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Macronutrient Suppression in Nutrient Solution Alters the Growth and Citral Content of *Cymbopogon flexuosus*

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Abstract

Cymbopogon flexuosus Stapf is a medicinal species cultivated on several continents. The essential oil extracted from its leaves has relevant commercial value and is widely used in flavoring agents, fragrances, perfumery, cosmetics, soaps, and detergents as well as in the pharmaceutical industry. This study evaluated the effect of macronutrient suppression on the growth, visual diagnosis, content, and chemical composition of *C. flexuosus* essential oil in a hydroponic culture. A completely randomized design with four replicates was used, with three plants per pot in each replicate. The treatments were characterized by suppressing the macronutrients, N, P, K, Ca, Mg, and S, under the missing element technique. After 90 days of cultivation, the deficiency symptoms were photographed and characterized. The dry biomass of the roots and shoot, root-to-shoot ratio, number of tillers, leaf analysis, content, yield, and chemical composition of the essential oil were evaluated. Macronutrient suppression in a hydroponic culture influenced growth and chemical composition of *C. flexuosus* essential oil. Total biomass production was more limited in potassium and magnesium omission. Suppressing sulfur promoted an increase in content and yield of essential oil. The highest citral content was observed in phosphorus and nitrogen omission.

Keywords: aromatic plant, nutrition, hydroponics, lemongrass

1. Introduction

Cymbopogon flexuosus Stapf, family Poaceae (Gramineae), is popularly known as Indian lemongrass, Cochin grass, or Malabar grass (Marigowda et al., 2016). It grows on several continents and originates in south Asia, southeast Asia, and Australia (May et al., 2008). The essential oil extracted from this species is widely used and marketed in the pharmaceutical, food, hygiene, and cosmetics industries (Ganjewala & Luthra, 2010). The estimated world production is 800 to 1300 tons of the oil per year, with 40% of this production coming from China and Indonesia (Joyce et al., 2015).

Lemongrass oil has sedative, digestive, antirheumatic, calming, antifebrile, carminative, stomach, analgesic, antispasmodic, and antimicrobial activities (May et al., 2008; Meena et al., 2016). In addition, it is a good candidate for innovative therapeutic strategies against cancer because of its cytotoxic and anticancer potential (Sharma et al., 2009; Marigowda et al., 2016). The citral monoterpene, a racemic mixture of the geranial and neral isomers, corresponds to 75-85% of the oil's chemical composition. Its biological properties, including insecticidal and larvicidal activities against *Aedes aegypti* (Vera et al., 2014), antifungal activity (Ganjewala & Luthra, 2010), and bactericidal and antimicrobial activities (Adukwu et al., 2016), are attributed to this major constituent of

lemongrass essential oil. According to Joyce et al. (2015), this monoterpene inhibits *Saccharomyces cerevisiae* fermentation in alcohol biorefineries.

Mineral nutrients perform essential and specific functions, and their deficiencies or toxicities are observed via symptoms often characteristic of each nutrient (Oliveira et al., 2009). The nutrient solution and the missing element technique are used in scientific research mainly to obtain symptomatological information on a plant's deficiency of a certain nutrient or toxicity caused by that nutrient, aiding in improving the production system (Andriolo et al., 2002; Alves et al., 2008). Maia et al. (2014) observed that the number of studies on culturing medicinal plants in hydroponics is increasing in scientific research, and managing these cultures is feasible. These authors found that the hydroponic system enables seedling production without agrochemical residues and increases the content of active principles of interest. Studies on nutrient solutions have been conducted on several medicinal species, including *Mentha arvensis* (Carvalho et al., 2016), *Achillea millefolium* (Alvarenga et al., 2015), *Coriandrum sativum* (Daflon et al., 2014), *Ocimum* sp. (Amaral et al., 2015), *Maytenus ilicifolia* (Benedetti et al., 2009), and *Arrabidaea chica* (da Silva Júnior et al., 2011).

In this context, this study evaluated the growth, production, and chemical composition of essential oil from *C. flexuosus* and characterized the visual symptoms of macronutrient deficiency in hydroponic cultures using the missing element technique.

2. Material and Methods

2.1 Procedure, Harvesting, and Vegetative Analysis

The experiment was conducted in an 18.5 × 13 m plastic greenhouse, located in a north-south position at the Laboratory of Culture of Vegetable Tissues and Medicinal Plants of the Department of Agriculture of the Federal University of Lavras (DAG/UFLA), in the southern region of Minas Gerais state, at an altitude of 918 m, latitude 21°14′ S and longitude 45°00′ W.

Cymbopogon flexuosus mother plants belonging to the Medicinal Plants Garden of UFLA were identified and compared with the exsiccate deposited by the Agronomic Institute of Campinas IAC Herbarium under the number 45335. The seedlings were propagated by clump division, and each tiller was approximately 8 cm high. The propagules were cultured in trays of expanded polystyrene containing the commercial substrate Plantmax® and were kept in a greenhouse with 60% shading. After 60 days, the plants were adapted in 25 and 50% Hoagland and Arnon (1950) nutrient solution, for one week each. They were then placed in individual 6-liter pots containing 100% of the solution's ionic strength, and the missing element technique was applied for 90 days. A completely randomized experimental design was used with seven treatments and four replications. The treatments were the complete solution (control) and macronutrient suppression of N, P, K, Ca, Mg, and S. The experimental plot comprised three plants per pot.

The solutions were prepared with pure reagents. A modified Hoagland and Arnon solution (1950) was used containing the following concentrations in the complete solution: 210.105 mg L^{-1} N, 30.974 mg L^{-1} P, 156.392 mg L^{-1} K, 160.4 mg L^{-1} Ca, 48.61 mg L^{-1} Mg, 32.06 mg L^{-1} S, 500 µg L^{-1} B, 20 µg L^{-1} Cu, 648 µg L^{-1} Cl, 5022 µg L^{-1} Fe, 502 µg L^{-1} Mn, 11 µg L^{-1} Mo, and 50 µg L^{-1} Zn. In the treatment solutions, the nutrient concentrations were identical to those of the complete solution, except for the suppressed nutrient. The solutions were renewed fortnightly and replenished by adding deionized water when necessary. The pots were black plastic, and their exteriors were painted with aluminum paint to prevent heating the nutrient solution. An air compressor was used for aeration, and a cylindrical porous stone measuring 1 cm in diameter and 2 cm long was placed at the end of each hose.

The symptoms were observed, described and photographed at the end of the experimental period. The harvested material was dehydrated in a forced ventilation oven at 40 °C for three days. Leaf dry biomass (LDB), leaf base dry biomass (LBDB), shoot dry biomass (SDB), root dry biomass (RDB), and total dry biomass (TDB) were evaluated in grams per plant. The leaf base dry biomass (LBDB) corresponds to the sectioned region between the root and leaf. Shoot dry biomass (SDB) was calculated by the sum of the leaf dry biomass and leaf base dry biomass (LDB+LBDB). Root-to-shoot ratio (R:S), number of tillers (NT) and the percentage of relative production of total dry biomass (RPTDB) were also determined, and the complete treatment corresponded to 100%. The root-to-shoot ratio (R:S) was obtained by dividing the root dry biomass by the shoot dry biomass (RDB/SDB).

2.2 Leaf Analysis

The samples collected to evaluate the dry biomass were ground in a Wiley mill and sent to the Laboratory of Leaf Analysis of the Chemistry Department of UFLA to determine the N, P, K, Ca, Mg, S, B, Cu, Mn, Zn, and Fe contents using the methodology proposed by Malavolta, Vitti, and Oliveira (1997).

2.3 Extraction and Chemical Analyses of the Essential Oil

The *C. flexuosus* essential oil was extracted by hydrodistillation in a modified Clevenger apparatus using a 10 g sample of leaf dry biomass in 1 L of distilled water for 90 min. The essential oil was purified by liquid-liquid partition with dichloromethane (3 × 5 mL). The organic phase was combined and treated with approximately 5 g of anhydrous magnesium sulfate for 30 min. Next, the solution was filtered, and the solvent evaporated at room temperature under a gas exhaust hood. Five replicates were performed per essential oil treatment from samples composed of dry biomass and stored in amber flasks. The essential oil content (%) and yield (mg plant^{-1}) were determined. Each sample's content represents the oil weight (mg) in 100 mg of leaf dry biomass, and the yield is the product of the content multiplied by the leaf dry biomass.

For the quantitative analysis, the essential oil was analyzed in an Agilent 5890A system equipped with a flame ionization detector (FID), using an HP-5MS column (30 cm long × 250 µm internal diameter × 0.25 µm thick). Helium was used as the carrier gas with a flow rate of 1.0 mL min^{-1}. The injector and detector temperatures were 220 and 240 °C, respectively. The initial oven temperature was 60 °C, which was maintained for 1 min, followed by a temperature increase of 3 °C min^{-1} to 150 °C, then subsequently increased at 10 °C min^{-1} to 250 °C. The essential oil was diluted in ethyl acetate (1%, v/v), and 1 µl was injected in *split* mode at a 1:50 ratio. The analyses were performed in triplicate, and the results are expressed as the mean percentage of the relative normalized area of the chromatographic peaks ± standard deviation.

Qualitative analyses were performed on an Agilent® 7890A Chromatograph coupled to an Agilent® MSD 5975C mass selective detector (Agilent Technologies, California, USA) operated by electronic impact ionization at 70 eV in sweep mode at a speed of 1.0 scan/s, with a mass acquisition interval of 40-400m/z. The operating conditions were the same as those used in gas chromatography-flame ionization detector (GC-FID) analyses. The components were identified by comparing their retention indices (IK_c) calculated using mass spectra data and retention indices (IK) from the literature (Davies, 1990; Adams, 2007) and by comparing mass spectra from the NIST/EPA/NHI library database (NIST, 2008) and the literature (ADAMS, 2007). The retention indices were calculated using the Van den Dool and Kratz equation (1963), and retention indices cited in the literature were used for the attributions (Adams, 2007).

2.4 Statistical Analysis

Statistica® software, version 13.3 (StatSoft; Tulsa, OK, USA), was used to statistically analyze the experimental data. The observed values were submitted to analysis of variance (ANOVA, $p < 0.05$) and compared using Tukey's test ($p < 0.05$). Principal component analysis (PCA) was used to study the major essential oil compounds.

3. Results and Discussion

Dry biomass production was higher in plants grown in complete nutrient solution. All means of the variables analyzed differed statistically due to the macronutrient suppression (Table 1). The number of tillers under S suppression (67) was similar to that of the complete solution (76); however, the other suppressions greatly reduced the *C. flexuosus* tillering capacity (Table 1). The greatest limitation to root growth (RDB) relative to the complete treatment was verified by suppressing K, Ca, and Mg, with reductions of 93%, 75%, and 89%, respectively. However, RDB was unaffected by the absence of P and S. Daflon et al. (2014) also observed that S suppression did not reduce the dry matter in *Coriandrum sativum* roots.

The LDB and LBDB production were more affected by N, K, and Mg macronutrients (Table 1). Consequently, SDB presented greater limitations after suppressing the same nutrients. The reduced dry biomass production was related to these nutrients' functions in the photosynthetic apparatus structures and photosynthesis. Nitrogen deficiency inhibits plant growth because N is a constituent of many plant cell components, including amino acids and nucleic acids (Carvalho et al., 2016). Potassium acts mainly in regulating the osmotic potential, maintaining the pH in the cell, and controlling the stomata opening and closing (Malavolta, 2006). Magnesium is the central element of the chlorophyll molecule (Shaul, 2002).

All treatments with macronutrient suppression resulted in a total dry biomass lower than that of the complete treatment (Table 1). The nutrient limitation order for TDB was K = Mg > N = Ca = P > S. The relative total dry biomass (RPTDB) produced was lower after suppressing K (6.5%) and Mg (8.5%), which reduced the biomass gained by 93.5% and 91.5%, respectively. Puga et al. (2010) studied the effect of macronutrient suppression on chicory and also verified better total dry biomass yield in the complete solution and when suppressing sulfur. Per Sanchez and Uehara (1980), dry mass production below 40% relative to the complete treatment is considered a severe deficiency. Thus, suppressing N, P, K, Ca, and Mg induced severe deficiencies in *C. flexuosus* at 90 days.

Table 1. Mean values of the number of tillers (NT), root dry biomass (RDB), leaf dry biomass (LDB), leaf base dry biomass (LBDB), shoot dry biomass (SDB), total dry biomass (TDB), relative production of total dry biomass (RPTDB), and root-to-shoot ratio (R:S) in *C. flexuosus* under macronutrient suppression in a hydroponic culture

	NT	RDB	LDB	LBDB	SDB	TDB	RPTDB	R:S
		---------- g plant^{-1} ----------					%	
Complete	76 a*	13.22 a	44.24 a	7.56 a	51.80 a	65.02 a	100	0.26 c
-N	18 b	6.36 b	5.47 d	2.80 b	8.27 d	14.36 c	22.1	0.76 b
-P	23 b	12.53 a	8.76 c	3.81 b	12.58 c	25.11 c	38.6	1.00 a
-K	7 b	0.94 c	2.40 d	0.91 c	3.31 d	4.25 d	6.5	0.29 c
-Ca	20 b	3.37 c	10.38 c	3.23 b	13.60 c	16.97 c	26.1	0.24 c
-Mg	11 b	1.39 c	3.12 d	1.03 c	4.15 d	5.54 d	8.5	0.34 c
-S	67 a	12.35 a	36.77 b	6.04 a	42.76 b	55.11 b	84.8	0.29 c
CV(%)	27.15	28.55	22.17	36.28	24.41	22.41	-	24.19

Note. * Means followed by the same letter in a row do not differ statistically from each other by Tukey's test at 5% probability.

The R:S ratio was higher in plants grown under phosphorus suppression, followed by nitrogen. The other treatments presented smaller and statistically similar values (Table 1). This result indicates that the complete solution and the K, Ca, Mg, and S suppressions direct the biomass distribution to the plant shoot.

Analysis of the leaves (Table 2) revealed that the percentage of each suppressed macronutrient, except S, was below the limit that Malavolta, Vitti, and Oliveira (1997) suggested for grasses. In the sulfur suppression treatment, the leaves' nutrient content (0.20%) was similar to that of the complete solution (0.22%). Sulfur mobility in the plant is practically nonexistent, in addition to being a constituent of proteins, essential amino acids, cell membranes and other compounds (Marschner, 2012). Thus, early incorporation of S into organic molecules that are essential to the plant's initial development may have occurred during the adaptation period.

Table 2. Leaf analysis of *C. flexuosus* under macronutrient suppression in a hydroponic culture for 90 days

		N	P	K	Ca	Mg	S	B	Cu	Mn	Zn	Fe
		---------- % ----------						---------- mg kg^{-1} ----------				
Complete		2.19	0.99	2.40	0.35	0.25	0.22	19.18	7.39	37.58	12.33	165.23
-N		0.72	0.72	1.77	0.24	0.17	0.42	38.19	3.89	146.12	17.69	263.93
-P		0.66	0.04	1.66	0.98	0.33	0.28	52.53	4.28	50.72	13.29	307.41
-K		2.46	1.99	0.19	0.86	0.59	0.43	41.69	8.75	70.73	18.55	339.12
-Ca		2.26	1.73	2.83	0.09	0.51	0.43	65.03	7.24	95.31	16.74	251.64
-Mg		1.98	1.29	2.60	0.86	0.08	0.21	44.52	6.27	64.83	13.79	244.29
-S		2.32	1.23	2.17	0.49	0.32	0.20	34.19	8.37	46.12	12.09	153.78
Range of sufficiency*	Low	1.2	0.08	1.10	0.3	0.1	0.1	15	5	80	20	100
	High	1.5	0.12	1.50	0.6	0.2	0.2	30	15	300	50	200

Note. * Malavolta et al. (1997).

Notably, P suppression considerably reduced the leaf N content (0.66%) compared with that of the complete solution (2.19%). The opposite relationship was also verified, where N suppression reduced the P content relative to the other treatments. Ågren, Wetterstedt, and Billberger (2012) reported that nitrogen fertilization may stimulate extracellular phosphatase activity and thus increase P uptake. Daflon et al. (2014) reported the importance of evaluating the nutritional contents in plant tissues. These researchers observed that potassium content in plants treated with K suppression was lower than that in the complete treatment even without visual symptoms. Therefore, leaf analyses enable more precise inferences from the data. The symptoms of N, P, Ca, and S deficiencies in the leaves and roots were photographed at 90 days after applying the treatments (Figure 1). The plants with K and Mg suppression died after 35 days; thus, they could not be recorded at the end of the experimental period.

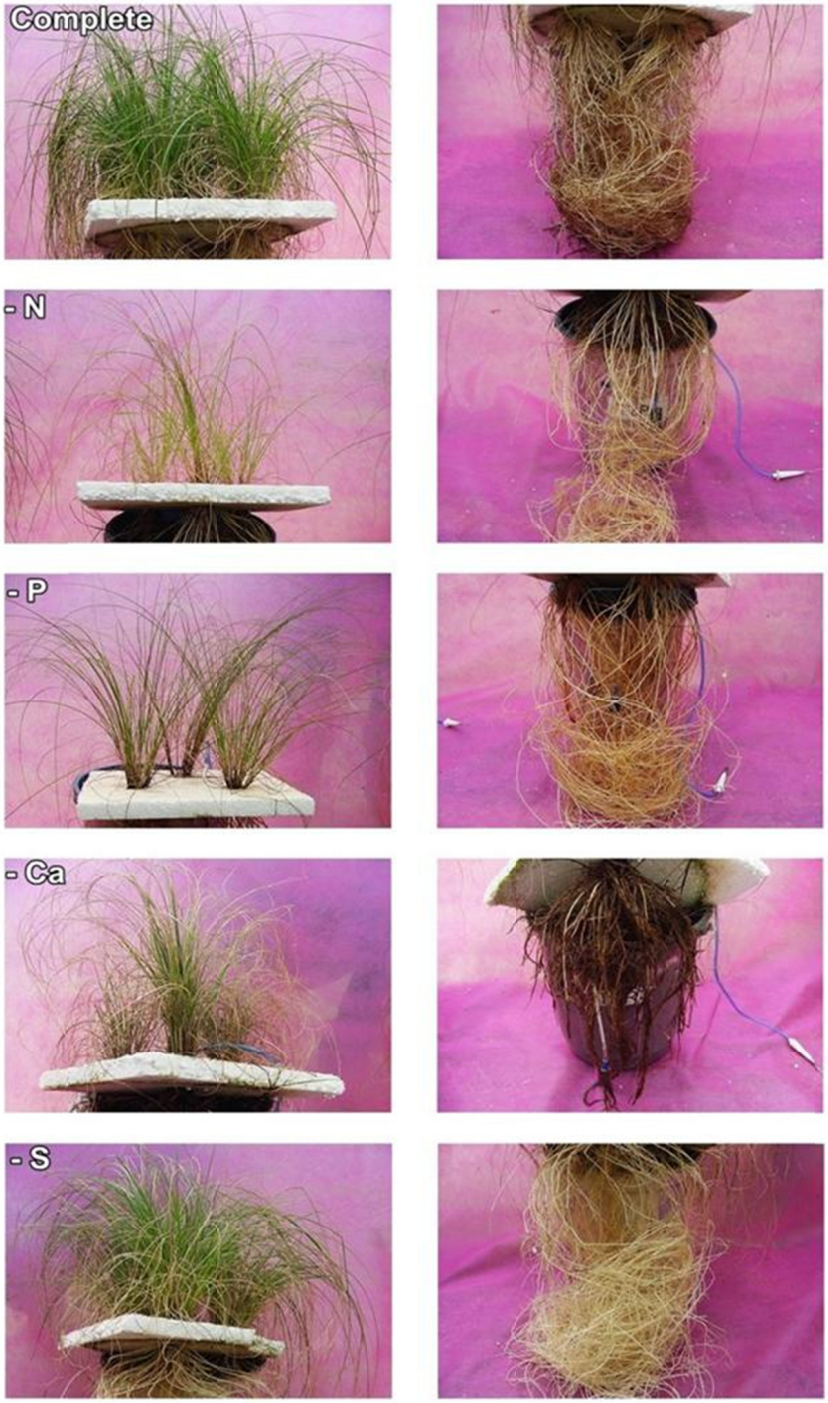

Figure 1.Visual diagnosis of the shoot and root system of *C. flexuosus* cultivated for 90 days in a complete solution and under N, P, Ca, and S suppressions

Over the 90-day experimental period, the complete solution provided plants with darker green leaves and more abundant root systems than those of the other treatments (Figure 1). Nitrogen suppression resulted in generalized chlorosis of old leaves after 40 days. The entire plant became chlorotic, with fewer tillers and a less abundant root system with the culture advance. Generalized chlorosis was observed in old leaves due to the plants' lower chlorophyll production and high nutrient mobility (Marschner, 2012).

P-deficient plants also showed smaller and fewer tillers. After 30 days, the leaves appeared dark green (Figure 1). Coelho et al. (2011) observed that P-deficient *Tagetes erecta* presented necroses on the edges of new leaves, browning leaf blades and smaller young leaves. As with K suppression, the absence of Mg resulted in the plants

dying at 35 days. Initially, the leaves developed typical symptoms with internerval chlorosis of the old leaves. According to Taiz et al. (2017), this type of chlorosis occurs because chlorophyll in the vascular bundles remains unchanged for longer periods than does chlorophyll in the cells between bundles. New chlorotic leaves and abundant root systems with whitish coloring were observed with S suppression at the end of the culturing. Per Marschner (2012), S is a constituent element of essential amino acids and has little mobility; thus, its symptoms occur in younger leaves.

In addition, suppressing macronutrients in the nutrient solution influenced the content and yield of the *C. flexuosus* essential oil (Figure 2). The highest mean essential oil content was verified for sulfur suppression (1.92%), followed by the complete solution (1.67%) (Figure 2a). The increased essential oil production after S suppression may be related to its incorporation during the plants' adaptation period to the nutrient solution. This leaf S content was within the content suggested by Malavolta, Vitti, and Oliveira (1997). Considering that S is related to the primary metabolism's protein, its absence did not reduce the secondary metabolite production. In turn, the nutrient solution with N suppression was the solution that most affected the essential oil content, which was reduced by 38%.

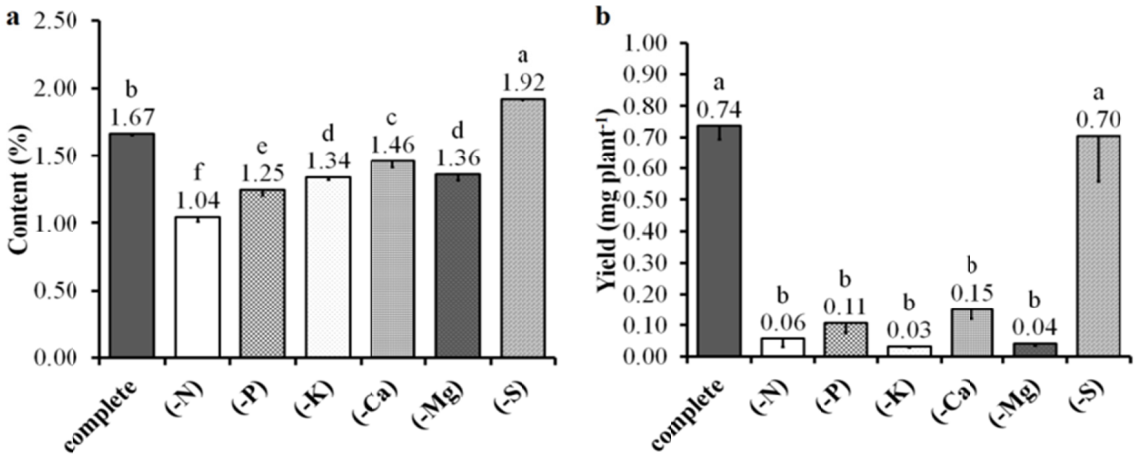

Figure 2. Mean values of a: content (%) and b: yield (mg plant^{-1}) of *C. flexuosus* essential oil under macronutrient suppression in a hydroponic culture. Means followed by the same letter do not differ statistically from each other by Tukey's test at 5% probability

Despite the higher essential oil content after suppressing S, the complete solution resulted in an oil yield statistically similar to that of the S suppression treatment (Figure 2b). In calculating the oil yield, the oil content is related to the leaf dry biomass production. The complete solution provided the highest mean LDB. Compared with the complete solution, the N, P, K, Ca, and Mg suppressions provided lower essential oil yields, which were reduced by 92, 85, 96, 80, and 95%, respectively.

Five major constituents were identified by chemically analyzing the *C. flexuosus* essential oil: linalool (3.08-3.59%), neral (9.54-27.79%), geraniol (3.31-16.21%), geranial (14.31-50.83%), and geranial acetate (6.56-45.50%) (Table 3).

Table 3. Content±standard deviation (%) of the major chemical constituents of *C. flexuosus* essential oil

Constituents	Complete	-N	-P	-K	-Ca	-Mg	-S
Linalool	3.50±0.00	3.08±0.02	3.30±0.00	4.88±0.03	3.59±0.04	3.42±0.00	3.47±0.00
Neral	14.99±0.05	21.54±0.08	27.79±0.09	10.62±0.03	9.54±0.07	20.59±0.02	18.86±0.01
Geraniol	12.73±0.04	4.44±0.03	3.31±0.00	15.99±0.00	16.13±0.13	16.21±0.02	11.23±0.01
Geranial	23.31±0.09	48.36±0.14	50.83±0.07	15.55±0.10	14.31±0.10	33.77±0.00	30.11±0.01
Geranyl acetate	37.61±0.13	10.06±0.05	6.56±0.01	45.50±0.08	16.50±0.09	12.12±0.00	29.32±0.02
Total (%)	92.14	87.48	91.79	92.54	60.07	86.11	92.99

Suppressing the nutrients promoted quantitative changes in the essential oil's chemical composition. Principal component analysis (PCA) of the four major chemical compounds and citral (PC1+PC2) showed an approximate adjustment of 94.91% (Figure 3). Notably, neral, geranial and citral were positively correlated with each other and negatively correlated with geraniol. Ganjewala and Luthra (2010) studied the biosynthetic route of *C. flexuosus* and concluded that geraniol is oxidized via geraniol dehydrogenase to produce geranial, which is converted to neral by an isomerase. Citral monoterpene is a racemic mixture of the geranial and neral isomers (Gupta & Ganjewala, 2015).

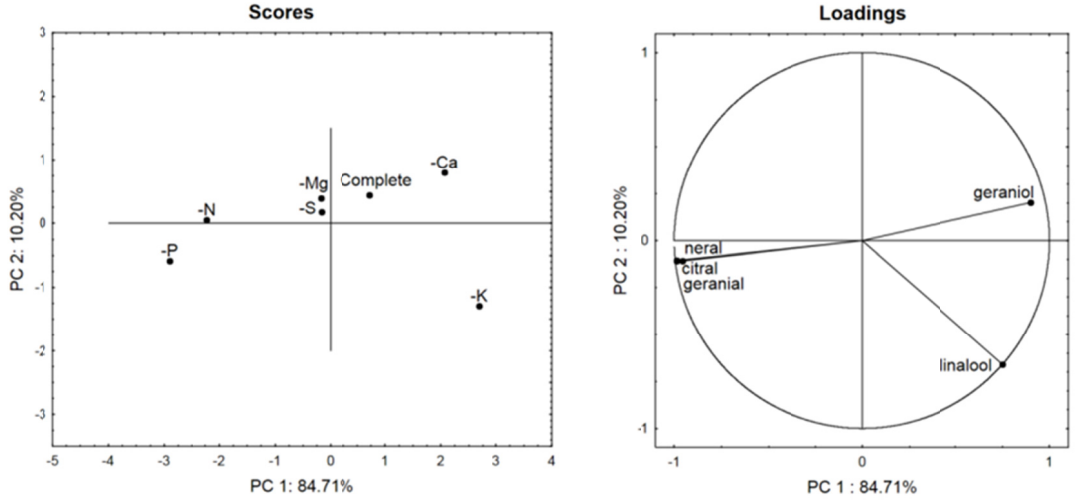

Figure 3. Principal component analysis (PCA) of the major constituents of *C. flexuosus* essential oil

The neral, geranial and citral levels were increased in the plants cultivated under phosphorus and nitrogen suppression. The absence of K in the nutrient solution positively influenced the linalool and geranyl acetate contents. Calcium suppression favored a higher geraniol content (Figure 3). These nutrients are related in their synthesis of secondary compounds as enzyme catalysts or their presence in their structure. Alvarenga et al. (2015) also verified quantitative changes in the major components of *Achillea millefolium*.

4. Conclusions

Macronutrient suppression in the nutrient solution of *Cymbopogon flexuosus* resulted in visual symptoms of the plant's nutritional deficiency; these symptoms are also common in other species. Potassium and magnesium are the most limiting macronutrients for biomass production, and the limiting order of TDB is K = Mg > N = Ca = P > S. The highest essential oil content occurred under sulfur suppression, and a similar yield was verified for the complete solution. Chemical analysis of the essential oil was quantitatively altered in the hydroponic culture. P and N suppression promoted an increase in citral contents.

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