SOIL FERTILITY AND ESSENTIAL OIL PRODUCTION IN CITRONELLA CULTIVATION IRRIGATED WITH DAIRY CATTLE WASTEWATER (DCW) †

[FERTILIDAD DEL SUELO Y PRODUCCIÓN DE ACEITES ESENCIALES EN EL CULTIVO DE CITRONELLA RIEGO CON AGUAS RESIDUALES DE GANADO LECHERO (DCW)]

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SUMMARY

Background. Due to the negative impacts caused by the inadequate disposal of dairy cattle wastewater (DCW), alternatives are needed to reduce the environmental impact. **Objective.** The aim of this study was to evaluate the effects of applying DCW via fertigation on the physical and chemical characteristics of the soil and on the production of citronella essential oil (*Cymbopogon nardus* (L.) Rendle). **Methodology.** Nitrogen (N) was adopted as a reference element and DCW equivalent amount to be applied so as to replace this element was calculated. Doses equivalent to 100%, 200%, 300% and 400% of the recommended N concentration were applied. After six months of cultivation, the availability of nutrients for the soil and its relationship to the production of essential oil were evaluated. **Results.** The results showed that DCW supply for citronella cultivation was effective and, in most parameters, equivalent to the treatment with mineral fertilization. Besides, there was no significant difference between the treatments, regarding the quantity and quality of essential oil production. **Implications**: DCW does not increase the yield of essential oil, but it is an excellent alternative for the supply of nutrients in degraded soils. **Conclusions.** Therefore, DCW use for fertigation of citronella cultivation is a valid alternative, helping to mitigate the impacts related to its disposal. However, new studies with a longer evaluation period and other types of soil are suggested.
Keywords: citronelal; citronelol; *Cymbopogon nardus* L.; fertigation; nitrogen fertilization.

RESUMEN

Antecedentes: Debido a los impactos negativos causados por la disposición inadecuada de las aguas residuales del ganado lechero (DCW), se necesitan alternativas para reducir el impacto ambiental. **Objetivo:** El objetivo de este estudio fue evaluar los efectos de la aplicación de DCW vía fertirrigación sobre las características físicas y químicas del suelo y sobre la producción de aceite esencial de citronela (*Cymbopogon nardus* (L.) Rendle). **Metodología:** Se adoptó el nitrógeno (N) como elemento de referencia y se calculó la cantidad equivalente de DCW a aplicar para reemplazar este elemento. Se aplicaron dosis equivalentes al 100%, 200%, 300% y 400% de la concentración de N recomendada. Luego de seis meses de cultivo, se evaluó la disponibilidad de nutrientes para el suelo y su relación con la producción de aceite esencial. **Resultados:** Los resultados mostraron que el suministro de DCW para el cultivo de citronela fue efectivo y, en la mayoría de los parámetros, equivalente al tratamiento con fertilización mineral. Además, no hubo diferencia significativa entre los tratamientos, en cuanto a la cantidad y calidad de producción de aceite esencial. **Implicaciones**: DCW no aumenta el rendimiento de aceite esencial, pero es una excelente alternativa para el aporte de nutrientes en suelos degradados. **Conclusiones:** Por tanto, el uso de DCW para fertirrigación del cultivo de citronela es una alternativa válida, que ayuda a mitigar los impactos relacionados con su eliminación. Sin embargo, se sugieren nuevos estudios con un período de evaluación más largo y otros tipos de suelo.
Palabras clave: citronelal; citronelol; *Cymbopogon nardus* L.; fertirrigación; fertilización con nitrógeno.

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INTRODUCTION

Wastewater discharges into water bodies are a major cause of water pollution, contributing to the increase in biochemical oxygen demand (BOD) and consequently causing negative impacts to aquatic ecosystems (Morrison *et al*., 2001). In relation to the soil-plant system, when agronomic and environmental criteria are not considered at the moment of wastewater disposal, it can cause problems such as toxicity to plants and contamination of both soil and water (surface and underground). However, if done properly, fertigation with wastewater is beneficial, as a source of water and nutrients for plants, and consequently reducing the use of fertilizers and the polluting impact (Fonseca et al., 2007; Erthal *et al*., 2010).

In this context, the search for alternatives to reduce environmental impact related to existing activities, as well as the search for other forms of income generation, become fundamental to boost production, reducing the economic and environmental fragility related to properties, particularly small ones. Within this scope, among many typical activities of family farming, dairy cattle is present in most properties, throughout Brazilian national territory, but it is also a great generator of waste, especially due to the huge amount of solid and liquid waste produced daily. Therefore, several studies aim to mitigate the impact caused by dairy cattle wastewater (DCW).

It should also be highlighted that one of the markets that has become viable for small producers is the medicinal or aromatic plants production, particularly citronella grass (*Cymbopogon nardus* (L.) Rendle), due to its rusticity and its ability to adapt to different types of soils and climates (Sivashanmugam *et al*., 2009; Faria *et al*., 2018; Zhang *et al*., 2019), besides presenting economic value related to the essential oil extracted from it (Perini *et al*., 2011). Thus, the interest and development of studies about it have been highlighted (Castro *et al*., 2007; 2010).

Citronella grass has volatile substances in its leaves, such as β-citronelal, β-citronelol, eugenol, geraniol and limonene, among others, generally called monoterpenes (Seixas *et al*., 2011; Kpoviessi *et al*. 2014). These substances act as a chemical defense of the plant against predators (Castro *et al*., 2007). Citronellal is used as a basic material for the synthesis of important chemical compounds (Furlan *et al*., 2010; Perini *et al*., 2011). Essential oils have a complex chemical composition, especially the presence of terpenes and phenylpropanoids. These substances are volatile and are mainly contained in citronella leaves, and are related to several survival functions, such as defense against biotic stresses (Oliveira *et al*., 2011).

Citronella essential oil is of economic interest due to substances it contains which have biological activities, such as insecticide (Hernandez-Lambraño *et al*., 2015; Smitha and Rana, 2015), acaricide (Agnolin *et al*., 2010) fungicide (Seixas *et al*., 2011; Veloso *et al*., 2012) and bactericide (Silva *et al*., 2010; Nascimento *et al*., 2011), besides being used in the form of tea as a tranquilizer and digestive (Castro *et al*., 2010; Kpoviessi *et al*., 2014) and in cosmetics and flavorings.

Citronella grass cultivation can become an alternative for diversification and use of areas within the family farmer’s property, including those with less fertility, due to its rusticity. In addition, the use of DCW fertigation can be an alternative for reducing the acquisition of external inputs and the production seasonality throughout the year, promoting, within an agroecological premise, the conservation of natural resources and greater sustainability of productive agroecosystems (Primavesi, 1984; Nodari & Guerra, 2015; Oliveira *et al*., 2019).

Thus, in order to propose an alternative use of DCW that reduces the environmental impact generated by the inadequate disposal of residues from dairy production and also to evaluate a production alternative that can be developed by small agricultural properties, this study aimed to assess the effects of DCW application, via fertigation, on the physical and chemical characteristics of the soil and on the production of citronella essential oil.

MATERIAL AND METHODS

The experiment was carried out between March and November 2017 (autumn to spring), in the experimental area Banco Ativo de Germoplasma, at the Campus Gragoatá, at Universidade Federal Fluminense – Niterói, Rio de Janeiro, Brazil, whose coordinates are latitude 22º54'00 ”S, longitude 43º08'00” W and altitude 8 m. Climatically the region is classified as “Aw” climate, according to Köppen, i.e., tropical climate with dry winter and rainy summer, with an average annual temperature of 23ºC and an average annual rainfall of 1,200 mm.

The citronella grass seedlings *Cymbopogon nardus* (L.) Rendle, that belong to the Poaceae family, with approximately 20cm high, were acquired in the Centro de Abastecimento do Estado da Guanabara (Cadeg – Rio de Janeiro) and remained in the greenhouse for one month to be acclimatized to light conditions, relative humidity and local temperature. Before transplanting to 18L polyethylene pots, pruning was performed on the aerial part to standardize the plants. To ensure good drainage, 2 to 3 cm of gravel 0 (zero) was placed at the bottom of the pots and they were covered with a geotextile blanket and the rest filled with soil (25 kg of soil per pot). 12 pots were used per treatment, with one plant per pot.

The physicochemical characterization of the soil used in the pots was carried in Soil Analysis Laboratory according to methods described by Embrapa ("Empresa Brasileira de Pesquisa Agropecuária" - Brazilian Agricultural Research Company) (1997).

Soil analysis

The result of the physicochemical analysis (done before the experiment and also after) showed that the soil before the experiment, was acidic, dystrophic, of low fertility and sandy texture, compatible with a degraded soil. The levels of phosphorus (P) and potassium (K) were low (P < 10 and K < 45 $mg.dm^{-3}$, respectively). The high level of aluminum and the low levels of calcium and magnesium (Al > 0,3 and Ca + Mg < 2 $cmol.dm^{-3}$) indicate the need for liming (Embrapa, 2013). Thus, correction and fertilization were indispensable for the development of this cultivation in this soil.

Chemical characterization of the soil used in the experiment, with its values: Hydrogenionic potential (pH): 4.2 in water, K: 14.0 $mg.dm^{-3}$, P: 9.45 $mg.dm^{-3}$, Ca: 0.40 $cmol.dm^{-3}$, Mg: 0.10 $cmol.dm^{-3}$, Al: 0.90 $cmol.dm^{-3}$, H+Al: 0.54 $cmol.dm^{-3}$, Sum of exchangeable bases (SB): 0.54 $cmol.dm^{-3}$, Effective Cation Exchange Capacity (t): 1.44 $cmol.dm^{-3}$, T - Cation Exchange Capacity at pH 7.0 (T): 2.62 $cmol.dm^{-3}$, Base Saturation Index (V) 20.4%, Aluminum Saturation Index (m): 62.50%, Organic Matter (MO): 1.41 $dag.kg^{-1}$, Remaining Phosphorous (P-rem): 35.09 $mg.l^{-1}$, Zn: 1.18 $mg.dm^{-3}$, Fe: 69.08 $mg.dm^{-3}$, Mn: 1.30 $mg.dm^{-3}$, Cu: 0.04 $mg.dm^{-3}$, B: 0.13 $mg.dm^{-3}$, S: 8.64 $mg.dm^{-3}$. Physical characterization of the soil used in the experiment, with its values: Clay: 9 $dag.kg^{-1}$, Silt: 1 $dag.kg^{-1}$, Sand: 90 $dag.kg^{-1}$, Soil classification: Sandy texture.

Thus, 85.0% of dolomitic agricultural limestone Total Neutralization Relative Power (TNRP), in the proportion of $210g{\cdot}m^{-2}$, was applied to all the soil used in the experiment to correct acidity and add magnesium. A homogenization was carried out manually in a container with the soil, which was later placed in the pots, where it remained for 45 days before transplanting. To ensure good drainage, 3cm of gravel 0 (zero) was placed at the bottom of the pots and covered with a geotextile fabric.

On the day the seedlings were transplanted into the pots, phosphorus and potassium were fertilized with the application of K_2O (potassium chloride), content of 60% in the concentration of $10g.plant^{-1}$, and P_2O5 (simple superphosphate), content of 18% in the concentration of $37g{\cdot}plant^{-1}$, respectively, following the results of the soil analysis and the recommendations according to Embrapa (2013), in order to guarantee macronutrients, supply in all treatments.

The pots were arranged in the greenhouse, in an area of 112 m^2 and covered with agricultural plastic of 150 µm, in addition to shading mesh with a 50% level of shading on the sides and roof, with spacing of 1.0 m between lines and 0.50 m between pots.

DCW Characterization

The parameters involved in the DCW characterization, according to the ABNT ("Associação Brasileira de Normas Técnicas" - Brazilian Association of Technical Standards) NBR: 1986 method, the DCW chemical characterization used in the experiment: pH: 6.51 upH, Conductivity (Cond.): 14.00, Total Solids: 9.45 $mg .l^{-1}$, Total N: 0.40 $mg.l^{-1}$, Organic Nitrogen: 0.10 ($mg.l^{-1}$), Chemical Oxygen Demand (COD): 0.90 $mg.l^{-1}$, Biochemical Oxygen Demand (BOD): 2.08 $mg.l^{-1}$, Total Phosphorus: 0.54 $mg.l^{-1}$, Total Zinc: 1.44 $mg.l^{-1}$, Total Iron: 2.62 $mg.l^{-1}$, Total Copper (value lower than the detection limit of the used method): 20.45 $mg.l^{-1}$, Calcium: 62.50 $mg.l^{-1}$, Potassium: 1.41 $mg.l^{-1}$, Mg: 35.09 $mg.l^{-1}$, Sodium: 1.18 $mg.l^{-1}$, Fe: 69.08 $mg.l^{-1}$, Mn: 1.30 $mg.l^{-1}$, Cu: 0.04 $mg.l^{-1}$, B: 0.13 $mg.l^{-1}$, S: 8.64 $mg.l^{-1}$.

The first DCW application was performed 79 days after transplanting (DAT) of the seedlings and, from then on, once a week. The DCW used in this experiment was prepared from fresh material (cattle feces and urine) collected after the scraping of a privately owned corral. To maintain the conditions presented by Erthal *et al*. (2010) and with the concern of having a final compatible volume to be applied, 70% water without chlorine was mixed with 30% fresh manure to prepare the DCW used in the experiment.

The calculation for determining the DCW volume equivalent to the recommended Nitrogen concentration for citronella cultivation was according to Matos (2006), in addition to the reference of the Nitrogen concentration required for citronella cultivation (SBCS, 2004), according to Equation 1:

$$TA_{AR} = 1000 \cdot \frac{\left[N_{abs} - \left(T_{m1} \cdot MO \cdot \rho \cdot 10^{7} \cdot 0{,}05 \cdot \frac{n}{12} \right) \right]}{\left[T_{m2} \cdot N_{org} + \left(N_{amoniacal} + N_{nitrato} \right) TR \right]} \tag{1}$$

In which:

TA_{AR} = annual application rate, $m^3 \cdot ha^{-1}$;
N_{abs} = nitrogen absorption by the cultivation to obtain the desired productivity, $kg \cdot ha^{-1}$;
T_{m1} = annual rate of organic matter mineralization previously existing in the soil, $kg \cdot kg^{-1}$;
MO = soil organic matter content, $kg \cdot kg^{-1}$;
ρ = soil specific mass, $ton \cdot m^{-3}$;
n = number of months of cultivation;
T_{m2} = annual rate of organic nitrogen mineralization, $kg \cdot kg^{-1}.year^{-1}$;
N_{org} = organic nitrogen available by the applied residue, $mg \cdot L^{-1}$;
$N_{amoniacal}$ = ammoniacal nitrogen available by the applied residue, $mg \cdot L^{-1}$;
$N_{nitrato}$ = nitric nitrogen available by the applied residue, $mg \cdot l^{-1}$; e
TR = recovery rate of mineral nitrogen by the cultivation, $kg \cdot kg^{-1}.year^{-1}$.

The applied treatments are shown in Table 1.

Table 1. Treatments evaluated in the experiment.

Treatment	Dose (%)
T1	100% of the recommended N concentration provided via mineral fertilization
T2	100% of the recommended N concentration provided via DCW fertigation
T3	200% of the recommended N concentration provided via DCW fertigation
T4	300% of the recommended N concentration provided via DCW fertigation
T5	400% of the recommended N concentration provided via DCW fertigation

The application percentages of DCW volume were defined from the calculated volume (T2). For all treatments, complementary mineral fertilization of phosphorus and potassium were applied. The N concentration applied was 80 $kg \cdot ha^{-1}$ or 5 $g \cdot plant^{-1}$. As the urea content (the Nitrogen source used) was 45%, the final total was 11 $g \cdot plant^{-1}$, divided into two applications: the first at 30 DAT and the second at 90 DAT.

The application of different DCW dosages in the soil was done manually, using graduated cylinders with different volume graduations to differentiate treatments, i.e., the irrigation system was not used for DCW application, as, in addition to the potential clogging of the sprinklers, the system was set up so that water application was identical for all plants.

The crop irrigation was carried out by a localized irrigation system using drippers. The system consists of: motor-pump set; manometer; PVC pipes (suction line, discharge, main and bypass lines); 16 mm diameter polyethylene hose; drippers with an operating pressure of 10 mH_2O and a flow of 4 $L \cdot h^{-1}$, which were integrated in the 0.50 m spacing, with only one sprinkler per plant.

Before it was used in the experiment, an evaluation of the water distribution uniformity of the installed irrigation system was carried out. For that, the Christiansen test (1942) was applied, according to which the four lateral lines must be evaluated – the first and the last, in addition to those located at 1/3 and 2/3 of the derivation line. As the installed system was small, we decided to collect the flow rates of all the drippers in each of these lines. The volume applied by each sprinkler for a period of 5 minutes was collected in Becker glasses, and then the measurement was made in a graduated cylinder. Christiansen Uniformity Coefficient (CUC) was calculated by Equation 02:

$$CUC = 100\left[1 - \frac{\sum_{i=1}^{n}|q_i - \overline{q}|}{n_e - \overline{q}}\right] \quad (2)$$

In which:

q_i= flow rate of each dripper, $L \cdot h^{-1}$;
$\overline{q}$ = average dripper flow, $L \cdot h^{-1}$;
n_e= number of sprinklers.

In the irrigation management, the chosen condition was to keep the potential matrix of pots always between the field capacity (FC) and the critical humidity, using the soil moisture retention curve as reference. The current humidity was obtained through the use of a properly calibrated TDR (Time Domain Reflectometer) and, from this point on, the necessary volume was calculated to reach the FC again.

On the days when DCW was applied in the different treatments, irrigation was performed manually using a graduated cylinder in order to supply only the amount of water needed, considering the volume already provided via DCW, so as not to alter the amount of water in the different treatments.

Essential oil extraction

For the citronella essential oil extraction obtained in each treatment, the raw material (leaves) was collected at 183 days after the uniformization pruning, totaling three replicates per treatment and stored in a freezer at -20ºC.

For the essential oil extraction, 250g of fresh citronella leaves were used. The leaves were cut into 2cm pieces and placed in five-liter round-bottomed flasks placed on heating mantles. The raw material was then subjected to hydrodistillation with the addition of 3L of distilled water using modified Clevenger type device for 4 hours, timed from the beginning of the boiling point (Bruneton, 1993).

After the extraction period, the essential oil from each sample was combined in separation funnels and subjected to liquid-liquid partition with 200mL of n-hexane. The organic phases obtained were transferred to Erlenmeyers, to which an excess of anhydrous magnesium sulfate was added in order to ensure the removal of remaining water droplets from the separation process of the organic and inorganic phases. Then, the organic phase was filtered through filter paper and subjected to evaporation under reduced pressure using a rotary evaporator at 30oC until complete solvent evaporation. The essential oil obtained was then transferred to previously weighed bottles and the yield calculated by difference in order to determine the percentage yield (w/w). Soon after weighing, the bottles were wrapped in aluminum foil and frozen for further analysis.

Chemical analysis of essential oil

The obtained essential oil was diluted to 0.1% (v/v) in dichloromethane and analyzed in a gas chromatograph coupled to the mass spectrometer (Shimadzu QP 5000). Mass spectrum was obtained by electron impact ionization (70eV; 1 scan s^{-1}). The analysis conditions were as follows: injector temperature, 260°C; detector temperature, 290°C; mobile phase, helium; mobile phase flow, 1 mL/min; injection in Split mode (1:40); initial oven temperature, 60°C; heating rate, 3°C min^{-1} to 290°C; injection volume, 1µL; capillary column RTX-5 (0.25mm x 30m x 0.25µm).

The percentage composition of essential oil was calculated using the CG-DIC peak area normalization method. The identification of the major substances was carried out by comparing the Arithmetic Index (AI), determined in relation to the retention time of a series of n-alkanes (C7-C40, Sigma-Aldrich) and the fragmentation pattern of the mass spectral was compared with NIST mass spectral libraries.

Experimental design and/or statistical analysis

After the analysis of the essential oil and subsequent calculations, the data were subjected to statistical analysis. The Shapiro-Wilk and Kolmogorov-Smirnov normality tests were used, however, for the present study, the Shapiro-Wilk (W) normality test was adopted as a function of the sample size. The chi-square test was used to determine the homogeneity of the sample at the level of 5%, using the statistical program Sisvar V.5.6 (Ferreira, 2014).

The experimental design used was in randomized blocks (DBC) with 4 (four) repetitions. The sources of variation chosen were the concentrations of applied wastewater (treatment 1 (DCW-100% Nitrogen), treatment 2 (DCW-200% Nitrogen), treatment 3 (DCW-300% Nitrogen) and treatment 4 (DCW-400% Nitrogen)) and the control (mineral control). The variables chosen to be analyzed were the amounts of essential oils (g), namely: Citronelol Acetate, β-Citronelal, β-Citronelol and trans-Geraniol present in each treatment.

RESULTS AND DISCUSSION

Soil fertility

Soil fertigation with DCW application in the treatments promoted a greater availability of nutrients for the plants, which caused an improvement in soil fertility (Table 2), also noticeable by the visual analysis of the plants. All treatments with DCW application presented a uniform growth aspect when compared to the treatment of fertilization application only via mineral. This is confirmed by the data of total dry mass in which there was no difference between treatments (Hamacher *et al*., 2019). Thus, the supply of mineral nutrients via DCW to citronella plants can be indicated, as it reflected in the same biomass gain when compared to mineral fertilization, according to data previously published by the research group, analyzing the growth of this species under these irrigation depths with DCW (Hamacher *et al*., 2019).

On the other hand, if provided inappropriately, DCW can have negative effects, especially those caused by the high concentration of salts in these waters. The increase in the concentration of salts in the soil, caused by the application of wastewater, makes the plants need osmotic adjustment in order to maintain the water flow in the soil-leaf direction (Erthal *et al*., 2010), which may promote water deficit in plants and a need for osmotic adjustment by them (Taiz *et al*., 2017), which is why it is so important to correctly calculate the availability of the DCW application volume, as it must be according to the capacity of the soil-plant system in order to absorb the applied residue without compromising the quality of the soil, the plant or the groundwater (Erthal *et al*., 2010). Also depending on what type of wastewater is available for use, it should be directed to cultivations that do not have direct human consumption use, as is the case of fertigation with domestic wastewater (Silva *et al*., 2016).

Table 2. Chemical characterization of the soil, immediately after the removal of the plants, in all treatments, at the end of the experiment.

Parameters	Unit	T1	T2	T3	T4	T5
pH		4.90	5.50	5.60	6.00	6.10
Ca	$cmol.dm^{-3}$	3.35	4.35	3.92	3.67	2.79
Mg	$cmol.dm^{-3}$	0.16	0.24	0.34	0.61	0.49
Al	$cmol.dm^{-3}$	0.22	0.16	0.08	0.09	0.05
H+Al	$cmol.dm^{-3}$	2.90	2.40	1.64	1.56	1.14
V	%	56.25	69.42	75.60	76.62	78.25
M.O.	$dag.kg^{-1}$	1.16	1.35	1.15	1.41	1.23
P-Rem	$mg.l^{-1}$	58.94	56.83	58.70	58.94	58.47
Zn*	$mg.dm^{-3}$	3.40	4.69	4.42	4.73	4.31
Fe	$mg.dm^{3}$	66.72	82.27	76.37	78.65	69.26
Mn	$mg.dm^{-3}$	2.21	4.43	4.84	9.06	7.50
Cu*	$mg.dm^{-3}$	0.72	0.83	0.96	0.78	0.79
B	$mg.dm^{-3}$	0.07	0.08	0.12	0.11	0.09
S	$mg.dm^{-3}$	212.47	244.43	243.76	165.44	76.31
N	$g.kg^{-1}$	1.46	1.46	1.50	1.52	1.58

P-rem = Remaining phosphorus; V = Base Saturation Index; M.O. = Organic matter.

In general, plants are not very sensitive to variations in the relations between cations determined in soil analysis. It means that in the management of soil fertility it is more important to maintain individual levels of the main cations at a sufficient level (Broch and Ranno, 2013). In the chemical characterization of the soil, specifically in relation to the pH value, it was observed that it was acidic before any treatment and, even after correction with dolomitic limestone (T1) it remained acidic (Table 2). However, as the N concentration was increased via DCW, the pH value increased until it reached 6.10 in the T5 treatment (Table 2). It was possible to observe an increase of up to 1.2 points in relation to the T1 treatment. As a consequence, in relation to the T1 treatment, in which only the recommended liming was done, aluminum was reduced by up to -77.27%, in T5. The DCW application contributed to raise the pH and reduce the aluminum content in the soil, in addition to the correction with standard and uniform limestone in all treatments (Table 2).

The V% increase was linear in all treatments, reaching 78.25% in T5 (Table 2). This result shows the effect of DCW application in improving the fertility of the analyzed soil.

The soil organic matter (SOM) content can be considered as indicative of soil quality (Broch and Ranno, 2013). However, in the present study, it was found that it did not change in relation to the treatments.

In the present study, in the different treatments analyzed, the increase in N in the soil was not significant, possibly due to the fact that the plants have assimilated it in their metabolism or volatized. It also did not reflect on the biomass gain in citronella (Hamacher *et al*., 2019). However, Yasmeen *et al*. (2014), working with wastewater, found that fertigation promoted a moderate increase in N and increased growth and yield in *Vigna radiata* L.

Studies carried out with the application of treated sanitary wastewater (TSW) (Santos *et al*., 2017), found that soil nutrients and organic matter do not increase with doses of these effluents up to 0.8 m in depth. However, the pH of the soil and the exchangeable sodium increased linearly with the doses of treated wastewater, respectively, up to 0.6 and 0.8 m deep in the soil in the experiments by Santos *et al.* (2017). The same was verified for pH in this work, however, to a lesser extent. These same authors also suggest that fertigation can threaten the soil-plant system balance with continuous use, mainly due to the high sodium concentration TSW may contain, however, it was not verified in the present study.

In the study developed by Jorge *et al*. (2017), the authors concluded that the use of DCW fertigation for tomato cultivation demonstrated that there was an increasing linear effect on production, on accumulated productivity and on the accumulation of nutrients in the leaves as a function of the DCW concentration, and that the highest weekly and aggregated yield of tomatoes, as well as the highest leaf nutrient content, were observed when 400% of the nitrogen concentration recommended for tomatoes was used. However, the authors still indicated that nitrogen fertilization must be carried out in the organic cultivation of tomatoes, which can be done through DCW fertigation, but that it must be supplemented in mineral form, in order to provide the adequate amount of phosphorus and potassium for the plants.

Essential oil production

The substances identified in greater concentration in the essential oil of the species *Cymbopogon nardus* were: β-Citronelal, β-Citronelol, trans-Geraniol and Citronelol Acetate (Figure 1), which are classified as monoterpenes and sesquiterpenes. Among the analyzed substances, β-Citronelal was the major for all treatments, which was also found by Lacerda (2015) working with different substrate compositions and by Daflon (2016) working with different thinning proportions in the root system and in the aerial part of citronellae. However, there was no significant difference between the analyzed treatments (Table 3).

It is important to note that even though there is no statistical difference indicated by the Tukey Test, Although the values have a large discrepancy between them, the means found were not significantly different because the repetitions showed a very high deviation between them, resulting in a high Minimum Significant Difference (DMS) for there to be significance between treatments, however, for the production of essential oils in treatments, it is recommended to use wastewater for the cultivation of citronella, as it provides essential mineral nutrients for aqueded growth and development, even in soil degraded.

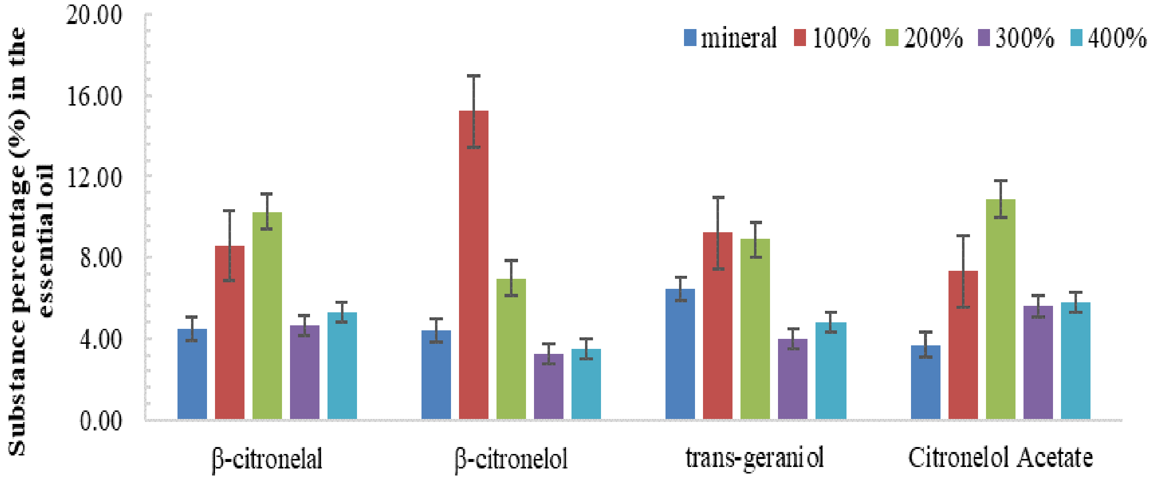

Figure 1. Substance percentage in each treatment, after 183 days of cultivation (mineral control), treatment 1 (DCW-100% Nitrogen), treatment 2 (DCW-200% Nitrogen), treatment 3 (DCW-300% Nitrogen) and treatment 4 (DCW-400% Nitrogen). No statistical difference between treatments. Bar indicates Standard Error (Standard error of means) (SEM).

Table 3. Amount of essential oil (g) obtained in each treatment.

Treatments	Essencial Oils				
	Citronelol Acetate	***β*-Citronelal**	***β*-Citronelol**	***trans*-Geraniol**	**(DMS) line analysis**
Mineral	3.71 Aa	4.48 Aa	4.42 Aa	6.43 Aa	11.431
100% DCW	7.34 Aa	8.58 Aa	15.19 Aa	9.22 Aa	11.431
200% DCW	10.88 Aa	10.26 Aa	6.96 Aa	8.89 Aa	11.431
300% DCW	5.60 Aa	4.67 Aa	3.23 Aa	3.98 Aa	11.431
400% DCW	5.78 Aa	5.32 Aa	3.50 Aa	4.79 Aa	11.431
DMS column analysis	12.180	12.180	12.180	12.180	

Averages followed by the same letter, uppercase in the column and lowercase in the row, do not differ by Tukey test at the significance level of 5%. No statistical difference between treatments. Standard error of means (SEM). Column and line analysis SEM: 3.014. Minimum Significant Difference (DMS).

Other studies developed with citronella (Castro *et al*., 2007; Mahalwal; Ali, 2002) that were carried out under different edaphoclimatic conditions, identified the constituents of the essential oil of the species *Cymbopogon nardus* by GC/MS and found, as constituents of the essential oil, compounds as monoterpenes and sesquiterpenes, the major constituents being Geraniol, Citronelol, Citronelal, elemol, (E)-nerolidol and beta-karyophylene. However, it is worth noting that, regarding raw material that has active principles, there are several factors that can change the chemical composition, such as the content of essential oils. This fact is pointed out by Verma *et al*. (2013), who describe that in winter periods there is a higher yield of the essential oil. Castro *et al.* (2010) also found that the content of the essential oil obtained 168 days after planting was the one with the highest production projection.

The production of secondary metabolites that are responsible for the bioactivity of plants is determined by several factors, one of the main being the climatic (Jamwal *et al*., 2018). Several studies with medicinal plants report that variations in the active principles can be altered by seasonality (Ncube *et al*., 2011), geographic distribution (Asase and Peterson, 2019), hydric stress (Zhang *et al*., 2017), harvest periods and management (Sharma and Kala, 2018), among other factors.

Mineral elements can also interfere in the production of secondary metabolites, such as manganese (Mn), which acts as an important cofactor for several key enzymes in the biosynthesis of secondary plant metabolites associated with the shikimic acid pathway (Massoni de Andrade *et al*., 2011). In this work, it was observed that Mn showed an increase in relation to the DCW application in the soil (Table 2), but it did not interfere in a greater production of active compounds, since the terpene production pathway is the mevalonate pathway and other alternative routes using, for example, pyruvate plus 3-phosphoglyceric acid (3PGA) (Taiz *et al.*, 2017).

By providing different volumes of DCW supply and mineral fertilization, in relation to the essential oil content, using gas chromatography data coupled to the mass spectrophotometer, the area of each substance was obtained in all samples and the percentage yields of each treatment (Tables 4 and 5).

The treatment in which DCW was not provided, that is, only with mineral fertilization, was the one with the greatest fresh mass, but it did not differ statistically from the others (Hamacher *et al*., 2019), and it also did not reflect in greater oil production, because the treatment that presented the highest oil yield was the application of 400% of the Nitrogen concentration via DCW supply, but with no difference for the treatment with mineral fertilization. The oil yield, under these experimental conditions, demonstrated that regardless of the treatment applied, oil production was equivalent for all treatments and did not show a concentration dependent relationship with the DCW application.

For the four analyzed substances, interference was not verified due to different nitrogen concentrations provided by the DCW. The responses to this control were equal to the control with mineral fertilization. However, it would be interesting to have a

Table 4. Substances present in citronella essential oil in greater quantities and their yields.

TR min	YIELD % (p/p)	1.22	0.95	1.01	1.03	1.29
	Treatments	**Mineral fertilization**	**100% N via DCW**	**200% N via DCW**	**300% N via DCW**	**400% N via DCW**
	Substance	signal area	signal area	signal area	signal area	signal area
14.0	*β*-Citronelal	50979984	97685401	116791714	53158768	60529825
17.1	*β*-Citronelol	17961006	23126580	28243042	13115917	14224740
18.3	*trans*-Geraniol	16775111	24064888	23190814	10388087	12518365
22.5	Citronelol Acetate	2615326	5175878	7672704	3950395	4077571

Table 5. Fresh mass used, essential oil and yields for each treatment.

Treatments	Fresh Mass Average (g)	Essential Oil Average (g)	Yield (%)
Mineral fertilization	253.33	3.078	1.22
100% N via DCW	250.00	2.382	0.95
200% N via DCW	238.67	2.406	1.01
300% N via DCW	247.33	2.541	1.03
400% N via DCW	247.33	3.198	1.29

treatment as zero control (natural soil fertility), i.e., without supplying any mineral elements, only natural soil. Lacerda (2015) found that harvest period has a major influence in this plant, even working with different substrates. The author found that, depending on the harvesting period, there was an interference with treatments, thus indicating that the age of the plant, i.e., its development stage, interfered with the essential oil yield, which may have occurred in this study.

Thus, DCW fertigation in the production of citronella can be indicated, since it is presented in an equivalent way to mineral fertilization. In addition, it can be a viable alternative, mainly for the supply of nutrients, in the recovery of degraded soils and the promotion of water availability, in addition to the adequate and productive availability of the generated wastewater.

CONCLUSION

DCW application promotes soil fertilization, increasing the availability of nutrients in the soil for plants, as long as they are applied in volumes calculated for the crop. However, it does not interfere with the citronella essential oil production amount.

When using DCW with properly calculated volume, there is the possibility of not depending on external inputs (mineral fertilizer) to maintain productivity, consequently reducing costs related to this activity.

The use of DCW for citronella cultivation can be indicated as a good destination for a waste that is often underutilized and that becomes an environmental liability for soils and different water bodies in which disposal is usually carried out.

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