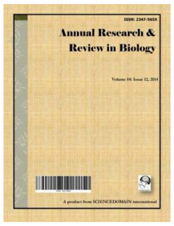

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Effect of Bio and Organic Fertilizers on Oil Production and Chemical Composition of Lemongrass Plant

Abd El Ghafor A. El-Sayed[1], Ahmed S. El-Leithy[1], Hend M. Swaefy[1*] and Zakia F. M. Senossi[2]

[1]Department of Ornamental Horticulture, Faculty of Agriculture, Cairo University, Egypt.
[2]Department of Horticulture, Faculty of Agriculture, Omar Al-Mukhtar University, Libya.

Authors' contributions

This work was carried out in collaboration between all authors. Author AEGAES designed the study. Author ASEL wrote the protocol and managed literature searches. Author HMS managed the analyses of the study and wrote the first draft of the manuscript. Author ZFMS performed the field research. All authors read and approved the final manuscript.

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ABSTRACT

This study was carried out at the Department of Ornamental Horticulture, Faculty of Agriculture, Cairo University during the two successive seasons of 2015 and 2016. The experiment was designed using a complete randomized blocks design. The objective of this study was to investigate the effect of NPK (recommended dose), biofertilizers (Nitrobien and Phosphorien) at 1,2 and 4 g/plant, compost and poultry manure at the rates of 5, 10 and 15 ton/ feddan (feddan=4200 m^2) on essential oil production and biochemical compositions of *Cymbopogon citratus* plants. The results showed that the average percentage of essential oil ranged between 0.140-0.300% at the first cut and 0.133-0.283% at the second cut of the two seasons, respectively. First cut (in August) resulted in more essential oil production than second cut (in October). Poultry manure produced the maximum essential oil yield/plant compared to control and the other fertilizer treatments. The main

*Corresponding author: E-mail: swaefyhend@yahoo.com;

components in essential oil of lemongrass were β. myrcene, linalool, neral (citral b), geranial (citral a) and geranyl acetate identified by GC. Arranged in descending order, the major constituents were citral a and citral b which reached to 69.72- 81.39% in the oil. Compost at the rate of 10 ton/ feddan was the most effective application in citral content which reached to 81.39%. The organic applications positively affected pigments content of lemongrass plant. The highest herb content of phosphorus and potassium resulted from the plants treated by compost at the rate of 10 ton/ feddan and nitrobien at the rate of 2 g/plant.

Keywords: Biofertilizers; citral; Cymbopogon citratus; essential oil; organic fertilizers.

1. INTRODUCTION

Cymbopogon is a genus of about 55 species cultivated in South and Central America, Africa and other tropical countries [1]. *Cymbopogon citratus*, Stapf (Lemongrass) plant belongs to the family Poaceae (Gramineae). Freshly cuts are the source of the essential oil through steam distillation.

Cymbopogon citratus possesses various pharmacological activities such as anti-amoebic, anti-bacterial, anti-diarrheal, anti-filarial, anti-fungal and anti-inflammatory properties [2]. The estimated world consumption of lemongrass is between 100 and 500 tons per annum. Citral is composed of an isomeric mixture of geranial (E-3, 7-dimethyl-2, 6-octadienal) and neral (Z-3, 7-dimethyl-2, 6-octadienal). In the isomeric mixtures, geranial is usually the predominant isomer [3]. Because of its intense lemon aroma and flavor, citral has been used extensively in flavoring of food, cosmetic and detergent industries. Also, lemongrass inhibited the viral replication completely even at a concentration of 0.1%. Citral is used as a raw material for the production of ionone, vitamin A and beta-carotene [4].

Cymbopogon. citratus essential oil exhibited high repellency against *Aedes aegypti* , *Anopheles dirus* and *Culex quinquefasciatus*. The percentage repellency increased when the concentration of essential oil increased [5]. The repellent activity of *Cymbopogon citratus* essential oil against the sandfly, *Phlebotomus duboscqi* was tested. The results of GC–MS demonstrated that the oil was dominated by monoterpene hydrocarbons. The monoterpene fraction for *C. citratus* was characterized by a high percentage of geranial (20.45%), myrcene (14.24%), neral (11.57%) and verbenene (9.26%). The essential oil of *C. citratus* at three concentrations (1,0.75 and 0.5 mg/ml) provided the high repellency with 100, 87.67 and 89.13%, respectively at 180 min. [6]. The major constituents of *Cymbopogon citratus* essential oil were geranial and neral. They found that *C. citratus* essential oil was effective in spinach disinfection, reducing *E. coli*, *Salmonella choleraesuis*, *Listeria monocytogenes* and *Staphylococcus aureus* [7].

Lemongrass plant is a fast growing, perennial grass and considered heavy feeder and has a large vegetative growth which needs an adequate supply of nitrogen to show good yield with high quality. Chemical fertilizers had an adequate supply of nutrients but also had a side effect on human and environment.

Using biofertilizer possesses ecological benefits because it contains effective microorganisms which help in nitrogen fixation and improve soil fertility without any pollutant. Bio fertilizers are used in a small quantity as opposed to chemical fertilizers which are added to the soil in large quantities. Biofertilizers help in stimulating the plant growth hormones providing better nutrients uptake. A small dose of biofertilizers is sufficient because each gram of carrier of biofertilizers contains at least 10 million viable cells of a specific strain [8]. N_2 –fixing bacteria can benefit plant growth through the production of secondary metabolites such as antibiotics and plant hormones like substances [9]. Also, *Rhizobium leguminosarum* bv. *phaseoli* and *Azotobacter chroococcum* showed a positive reaction for IAA, siderophores. Whereas, *Bacillus megatherium* var. *phosphaticum* showed a highly positive reaction for P- solubilizers [10].

Organic fertilizers such as compost and poultry manure maintain the soil fertility and physical properties because they contain a large amount of nutrients which released consistently. Poultry manure is available in Egyptian village, so it is cheap compared to chemical fertilizers. Organic manure application resulted in the balanced supply of nutrients, including micronutrients, increased soil microbial activity, the decomposition of harmful elements and increased soil water availability [11]. Organic amendments improved performance over time, proving that their fertilizing effect lasts longer [12]. Also, organic amendment increased Phosphorus-use efficiency by the crop compared with mineral P fertilizer [13].

The objective of this study was to investigate the effect of NPK (recommended dose), biofertilizers (Nitrobien and Phosphorien) compost and poultry manure on essential oil production and biochemical compositions of *Cymbopogon citratus* plants.

2. MATERIALS AND METHODS

This study was carried out at the Department of Ornamental Horticulture, Faculty of Agriculture, Cairo University during the two successive seasons of 2015 and 2016. This work aims to replace chemical fertilizers by bio fertilizers and organic fertilizers.

2.1 Layout of the Experiment

This experiment was designed using a complete randomized blocks design.

2.2 Experimental Procedures

2.2.1 Plant material

A uniform tillers of lemongrass (*Cymbopogon citratus*) plants were obtained from Medicinal and Aromatic Plants Department, Agriculture Research Center, El-Dokki, Cairo, Egypt. The rooted tillers were pruned and inserted in prepared soil plots on 4th April 2015 and 2016 seasons. The physical and chemical characteristics of the soil experiment field were determined according to [14] and are shown in Table 1.

2.2.2 Soil preparation

The soil was prepared on 2nd March 2015 and 2016 for the first and second seasons, respectively. Compost and poultry manure fertilizers were added at 5, 10 and 15 ton/fed during soil preparation. The chemical analysis of the used compost and poultry manure are shown in Table 2.

2.2.3 Cultivation procedures

The experiment included 14 treatments, each experimental unit (plot) was 2×1.5 m (3.0 m^2) and divided into 3 rows with 60 cm apart and 50 cm between the plants, i.e. the plots contained 12 plants. Then, the treatments replicated three times (42 plots).

2.2.4 Fertilization treatments

NPK fertilizers at different levels of 400 kg ammonium sulphate (20.5 %N): 200 kg calcium superphosphate (15.5% P_2O_5):100 kg potassium sulphate (48% K_2O)/ fed. were added in two doses, the first dose was added 45 days after transplanting, while the second dose was added after half month from the first cut. Biofertilizers were obtained from General Organization for Agricultural Equalization, Agricultural Research Center, Giza, Egypt. Nitrobein contains nitrogen fixing bacteria (*Azotobacter spp.* and *Azospirilum spp.*). Phosphorein, contains *Bacillus megatherium*. Biofertilizers (both Nitrobien and Phosphorien) were added at the rate of 1, 2 and 4 g/plant, and divided into two portions, the first was added after 45 days from transplanting and the second was added after half month from the first cut, in the first and second seasons.

Table 1. Mechanical and chemical analysis of the experimental soil

Mechanical analysis of the samples taken from the experimental soil										
Year		**Sand**			**Silt**			**Clay**	**Texture**	
2015		56.4			19.75			23.85	SCL	
Chemical analysis of the samples taken from the experimental soil										
Year	**pH**	**E.C.**	**Millie equivalent/Liter**							
2015		**(dSm^{-1})**	**Cations**				**Anions**			
	(2.5:1)	(5:1)	Ca^{++}	Mg^{++}	Na^+	K^+	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}
	7.39	5.57	25	11	23.7	1.3	-	4.0	28.0	29
		mg/ kg		%						
	N	P	K	O.M.						
	149.6	6.2	420	0.6						

Table 2. The chemical analysis of the used compost and poultry manure during 2015 and 2016

Chemical characters	Compost		Poultry	
	2015	2016	2015	2016
pH	7.57	6.99	7.50	7.80
Total nitrogen (%)	0.92	1.28	3.21	2.80
Total phosphorus (%)	0.12	0.31	0.71	0.80
Total potassium (%)	1.42	0.56	1.15	1.17
C/N ratio	17 :1	18:1	18:1	19:1
Organic matter (%)	26.5	39.4	63.6	60.6

The experiment contained 14 treatments as following:

1. Control (without fertilizer)
2. NPK (recommended dose) at (400:200:100 kg/ fed., respectively)
3. Nitrobien at 1 g/plant.
4. Nitrobien at 2 g/plant
5. Nitrobien at 4 g/plant
6. Phosphorien at 1 g/plant
7. Phosphorien at 2 g/plant
8. Phosphorien at 4 g/plant
9. Compost at 5 ton/fed.
10. Compost at 10 ton/fed.
11. Compost at 15 ton/fed.
12. Poultry manure at 5 ton/fed.
13. Poultry manure at 10 ton/fed.
14. Poultry manure at 15 ton/fed.

2.3 Recorded Data

The plants were harvested two times (on 10th August and 5th October, for the first and the second cuts, respectively, in the first season 2015 and on 8th August and 6th October for the first and the second cuts, respectively, in the second season 2016). The plants were harvested by cutting vegetative parts at 20 cm above the soil surface.

Data were recorded in the two seasons as the following:

2.3.1 <u>Essential oil</u>

1. Essential oil percentage in fresh herb.
2. Essential oil yield (ml /plant)
3. Essential oil components by GC.

2.3.1.1 Essential oil percentage in the fresh herb

The oil percentage was determined in fresh herb in both seasons using the hydro-distillation method by Clevenger apparatus according to [15]. A known weight of fresh herb (100 g) was placed in a flask of 1 L capacity for distillation, and an adequate amount of water was added. A proper essential oil trap and condenser were attached to the flask and enough water was added to fill the trap. The distillation continued for 3.0 hours until no further increase in the oil was observed. After finishing the distillation process the apparatus was left to be cooled, and the essential oil percentage was estimated as follows:

> Essential oil % = {Essential oil vol. (Measuring pipette reading) / Weight of sample} ×100

The oil was dried by sodium sulphate anhydrous.

2.3.1.2 Essential oil yield/ plant (ml)

> Essential oil yield per plant = oil percentage × herb fresh weight/plant.

2.3.1.3 Essential oil components

Samples taken from the essential oil obtained in the two cuts of the first season were analyzed using GC analysis, to determine their main constituents. The use of GC in the quantitative determinations was performed using the methods described by [16].

The GC analysis of the essential oil samples were carried out using gas chromatography instrument stands at the Laboratory of Medicinal and Aromatic Plants, National Research Center with the following specifications.

Instrument: capillary GC-2010 plus Gas Chromatographs (Shimadzu Corp., Japan), coupled with a Shimadzu FID 2010 Plus detector (Flame Ionization Detector). The GC system was equipped with a Stabilwax column (30 m x 0.25 mm i.d., 0.25 μm film thickness). Analysis were carried out using helium as carrier gas at a flow rate of 1.0 mL/min at a split ratio of 1:10 and the following temperature program: 40ºC for 1 min; rising at 4.0ºC/min to 150ºC and held for 6 min;

rising at 4ºC/min to 210ºC and held for 1min. The injector and detector were held at 210ºC and 250ºC, respectively. Diluted samples (1:10 hexane, v/v) of 0.2 μL of the mixtures were always injected. Most of the compounds were identified using GC standards. The obtained chromatogram and analysis report for each sample were analyzed to calculate the percentage of the main volatile oil components. The area of each peak was first calculated by an automatic integrator. The areas were then summed, and the total area of the peaks represented the whole sample. The percentage of each component was the ratio between its peak areas to the total peak areas, multiplied by 100.

2.3.2 Chemical constituents

2.3.2.1 Pigment contents

Determination of chlorophyll a, b and total carotenoids (mg/g fresh leaves): Chlorophyll a, b and total carotenoids contents in the fresh leaves were determined in leaf samples (mg/g fresh matter) according to [17]. The determination was conducting at wave lengths of 660, 640 and 440 nm, respectively.

The contents of the different leaf pigments were calculated using the following formulas:

Chl. A mg/L = 9.784 E 660 - 0.99 E 640
Chl. B mg/L = 21.426 E 640 - 4.65 E 660
Carotene mg/L = 4.695 E 440 - 0.268 (a + b)

2.3.2.2 Elements determination

The dried leaves of samples in the two seasons were grounded and kept in desiccators for chemical analysis.

Dry herb samples were oven- dried at 70ºC until a constant weight was obtained. The dried samples were then digested for extraction of nutrients, using the method described by [18]. The percentages of the three main nutrients (N, P and K) in the extract were determined as follows:

Nitrogen determination was carried out using the modified micro-Kjeldahl method, as described by [19]. The phosphorus content was estimated, as recommended by [20]. Potassium was determined by using a "Pye Unicam, Model SP-1900" atomic absorption spectrophotometer with a boiling air-acetylene burner according to [21].

2.4 Statistical Analysis of Data

Data recorded on oil percentage, oil yield (ml/ plant), Chlorophyll a, b and carotenoids content (mg /g. F. W.) were statistically analyzed, and separations of means were performed using Duncan test as described by [22].

3. RESULTS AND DISCUSSION

3.1 Essential oil Percentage

The presented data in Table 3 showed the effect of fertilization treatments on essential oil percentage. The average percent of essential oil ranged between 0.140-0.300% at the first cut and 0.133-0.283% at the second cut of the two seasons, respectively.

The plants at the first cut had essential oil percentage more than the plants at the second cut in both seasons, except with nitrobein treatment in the second season. The highest essential oil percentage (0.300) was obtained with the high rate of poultry (4g/plant), low rate of compost (5 ton/fed.) and nitrobein2 (2g/plant) treatments at the first cut of the first season. Also, results revealed that most of fertilization treatments had no significant effect on essential oil percentage compared to control plants in both cuts of the two seasons. In general, the essential oil percentage in all treatments at the first cut (August) were higher than those at the second cut (October) in both seasons, this effect presumably due to longer light duration, higher temperature and higher light intensity previously dominated during summer growing months (July-August), this can be the most condition for oil synthesis and accumulation in the leaves. This result agreed with some investigators on *Artemisia dracuncutus* [23] and on summer savory, *Satureja hortensis* L. plants [24] Also, [25] mentioned that the lemongrass leaves are the main source of essential oil production, which exhibited essential oil content of 0.3-0.45 %.(w/w; F.W.basis). The leaf-blade produced more essential oil (0.42%) compared to leaf-sheath (0.13%) [26] Also, on (*Vetiveria zizanioides*, L. Nash) the fertilization with compost (2 ton/fed.) + Chicken manure (2 ton/fed.) + 100kg feldspar + 100 kg rock phosphate mixed inoculation with *Bacillus megatherium* var. phosphaticum and *B. circulanse* resulted in the highest values of oil % and oil yield [27]. On the other hand, the effect of four nitrogen sources (composting of bovine manure, goat manure, poultry manure and urea)

Table 3. Effect of NPK, Bio and organic fertilizers on essential oil percentage (%) of *Cymbopogon citratus* during 2015 and 2016 seasons

Treatment	First season, 2015		Second season, 2016	
	1st cut	2nd cut	1st cut	2nd cut
Control	0.200 ab	0.133 a	0.167 ab	0.133 c
NPK	0.267 ab	0.183 a	0.217 ab	0.197 bc
Nitro1	0.217 ab	0.150 a	0.170 ab	0.193 bc
Nitro2	0.300 a	0.200 a	0.140 b	0.283 a
Nitro3	0.267 ab	0.167 a	0.170 ab	0.250 ab
Phosph1	0.200 ab	0.183 a	0.217 ab	0.173 bc
Phosph2	0.183 ab	0.183 a	0.223 ab	0.183 bc
Phosph3	0.167 b	0.150 a	0.277 a	0.200 bc
Compost1	0.300 a	0.133 a	0.267 a	0.167 bc
Compost2	0.183 ab	0.150 a	0.217 ab	0.183 bc
Compost3	0.200 ab	0.150 a	0.167 ab	0.140 bc
Poultry1	0.183 ab	0.183 a	0.233 ab	0.183 bc
Poultry2	0.217 ab	0.183 a	0.240 ab	0.233 ab
Poultry3	0.300 a	0.217 a	0.233 ab	0.217 abc

Control: Without fertilization NPK: Recommended dose Nitro: Nitrobein 1, 2 and 3 (1, 2, 4 g/ plant)respectively Phosph: Phosphorein 1, 2 and 3 (1, 2, 4 g/ plant)respectively compost: 1, 2 and 3 (5, 10, 15 ton/ feddan) Poultry: 1, 2 and 3 (5, 10, 15 ton/ feddan), respectively

showed that the nitrogen sources did not affect the essential oil yield and composition of *C. citratus*. The increase in essential oil production derived by N fertilization was due to an increase of leaf biomass [28]. According to [29] young lemongrass leaves have the ability to metabolize sucrose. Excess sucrose from metabolic processes was converted to monoterpenes and finally to accumulation of essential oil. Also, essential oil metabolism was controlled by the balance between photosynthesis and the utilization of photosynthate (sucrose) or the growth differentiation balance [30].

3.2 Essential Oil Yield (ml /plant)

As shown in Table 4 the data indicated that, in general, first cut resulted in more essential oil yield production than the second cut except with compost at the medium rate in the first season and the treatment of control and nitrobein in the second season. The average essential oil yield per plant ranged between 1.086-6.170 ml per plant in the first cut and 1.079-3.889 ml per plant in the second cut for both seasons. Concerning to the effect of different fertilizers on total essential oil yield per plant, in the first season, the high rate of poultry, nitrobein2 (2g/plant),medium rate of poultry and NPK treatments resulted in best production compared with the other treatments, with the mean values 7.849, 6.336, 6.166 and 6.045ml per plant ,respectively. While, in the second season medium rate of poultry produced the maximum total essential oil yield per plant followed by the high rate of poultry with the mean values; 10.059 and 8.536 ml per plant, respectively. The lowest essential oil yield per plant was obtained from control plants in both cut of two seasons. The percentage of essential oil depends on the physiological state of the whole plant, while the essential oil yield depends mainly on the amount of vegetative yield. These results are in agreement with [31] who found that essential oil content of lemongrass decreased with the increase in maturity stages at harvest. Also, [27] on *Vetiveria zizanioides* and [32] on *Ocimum* species, they pointed out that poultry manure application resulted in significantly increment in essential oil yield per plant compared to control plants.

3.3 Essential Oil Components

The components of essential oil of *Cymbopogon citratus* are represented in Table 5 and Fig. 1 (A and B). The identified compounds were β myrcene, linalool, neral, geranial and geranyl acetate. These results agree with those mentioned by [33] who analyzed the essential oils of *Cymbopogon citratus* (DC.) Stapf grown in three different locations in South India and found that the main constituents of the oils were β-myrcene, neral, geranial and geraniol. The oil from Nadugani was found to contain the major constituents' neral, geranial, geraniol and geranyl acetate.

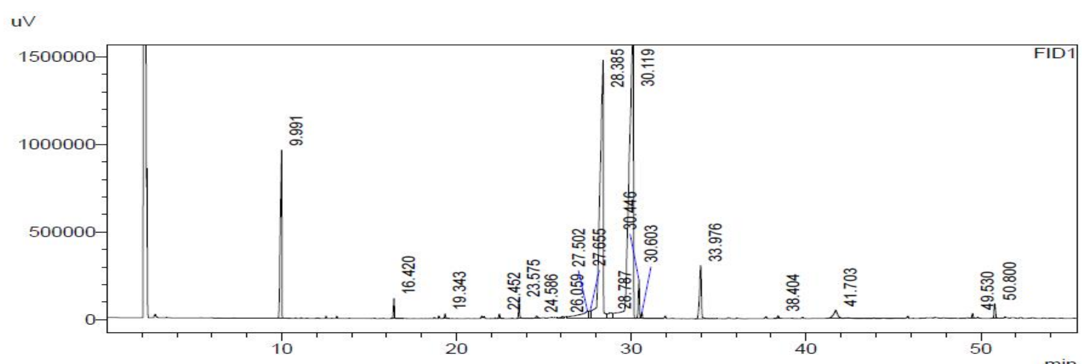
Table 4. Effect of NPK, Bio and organic fertilizers on oil yield (ml /plant) of *Cymbopogon citratus* during 2015 and 2016 seasons

Treatment	First season, 2015			Second season, 2016		
	1ST cut	2nd cut	Total	1ST cut	2nd cut	Total
Control	1.587g	1.079 h	2.666 h	1.086 f	1.217 j	2.303 i
NPK	4.288 a	1.757 ef	6.046 b	2.967 d	2.342 c	5.309 de
Nitro1	2.544 de	1.247 g	3.791 fg	2.166 e	2.333 c	4.499 f
Nitro2	4.518 a	1.818 e	6.336 b	1.804 e	2.084 de	3.888 g
Nitro3	3.694 b	1.600 f	5.293 c	1.927 e	2.330 c	4.258 fg
Phosph1	2.569 de	1.704 ef	4.272ef	1.867 e	1.345 ij	3.212 h
Phosph2	1.973 fg	1.744 ef	3.717 fg	3.171 d	1.507 hi	4.678 ef
Phosph3	1.884 g	1.380 g	3.264 g	2.831 d	1.917 ef	4.747 ef
Compost1	3.234 bc	1.770 ef	5.004 cd	3.183 d	1.692 gh	4.875 ef
Compost2	2.023 efg	2.067 d	4.090 ef	4.050 c	1.784 fg	5.835 d
Compost3	2.456 efg	2.166 cd	4.622 de	3.041 d	1.715 fgh	4.756 ef
Poultry1	3.010 cd	2.319 c	5.329 c	4.492 c	2.223 cd	6.716 c
Poultry2	3.533 bc	2.633 b	6.166 b	6.170 a	3.889 a	10.059 a
Poultry3	4.668 a	3.181 a	7.849 a	5.368 b	3.168 b	8.536 b

Control: Without fertilization NPK: Recommended dose Nitro: Nitrobein 1, 2 and3 (1, 2, 4 g/ plant) respectively Phosph: Phosphorein 1, 2 and3 (1,2, 4 g/ plant)respectively compost: 1, 2 and3 (5,10, 15 ton/ feddan) Poultry: 1, 2 and3 (5, 10, 15 ton/ feddan), respectively

First season - First cut

(A): Control (Without fertilization)



(B): Poultry 2 (10 ton/ feddan)

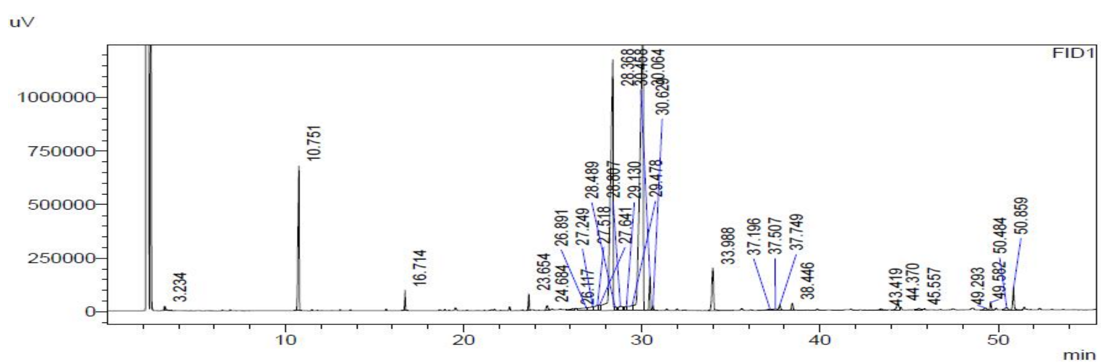


Fig. 1. Gas chromatogram of lemongrass essential oil

Table 5. Effect of NPK, Bio and organic fertilizers on essential oil components of lemongrass plant by GC at both cuts in the first season

Treatment	β Myrcene		Linalool		Neral (Citral b)		Geranial (Citral a)		Geranyl acetate		Citral a + Citral b		Total identified	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Control	8.404	9.023	0.675	0.542	30.951	29.205	46.036	44.783	3.499	3.724	76.99	73.99	89.57	87.28
NPK	8. 742	9.521	0.549	0.469	30.503	29.828	44.708	44.576	4.526	4.076	75.21	74.40	89.03	88.47
Nitro1	7.785	7.726	0.677	0.561	29.565	31.482	44.058	43.935	4.076	3.931	73.62	75.42	86.16	87.64
Nitro2	7.925	7.906	0.514	0.511	30.584	33.161	46.101	45.701	3.541	3.626	76.69	78.86	88.67	90.90
Nitro3	8.430	5.998	0.615	0.459	32.015	27.902	44.655	41.522	3.799	3.514	76.67	69.42	89.51	79.39
Phosph1	3.934	7.544	0.493	0.527	29.700	29.562	48.543	45.692	3.106	3.806	78.24	75.25	85.77	87.13
Phosph2	5.200	8.849	0.901	0.534	31.456	30.590	49.788	43.169	3.647	4.010	81.24	73.76	90.99	87.15
Phosph3	4.908	9.899	0.576	0.556	30.853	31.363	48.518	44.861	3.776	3.496	79.37	76.22	88.63	90.17
Compost1	8.083	6.455	0.701	0.534	31.626	30.342	44.511	44.319	3.921	3.798	76.14	74.66	88.85	85.45
Compost2	2.668	8.393	0.748	0.510	33.722	31.859	47.663	43.041	3.602	3.800	81.39	74.90	88.41	87.60
Compost3	4.441	7.518	0.682	0.477	29.521	29.825	44.724	43.960	3.260	3.817	74.25	73.79	82.63	85.60
Poultry1	6.872	8.153	0.743	0.569	31.872	32.084	44.045	42.970	3.608	4.380	75.92	75.05	87.14	88.15
Poultry2	7.388	8.364	0.815	0.565	29.888	28.178	42.960	41.544	3.333	3.491	72.85	69.72	84.39	82.14
Poultry3	7.685	7.266	0.593	0.409	30.146	30.770	45.930	44.731	3.799	4.940	76.08	75.50	89.91	88.47

Control: Without fertilization NPK: Recommended dose Nitro: Nitrobein 1, 2 and3 (1, 2, 4 g/ plant) respectively. Phosph: Phosphorein 1, 2 and3 (1,2, 4 g/ plant) respectively compost: 1, 2 and3 (5,10, 15 ton/ feddan) Poultry: 1, 2 and3 (5, 10 ,15 ton/ feddan), respectively

Results showed that the total identified compounds percentage ranged between 82.63-90.99% at the first cut and 79.39-90.90% at the second one.

The quality characteristic of *Cymbopogon citratus* essential oil is based on citral content. Citral is a mixture of neral (citral b) and geranial (citral a). [34] found that citral was the major component of *Cymbopogon citratus* essential oil by GC-MS evaluation. [35] stated that the Brazilian Pharmacopeia recommends a minimum value of 60% of citral in the essential oil in lemongrass Brazilian plantations.

Results revealed that, at the first cut citral content was higher than at the second cut with all treatments except with nitrobein 1 and 2. The highest citral content 81.39% was obtained from plants treated with compost at medium rate (10 ton/fed.) followed by phosphorein at the rate 2 g/plant with the mean value 81.24%. These results meet the minimum citral content (75%) of *Cymbopogon citratus* in the International Organization for Standardization [36]. The highest neral content 33.722% was obtained from plants treated with compost at medium rate, while the highest geranial content 49.788% was found with the treatment of phosphorein(2 g/plant). The treatments of phosphorein had the highest percentages of β myrcene compared to the first one, phosphorein3 (4 g/plant) which resulted the maximum percentage of β myrcene (9.899%). The maximum percentages of linalool were found at the first cut compared to the second one, phosphorein 2 (2 g/plant) treatment resulted the maximum percentage of linalool at the first cut (0.901%). Plants treated with the high rate of poultry manure produced the highest geranyl acetate (4.940%) at the second cut. A similar composition was reported by [37,7] and [38]. The finding result was in harmony with [31] who concluded that the essential oil and citral content showed significant differences when lemongrass was harvested at different maturity stages. The optimum percentage of essential oil was obtained when lemongrass was harvested at 5.5 months after planting. The percentage of citral content was higher when lemongrass was harvested at 6.5 months after planting. The estimated optimum percentage of citral content was obtained at 6.7 ± 0.3 months after planting. Thus, lemongrass should be harvested between 6.5 to 7.0 months after planting to achieve optimum essential oil with a high composition of citral.

3.4 Chlorophyll A & B Content (mg /g. F. W.)

As shown in Table 6 it can be noticed that the treatments which supplied plants with an abundant amount of nitrogen such as compost and poultry manure, positively affected pigments contents of lemongrass plant. The plants at the first cut in the first season had more chlorophyll A content than the second season.

The highest chlorophyll A content (1.063 mg/g f.w.) was obtained from plants treated with poultry manure at the medium rate at the first cut in the first season. Also, in the second season, the highest chlorophyll A content (0.612 mg/g f.w.) was obtained from poultry at the medium rate compared to other treatments.

A high N productivity (the ratio of relative growth rate to whole plant nutrient concentration in the plant tissue) is associated with a relatively large investment of N in photosynthesizing tissue, an efficient use of the N invested in the leaves for the process of photosynthesis and relatively low carbon use in respiration [39].

Concerning chlorophyll B content, the result showed that, in the first season, the highest chlorophyll B content (0.610 mg/g f.w.) was recorded with the medium rate of compost at the second cut compared to the other treatments of both seasons. Also, nitrobein at medium rate had a positive effect on chlorophyll B content with mean values 0.267 and 0.567 mg/g f.w. at the first and second cut, respectively. In the second season, the highest chlorophyll B content (0.563 mg/g f.w.) was recorded with the medium rate of poultry at the second cut and followed by the medium rate of compost with mean value 0.378 mg/g f.w. compared to the other treatments. Finding result was in harmony with [35] on lemongrass (*Cymbopogon citratus*) plants who found that the addition of the compost increased the levels of nutrients in the plants, mainly nitrogen, positively influencing the chlorophyll contents.

3.5 Carotenoids Content (mg/g. F. W.)

Data presented in Table 7 revealed that in the first season, the plants treated with the recommended dose of chemical fertilizers (NPK) had the highest carotenoids content with values 0.390 and 0.218 mg /g f. w. at the first and second cut, respectively compared to the other treatments. In the second season, the highest carotenoids content was obtained from organic fertilizers treatments followed by NPK treatment at the first cut. While at the second cut the highest carotenoids content (0.616 mg /g f. w.) was recorded with the medium rate of nitrobein compared to the other treatments.

Carotenoids protect chlorophyll from photo damage (photo oxidation) by absorbing the high intensity light. They play an important role as antioxidants and pro-vitamin A [40].

It could be noticed from the previous results that the highest carotenoids content was obtained from the same treatment which had the high percentage of monoterpenes. In the first season at the first cut, NPK treatment recorded the highest carotenoids content and also β-myrcene and geranyl acetate percentage as shown in Table 5. The medium rate of compost recorded high carotenoids content and the highest total citral percentage as shown in Table 5. [41] mentioned that carotenoids and volatile organic compounds share the initial step of their synthesis. They modified the light environment of *Quercus ilex* and studied the capacity of leaf monoterpene emission and carotenoids content. They found a significant and positive correlation between levels of pigment contents and monoterpene emission capacity.

Table 6. Effect of NPK, Bio and organic fertilizers on chlorophyll A and B content (mg /g. F. W.) in leaves of *Cymbopogon citratus* during 2015 and 2016 seasons

Treatment	Chlorophyll A		Chlorophyll B	
	First season			
	1st Cut	2nd Cut	1st Cut	2nd Cut
Control	0.290 ef	0.125 g	0.124 c	0.243 d
NPK	0.670 bcde	0.279 bcde	0.215 abc	0.448 abc
Nitro1	0.599 bcdef	0.129 g	0.178 abc	0.404 bcd
Nitro2	0.415 cdef	0.251 cdef	0.267 a	0.567 abc
Nitro3	0.415 cdef	0.184 efg	0.199 abc	0.291 cd
Phosph1	0.598 bcdef	0.227 defg	0.221 abc	0.347 cd
Phosph2	0.235 f	0.226 defg	0.176 abc	0.303 cd
Phosph3	0.352 def	0.160 fg	0.153 bc	0.459 abc
Compost1	0.683 bcd	0.361 b	0.125 c	0.362 cd
Compost2	0.528 cdef	0.320 bcd	0.192 abc	0.610 a
Compost3	0.445 cdef	0.490 a	0.210 abc	0.303 cd
Poultry1	0.916 ab	0.278 bcde	0.191 abc	0.389 cd
Poultry2	1.063 a	0.341 bc	0.243 ab	0.391 cd
Poultry3	0.789 abc	0.296 bcd	0.186 abc	0.304 cd
	Second season			
Control	0.192 f	0.149 g	0.024 e	0.128 e
NPK	0.269 def	0.284 cde	0.092 cde	0.131 e
Nitro1	0.239 ef	0.195 fg	0.072 de	0.146 e
Nitro2	0.239 ef	0.236 def	0.058 e	0.272 cd
Nitro3	0.299 cde	0.216 efg	0.122 bcde	0.234 d
Phosph1	0.193 f	0.196 fg	0.163 bcde	0.227 d
Phosph2	0.234 ef	0.242 def	0.047 e	0.247 d
Phosph3	0.340 cd	0.283 cde	0.149 bcde	0.198 de
Compost1	0.372 c	0.236 def	0.280 ab	0.229 d
Compost2	0.359 c	0.302 cd	0.368 a	0.378 b
Compost3	0.511 b	0.418 a	0.242 abcd	0.504 a
Poultry1	0.495 b	0.328 bc	0.163 bcde	0.354 b
Poultry2	0.612 a	0.384 ab	0.259 abc	0.563 a
Poultry3	0.480 b	0.315 bcd	0.117 bcde	0.335 bc

Control: Without fertilization NPK: Recommended dose Nitro: Nitrobein 1, 2 and3 (1, 2, 4 g/ plant) respectively Phosph: Phosphorein1, 2 and3 (1 ,2, 4 g/ plant)respectively compost: 1, 2 and3 (5, 10, 15 ton/ feddan) Poultry: 1, 2 and3 (5, 10, 15 ton/ feddan), respectively

Table 7. Effect of NPK, Bio and organic fertilizers on carotenoids content (mg /g. F. W.) in leaves of *Cymbopogon citratus* during 2015 and 2016 seasons

Treatment	Carotenoids (mg/g. F. W.)			
	First season		Second season	
	1st cut	2nd cut	1st cut	2nd cut
Control	0.194 d	0.143 c	0.226 f	0.303 e
NPK	0.390 a	0.218 a	0.397 bcd	0.451 bc
Nitro1	0.309 b	0.173 abc	0.347 de	0.496 b
Nitro2	0.245 cd	0.200 abc	0.282 ef	0.616 a
Nitro3	0.333 ab	0.150 bc	0.393 cd	0.369 cde
Phosph1	0.305 b	0.168 abc	0.390 cd	0.398 cd
Phosph2	0.321 b	0.198 abc	0.374 cde	0.330 de
Phosph3	0.281 bc	0.211 ab	0.337 de	0.387 cde
Compost1	0.320 b	0.177 abc	0.402 bcd	0.310 e
Compost2	0.340 ab	0.158 abc	0.528 a	0.432 bc
Compost3	0.314 b	0.158 abc	0.497 ab	0.490 b
Poultry1	0.296 bc	0.203 abc	0.453 abc	0.322 de
Poultry2	0.337 ab	0.162 abc	0.458 abc	0.318 de
Poultry3	0.315 b	0.217 a	0.510 a	0.375 cde

Control: Without fertilization NPK: Recommended dose Nitro: Nitrobein 1, 2 and3 (1, 2, 4 g/ plant) respectively Phosph: Phosphorein1, 2 and3 (1,2 , 4 g/ plant), respectively compost: 1, 2 and3 (5,10, 15 ton/ feddan) Poultry: 1, 2 and3 (5, 10, 15 ton/ feddan), respectively.

Table 8. Effect of NPK, Bio and organic fertilizers on total carbohydrates % in leaves of *Cymbopogon citratus* during 2015 and 2016 seasons

Treatment	Carbohydrate %			
	First season		Second season	
	1st cut	2nd cut	1st cut	2nd cut
Control	22.5	20.6	21.4	23.6
NPK	25.6	24.6	24.4	27.0
Nitro1	24.4	25.2	28.4	25.2
Nitro2	28.8	22.2	25.6	28.7
Nitro3	28.2	22.2	25.0	25.8
Phosph1	25.1	25.4	27.1	24.8
Phosph2	25.3	26.4	26.9	24.5
Phosph3	26.0	27.5	30.0	29.2
Compost1	27.6	25.8	24.5	26.7
Compost2	28.8	21.0	22.2	23.7
Compost3	29.3	29.7	23.3	26.9
Poultry1	23.1	23.7	26.4	28.0
Poultry2	22.5	23.3	31.4	30.9
Poultry3	24.6	21.3	25.1	29.0

Control: Without fertilization NPK: Recommended dose Nitro: Nitrobein 1, 2 and3 (1, 2, 4 g/ plant) respectively Phosph: Phosphorein1, 2 and3 (1, 2, 4 g/ plant)respectively compost: 1, 2 and3 (5, 10, 15 ton/ feddan) Poultry: 1, 2 and3 (5, 10,15 ton/ feddan), respectively

3.6 Total Carbohydrates % in the Dry Leaves

Data presented in Table 8 revealed that applying the high rate of compost to lemongrass plants resulted in the highest carbohydrates percentage (29.3%) at the first cut in the first season followed by nitrobein or compost at the medium rate with value (28.8%). While, highest carbohydrates percentage at the second cut was obtained from the high rate of compost and phosphorein with values 29.7 and 27.5%, respectively.

Table 9. Effect of NPK, Bio and organic fertilizers on N, P and K contents of *Cymbopogon citratus* during 2015 and 2016 seasons

Treatment	N%				P%				K%			
	First season		Second season		First season		Second season		First season		Second season	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Control	0.97	0.65	0.62	0.76	0.12	0.15	0.11	0.15	1.93	1.93	1.17	1.14
NPK	1.38	1.23	0.91	0.82	0.18	0.20	0.16	0.21	2.28	2.01	1.40	1.49
Nitro1	1.26	1.18	0.79	0.88	0.17	0.17	0.16	0.20	1.93	2.19	1.17	1.23
Nitro2	1.06	1.19	0.71	0.76	0.25	0.26	0.19	0.23	2.45	2.36	1.49	1.40
Nitro3	1.41	1.06	0.88	1.12	0.23	0.19	0.16	0.17	1.93	2.28	1.49	1.31
Phosph1	1.00	0.85	0.82	0.82	0.21	0.19	0.14	0.21	2.01	1.93	1.49	1.40
Phosph2	1.23	0.94	0.88	1.00	0.22	0.17	0.11	0.15	2.10	2.19	1.49	1.31
Phosph3	1.18	1.06	1.06	0.91	0.20	0.18	0.13	0.15	2.19	2.01	1.49	1.31
Compost1	1.29	1.12	1.12	0.94	0.21	0.16	0.16	0.20	2.36	2.10	1.49	1.49
Compost2	1.23	1.15	1.03	0.94	0.25	0.26	0.17	0.23	2.45	2.36	1.49	1.58
Compost3	1.03	1.59	1.00	1.06	0.16	0.19	0.16	0.22	2.10	2.01	1.17	1.49
Poultry1	1.53	1.12	1.06	1.15	0.18	0.17	0.17	0.18	2.19	2.01	1.84	1.31
Poultry2	1.47	1.47	0.94	1.03	0.16	0.24	0.14	0.22	2.45	2.10	1.75	1.31
Poultry3	1.18	1.00	1.09	1.21	0.16	0.19	0.17	0.21	2.45	2.19	2.01	1.30

Control: Without fertilization NPK: Recommended dose Nitro: Nitrobein 1, 2 and3 (1, 2, 4 g/ plant) respectively Phosph: Phosphorein 1, 2 and3 (1, 2, 4 g/plant) respectively compost: 1, 2 and 3 (5,10, 15 ton/ feddan) Poultry: 1, 2 and3 (5, 10, 15 ton/ feddan), respectively

In the second season, the highest carbohydrates percentage was obtained from the medium rate of poultry manure with values 31.4 and 30.9% at the first and second cuts, respectively followed by the treatment of phosphorein at the high rate with value 30.0 and 29.2% at the first and second cut, respectively compared to other treatments. This result was in harmony with [42] who found that adding compost at 8 ton/ fed to *Rosmarinus officinalis*, L. plants, resulted in highest total carbohydrates compared to other compost treatments and bio fertilizers treatments.

3.7 NPK Content

Data presented in Table 9 indicated that the different application treatments increased the values of herb mineral contents compared to control plants in both seasons. The average percentage of nitrogen content ranged between 0.62-1.53% at the first cut and 0.65-1.59% at the second cut of the two seasons, respectively. The highest nitrogen content (1.59%) was obtained from compost treatment at the high rate at the second cut in the first season. Regarding the phosphorus content, in both seasons, applying the medium rate of nitrobein and compost resulted in the highest phosphorus content in the dry herb of lemongrass at both cuts.

Concerning the potassium content, the results indicated that in the first season, most of organic fertilizers treatments and nitrobein at medium rate had the higher potassium content at the first cut compared to the other treatments. As well as, the medium dose of compost and nitrobein had the highest potassium content at the second cut. The highest potassium contents in the second season were obtained from the poultry manure treatments at the first cut and from the medium dose of compost at the second one compared to the other treatments. This result was in agreement with [35] on lemongrass (*Cymbopogon citratus*) plants grown in soil containing different organic compost doses (0, 5, 10, 20, 40 and 60 t ha^{-1}), formed from the sewage sludge composting process and waste of urban vegetation pruning. The results showed that the addition of the compost increased the levels of nutrients in the plants, mainly nitrogen, positively influencing the chlorophyll contents in the first season.

4. CONCLUSION

From previous results, it could be concluded that poultry manure and compost at the rate of 10 ton/ feddan were the most effective application to increase the essential oil yield/plant and citral content. The organic applications positively affected pigments content of lemongrass plant. The highest herb content of phosphorus and potassium resulted from the plants treated by compost (10 ton/ feddan) and nitrobien (2 g/plant). Also, it can be recommended that the aim of this work is to replace chemical fertilizers by bio and organic fertilizers for clean production of medicinal and aromatic plants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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