essential oils was detected against some pathogenic microorganisms namely: *Escherchia coli, Salmonella typhi, Staphylococcus aureus, Pseudomonas areuginosa, Fusarium oxysporum* and *Rhizoctonia solani.* The antimicrobial activity was determined by agar diffusion technique and measured according to [31].

The differences between means were assessed using the least significance difference (LSD) test according to [32]. Soil, water and compost manure analyses are shown in Tables (A, B, C and D).

Table A: The mechanical analysis of the experimental soil area.

Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Soil texture
0-30	92.91	5.21	1.88	Sandy

Table B: The chemical analysis of the experimental soil area.

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 C: The chemical analysis of irrigation water.

pH	E.C.	Soluble anions (meq/l)				Soluble cations (meq/l)			
	ppm	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
7.47	2422.94	-	2.80	20.12	14.73	8.27	6.07	22.37	0.94

Table D: The chemical analysis of 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

I- Growth and yield characters

The presented data in Tables (1, 2, 3, 4 and 5) show the effect of planting density, biofertilization and their interaction on growth and yield characters of plants. Concerning the effect of planting density, in both cuts of both seasons, increasing the distance within rows from 50 to 75 cm significantly increased plant height, fresh and dry weights of herb per plant but decreased fresh and dry weights of herb per feddan. Similar results were obtained by [33- 35] who reported that closer plant spacing resulted in higher herb yield per unit area while wider spacing gave higher herb yield per plant which might be attributed to the opportunity of wider-spaced plants to increase the synthesis of metabolites, resulting in growth of more stems and roots. As for the effect of biofertilization on growth and yield parameters, in both cuts of both seasons, inoculated plants significantly superpassed uninoculated ones in fresh and dry weights of herb per plant as well as per feddan. The increase in herb yield with biofertilization was in agreement with the results reported by [36]. Regarding the effect of interaction between planting density and biofertilization, in both cuts of both seasons, the lowest planting density (75x50cm=11200 plants/feddan) with biofertilization recorded significantly highest fresh and dry weights of herb per plant while, the highest planting density (50x50cm=16800 plants/feddan) with biofertilization recorded significantly highest fresh and dry weights of herb per feddan as compared to control treatment (50x50cm without biofertilization). The increment in growth and yield characters by applying the biofertilizer may be due to the effect of different microbial strains of the biofertilizer such as nitrogen fixing bacteria (*Azotobacter chroococcum*) which led to nitrogen fixation, synthesis of vitamins, amino acids, auxins and gibberellins which stimulate the plant growth. Also, phosphate solubilizing bacteria (*Bacillus megaterium*) which is effective on releasing P from inorganic and organic pools of total soil P through solubilizing and mineralization as well as the production of growth promoting substance. Moreover, bread yeast (*Saccharomyces cerevisiae*) causes various promotive effects on plants and it is a natural source of cytokinins, protein, amino acids, nucleic acid, vitamin-B and releases CO_2 [37- 43]. These results indicate the importance of using biofertilizers as a promising alternative to reduce the amounts of mineral fertilizers also support an effective tool for desert development and sustainable agriculture.

Table 1: Effect of planting density, biofertilization and their interaction on plant height (cm) of *Cymbopogon citratus* (DC.) Stapf. during the two successive seasons of 2013/2014 and 2014/2015.

Biofertilization / Density	First season						Second season					
	1st cut			2nd cut			1st cut			2nd cut		
	without	with	Mean	without	with	Mean	without	with	Mean	without	with	Mean
50 cm row spacing (16800 plants/fed.)	51.57	61.02	56.30	46.36	48.88	47.62	52.11	61.61	56.86	42.98	47.03	45.01
75 cm row spacing (11200 plants/fed.)	57.09	61.75	59.42	49.72	50.67	50.20	59.27	62.29	60.78	47.56	47.80	47.68
Mean	54.33	61.39		48.04	49.78		55.69	61.95		45.27	47.42	
L.S.D. at 0.05												
Density	0.90			2.37			2.04			NS		
Biofertilization	1.33			NS			2.44			NS		
Density x Biofertilization	1.89			NS			3.45			NS		

Table 2: Effect of planting density, biofertilization and their interaction on fresh weight of herb/plant (g) of *Cymbopogon citratus* (DC.) Stapf. During the two successive seasons of 2013/2014 and 2014/2015.

Biofertilization / Density	First season						Second season					
	1st cut			2nd cut			1st cut			2nd cut		
	without	with	Mean	without	with	Mean	without	with	Mean	without	with	Mean
50 cm row spacing (16800 plants/fed.)	63.15	79.66	71.41	57.23	69.34	63.29	67.30	85.97	76.64	53.41	62.37	57.89
75 cm row spacing (11200 plants/fed.)	91.28	105.28	98.28	76.84	87.28	82.06	100.05	110.46	105.26	71.17	80.42	75.80
Mean	77.22	92.47		67.04	78.31		83.68	98.22		62.29	71.40	
L.S.D. at 0.05												
Density	8.46			7.91			7.23			5.73		
Biofertilization	4.18			4.21			9.03			5.67		
Density x Biofertilization	5.92			5.95			12.77			8.03		

Table 3: Effect of planting density, biofertilization and their interaction on fresh weight of herb/feddan (kg) of *Cymbopogon citratus* (DC.) Stapf. During the two successive seasons of 2013/2014 and 2014/2015.

Biofertilization / Density	First season						Second season					
	1st cut			2nd cut			1st cut			2nd cut		
	without	with	Mean	without	with	Mean	without	with	Mean	without	with	Mean
50 cm row spacing (16800 plants/fed.)	1060.92	1338.29	1199.61	961.46	1164.91	1063.19	1130.64	1444.30	1287.47	897.29	1047.82	972.56
75 cm row spacing (11200 plants/fed.)	1022.34	1179.14	1100.74	860.61	977.54	919.08	1120.56	1237.15	1178.86	797.10	900.70	848.90
Mean	1041.63	1258.72		911.04	1071.23		1125.60	1340.73		847.20	974.26	
L.S.D. at 0.05												
Density	NS			92.40			88.40			64.80		
Biofertilization	45.52			27.60			98.38			62.20		
Density x Biofertilization	64.38			39.03			139.13			87.97		

Table 4: Effect of planting density, biofertilization and their interaction on dry weight of herb/plant (g) of *Cymbopogon citratus* (DC.) Stapf. during the two successive seasons of 2013/2014 and 2014/2015.

Biofertilization / Density	First season						Second season					
	1st cut			2nd cut			1st cut			2nd cut		
	without	with	Mean	without	with	Mean	without	with	Mean	without	with	Mean
50 cm row spacing (16800 plants/fed.)	26.47	31.57	29.02	19.22	21.43	20.33	28.23	34.01	31.12	16.16	19.88	18.02
75 cm row spacing (11200 plants/fed.)	36.21	42.39	39.30	24.01	27.27	25.64	39.69	45.48	42.59	19.40	23.34	21.37
Mean	31.34	36.98		21.62	24.35		33.96	39.75		17.78	21.61	
L.S.D. at 0.05												
Density	3.03			3.78			2.97			1.20		
Biofertilization	0.95			1.91			3.71			1.67		
Density x Biofertilization	1.34			2.70			5.25			2.36		

Table 5: Effect of planting density, biofertilization and their interaction on dry weight of herb/feddan (kg) of *Cymbopogon citratus* (DC.) Stapf. during the two successive seasons of 2013/2014 and 2014/2015.

Biofertilization / Density	First season						Second season					
	1st cut			2nd cut			1st cut			2nd cut		
	without	with	Mean	without	with	Mean	without	with	Mean	without	with	Mean
50 cm row spacing (16800 plants/fed.)	444.70	530.38	487.54	322.90	360.02	341.46	474.26	571.37	522.82	271.49	333.98	302.74
75 cm row spacing (11200 plants/fed.)	405.55	474.77	440.16	268.91	305.42	287.17	444.53	509.38	476.96	217.28	261.41	239.35
Mean	425.13	502.58		295.91	332.72		459.40	540.38		244.39	297.70	
L.S.D. at 0.05												
Density	39.12			50.55			3615			14.45		
Biofertilization	13.41			30.27			40.62			19.40		
Density x Biofertilization	18.97			36.13			57.45			27.44		

II- Chemical analyses

- **Essential oil percentage:** Data of the effect of planting density, biofertilization and their interaction on essential oil percentage is presented in Table (6). The average percent of essential oil in the air dried herb ranged between 2.01-2.10% in the first cut and 1.46-1.89% in the second one of the two seasons, respectively. Concerning the effect of planting density, the significantly maximum oil percentage was detected by the lowest planting density in the second cut while the differences were insignificant in the first cut in both seasons. In addition, results showed that biofertilization treatments did not exert significant effects on oil percentage in both cuts of the two seasons. The interaction between treatments revealed that the lowest planting density with biofertilization recorded highest essential oil percentage in the second cut of both seasons while the differences were non-significant in the first cut of both seasons. As shown in the abovementioned results, the different agronomic treatments had a slight effect on essential oil percentage of lemongrass plants.

Table 6: Effect of planting density, biofertilizatin and their interaction on essential oil percentage *of Cymbopogon citratus* (DC.) Stapf. during the two successive seasons of 2013/2014 and 2014/2015.

Biofertilization / Density	First season						Second season					
	1st cut			2nd cut			1st cut			2nd cut		
	without	with	Mean	without	with	Mean	without	with	Mean	without	with	Mean
50 cm row spacing (16800 plants/fed.)	2.03	2.10	2.07	1.54	1.57	1.56	2.01	2.06	2.04	1.46	1.49	1.48
75 cm row spacing (11200 plants/fed.)	2.13	2.07	2.10	1.85	1.89	1.87	2.02	2.03	2.03	1.75	1.81	1.78
Mean	2.08	2.09		1.70	1.73		2.02	2.05		1.61	1.65	
L.S.D. at 0.05												
Density	NS			0.07			NS			0.08		
Biofertilization	NS			NS			NS			NS		
Density x Biofertilization	NS			0.07			NS			0.23		

Essential oil yield per plant and per feddan

Data presented in Tables (7 and 8) represent the effect of planting density, biofertilizatin and their interaction on essential oil yield per plant and per feddan. The average essential oil yield produced per plant ranged between 0.54-0.92 ml / plant in the first cut and 0.24-0.52 ml / plant in the second one for both seasons. Also, the mean essential oil yield per feddan varied between 8.62-11.76 l/feddan in the first cut and 3.81-5.82 l/feddan in the second one for both seasons. With respect to the effect of planting density, in both seasons, the significantly highest oil yield per plant was obtained by wider spacing of 75 x 50 cm while significantly maximum oil yield per feddan was obtained by narrow spacing of 50 x 50 cm in the first cut. However, differences were non-significant between the two densities in the second cut. These results agreed with those stated by [33, 35, 44] who pointed out that closer plant spacing resulted in higher oil yield per unit area while wider spacing resulted in higher oil yield per plant. For the effect of biofertilization, in both cuts of both seasons, the treatments with inoculation produced the significantly highest oil yield per plant and per feddan while uninoculated plants produced lowest yield. The promotive effect of the biofertilizer on oil yield was in harmony with those found by [36] on lemongrass. As for the effect of interaction between treatments, in both cuts of both seasons, the lowest planting density with biofertilization recorded significantly the highest oil yield per plant while the highest planting density with biofertilization produced significantly the highest oil yield per feddan in comparison to control plants. The stimulatory effect of the treatment (cultivation at 50 cm within rows with biofertilization) on oil yield per feddan may be attributed to the increase in dry herb yield per feddan as mentioned before. Similar results were observed by [34]. Also, lemongrass plants growing in sandy soils have higher leaf oil yield [45].

Table 7: Effect of planting density, biofertilizatin and their interaction on essential oil yield / plant (ml) of *Cymbopogon citratus* (DC.) Stapf. during the two successive seasons of 2013/2014 and 2014/2015.

Biofertilization / Density	First season						Second season					
	1st cut			2nd cut			1st cut			2nd cut		
	without	with	Mean	without	with	Mean	without	with	Mean	without	with	Mean
50 cm row spacing (16800 plants/fed.)	0.54	0.66	0.60	0.30	0.34	0.32	0.57	0.70	0.64	0.24	0.30	0.27
75 cm row spacing (11200 plants/fed.)	0.77	0.88	0.83	0.44	0.52	0.48	0.80	0.92	0.86	0.34	0.42	0.38
Mean	0.66	0.77		0.37	0.43		0.69	0.81		0.29	0.36	
L.S.D. at 0.05												
Density	0.06			0.06			0.08			0.04		
Biofertilization	0.06			0.03			0.11			0.06		
Density x Biofertilization	0.08			0.05			0.16			0.08		

Table 8: Effect of planting density, biofertilization and their interaction on essential oil yield / feddan (l) of *Cymbopogon citratus* (DC.) Stapf. during the two successive seasons of 2013/2014 and 2014/2015.

Biofertilization / Density	First season						Second season					
	1st cut			2nd cut			1st cut			2nd cut		
	without	with	Mean	without	with	Mean	without	with	Mean	without	with	Mean
50 cm row spacing (16800 plants/fed.)	9.07	11.09	10.08	5.04	5.71	5.38	9.58	11.76	10.67	4.03	5.04	4.54
75 cm row spacing (11200 plants/fed.)	8.62	9.86	9.24	4.93	5.82	5.38	8.96	10.30	9.63	3.81	4.70	4.26
Mean	8.85	10.48		4.99	5.77		9.27	11.03		3.92	4.87	
L.S.D. at 0.05												
Density	0.68			NS			1.02			NS		
Biofertilization	0.54			0.54			1.43			0.68		
Density x Biofertilization	0.77			0.76			2.02			0.97		

- **Essential oil constituents**

Data of the effect of interaction between treatments on chemical constituents of essential oil are presented in Table (9). Nine components were identified in volatile oil and citral was the chief component of the oil. In the first cut the detected compounds were citral a (45.85-52.23%), citral b (26.86-33.12), d- limonene (7.03-11.95%), beranyl acetate (0.55-4.82%), borneol (1.21-3.94%), linalool (0.57-3.02%), nerol (0.97-1.28%), methyl geranate (0.12-0.42%) and n-hexadecane (0.16-0.34%). In the second cut the observed constituents were citral a (41.27-46.48%), citral b (24.05-29.91), d- limonene (5.27-13.18%), beranyl acetate (0.94-8.84%), borneol (0.69-3.70%), linalool (1.01-1.75%), nerol (0.91-2.41%), methyl geranate (0.57-5.56%) and n-hexadecane (0.21-0.18%). The most important quality characteristic of lemongrass oil is based on the citral content, the aldehyde responsible for the lemon odor. This important component is extensively used in perfumery, manufacturing of vitamin A and pharmacy industries [46- 48]. The former results indicated the good quality properties of the produced lemongrass oil in Siwa Oasis as the composition of most samples meet the minimum citral requirement (75%) in the International Standard of Lemongrass Essential Oil (ISO 3217, 1974) [49]. Regarding the effect of agricultural practices on quality of lemongrass oil, in the first cut the highest citral concentration was determined by cultivation at 75 cm row spacing with biofertilization followed by 50 cm row spacing with biofertilization over control plants. These results coincided with the opinion of [50] who found that biofertilizer treatments produced plants containing an essential oil with higher citral percent. Also, these findings were in harmony with those mentioned by [45] who showed that citral content in lemongrass is affected by environmental conditions and cultivation practices.

Table 9: Effect of the interaction between treatments on chemical constituents (%) of essential oil of *Cymbopogon citratus* (DC.) Stapf. in two cuts of the second season (2014/2015).

No	Compound	50 cm row spacing without biofertilization	50 cm row spacing with biofertilization	75 cm row spacing without biofertilization	75 cm row spacing with biofertilization
		1st cut			
1	D- Limonene	8.63	9.99	11.95	7.03
2	Linalool	0.79	0.79	3.02	0.57
3	Nerol	1.02	1.06	1.28	0.97
4	Borneol	1.35	1.31	3.94	1.21
5	Citral B (Neral)	32.48	33.12	26.86	32.51
6	Citral A (Geranial)	49.04	49.69	45.85	52.23
7	Methyl geranate	0.42	0.12	-	0.38
8	Beranyl acetate	2.46	0.55	4.82	2.04
9	n-hexadecane	0.16	0.20	0.34	-
	Total citral content	81.52	82.81	72.71	84.74
2nd cut					
1	D- Limonene	13.18	12.02	5.27	10.70
2	Linalool	1.29	1.75	1.01	1.31
3	Nerol	0.91	1.68	2.41	0.94
4	Borneol	1.29	3.70	0.69	1.09
5	Citral B (Neral)	29.91	28.67	24.05	29.06
6	Citral A (Geranial)	46.01	43.22	41.27	46.48
7	Methyl geranate	0.58	-	5.56	0.57
8	Beranyl acetate	1.03	2.41	8.84	0.94
9	n-hexadecane	0.46	0.23	1.18	0.21
	Total citral content	75.92	71.89	65.32	75.54

III- Microbiological analyses

- **Isolation, characterization and Identification of *Azotobacter* (Az) and phosphate dissolving bacteria (PDB) isolates:** Different soil samples were collected from different sites of study area and used as about twenty *Azotobacter* (Az) and phosphate dissolving bacteria (PDB) isolates were obtained. Cultures of *bacterial* isolates were purified and microscopical examination was carried out to check the purity of cultures.
- **Identification of most active isolates using 16S rRNA genes sequencing:** The 16S rRNA gene sequence of BPR7 comprised of 1446bp (NCBI gene bank accession number JN208240). It showed the maximum sequence similarity (100%) to Azotobacter sp., *Azotobacter chroococcum* strain DSM 2286 16S ribosomal RNA gene partial sequence and Bacillus sp., *Bacillus circulans* strain ATCC 4513 16S ribosomal RNA gene partial sequence.
- **Biochemical activities of the selected bacterial isolates:** Microbes under study known to produce a number of secondary metabolites which may affect growth, health of plants, relationships between rhizosphere soil microorganisms, plant growth regulators (quantitative (HPLC) / µg/ml) and enzyme production. Table (10) showed the biochemical activities of *Azotobacter chroococcum* and *Bacillus megaterium* used in the trial for production of plant hormones and enzymes. As shown in Table (10) microorganisms exhibited biochemical and hormonal activities *in vitro* that could result in beneficial action in the field [51].
- **Effect of treatments on microbial activities in rhizosphere of lemongrass:** Initial total microbial counts in soil of siwa were $30x10^5$cfu/g dry soil. Data recorded in Table (11) showed that total microbial counts in lemongrass rhizosphere tended to increase in inoculated treatments compared to uninoculated. The highest total microbial counts were obtained with biofertilization treatments and 75 cm row spacing. Initial CO_2 mg CO_2/100g dry soil/24hr was 4.1×10^5cfu/g. The soil respiration (CO_2 evolution) was increased suggesting that microbial activity is higher in the inoculated treatments. The generation of carbon dioxide (CO_2) was determined as an indication of the biological activity in plant rhizosphere. Results in Table (11) clearly showed that biofertilization treatment and 75 cm within row spacing gave higher rate of CO_2 evolution than all other treatments. Data of CO_2 evolution were almost in harmony with those of total microbial counts discussed before. Concerning the effect of biofertilization on phosphate dissolving bacteria (PDB) counts, Azotobacter densities and yeast counts, results clearly showed that initial densities of Azotobacter was $7.4x10^2$cfu/g cells/ml, initial counts of PDB and yeast in rhizosphere of lemongrass plant were 3.7 and $1.6x10^2$cfu/g respectively. These counts tended to increase in most of samples with biofertilizer application and the highest counts of most studied microorganisms were recorded with the second planting distance of 75cm.
- **Antimicrobial activity of lemongrass essential oil:** Antimicrobial activity of lemongrass oil was examined against some human and plant pathogenic microbes was detected and represented in (Table 12). *Pseudomonas areuginosa* was most resistant to lemongrass oil, the other tested pathogen were sensitive to oil as shown in Table12. Biofertilizers application increased the antagonistic activity of lemongrass oil against some pathogenic microbes. This result agreed with the finding of [52].

Table 10: Biochemical activities of microbial isolates from the different sites of study area.

Isolate	Total N (ppm)	P. Inhibition zone (cm)	Hormonal activity quantitative (HPLC) / µg/ml			Enzyme activity*			
			IAA	GA_3	Cytokinin	Amylase	Cellulase	Phosphatase	Protease
Azotobacter	155	2.1	0.22	2.69	25.3	++	-	+	+
Bacillus	-	3.8	0.29	1.81	14.92	+	+	++	++

Table 11: Effect of biofertilizers applied on microbial activities in the rhizosphere of lemongrass.

Treatments		Total microbial counts×10^5cfu/g		PDB counts (×10^2cfu/g dry soil)		*Azotobacter* densities (×10^3cells/g dry soil)		Yeast Counts (×10^2cfu/g dry soil)		CO_2 ($mgCO_2$/100g dry soil/24hr)	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
First season											
50 cm row spacing	Without biofertilization	39.2	39.7	6.3	5.2	10.2	10.9	2.08	2.29	4.73	4.85
(16800 plants/fed.)	With biofertilization	41	44.2	5.6	5.8	10.3	12.8	1.90	2.05	4.96	5.36
75 cm row spacing	Without biofertilization	38	40.1	6.2	5.2	11.3	11.5	2.13	2.45	4.91	5.18
(11200 plants/fed.)	With biofertilization	42.3	46	5.2	5.7	12.1	11.6	2.16	2.28	5.22	5.71
Second season											
50 cm row spacing	Without biofertilization	68	69.3	8.9	9.1	12.8	13.2	3.5	3.88	8.3	8.75
(16800 plants/fed.)	With biofertilization	76	78.2	8.2	8.9	13.7	14.5	3.8	3.96	8.6	8.81
75 cm row spacing	Without biofertilization	75	76.8	8.4	9.2	13.9	14.3	3.96	4.19	8.96	9.29
(11200 plants/fed.)	With biofertilization	79.1	77.5	9.5	9.3	14.2	15	4.2	4.35	9.2	9.46

Table 12: Antimicrobial activity of lemongrass oil on some pathogenic microorganisms (inhibition zone diameter in cm).

Treatments		*Escherichia coli*	*Salmonella typhi*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Fusarium oxysporum*	*Rhizoctonia solani.*
1st cut							
50 cm row spacing	Without biofertilization	3.1	3.5	3.4	3.1	4.7	3.9
(16800 plants/fed.)	With biofertilization	4.8	5.8	4.2	3.4	5.9	4.6
75 cm row spacing	Without biofertilization	2.9	2.7	3.4	2.6	3.6	3.5
(11200 plants/fed.)	With biofertilization	4.2	5.3	3.8	2.9	3.9	4.2
2nd cut							
50 cm row spacing	Without biofertilization	3	3	3.1	2.8	3.8	3.6
(16800 plants/fed.)	With biofertilization	3.2	5.1	3.9	3.2	4.6	4.5
75 cm row spacing	Without biofertilization	2.5	2.3	2.9	2.1	3.3	3.5
(11200 plants/fed.)	With biofertilization	3.7	4.8	3.5	2.4	3.7	3.9

From the aforementioned results, it is remarkable that quantity and quality of lemongrass was affected by environmental conditions prevailing in the habitat i.e. the parameters of fresh and dry weights of herb per plant and per feddan, oil percentage, oil yield per plant and per feddan, citral content as well as oil biological activity were higher in the first cut (November cut) where the average monthly temperatures was higher, while these parameters were declined in the second cut (February cut) where the average monthly temperatures were dropped [53].

Finally, the cultivation of medicinal and aromatic plants in Siwa Oasis contributes to the development and diversification of agricultural activity in the Oasis, which depends mainly on the cultivation of olive and dates thereby, increases the income of local residents.

Conclusion

Under Siwa Oasis conditions, *Cymbopogon citratus* (DC.) Stapf. plants should be cultivated at narrow row to row spacing (50 cm) and should be inoculated with a biofertilizer mixture of several microorganisms strains of *Azotobacter chroococcum*, *Bacillus megaterium* and *Saccharomyces cerevisiae* after planting and after each cut to obtain the highest yields of herb and oil per feddan.

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