Management of citronella (*Cymbopogon winterianus* Jowitt ex Bor) for the production of essential oils

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Abstract

Citronella (*Cymbopogon winterianus*) is a plant with repellent properties attributed to the presence of monoterpenes. The essential oils produced by the plant are influenced by agronomic and environmental factors. This work aimed to verify the interference of water stress and mineral nutrition in the development of citronella plants and oil production. Citronella was grown in pots with average capacity of 2.7 kg of soil and induction of water stress for 10 days, and in different doses of nitrogen and phosphate nutrients. The work was carried out in a greenhouse with a completely randomized design and four replications and a $2 \times 3 \times 2$ factorial scheme: two levels of water stress (with and without), three levels of nitrogen (0, 122 and 244 kg ha⁻¹) using urea fertilizer and two levels of phosphate (0 and 300 kg ha⁻¹) using sodium monobasic phosphate. Citronella seedlings were used for planting. The maximum average temperature in the greenhouse was 35 °C. After 90 days of planting, water stress was implanted for 7 days, returning with irrigation after this period. Dry weight of aerial part, roots, number of leaves, photosynthetic rate and production of essential oils were evaluated. The results showed that the nitrogen and phosphate fertilizers intensified the production of the aerial part of the plants. The roots of the plants performed better without water stress and with the application of phosphate fertilizer. The increase in the photosynthetic rate was induced by water stress. The water stress and the use of nitrogen and phosphate fertilizers promoted the increase the essential oils concentration in the aerial part, demonstrating the citronella's physiological adaptation capacity.

Keywords Plants · Secondary compounds · Mineral nutrition · Water stress · Plant physiology

1 Introduction

Java citronella (*Cymbopogon winterianus*) is a tropical herbaceous plant, of perennial cycle [1], adapted to hot climates, fertile and well-defined soils. They have a large production of biomass and are widely used in the production of natural products [2, 3], often used as insect repellent or as a starting point for the synthesis of several active principles [4]. Its repellent property attributed to the presence of monoterpenes in its leaves, especially to citronellal, geraniol, citronellol, limonene and eugenol [5].

In Brazil the cultivation of citronella is driven by the economic value of its essential oil. Although it presents a large volume of biomass, in the country the yield of essential oil is relatively low, varying from 0.3 to 0.6% in relation to the weight of the plant which is insufficient to meet market demand [6].

Essential oils are secondary compounds, which, different from primary metabolites, have a frequency lower than 1% of total carbon [7] playing an important role in the environmental adaptation of the plant [8]. Essential oils are mainly composed of mono and sesquiterpenes, and

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phenylpropanoids, which give them organoleptic characteristics [7]. They act as chemical regulators and messengers in plant biochemistry, playing a fundamental role in fertilization, pollination and environmental adaptation of the plant [9].

The biosynthesis of essential oils is genetically controlled, associated with plant physiology and dependent on its metabolic state [10] but strongly influenced by environmental factors, by harvest and post-harvest. Agricultural factors have a critical effect on the growth and productivity of citronella grass. Temperature, light and humidity affect the quality and quantity of its volatile active components, in particular geraniol, citronellal and citronellol [11, 12]. The search for optimal agronomic factors for its cultivation allows the highest yield of its secondary metabolites, significantly increasing its repellent action [13].

Water stress also significantly interferes at the development of plants. Munns [14] mentions that water stress reduces the capacity of plants to absorb water and mineral nutrients, promoting reduced growth and metabolic rates. The interference of water stress on plants is conditioned by factors such as variety, duration of stress and stage of development of plants.

Sangwan et al. [10] describe that the use of fertilizers in the cultivation of aromatic plants affects the production of essential oils, and it is necessary to determine the proper management of fertilization. The addition of nutrients, particularly nitrogen, is generally used to increase biomass production. Authors, Carvalho et al. [15], Martuscello et al. [16], Fagundes et al. [17] and Patês et al. [18], discuss the use of nitrogen in soil, and agree with its importance in the formation and development of leaves, in addition to the increase in plant biomass. In general, nutritional stress promotes an increase in the concentration of secondary metabolites, except for nitrogen and sulfur deficiency, resulting in a considerable reduction of metabolites containing these elements. In this same sense, the levels of phosphorus and potassium can have effects on the production of nitrogen metabolites [19].

This work aimed to evaluate the vegetative development and oil production of citronella plants under water stress and with the application in the soil of two essential nutrients nitrogen and phosphorus in different dosages.

2 Materials and methods

The experiment was carried out in laboratory and greenhouse of the Vale do Rio Doce University, in Governador Valadares-Minas Gerais, Brazil. In this experiment were used the reproductive structures (tillers) of the Java citronella (*Cymbopogon winterianus*). The plants were removed in

the experimental area of Univale, and the seedlings were prepared by dividing the tiller plants of parent plants. Each matrix plant had its leaves pruned at the time of the limbo-sheath transition and the clump collected and separated into seedlings ranging in size from 20 to 30 cm. The seedlings planted in pots with average capacity of 2.7 kg of soil.

The soil used here was collected from a superficial horizon of alluvial deposited the experimental field of Univale, dried and sieved in 5.0 mm mesh sieves. Sample of this soil (200 g) was collected for textural analysis, and its granulometric characteristics were determined according to the methodology of Embrapa [20] (Table 1) and routine chemical analysis (determination of pH, phosphorus, calcium, magnesium, aluminum, potential acidity, organic matter, total CTC, effective CTC, sum of bases, base saturation and aluminum saturation) (Table 2) was executed. The soil was characterized as having a sandy texture [21].

After soil preparation, citronella seedlings were planted. Nine days after planting, mineral nutrients were applied by solution, according to the established recommendations for the use of correctives and fertilizers. Three doses of nitrogen were applied (N0, N1 and N2) representing the control treatment (0 kg N ha⁻¹), the recommended dose N1 (122 kg N ha⁻¹) and twice the recommended dose N2 (244 kg N ha⁻¹), respectively. Another factor evaluated was the phosphorus dose (P0 and P1) represented by the control treatment P0 (0 kg P ha⁻¹) and the recommended dose P1 (300 kg P ha⁻¹). The used phosphate fertilizer was a monobasic sodium phosphate (NaH₂PO₄) with a concentration 22.46% of phosphorus. The solutions were prepared with distilled water and applied individually to the vessels. The proportion of phosphorus used was equivalent to 300 kg of P₂O₅ ha⁻¹ (dose P1). The source of nitrogen used was urea [(NH₂)₂CO] containing 50% nitrogen. The urea was prepared in aqueous solution, diluting 48.9 g to 2000 mL of distilled water, applying doses equivalent to 122 kg N ha⁻¹ and 244 kg N ha⁻¹, called doses N1 and N2, respectively.

The soil of the pots was maintained with a humidity level above 80% of the field capacity until the moment of the application of water stress. The experimental design used was completely randomized with four replications.

After 90 days of planting, water stress was implanted in half of the pots by interrupting the irrigation water supply. Water stress was maintained for a period of 7 days. After this period, irrigation was resumed normally. During water stress, the chlorophyll rate of all leaves was assessed using the OSI CCM chlorophyll quantity meter.

Table 1 Textural analysis of the soil used in plant development

Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)
65.73	13.61	8.77	11.89

Table 2 Chemical analysis of the soil used in the experiment

pH	P (Mg dm ⁻³)	K (Mg dm ⁻³)	Ca (Cmolc dm ⁻³)	Mg (Cmolc dm ⁻³)	Al (Cmolc dm ⁻³)	H + Al (Cmolc dm ⁻³)	M.Org (dag kg ⁻¹)	S (Cmolc dm ⁻³)	T (Cmolc dm ⁻³)	t (Cmolc dm ⁻³)	V (%)	m (%)
5.30	10.4	115.7	1.8	0.6	0.5	2.9	1.12	2.7	5.6	3.2	48.4	15.6

P = Phosphor, K = potassium, Ca = calcium, Mg = magnesium, Al = aluminum, H + Al = acidez potencial, Mat. Org = organic matter, Prem = residual phosphor, S = sum of bases, T = Total cation change capacity, t = Effective cation change capacity, V = base saturation, m = aluminum saturation

Ten days after the end of the water stress, the plants were collected and were cut separately in their parts. Total number of leaves, dry weight of shoots and roots were weighted and evaluated. The number of leaves was obtained by manual counting considering the formed and in formation leaves that presented green color, with photosynthetic activity. The dry weight of the plants was determined after 72 h in an oven at 60 °C, as described by Borges et al. [22].

The roots were washed in running water until the total removal of organic particles and minerals present on roots and then packed in paper bags and dried at a temperature of 60 °C for 72 h.

The aerial part of the plants was manually chopped and placed in a glass flask together with distilled water over hydrodistillation performed in a Clevenger-type apparatus according to technique II of the Farmacopéia Brasileira [23].

The oil was extracted from the distillate with a separating funnel using 40 ml of pentane as a solvent. Excess anhydrous magnesium sulfate was added to remove the organic phase in order to remove the water present. The organic phase was filtered and the pentane removed on a rotary evaporator at 40 °C, according to Seixas [24].

The oil it was calculated with the aid of the formula used by Scherer [25], where the yield was determined in percentage, dividing the volume of oil obtained in liters by the dry mass used in kilograms.

The oil mass obtained was determined by weighing on an analytical balance. The oil obtained was transferred to amber glass and stored at -5 °C. The total oil production per leaf weight was calculated, in percentage.

The experiment was carried out in a 3 × 2 × 2 factorial model using the following factors: three doses of nitrogen, two doses of phosphorus and implantation of an environment with and without water stress in the soil. The experimental design used was in randomized blocks with four replications. The results were subjected to analysis of variance (ANOVA) and the averages to the Tukey test at 5% probability. In the statistical analysis, the SAEG software developed by the Federal University of Viçosa [26] was used.

3 Results and discussion

Citronella plants have a C4 photosynthetic system, which contributes positively to the water stress tolerance process [27]. Although there was no significant difference between the results of the dry weight of the aerial part of plants with and without induction to water stress, we observed a tendency toward less dry mass production of leaves subjected to water scarcity (Fig. 1). Regarding the

Fig. 1 Dry weight of aerial part of citronella plants grown in sandy soil under water with different and doses of nitrogen and phosphorus (CV=38.40%)

Fig. 2 Number of leaves of citronella plants grown in sandy soil under water stress and with different doses of nitrogen and phosphorus (CV=38.6%)

use of phosphate fertilizer, it appears that the application of this element promoted an increase in the dry matter production of the leaves by 28.47% in relation to the treatment without the use of phosphorus. This same trend of behavior of accumulation of dry matter in the leaves was observed with the use of nitrogen, in which the highest dose of this element (N2) was superior to the treatment without nitrogen (N0) with an increase of 48%. These results confirm the importance of the presence of these macronutrients in the development of citronella plants in poor nutrient soils. In comparison with experiments carried out by Seixas [24], it was found that treatments with 150% of the recommended dose of mineral fertilizer showed growth rates in the dry mass variable of 49.6% (4.33 g day^{-1}), in relation to witness.

In the production of the number of leaves of citronella plants, statistically positive effects of the application of mineral nutrients and the tendency of the effect of water stress were identified (Fig. 2). Regarding the water stress variable was observed that plants developed without the presence of stress during their cycle had a higher number

of leaves than plants subjected to water stress. Despite not observing significant differences, the absence of water in a period of high temperature compromised the development and the meristematic differentiation of the apical bud, reducing the rate of leaf development. According to Santos and Carlesso [28] when the plant suffers water restriction after a development of the leaf area, there may be a reduction in leaf expansion, a decrease in the leaf area index, senescence and an increase in leaf abscission.

Regarding the use of phosphate fertilizer, the plants produced a larger number of leaves that grew in soil that received increased doses of fertilizers. Phosphorus is an essential macronutrient responsible for several physiological processes in the plant, including the development of cell membranes and the production of organic energy complexes (Fig. 2). Similar results were obtained by Gusmaini [29], evaluating the effect of phosphate fertilization in Andosol soil, poor in phosphorus. He found that phosphate fertilization promoted a 21.1% increase in the biomass production of the plants as well as their oil content.

Regarding the use of nitrogen compounds, it was observed that treatments with the use of this element stimulated the production of new leaves in citronella plants. Nitrogen is a fundamental element of the chlorophyll molecule, stimulating the photosynthetic process and the production of organic compounds aimed also at the growth of the leaves. At the N1 dose, the number of leaves on the plants was almost 50% higher than the plants of the control treatment, without the use of nitrogen.

Seixas [24] had found that nitrogen at a dose 150% higher than the recommended, in addition to increasing the dry mass of the aerial part, also provided a significant increase in the number of leaves and tillers of citronella plants. Rao et al. [30] demonstrated that the application of nitrogen to the soil promoted an increase of 42% in green mass and 36% in the production of oil in citronella plants.

The production of dry mass of citronella roots was higher in plants that did not suffer water stress (Fig. 3). Plants that submitted to stress showed values 23% lower than plants not submitted to treatment. The period of water stress was short (7 days), but enhanced by the high temperature in the greenhouse, which reached values above 35°C . The reduction in these values probably occurred due to the intensification of perspiration in periods of high temperature, consuming its reserves and reducing its mass.

In the evaluation of phosphorus application, the use of this element promoted a significant increase in the production of dry mass of the roots, an expected process considering the role of phosphorus in the cellular development and energy production of plants. The original phosphorus content in the soil (10.4 mg dm^{-3}), considered low,

Fig. 3 Dry weight of roots of citronella plants grown in sandy soil under water stress and with different doses of nitrogen and phosphorus (CV=36.25%)

intensified the response of plants in the use of fertilizer. Regarding the application of nitrogen, despite not identifying significant results between treatments, there is a tendency to increase the dry mass of citronella roots with the increase in the applied nitrogen dose.

The level of chlorophyll is one of the main phytotechnical indicators of changes in the physiological behavior of plants due to external variables, such as climatic conditions and soil fertility. The induction of hydric stress in the soil promoted an increase in the photosynthetic rate of plants when compared to treatment without stress. It appears that there were no effects of phosphate and nitrogen fertilization, although we observed a tendency to increase photosynthesis in the presence of nitrogen (Fig. 4). Mohd Idrees [31] found that, in the presence of salicylic acid applied to plants subjected to water stress of 50% of field capacity, the total chlorophyll content in citronella plants was reduced, differently from what was observed in this article.

It can be seen in Fig. 5, that the effect of hydric stress induced a greater accumulation of essential oils in plants

Fig. 4 Chlorophyll rate of citronella plants grown in sandy soil under hydric stress and with different doses of nitrogen and phosphorus (CV=42.84%)

compared to plants that did not suffer stress. The presence of phosphate fertilizer as well as nitrogen fertilizer also potentiated the production of essential oils in the plant. The plants responded positively to the external factors applied in relation to the production of essential oils, indicating the potential of the plants in response to the application of chemical fertilizers.

The environmental factors associated with genetic determination are fundamental in the production and concentration of oils in citronella plants. Environmental stress tends to promote the metabolism of plants in the intensification of secondary metabolic routes that induce the production of protective compounds, which is in accordance with Taiz and Zeiger [27] who describe the ability of essential oils to repel potential herbivores, even before they attack the plant. Studies carried out by Morais [32] about the increase in the production of essential oil under hydric stress conditions corroborate these observations. Sanguan et al. [33] found that citronella plants are relatively resistant to hydric stress, increasing the amount of essential oils in their leaves. Farooqui et al. [34] also reported in their article there is increase in oil concentration in citronella species under water stress.

Mirzaie et al. [35] found that citronella plants subjected to soil hydraulic stress at 50% field capacity showed increases in the production of essential oils, but with decreases in production when the soil moisture content was reduced to 25% of field capacity.

It appears that the hydric stress imposed on the plants for 7 days was sufficient to induce the production of a significantly greater amount of oil in relation to plants not subjected to hydric stress, although the dry mass of the aerial part does not differ statistically between treatments with and without stress (Fig. 6). Under stress, plants tend to be more vulnerable to attack by pests and diseases. Due to this, some plants promote the production of a greater amount of secondary compounds accumulated in the

Fig. 5 Yield of essential oil from the aerial part of citronella plants, grown in sandy soil under hydric stress and with different doses of nitrogen and phosphorus (CV=12.34%)

Fig. 6 Total essential oil produced in the aerial part of citronella plants, grown in sandy soil under hydric stress and with different doses of nitrogen and phosphorus (CV=40.5%)

cells, in trichomes, usually present in the epidermis and in the leaf mesophyll including the formation of oil-producing glands [36] that will act to prevent cell damage that occurs due to oxidation processes [37]. Although expected that the highest production of secondary metabolites would occur with the greatest availability of hydric, in many species the opposite occurs [38]. Studies show that despite the reduction in biomass, there is an essential oil yield twice as high [32]. In the species *Ocimum basilicum* (basil), *Anethum graveolens* (dill) and *Artemisia dracuncululus* (tarragon) the increase in hydric stress is accompanied by the corresponding increase in oil yield [39].

The use of phosphorus promoted a significant increase in oil production, about 41% more than plants that grew in soils without the application of this element. Phosphorus is part of several metabolic routes in plants, and its presence has intensified biochemical activity and, consequently, the oil production.

Nitrogen also stimulated oil production by citronella plants. At dose N1 in relation to control N0, the increase in the amount produced was approximately 57%. In the highest dose (N2), the increase in production in relation to the control was 121%, higher than the production and dry matter of the aerial part of the plants, indicating not only an increase in production in relation to the dry mass, but also of the concentrations as seen in Fig. 5. These results are similar to those obtained by E-Sayed et al. [40] where they verified positive effects of the use of phosphorus and nitrogen in the development of citronella plants and in the production of oil.

When increasing the doses of mineral fertilizer, some authors observed an increase in the levels of essential oil, mainly when increasing the levels of nitrogen. The increase was justified because nitrogen is part of the chlorophyll molecule that promotes an increase in photosynthetic activity. Phosphorus, in turn, provides energy for the various metabolic processes linked to plant growth. According

to Figueiredo et al. [41], the scarcity of hydric common in winter causes an increase in plant stress, favoring secondary metabolism, increasing the levels of essential oils.

4 Conclusion

With the obtained results, it can be concluded that the nitrogen and phosphate fertilizers intensified the production of the aerial part of the *Cymbopogon winterianus* plants and their roots responded to the application of phosphorus with the increase in the photosynthetic rate induced by hydric stress and not undergoing changes with the use of chemical fertilizers. *Cymbopogon winterianus* plants have a higher concentration and production of essential oils when subjected to hydric stress and with increased doses of phosphorus and nitrogen.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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