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Producción de biomasa verde y calidad de aceites esenciales de palmarosa (*Cymbopogon martini* Roxb.) con aplicación de fertilizantes de síntesis y orgánicos

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Abstract

The use of mineral fertilizers in *Cymbopogon martini* Roxb. (Palmarosa) crops has been implemented in order to increase biomass and essential oil yield. Palmarosa oil is rich in geraniol and geranyl acetate, molecules with high value in the cosmetic and pharmaceutical industries. Plants were obtained from the Seed Bank of the Centro Nacional de Investigación Agroindustrial de Plantas Aromaticas y Medicinales de la Universidad Industrial de Santander (UIS) (Cenivam), Santander, Colombia. Seedlings were maintained under conditions of nursery for two months and then were taken to the experimental field, in which the survival rate in the new crop conditions was calculated. The crop was carried out in the Cenivam experimental area (07° 08' 31.68"N, 73° 07' 06.14W; 988 m.a.s.l.), in a sandy-soil clay loam. The aim of the study was to evaluate the biomass productivity and analyze the quality and yield of the essential oil obtained of the *C. martini* crop under three types of fertilizers: Nutrimon® 14-14-14, mineral NPK granular fertilizer for agricultural use for land application composed of ammoniacal nitrogen 10%, nitric nitrogen (4%), assimilable phosphorus (P_2O_5) (14%), and water soluble potassium (K_2O) (14%), for treatments, 2, 4 and 6 g/plant were applied locally; and a treatment with 400 g of organic fertilizer were applied. A complete random block design, with thirteen treatments and three harvests and each one, consisting of three plants as replications was used. Data were analyzed using analysis of variance, comparing the means by Tukey test at 5% probability. No correlations between biomass and essential oil yield, according to the type of fertilizer applied, were observed, that suggests that treatments that increased the biomass did not necessarily represent higher essential oil yield, and treatments whose essential oil yield increments were significant, not necessarily corresponded to the largest biomass. It was found that organic or green fertilizers could be ideal for these crops, as they allowed relatively high oil yields, with higher percentages of geraniol and its acetate.

Key words: Mineral fertilizer, organic fertilizer, green manure, essential oil.

Resumen

El uso de fertilizantes minerales en cultivos de *Cymbopogon martini* Roxb. (Palmarosa) con el fin de incrementar la biomasa y el rendimiento de aceite esencial (AE) es una práctica cada vez más frecuente. Este aceite es rico en geraniol y acetato de geranilo, moléculas con alto valor en las industrias de productos cosméticos y farmacéutica. Las plantas fueron obtenidas del Centro Nacional de Investigación Agroindustrial de Plantas Aromaticas y Medicinales de la Universidad Industrial de Santander (UIS) (Cenivam) (07° 08' 31.68"N, 73° 07' 06.14O; 988 m.s.n.m.), Santander, Colombia.

El objetivo del estudio fue evaluar la productividad de biomasa y analizar la calidad y el rendimiento de los aceites esenciales (AE) de este cultivo con la aplicación de tres tipos de fertilizantes químico: Nutrimon® 14-14-14, NPK granulado (10% N, 14% P_2O_5 , 14% K_2O), en dosis de 2, 4 y 6 g/planta, más un tratamiento con 400 g de un fertilizante orgánico,. Se utilizó un diseño de bloques completos al azar, con trece tratamientos y tres cosechas de tres plantas cada una como repeticiones. Los datos fueron sometidos a análisis de variancia con comparación de las medias por la prueba de Tukey a 5% de probabilidad. No se observaron correlaciones entre biomasa y rendimiento del AE en función del tipo del fertilizante aplicado, lo que sugirió que los tratamientos que incrementaron la producción de biomasa no necesariamente representaron mayor rendimiento del AE y viceversa. Se encontró que los fertilizantes orgánicos son ideales para este cultivo, ya que favorecieron rendimientos relativamente altos en aceite, con altos porcentajes de geraniol y acetato de geranilo.

Palabras clave: Fertilizante mineral, fertilizante orgánico, fertilizante verde, aceite esencial.

Introduction

Cymbopogon martini (Roxb.) Wats. var. motia, known as palmarosa, is a perennial herb, widely distributed in tropical and subtropical regions (Khanuja *et al.*, 2005). It contains essential oil, whose main components are geraniol and geranyl acetate (Khanuja *et al.*, 2005). The essential oil from *C. martini* is widely used as a flavoring for food, beverages and snuff products, as a valuable component for perfumes, cosmetic and pharmaceutical products, and against the action of various bacteria, fungi and microorganisms (Rajeswara *et al.*, 2009, Teixeira *et al.,* 2005). Due to these applications, essential oil demand in the domestic and international market has increased, what has stimulated its cultivation. In this work, have implemented the use of mineral fertilizers in order to increase biomass and essential oil yield, which can generate higher profits. in Colombia, these studies are carried out to optimize the agronomic management of *C. martini* and to establish lines of action for the technical development of the crop. For this purpose, it is important to consider the regional soil and climate conditions, crop environment, harvest time, plant maturity, degree of wilting and even, the distillation process variables, since all the above-mentioned conditions affect both the quantity and quality of the essential oil obtained (Joy *et al.*, 2001). The fertilizer type is a variable that must also be considered, since the excess or deficiency of nutrients is directly related to the essential oil yield. The idea was to eliminate completely the need for any type of mineral inputs, in order to increase the *C. martini* essential oil yield, when this essential oil is a final product for human consumption. For this reason, in the present study, was evaluated the effect of fertilizer types in the *C. martini* crop and related the biomass production to the quantity and quality of the essential oil obtained.

Materials and methods

Cymbopogon martini plants were obtained from the seed bank of the National Research Centre for Agro-industrialization of Aromatic and Medicinal Tropical Plants (Cenivam) - Industrial University of Santander (UIS), Santander, Colombia. Seedlings were maintained under conditions of nursery for two months and then were taken to the experimental field, in which the survival rate in the new crop conditions was calculated. The crop was carried out in the Cenivam experimental area (07° 08’ 31.68” N, 73° 07’ 06.14" W; 988 m.a.s.l.) in a sandy-soil clay loam (16% silt, 20% clay and 64% sand), whose physical and chemical properties were as follows: pH 7.64, 12.2% moisture, 80.5% ashes, 0.17 oxidable total organic carbon (%C), 0.8% N, 0.21 ratio C/N, 0.15 P (%P_2O_5), 0.46 Ca (%CaO), 0.001% Cu, 0.50 Mg (%MgO), 0.84 K (%K_2O), 0.50% Na, 2.53% Fe, 0.05% Zn, 0.03% Mn. The land was divided into 13 plots of 7 x 6 m each, for an area of 42 m^2 / plot. For agricultural purposes, seven ridges were performed with a width of 0.70 m and spacing of 0.30 m. In each ridge, two rows of *C. martini* plants were designed with a system of triangular outline with 0.30 m between plants, for a total of 36 plants/ridge and a density of 252 plants/plot.

The following fertilizers were used: Nutrimon® 14-14-14, mineral NPK granular fertilizer for agricultural use for land application composed of ammoniacal nitrogen 10%, nitric nitrogen (4%), assimilable phosphorus (P_2O_5) (14%), and water soluble potassium (K_2O) (14%). For treatments, 2, 4 and 6 g/plant were applied locally, according to the dose recommended by Prakasa *et al.* (2001) and Rajeswara (2001), both at the time of planting, and after each harvest. Organic fertilizer was a composted mixture obtained from aromatic plants processed by microwave-assisted hydrodistillation and enriched with black soil, tanned bovine manure, magnesium sulfate, zinc sulfate, manganese sulfate, and borax according to the technical criteria of the Colombian Agricultural Institute - ICA, and with some enhancements at Cenivam, with the physicochemical properties as follows: pH 8.06, 40.8% moisture, 36.1% ashes, 14.2 oxidable total organic carbon (%C), 1.26% N, 0.52 P (%P_2O_5), 1.94 Ca (%CaO), 0.001% Cu, 0.43 Mg (%MgO), 1.1 K (%K_2O), 0.051% Na, 0.74% Fe, 0.005% Zn, 0.012% Mn, 0.14% S. For treatments, 400 g of organic fertilizer were applied at the dose recommended by Blank *et al.* (2007), localized in the area intercepted by the roots of the plant at the time of planting; green manure: incorporating local plant mass and bean plants with soil crushed into small pieces and mixed with soil of the corresponding experimental plot. The same soil was inoculated with commercial mycorrhizae Micorrizafer®, in order to enrich the soil microbiota and facilitate the availability of nitrogen and phosphorus to the crop. The treatments were as follows: treatment 1 = control (no fertilizer); treatment 2 = NPK, 2 g; treatment 3 = NPK, 4 g; treatment 4= NPK, 6 g; treatment 5 =NPK, 2 g + organic fertilizer; treatment 6 = NPK, 4 g + organic fertilizer; treatment 7 = NPK, 6 g + organic fertilizer; treatment 8 = organic fertilizer; treatment 9 = organic fertilizer + green manure; treatment 10:= green manure + NPK, 2 g; treatment 11: = green manure + NPK, 4 g; treatment 12 = green manure + NPK, 6 g; treatment 13 = green manure. The planting was conducted between October 2011 and March 2012, contemplating the rainiest seasons, October (average precipitation of 133 mm, average temperature of 22.5 °C); and the driest seasons, December (average precipitation of 73 mm, average temperature of 22.7 °C), of the region.

Three harvests of the aerial parts of the *C. martini* plants were performed, always in a vegetative state, with an interval of three months between them, contemplating rainy and dry seasons of the year. The harvests were conducted between 9:00 and 10:00 h, as recommended by Singh and Sharma (2001), who suggest that in the morning, the highest concentration of essential oil of *C. citratus* plant was obtained, because higher temperatures before noon, volatilize essential oil components, what affects its yield. Previous to steam distillation (multimode EQ21 essential oil distiller, 0.4 m^3, 80 psi vapor pressure), the plant material was chopped to 0.5 cm (Penagos® PE-800) and fresh mass was used to calculate the essential oil yield, using the following equation: *Yield(%) = (essential oil mass kg/harvest fresh mass kg) x 100.* The plant material was distilled for 2 h. In addition, three samples of 253 ± 3.0 g chopped plant material were taken for extraction of essential oil, using the microwave-assisted hydrodistilation (MWHD)(Stashenko *et al.*, 2004), and the yield was calculated similarly. The plant material was distilled three times for 20 minutes. The essential oil chemical composition, in terms of geraniol and geranyl acetate, was obtained by gas chromatography - mass spectrometry (GC-MS, Agilent Technologies 6890 Plus, Agilent Technologies MSD 5973 mass selective detector), split ratio (1:50); Agilent 7863 autosampler was used, HP-MS ChemStation G17001DA data system, D.00.01.27 version, 2002, including different mass spectra and retention indices databases WILEY 138K, NIST 2002 and ADAMS 2004).

A randomized complete block design was used with thirteen treatments and three harvests in the aerial part of the crop that formed the blocks, and at each harvest, three plants were used as replicates. Data were statistically analyzed using analysis of variance and means were compared by Tukey test at 5% probability. The correlation between biomass and production of essential oil was determined and the coefficient of variation (C.V.) each variable was calculated.

Results and discussion

Experimental conditions for *C. martini* crop recorded a survival rate of 74 ± 6.0% for all treatments, except for the green manure one (59 ± 4.0%). The highest rate of mortality was observed at the beginning of planting, when the plants were adapting to the new conditions, both the soil and the environment. In this regard, Singh and Sharma (2001) reported that *C. martini* grows relatively well in soils with neutral or moderately alkaline pH, with good water requirement and temperatures between 20 and 25 ^{o}C. Because the excess or deficit of water can affect the plant growth and, consequently, the essential oil yield (Singh and Sharma, 2001), the irrigation system adopted for the experimental crop was by sprinkling, operated when the soil environment merited it and considering that the field capacity of a sandy-clay loam soil is relatively high. The furrows designed allow quick drainage, preventing flooding of the plantation. It was found that with this type of drainage, the plants suffered no visible hydric stress, either deficit or excess of water, and growth and development proceeded normally during three months, time after which, the aerial part was harvested. Several studies have indicated the benefit of the nitrogen fertilizer application to increase biomass and essential oil yield in *C. martini* crops. However, the results have not been conclusive that there is indeed a direct relationship between the biomass increase in response to fertilizer application and the essential oil yield increase (Prakasa *et al.*, 2001; Rajeswara, 2001; Singh and Sharma, 2001). Furthermore, it is considered that prolonged use of nitrogen fertilizers reduces soil organic matter, leading to its degradation and causing environmental pollution problems (Rajeswara, 2001).

For the cultivation conditions employed, biomass and essential oil yield showed weak positive correlation (Pearson correlation coefficient = 0.401), suggesting that treatments that increased the vegetal mass in the *C. martini* crop did not necessarily represented the largest increase in the essential oil yield obtained, and, in turn, treatments whose essential oil yield increments were significant, not necessarily correspond to those with higher biomass. This could be observed in the biomass obtained under the 2nd harvest treatment NPK 6 + organic and 2nd and 3rd harvest of the treatment green manure + NPK 6, in which, no direct relationship was observed with the essential oil yield obtained (Figures 1 and 2). Between harvests obtained with the same treatment, increased biomass and essential oil yield, for some cases, were directly related (v. gr. 2nd harvest NPK4, 2nd and 3rd harvest NPK2 + organic, 3rd harvest NPK4 + organic, 3rd harvest organic, 3rd harvest green manure + organic, 3rd harvest green manure + NPK2, 3rd harvest green manure + NPK4, and 3rd harvest green manure + NPK6) (Figures 1 and 2). If a crop with a high essential oil yield, less biomass and without application of mineral fertilizers is intended, the application of organic fertilizer or green manure might be ideal because relatively high essential oil yield was observed in the present study for the 3rd organic harvest (0.25%) and 2nd green manure harvest (0.25%) (Figures 1 and 2). For the 2nd and 3rd harvests of control, relatively high essential oil yields (0.3 and 0.24%, respectively) (Figure 2) were also obtained. However, plant in a soil without fertilizer, will suffer a decrease in mineral supply and with time will succumb for nutritional detriment, losing vigor in growth and development, and therefore, a significant decrease in the essential oil yield will ensue. As for green manure, it was observed that, when supplemented with mineral fertilizer, it provided a better availability of nutrients for the next harvest, as was observed in the 2nd to the 3rd green manure harvests treatments, whose addition of NPK recovered the soil nutrients that were utilized in the previous harvest (Figures 1 and 2).

It is emphasized that in the green manure + organic and green manure treatments, the essential oil yield, obtained by microwave-assisted hydrodistilation (MWHD), were significant (Figure 3). However, Rodríguez *et al.* (2012) obtained 1.2% yield, using 300 g of fresh leaves, and commented that the oil yield was equal to that obtained in India

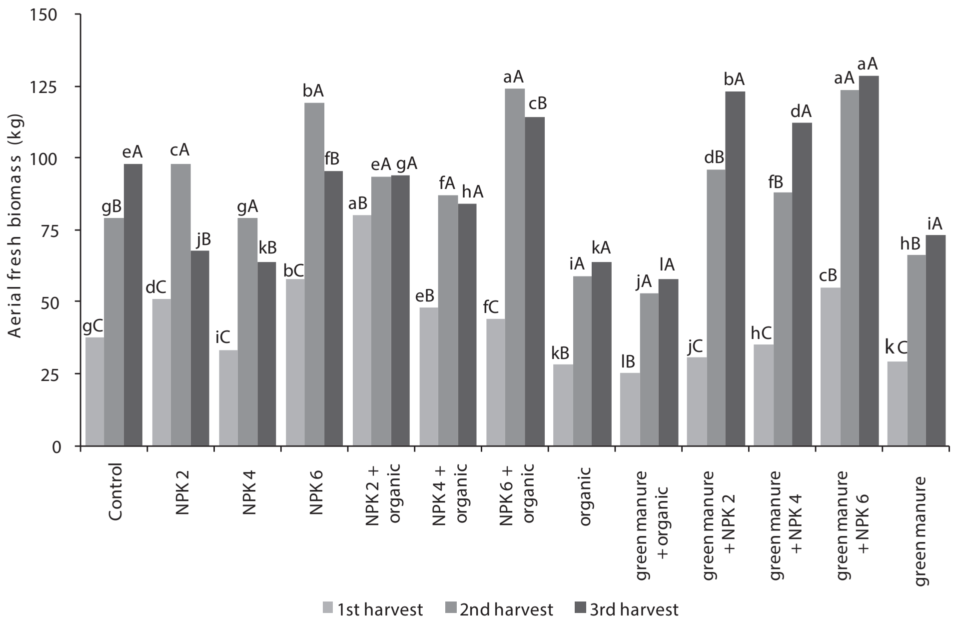

Figure 1. Aerial fresh biomass (kg) in three *Cymbopogon martini* Roxb. harvests subjected to treatments with three types of fertilizers. Lowercase letters compare the means of each harvest between treatments. Uppercase letters compare means between harvests each treatment (Tukey P ≤ 0.05). 1st harvest, C.V. = 36.6%; 2nd harvest, C.V. = 25.8%; 3rd harvest, C.V. = 26.6%.

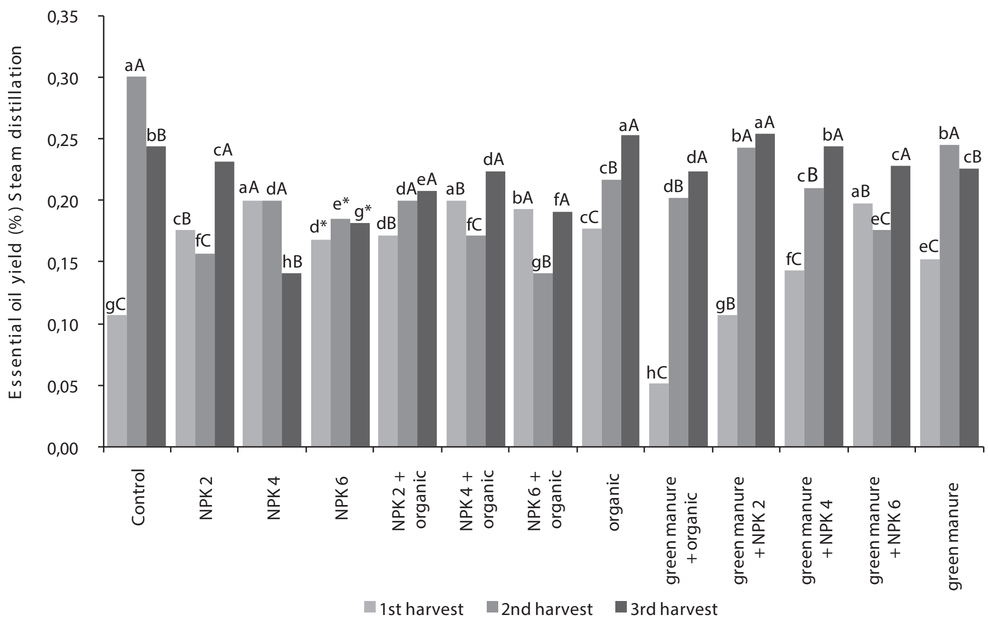

Figure 2. Essential oil yields (%) in three *Cymbopogon martini* Roxb. harvests subjected to treatments with three types of fertilizers. Steam distillation. Lowercase letters compare the means of each harvest between treatments. Uppercase letters compare means between harvests under each treatment. (*) not significant. (Tukey P ≤ 0.05). 1st harvest, C.V. = 28.6%; 2nd harvest, C.V. = 20.6%; 3rd harvest, C.V. = 14.6%.

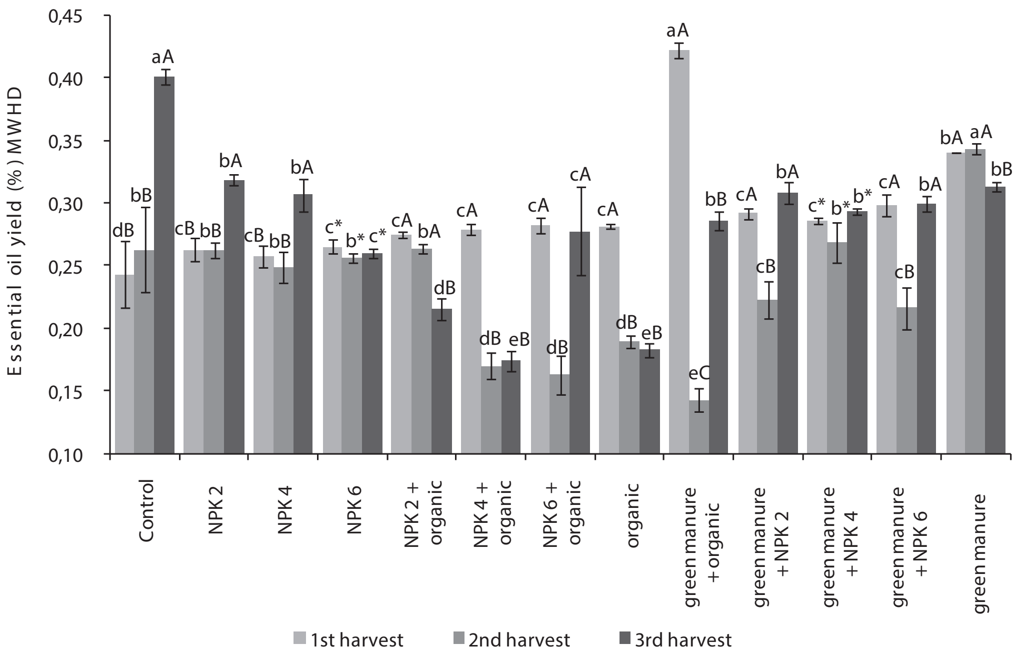

Figure 3. Essential oil yields (%) in three *Cymbopogon martini* Roxb. harvests subjected to treatments with three types of fertilizers. Microwave-assisted hydrodistilation (MWHD). Lowercase letters compare the means of each harvest between treatments. Uppercase letters compare means between harvests under each treatment. (*) not significant. (Tukey P ≤ 0.05). 1st harvest, C.V. = 15.7%; 2nd harvest, C.V. = 23.7%; 3rd harvest, C.V. = 21.8%.

(Khanuja *et al.*, 2005). It is considered that the *C. martini* essential oil yield may vary from 0.1 to 1.2% with the application of mineral or organic fertilizers (Joy *et al.*, 2001; Rajeswara 2001; Singh and Sharma, 2001; Teixeira *et al.*, 2005). According to Rajeswara *et al.* (2009), the stems of *C. martini* do not add essential oil and it is for this reason that in this study, stems were chopped and mixed with leaves of *C. martini* fresh material, which were distilled (MWHD), this contributed to the increase in plant mass, but decreased the essential oil yield.

The *C. martini* essential oil composition is characterized by high contents of geraniol (63 - 80%) and geranyl acetate (8 - 28%) (Khanuja *et al.*, 2005; Teixeira *et al.*, 2005). In their study, Joy *et al.* (2001), discovered in *C. martini* the presence of geraniol (95%) and geranyl acetate (5.7%), which characterize this specie as the best natural source of these compounds. In the present study, relatively high percentages of geraniol were obtained (2nd harvest organic and 3rd harvest NPK6 + organic, 84%; 2nd harvest NPK2, 83%, and 1st harvest NPK6, 82%, respectively) (Table 1).

Rodríguez *et al.* (2012) reported 69.6% for geraniol and 15.6% for geranyl acetate; in India, (Khanuja *et al.*, 2005; Rajeswara, 2001), 73 - 76% for geraniol was reported, and in Brazil (Teixeira *et al.*, 2005), 63.5%. In the present study, the high percentages of geranyl acetate were obtained from the first harvests with organic (33%), green manure + organic (31%), green manure + NPK2 (32%), green manure + NPK4 (30%), and green manure (32%), what corroborates the advantages of using organic or green manure for *C. martini* crops.

With organic or green manure, using fertilizer plant and/or animal origin, soil organic matter enhanced, providing macro and micronutrients for plants and correcting soil toxicity; this improves and determines the physicochemical and biological characteristics of the soil.

Table 1. Geraniol and geranyl acetate relative amounts (%), obtained from *Cymbopogon martini* Roxb. subjected to treatments with three types of fertilizers.

	Geraniol %			Geranyl acetate %		
Treatments	**1st harvest**	**2nd harvest**	**3rd harvest**	**1st harvest**	**2nd harvest**	**3rd harvest**
Control	73 fB	79 dA	78 hA	27 gA	21 cB	22 cB
NPK 2	78 bB	83 aA	79 eB	22 lA	17 eB	21 eA
NPK 4	75 dB	77 fB	80 dA	25 jA	23 bA	20 fB
NPK 6	82 aA	77 fB	83 bA	18 mB	23 bA	17 hB
NPK 2 + organic	77 c*	78 e*	79 f*	23 k*	22 c*	21 d*
NPK 4 + organic	71 gC	80 cA	77 iB	29 fA	20 dC	23 bB
NPK 6 + organic	74 eC	81 bB	84 aA	26 hA	19 dB	16 iC
organic	67 lC	83 aA	78 gB	33 aA	17 eC	22 cB
green manure + organic	69 iB	81 bA	81 cA	31 dA	19 dB	19 gB
green manure + NPK 2	68 jB	80 cA	80 dA	32 cA	20 dB	20 fB
green manure + NPK 4	70 hB	81 bA	81 cA	30 eA	19 dB	19 gB
green manure + NPK 6	75 dB	80 cA	80 dA	25 iA	20 dB	20 fB
green manure	68 kB	71 gA	71 jA	32 bA	29 aB	29 aB
C.V. (%)	6.1	4.0	3.8	16.3	15.3	14.5

Lowercase letters compare the means of each harvest between treatments. Uppercase letters compare means between harvests under each treatment. (*) not significant. (Tukey P ≤ 0.05). C.V. geraniol = 6.1%; C.V. geranyl acetate = 20.7%.

Conclusion

- Aerial fresh biomass and the essential oil yields obtained with the application of organic or green manure was not significantly different to those obtained with the mineral fertilizer.
- With organic or green manure best results were obtained for both the oil yield and quality, in terms of geraniol and geranyl acetate relative amounts.

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