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Effect of the fertilization, plant density and time of cutting on yield and quality of the essential oil of C*ymbopogon citratus* Stapf.

S. Linares[1*], N. Gonzalez[2], E. Gómez[1], A. Usubillaga[3], E. Darghan[1]

[1*]Departamento de Ingeniería Agronómica. Universidad Nacional experimental del Táchira. Avenida Universidad Paramillo.
[2]Laboratorio de Fitoquímica. UNET.
[3]Laboratorio de la Facultad de Farmacia. ULA-Mérida

Abstract

The aim of this study was to measure the effect of the fertilization, plant density and time of harvest time on the yield and quality of the essential oil extracted from *Cymbopogon citratus* (lemon grass). The experiment was conducted in the Experiment.Station at the University of Táchira. The quality and quantity of essential oil produced from freshly cut aerial part were determinaded for lemon grass cultivated under different condition of plant density, soil fertilization and harvest time. Results showed that highest yields were obtained by applying chemical fertilization (T12 17.21 l/ha), chemical plus organic fertilization (T21 16.43 l/ha) and organic fertilization (T30 15.19 l/ha). All these trails were performed with the same plant density of 0.7 x 0.7m and plant were first cut four months after sowing. The composition of the essential oil was established by gas chromatography coupled to mass spectrometry. The essential oil quality of was determined by adding the concentrations of neral, geraniol, geranial and acetate of geranilo. Use of chemical fertilization, organic fertilization or a mixture of both increased the quality up to 80.69%, 80.54 and 80.86 respectively. Cutting the plant four months after the sowing increased the quality of the essential oil up to 82.42%.
Key words: agronomic practices, *Cymbopogon citratus,* essential oil, GC-MS,

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[1]Autor para la correspondencia email: mfiguero@cantv.net

Introduction

The essential oils are natural products obtained from plants. These are formed by heterogeneous and complex volatile mixtures of chemical compounds, with predominance of terpene associated to aldehyde, alcohols and ketone which are deposit in several structures of the plant. These oils are employed in the pharmaceutical, cosmetic and food industries. Its yield varies from some thousandths by cent until 1-3% of the fresh weight of the plant (2, 4, 5, 7, 12).

Cymbopogon citratus Stapf (lemon grass), is sowed in many countries by its essential oil, with a high citral content (70 – 80%), which is used as raw matter for the synthesis of aromatic compounds and vitamin A, besides of its uses in the perfumery (10, 15).

In Venezuela, the specie is adapted to different climatic soils; it is commonly found in avenues, gardens, or familiar greenhouse as ornamental and medicinal plant. Lemon grass has not been studied in the country from the point of view of the agronomic handle. This research gives the chance to investigate other production areas with the purpose of processing raw matter for the extraction of essential oil, that at the same time can be used in the obtaining of other products with a higher aggregated value.

The experienced in other countries as Brazil, India, Argentina, Guatemala and Indonesia, where lemon grass is used as a commercial crop (8, 14), constitutes the principles of this research of agronomical practices, which purpose is to determine the effect of the fertilization, density of sow and time and cut of yield and quality of the essential oil extracted from *Cymbopogon citratus* Stapf (lemon grass).

Materials and methods

The essay was carried out in an established plot at the National Experimental University of Táchira (UNET), located at Táchira state, San Cristóbal municipality, San Juan Bautista parish. It has a high over the sea level of 1050 m. The mean annual precipitation is of 1200 mm. The annual average temperature is of 23°C. The soil had a thick texture (loamy sandy); with organic matter of 1.44% (low); low availability of phosphorus (3 ppm), calcium (106.95 ppm) and magnesium (32.55 ppm); mean quantity of potassium (110.55 ppm); normal calcium-magnesium relation (2.02606 Eq/Eq); pH 5; and low electrical conductivity (0.04 mmhos/cm), according to analysis of soils done prior establishing the study at the Soil Laboratory of UNET.

In the land area (2000 m^2), plots were market (4.00 x 4.00 m by side and 50 cm of division between them); the area was divided in three perpendicular blocks according to the soil fertility. Treatments were distributed at random in the plots (each treatment only done once per plot).

The material used as asexual "seed" originated from the procedure of separating the mother plant in the different units, and was collected from the properties of UNET. To these explants any disinfections treatments were done due to these were not necessary, explants were sowed immediately after were separated from the mother plant. It was proceeded to sow smallholdings using explants per point, the agronomic practices were done for the maintenance of smallholdings as a manual control of weed and irrigation control. The sow was done in the rainy period (June 1st, 2001).

The field investigation was organized in a block design of three factors with a randomized plot design. Density effect of sow (D), with three levels of sow distance between plants and rows: D1: 0.70 m x 0.70 m; D2: 0.70 m x 1.00 m; and D3: 1.00 m x 1.00 m. Effect of the fertilizer (F) to be applied in each treatment, with four levels: FO: Without application of fertilizers. F1: Fertilization of chemical origin. F2. Fertilization of chemical and organic origin. F3. Fertilization of organic origin. Age effect of the plant (EC) at the moment of the first cut with three levels: EC1: 60 days after the sow; EC2: 90 days after the sow; EC3: 120 days after the sow. The number of treatments was of 36 (table 1), each treatment was repeated three times, which represented 108 experimental units. Central plants of *C. Citratus* of each smallholding were considered as the experimental unit.

25 plants were established by smallholding for treatments with D1, 20 for D2 and 6 plants for D3. The application treatments of fertilizers were: FO: Without application of fertilizer. F1: A fertilizer of chemical origin in a dose of 250 kg/ha of the commercial fertilizer 15-15-15 (N-P-K), that gave the equivalent to the application of 37.5 P_2O_5 (pentoxide of phosphorus), of 37.5 kg of K_2O (oxide of potassium) and 37.5 kg of N (nitrogen) by hectare according to the recommendations of Wijesekera (15). F3: a fertilizer of organic origin, which was dehydrated dung, was used (Fertipollo®).

The employed doses were the equivalent of the application of 10 tons by hectare.

F2: In this case the fertilization of the chemical origin plus the fertilization of organic origin were combined. The mixture of two types of fertilizers of different origin was applied in the same dose of the corresponding treatments to the fertilizations F1 and F3. The application of fertilizers was done at the moment of the sow in only one dose.

Biometric information was taken every 30 days of the growth of central plants of each plot through the first month after the sow. The number of stalks by cane stools: number of leaves per plant (only green leaves) and height of the plant (measured in centimeters from the soil to the point of the longest leaves).

Plants were harvested for treatments that included the age of

Table 1. Identification of the applied treatments in the smallholdings sowed with *C. citratus*.

Treatments			
T1=F0D1EC1	T10=F1D1EC1	T19=F2D1EC1	T28=F3D1EC1
T2=F0D1EC2	T11=F1D1EC2	T20=F2D1EC2	T29=F3D1EC2
T3=F0D1EC3	T12=F1D1EC3	T21=F2D1EC3	T30=F3D1EC3
T4=F0D2EC1	T13=F1D2EC1	T22=F2D2EC1	T31=F3D2EC1
T5=F0D2EC2	T14=F1D2EC2	T23=F2D2EC2	T32=F3D2EC2
T6=F0D2EC3	T15=F1D2EC3	T24=F2D2EC3	T33=F3D2EC3
T7=F0D3EC1	T16=F1D3EC1	T25=F2D3EC1	T34=F3D3EC1
T8=F0D3EC2	T17=F1D3EC2	T26=F2D3EC2	T35=F3D3EC2
T9=F0D3EC3	T18=F1D3EC3	T27=F2D3EC3	T36=F3D3EC3

T1..36: Number of treatments; FO..4 Type of fertilization; D1..3: type of sow density; EC1..3: Type of age of cut.

the cut EC1 (at 60 days); EC2 (90 days) and EC3 (120 days).

Plants were cut very near the soil (including the foliar lamina and part of the stalk), the weighted and identified material was storage at 8ºC (for a maximum of 48 hours) for their posterior hydro-distillation.

The essential oil was extracted by hydro-distillation (7). The sample was cut in sections of 3 cm of longitude, and was put in the ball of the hydro-distiller. It was covered with water and was distilled (100ºC for three hours). The distilled material was collected using a Clevenger trap to separate oil from water.

The extracted oil was treated with anhydrous sodium sulfate to eliminate humidity. The quantity of oil was measured and was storage at 4ºC for its posterior chromatographic analysis. The yield in quantity of the obtained oil was determined on the base of the percentage in relation to the fresh matter of the foliage. The identification of the components of each samples of oil was done using a gas chromatograph compiled to a mass chromatograph (HP 5973), equipped with a capillary column HP-5MS (30mx0.2mmx0.25mm). The standard method of the preparation of the sample was applied, which consisted on taking 20 µl of the essential oil and diluting it with ether, then it was injected to a chromatograph. The temperature program was of: initial temperature 60ºC (5 min), increase of temperature: 4°C/min until 260°C. The mass spectrum of each components of oil was compared for their identification, to those reported in the data base of Wiley library (incorporated to the software of the chromatograph).

The identification criteria that was employed was the one of choosing the compounds that presented more than 90 percent of accuracy in the correlation of the mass spectrum with the patterns of the library. The quality for the perfumery of oil was evaluated in base of the addition of the phytoconstituents geraniol, Z-sitral (neral), E- citral (geranial) and acetate of geranyl that surpass the 75% of their total composition, as Wijesekera points out (15).

To the information obtained for the yield in quantity (ml of oil) was applied the Friedman test of the non parametric variance analysis for the blocks design, due to these did not follow a normal distribution when applying the corresponding statistics tests.

The experimental data obtained for the height of plants, number of leaves and number of stems, yield in fresh weight and quantity of essential oil extracted in function of: age of the cut, density of sow, and type of fertilization, the information was statistically analyzed using the statistical software STAT GRAPHIC PLUS, to determine their correlation through the correlation matrix of Spearman.

The chromatographic information referred to the chemical constitution of oil to determine the quality for perfumery, was analyzed by the variance analysis for a block design of three factors (density of sow, fertilization and age of cut), with an unbalanced block design and fixed effects (3). The statistical analysis was also helped by SAS System for window. Release 8.01. 1999-2000.

Results and discussion

The mean yield of the essential oil was higher in treatments T12 (17.21 l/ha=; T21 (16.43 l/ha) and T30 (15.19 l/ha) (figure 1). It is important to say that the cited treatments have in common the sow density of 0.70 per 0.70 m, age of the cut four months after the sow, and all these present chemical fertilization for T12; chemical fertilization and organic

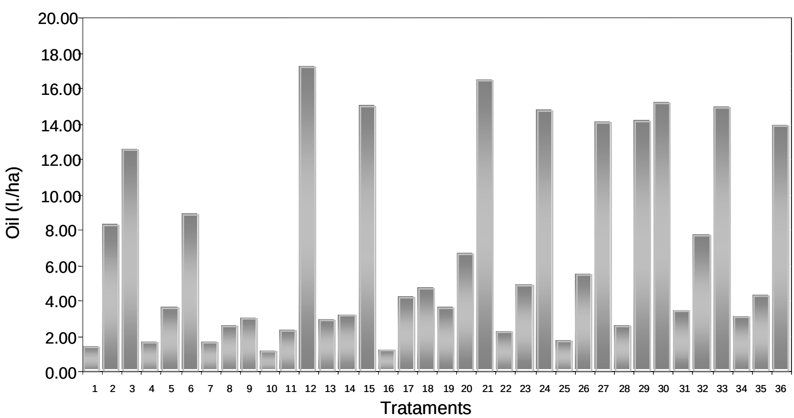

Figure 1. Yield in the essential oil extracted from the different essayed treatments.

fertilization for T21 and organic fertilization for T30.

From the mean analysis of treatments and according to its yield, categories from I to V and in an ascendant order of the yield were grouped (table 2).

In group V, ten treatments that did not have significant different between them were concentrated, besides this group was formed by the highest yields treatments in quantity of extracted essential oil (table 2).

Excepting T3 that appears in group V (table 2), to the other treatments some type of fertilization (chemical, organic or the mixture of both) was applied. It was observed that *C. citratus* plants responded to the application of fertilizers and when it was not used, the yield in the quantity of essential oil was lower. The obtained results do not agree to those obtained by Viturro *et al.* (14), where differences in yield and in quality were not found when the crop was fertilized or when it was not. It is important to mention that in the conditions of this research, the soil fertility was low, and any task tends to improve the obtained yields, as well as was proved in this research.

According to the influence of the age of cut on the yield of the quantity of oil it was observed (table 2, group V) that the highest yields were found when plants were cut being four months old. The atypical behavior T29 is exempted from this generalization, with an age of cut of three months after the sow (EC2). It is important to mention that Wijesekera (15) indicates, that the highest results in quantity of oil for *C. citratus* were obtained six months after the sow (in tropical conditions of Guatemala and Brazil). The obtained results in this research represent an advantage of two months in favor of production, if the first crop is done at four months, due to it implies that two cuts can be done in a year, because after have done the first cut each plant can be monthly sowed.

The results of treatments that are part of group V appear in table 3.

The best results are the correspondent to treatments T12, T21

Table 2. Formed groups for yield in quantity of the essential oil extracted from *C. citratus*, when applying the Friedman test of the non parametrical variance analysis for the blocks design.

Groups	Treatments								
I	T10	T16	T7	T1	T4	T25	T22		
II	T11	T8	T28	T9	T13	T34			
III	T14	T5	T31	T35	T17	T19	T23	T18	T26
IV	T20	T32	T2	T6					
V	T3	T27	T36	T15	T24	T29	T33	T21	T30	T12

(P> 0,05: Non significant)

Table 3. Yield in quantity of essential oil extracted from *C. citratus* for treatments that are part of the group V according to the statistical test of Friedman.

T	F	D	EC	P.F(g.)	A.E.(ml)	kg/ha	l/ha
T3	0	1	3	1527.31	5.52	3463.29	12.52
T12	1	1	3	2027.44	7.59	4597.38	17.21
T15	1	2	3	1539.17	6.30	3664.68	15.00
T21	2	1	3	2233.39	7.25	5064.38	16.43
T24	2	2	3	1579.86	6.17	3761.56	14.68
T27	2	3	3	1403.45	5.61	3508.62	14.03
T29	3	1	2	1557.45	6.21	3531.64	14.09
T30	3	1	3	1728.99	6.70	3920.61	15.19
T33	3	2	3	1729.84	6.26	4118.67	14.90
T36	3	3	3	1372.55	5.53	3431.38	13.83

T: Treatment, F: type of fertilization, D: type of density; EC: age of cut, P.F: fresh weight per hectare, A.E: essential oil extracted per sample. kg/ha: yield in fresh weight per hectare. L/ha: quantity of essential oil per hectare.

and T30. These findings agree to those obtained by Viturro *et al.* (14) with *C. citratus*, in relation that the highest yields of oil were obtained employing the sow density of 0.70 x 0.70 m and the age of cut that corresponds four months after the sow (EC3).

If the sow density is analyzed as a common factor among the three treatments with higher yields in quantity of essential oil, it can be inferred that with the highest density of sow, the highest yields were obtained, and this agrees to the reported by Ram *et al.* (9), about the yield of the essential oil obtained from *Artemisia annua* (plants with high oil content), because when increases the sow density also increases the yield of the oil extracted from this specie.

The average yield in percentage, based in the fresh weight of the vegetal material and in the quantity of extracted essential oil (0.40%), agrees to the values (0.44%) obtained by Viturro *et al.* (14) and to the interval (0.2 to 0.4%) reported by Wijesekera (15).

In table 4 the correlation that are between the biometrical variables of plants are presented, and these indicate that the number of stems, the number of leaves and height of the plant are correlated to the fresh weight and the quantity of essential oil; at the time that one of these increase also increases the yield in the produced fresh matter and the quantity of extracted oil.

These results agree to the reported by Ojeda *et al.* (4) and Domínguez (1), by the fact that when increases the aerial part of the plant, place where glands and the producer channel of oil are found, increases the

Table 4. Correlation matrix of Spearman for the biometrical description of *C. citratus* plants and the applied agriculture treatments.

	AP	D	EC	F	NH	NT	PF
AE	**0.5759	-0.1672	**0.6118	*0.2939	**0.7435	**0.7117	**0.9837
AP		0.0149	**0.4076	0.0958	**0.7648	**0.7268	**0.5904
D			0.0000	0.0000	0.0609	0.0708	-0.1259
EC				0.0000	**0.4889	**0.4376	**0.6580
F					0.1016	0.1552	**0.3212
NH						**0.9863	**0.7557
NT							**0.7243

AE: extracted essential oil (dependent variable). AP: high of the plant. D: sow density (independent variable). EC: age of cut (independent variable). F: type of fertilization (independent variable). NH: number of leaves. NT: number of stems. PF: fresh weight. **: Highly significant. *: Significant (trustable interval: 95%).

yield of these secondary metabolites.

Compounds already separated and identified by chromatography of gases with a detector of spectrometry of gases (CG-MS) are presented in table 5, where it is observed that the most abundant constituents, with quality for perfumery are the isomers Z-citral (32.81 -34.35%) and E-citral (40.95 - 43.16%); followed with b-mirceno (12.60 - 14.76%).

The quality for perfumery of the essential oil was fixed as was previously described. The obtained information of the addition of constituents (determined by their percentages of area), were analyzed applying a variance analysis for a block design of fixed effects and in unbalanced blocks, the ANOVA is shown in table 6.

The quality for perfumery was influenced by the interaction of factors, age of cut and the application of fertilization practices, as well as by the interactions between the age of cut and the density of sow, due to these present highly significant differences. (table 6, figures 2 and 3).

The anomalous behavior referred to the sudden fall of the quality of oil, when the age of cut two months after the sow interacts with the sow density of 0.70 x 1.00 m, can not be explained. Conversely, it is important to mention that the age of cut four months after the sow was the one that presented the highest quality of oil, independently to the type of density.

The sow density did not affect the quality of oil, because all treatments with different soil densities had similar quality (table 6), however, this result might be disguised by the sow density-age of cut interaction. This does not dismiss that the sow density affects the quality of oil, as well as happens in *Artemisia annua* (9), that when

Table 5. Main constituents of the essential oil of *C. citrates* in treatments with higher yields.

	Treatments					
	T12		T21		T30	
Constituents	TR(min)	A(%)	TR(min)	A(%)	TR(min)	A(%)
6-meti-5-hepten-2-ona	6.08	1.08	6.08	1.06	6.08	0.85
b mirceno	6.18	12.68	6.18	14.76	6.18	12.60
Linalool	9.19	1.10	9.19	1.07	9.19	0.29
Z-citral (neral)	13.69	34.35	13.68	32.81	13.68	33.25
Geraniol	14.07	4.74	14.07	5.06	14.07	5.39
E- citral (geranial)	14.65	42.16	14.64	40.95	14.65	43.16
Geranyl acetate	18.17	0.64	18.17	0.96	18.17	0.64

TR: time of retention. A%: percentage of area.

Table 6. Variance analysis for the dependent variable, quality of essential oil extracted from *C. citratus*

Source	GL	SC	Mean squares	Value of F	Pr > F
Density	2	7.9895789	3.9947895	0.32	0.7305
Age of cut	2	444.1055927	222.0527964	17.53	**<.0001
Fertility	3	276.4183012	92.1394337	7.28	**0.0003
Density x age of cut	4	300.4548578	75.1137145	5.93	**0.0004
Fertility x density	6	104.6222310	17.4370385	1.38	0.2361
Fertility x age of cut	6	375.2488255	62.5414709	4.94	**0.0003
Fertility x density x age of cut	11	214.8970851	19.5360986	1.54	0.1361

**: highly significant differences; GL: degrees of liberty; SC: addition of squares.

increased the sow density also increased the quality of oil.

The highest quality of oil was obtained with the age of cut, two months after the sow and with chemical fertilization; however, this response tends to reduce in the other ages of cut. This fact might be related to the high solubility of the applied fertilizer, thus it was rapidly absorbed and showed and immediate response, at the moment of running out the fertilizer through the time, values of the yield in quality of oil tend to reduce. Hence, it is recommendable to apply the fertilizer in a fractionated way to guarantee quality.

Values presented in the table of means in relation to the fertilization (table 7), establish two well defined groups, where the application of any type of fertilizers increases the quality of oil. These results, in the particular conditions of the research differ from those reported by Singh *et al.* (11), who express that the application of fertilizer does not affect the quality of oil of *Cymbopogon flexuosus*, other lemon grass specie with high production.

The quality of the essential oil extracted from lemon grass was affected by the age of cut; the highest yields were obtained with the cuts done four months after the sow. The high quality of oil at this age of cut (table 7) was higher than the obtained for *C. citratus* by Wijesekera, (15), who recommends to do the first cut passed six months.

Finally, the behavior of lemon grass crop reached its highest potential when some agriculture

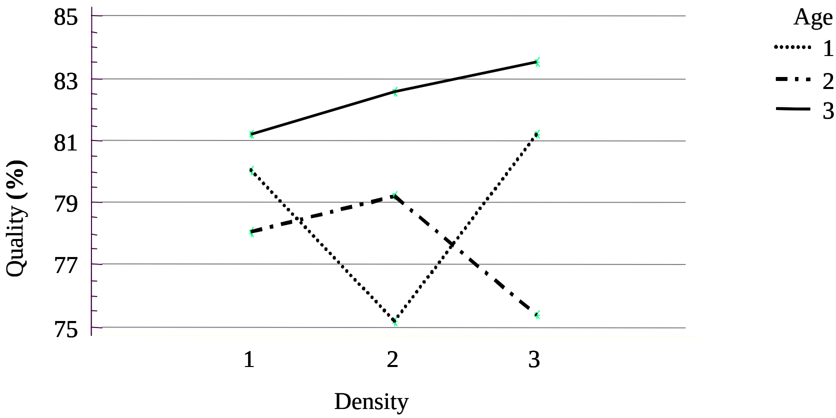

Figure 2. Quality of essential oil in function of the sow density and the age of cut of the aerial part of *C. citratus*

techniques as fertilization are applied, though when there were significant differences in relation to the quantity of extracted oil. It is not the same with the quality of it, due to two well differentiated groups were established, in which the best results (over 80.5% of quality for perfumery) were obtained, when any of treatments that included fertilization were applied, specially if the fertilizer of chemical origin was combined to the one of organic origin.

The age of plants at the moment

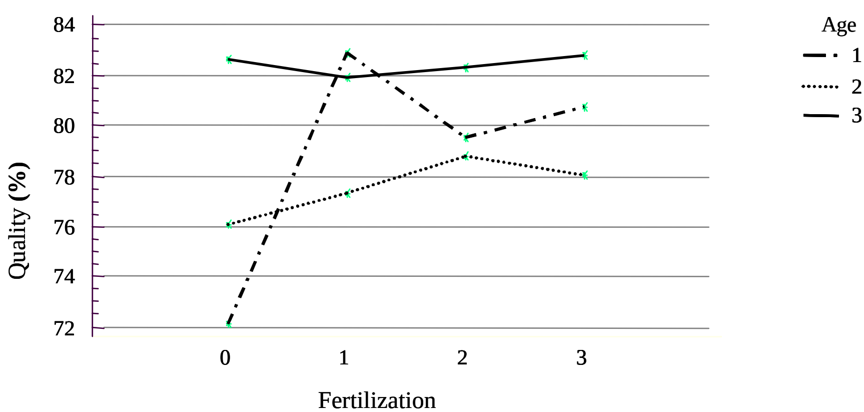

Figure 3. Quality of the essential oil in function of the fertilization and the age of cut.

Table 7. Means for the dependent variables, quality of the essential oil extracted from *C. citratus* in relation to the fertilization and age of cut.

	Fertilization				Age of cut		
	F0	F1	F2	F3	EC1	EC2	EC3
Calidad de aceite esencial (%)	76,9448	80,6859	80,8550	80,5433	79,1606	77,5486	82,4214
N	27	27	24	27	33	36	36

7: N: number of observations; mean: % components for perfumery.

of doing the first cut is also another important factor, due to it determines the quantity of extracted oil as well as its quality. For the previously described conditions when plants had its highest growth and therefore, a higher quantity of oil due to the high positive correlation of these variables and besides improved the quantity for perfumery.

On the other hand, the obtained results allow to conclude that the sow density as a factor, is also significant, because when increased the number of plants per smallholding, also increased the quantity of fresh matter by surface unit, hence a higher yield in the quantity of extracted oil, though the quality was not directly affected this variable.

The quality for perfumery of the extracted oil for the sowed lemon grass in this essay surpassed the international standards of quality, 80% in treatments response and 76% in those of lower response. It is important to mention that the agro-ecological conditions of the research were propitious for the crop of lemon grass, this fact indicates that the crop has a potential to be exploited in low fertility conditions, being a viable alternative of diversification for small, medium or big producers interested in producing aromatic plants for the extraction of essential oils.

Nowadays, the production of essential oils is very low and the national market of these products is covered by imports at very high costs. It is important to mention that the price of the essential oil of *C. citratus* is high (5 ml, 2 $ US) (13). If it is obtained an average yield of 17.21 l/ha of essential oil per cut, similar to the obtained in the best treatment of the research with approximately seven cuts per year, this might represent an important net income.

Conclusions

There is a high positive correlation between variables number of leaves per plant, number of stakes and height of the plant with the quantity of extracted essential oil, and the fresh weight of the sample. Therefore, if any of these parameters are measured, the quantity of oil that would be produced could be estimated.

The yield in oil (0.40%) in base of fresh weight is considered inside the normal parameters for the *C. citratus* specie, which was obtained when the cut of plants was done four months after the sow, and fertilizer was applied independently of its origin, hence it is considered that this practice is satisfactory for the crop.

The sow density did not statistically affect the quality of the extracted essential oil, however, when plants were sowed in a distance of 0.70 x 0.70 m months after the sow and with application of fertilizer, an increased in the quantity of extracted oil was deduced, this favors the final objective of the crops production, which is to obtain higher quantities of oil.

To obtain commercially acceptable yield and quality like those obtained in the agro-ecological conditions of this research (17.21 l/ha; 82.14% of citral), it is convenient to apply fertilizers of chemical origin doses equivalent to 37.5 kg of de P_2O_5, of 37.5 kg of K_2O and 37.5 kg of N; and/or organic matter in doses of 10 tons per hectare, in only one dose at the moment of the sow. Besides of doing the first cut four months after the sow.

The conditions of the investigation allowed to obtain *C. citratus* oil, with a high composition of isomers of citral (neral and geranial), of industrial application for perfumery.

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