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Liming on the growth and production of essential oil of basil grown in different light environments

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Abstract

Studies about the influence of agronomic factors in the growing and production of secondary metabolites of basil plants are scarce. Among the existing factors, the soil fertility and the light quality has a great influence in the vegetative production of this group of plants. This study aims to evaluate the impacts of different combinations of light quality and the absence and presence of liming on growth, content and yield of essential basil oil. The experimental design was completely randomized in a 5x2 factorial design arranged in subdivided plots. Five light environments were used: black mesh, thermo-reflective mesh, blue mesh, red mesh, and full sun, and two soil acidity correction conditions. We performed the analysis of plants growth at 50 days after thinning (DAT), besides the determination of the essential oil content and yield. We observed significant interaction between the light environment and the soil acidity correction for the variables root volume and dry mass, and trunk dry mass. Regarding the essential oil production, the greater content and yield were obtained in plats grown under thermo-reflective mesh and with limestone application, increasing the soil base saturation. The basil plants are influenced by the light quality and limestone application on the growth, and essential oil content and yield.

Keywords: Base Saturation, Luminosity, *Ocimum basilicum*

Introduction

According to the World Health Organization (WHO), medicinal plants are conceptualized as any plant that has, in one or more organs, substances used for therapeutic purposes (Oliveira et al., 2018). Among the aromatic and medicinal plants, basil (*Ocimum basilicum* L.), originally from India, stands out for its economic importance due to its in natura consumption and its industrial processing (Marques et al., 2015). The essential oil of this species is valued for having several substances of interest, among them linalool, which has commercial importance mainly for perfumery, cosmetics, food and pharmaceutical products (Luz et al., 2009).

The knowledge on the agronomic factors with regard to obtaining a higher production of dry matter and active principles of economic interest of medicinal plants is important, this one can vary according to the weather and environmental conditions. Thus, it is possible to recommend handling ways that aims to increase the production according to the purpose of use of the medicinal plant.

For Yellow Latosols with low levels of bases, liming is indispensable since it raises the calcium and magnesium contents, which influence the increase of soil base saturation, promoting an increase in nutrient availability in the soil solution, which in turn favors soil biota, root system development, and plant nutrient uptake (Nóia et al., 2014).

Studies have shown that both the quantity and quality of light influence many aspects of plant development (Melo & Alvarenga, 2009). Techniques that alter the quality of light by using colored meshes have led to improvements in the culture environment, directly affecting photosynthesis and leading to a greater efficiency of its use (Souza et al., 2011). Studies conducted with basil showed that it is possible to obtain

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high quality seedlings of these species, under blue and red light combination (Macedo et al., 2011). The vegetative growing and fresh biomass of basil plants associated to the pot cultivation was influenced by the use of red mesh and for essential oil extraction the best results were obtained under black mesh shade (Guerra et al., 2020).

Considering the above, the objective of this work is to evaluate the impacts of different combinations of light qualities and the absence and presence of liming in a Yellow Latosol on the growth, content and yield of essential oil of basil.

Material and Methods

Location of the experimental area

This study was carried out from September to November in 2017, in protected environment at the experimental area of the Universidade Federal do Recôncavo da Bahia, in the municipality of Cruz das Almas, BA, which is located geographically at 12°40'19'' S and 39°06'22'' W, at an altitude of 225 m.

Soil

The soil was a Dystrophic Yellow Latosol collected at the superficial layer (0.0-0.20 m). It was harrowed, air dried, passed through a 4-mm sieve, and later analyzed chemically, According to the proposed methodology by the lab (CAMPO – Centro de Tecnologia Agrícola e Ambiental). Based on the soil chemical analysis, an incubation was carried out by applying dolomitic limestone with 100% PRNT directly in each pot with a capacity of 6 dm^3 for a period of 90 days aiming to reach 70% of base saturation. The pots were then incubated for 90 days. After this period, soil analysis was performed to identify the chemical changes occurring due to the presence of limestone (Table 1).

Table 1. Chemical analysis result of the Yellow Latosols before and after the saturation increasing by bases through the liming collected in the city of Cruz das Almas, BA.

Characteristic	Soil conditions	
	Without limestone	With limestone
pH (H_20)	5.7	5.9
pH ($CaCl_2$)	5.0	5.2
Organic matter (%)	0.7	1.0
P (mg dm^{-3})	2.6	2.8
K (mg dm^{-3})	37.0	47.6
Ca^{2+} (cmol$_c$ dm^{-3})	1.0	1.4
Mg^{2+} (cmol$_c$ dm^{-3})	0.4	0.6
Al^{3+} (cmol$_c$ dm^{-3})	<0.1	0.0
H+Al (cmol$_c$ dm^{-3})	1.8	1.6
T (cmol$_c$ dm^{-3})	3.3	3.7
V (%)	45.0	57
m (%)	6.0	0.0
Ratios		
Ca/Mg	2.5	2.3
Ca/K	10.6	11.5
Mg/K	4.2	4.9
(Ca+Mg)/K	14.9	16.7
Saturation of the Exchange Complex		
K (%)	3.0	3.0
Ca (%)	30.0	38.0
Mg (%)	12.0	16.0
Na (%)	0.0	0.0
H+Al (%)	55.0	43.0

Experimental design and implementation

The experiment was completely randomized with a 2x5 factorial design and two soil correction conditions: absence and presence of liming, and five light conditions: 1) Red Mesh; 2) Blue Mesh; 3) Thermo-reflective Mesh; 4) Black Mesh (all the meshes with 50% of irradiance); and 5) Full Sun, with seven replications, arranged in a plot subdivided in space.

Based on the soil analysis report, proceed the fertilization recommendation using as reference the Mentha piperita (Raij et al., 1997), whose demands and uptakes are similar to the basil (Pravuschi et al., 2010), that doesn't have an specific recommendation. All pots received planting fertilization with N (100 kg ha^{-1}), P_2O_5 (60 kg ha^{-1}) and K_2O (60 kg ha^{-1}) as urea, single superphosphate and potassium chloride, respectively, together with 1 dm^3 of Vivatto Plus® at a ratio of 5:1. Three seeds per pot were used, these ones were certified to ensure homogeneity in the germination power and initial growing of basil seedlings and after 10 days proceed the thinning letting one plant per pot. The irrigation of pots was carried out manually at a necessary quantity according to the needs of plants.

Growth analysis

At 50 days after thinning, the plant height (PHe) was measured with a tape measure from the base to the apex of the plant (terminal bud); the stem diameter (SD) was measured at 1 cm from the substrate using a pachymeter with an accuracy of 0.01 mm; the number of leaves (NL) was counted manually considering leaves with at least 2/3 the size of a leaf already established; root length (RL) was measured with a tape measure from the upper base to the highest root concentration; and root volume (RV) was measured by the test tube method. Data of Chlorophyll A, chlorophyll B, total chlorophyll (CLO T) and chlorophyll A and B ratio index were collected using the Falker CFL1030-model electronic meter.

The leaves, stem and root were individually packed in paper bags, and placed in a greenhouse with forced air circulation at 40 ± 2°C until constant mass. Leaf dry matter (LDM), stem dry matter (SDM) and root dry matter (RDM) were determined using an analytical balance with an accuracy of 10^{-3}.

The total leaf area (LA) per plant was determined using the ratio between leaf dry matter and dry matter of 10 leaf discs. Leaf area ratio (LAR), leaf matter ratio (LMR) and specific leaf area (SLA) were determined using the mathematical formulas described by Peixoto (2011).

Extraction of the essential oil

The extraction of the essential oil was carried out by hydrodistillation by water vapor drag using a modified Clevenger apparatus in the Laboratory of Phytochemistry of the Federal University of Recôncavo da Bahia, Cruz das Almas, BA. 30 g of leaf dry matter were placed in 3-liter flasks, and distilled water was added until they were immersed. Then, the distillation process began by entraining the essential oil by water vapor.

To determine the moisture content (procedure performed in triplicate), 1 g of dry matter of the leaves of each treatment was separated. These samples were placed in a greenhouse with forced air circulation for 48 hours at a temperature of 40°C. Moisture was calculated using the Equation 1. For the determination of essential oil content (Santos et al., 2004), Equation 2 was used. The yield of the essential oil was obtained by multiplying the oil content by the leaf dry matter ((Content x LDM)/100).

$$U\% = (Wi - Wf)/(Wf \times 100) \qquad (1)$$

Where: U% = moisture content; Wi = initial weight; Wf = final weight.

$$Co = Vo/(Bm\ ((Bm \times U))/100) \times 100 \qquad (2)$$

Where: Co = oil content (%); Vo = volume of extracted oil; Bm = vegetal shoot biomass.

Statistical analysis

Growth data were submitted to analysis of variance and Tukey test at 5% probability using the "R" (R Development Core Team) statistical software.

For the essential oil content and yield, it was not possible to perform a statistical comparison by Tukey test. It was extracted from a composite sample formed by the grouping of replications of each treatment.

Results and Discussion

The results showed that there was a significant effect of the interaction between the soil correction conditions and the different light environments on root volume, stem dry matter and root dry matter (Table 2). In addition, light environments influenced plant height, stem diameter, leaf number, leaf dry matter, total dry matter, ratio between shoot dry matter and root dry matter, specific leaf area, leaf area ratio, leaf matter ratio and chlorophyll indexes. Regarding the isolated effects of soil correction conditions (absence and presence of limestone), they were significant for root length, leaf area, leaf area ratio and leaf matter ratio.

Basil plants grown in natural soil have a higher root volume when grown under a red mesh. It is significantly superior (64%) to plants cultivated under thermo-reflective mesh and 142% superior to plants that received limestone. This variable was not influenced by the presence of meshes, that is, shading effects, when plants were cultivated in a soil without limestone. However, for plants grown in a soil with limestone, root volume was significantly superior when grown in full sun in relation to different environments using meshes. These results can be explained because liming acts on the availability of Ca and Mg in the soil solution, increasing the pH, which in turn increases the availability of nutrients responsible for promoting root system modification (Favare et al., 2012). In addition, plants grown in full sun receive a higher luminous incidence than those grown under meshes, a condition that favors higher photosynthetic rates and, consequently, a higher plant growth.

In the unshaded environment, the root dry matter yield of basil plants was up to 86% higher when compared to plants grown under thermo-reflective mesh on natural soil. There was a reduction of 39% in root dry matter when plants were cultivated under a red mesh in a soil without limestone and 60% in a soil with limestone. These results indicate that the reduction in the intensity by using meshes limited the yield of roots, which can directly reflect in a lower absorption of water and nutrients by the plants.

According to the results obtained by Martins et al. (2008), there was a variation in the phytomass accumulation of roots under different meshes, since each species has its particularity as to the responses to light intensity and to the use of meshes.

Table 2. Interaction unfolding for root volume, root dry matter and stem dry matter of basil plants grown in the presence and absence of liming and different light environments.

Light environments	Root Volume (mL)	
	Without limestone	With limestone
Full sun	0.46 AB a	0.57 A a
Red mesh	0.51 A a	0.21 B b
Blue mesh	0.37 AB a	0.37 B a
Thermo-reflective mesh	0.31 B a	0.33 B a
Black mesh	0.46 AB a	0.37 B a
	Root Dry Matter (g)	
	Without limestone	With limestone
Full sun	2.73 A a	3.15 A a
Red mesh	1.67 C a	1.27 C a
Blue mesh	1.94 BC a	2.03 B a
Thermo-reflective mesh	1.47 C a	1.54 BC a
Black mesh	2.31 AB a	1.84 B b
	Stem dry matter (g)	
	Without limestone	With limestone
Full sun	6.37 A a	6.43 A a
Red mesh	4.85 CD a	5.39 B a
Blue mesh	5.12 BC a	5.28 B a
Thermo-reflective mesh	3.91 D b	4.63 B a
Black mesh	5.94 AB a	5.16 B b

* Means followed by the same uppercase letters in columns and lowercase letters in lines do not differ statistically by Tukey test ($p<0.05$)

Similar results were obtained by Chagas et al. (2013), who, evaluating the effects of photo-converting meshes on the growth of plants of Mentha arvensis, observed that the accumulation of dried biomass by roots was greater in plants grown under full sun. According to the same authors, the allocation of dry biomass to roots indicates that it occurs preferentially in function of light intensity. In these plants, such a greater accumulation of photoassimilates in roots, to the detriment of leaves, happens commonly as an adaptation strategy in increasing the capacity of the plant to absorb water and nutrients.

Plants cultivated under full sun and liming differed from the other environments as for stem dry matter, whereas in the absence of limestone in the soil, the plants grown under full sun did not differ from those grown under the black mesh, but they differed from the other environments (Table 2). According to Paulus (2016), heliophyte plants, such as basil, efficiently use high radiation intensities due to the high capacity of the electron transport system, and thus achieve greater photosynthetic gains.

The isolated effect of light environment, i.e., independent of liming, is presented in Table 3.

Light environments significantly influenced plant height. The highest heights were obtained when basil plants were cultivated under thermo-reflective and red meshes in relation to the other light environments, and the lowest heights were obtained in the full sun environment. Paulus et al. (2016), in a study with photo-converting meshes used for the growth of basil (*Ocimum basilicum* var. *citriodorum*), observed results similar to this study. Because it is a heliophyte species, stem elongation of basil plants is a response to avoiding shade, thus obtaining a greater uptake of light energy and indicating the involvement of phytochrome with the perception of shade (Taiz & Zeiger, 2013).

The variable stem diameter was better expressed when basil plants grew under red and black meshes, as well as under full sun. The temperature control was exerted by the thermo-reflective mesh and, in this environment, the plants have a lower height. Souza et al. (2014), studying rosemary (*Rosmarinus officinalis* L.), observed that plants grown under full sun showed a larger stem diameter. According to the same authors, a larger stem diameter is a desirable attribute, since it guarantees a greater support for shoots.

Table 3. Mean values for the variables plant height (PHe), stem diameter (SD), number of leaves (NL), leaf dry matter (LDM), total dry matter (TDM), ratio between shoot dry matter and root dry matter (SDM/RDM), specific leaf area (SLA), leaf area ratio (LAR), leaf matter ratio (LMR), chlorophyll A (CLO A), chlorophyll B (CLO B), total chlorophyll (CLO T) and chlorophyll A and B ratio (CLO A/B) of basil plants grown in different light environments independent of liming.

Variables	Light Environments				
	Full sun	Red mesh	Blue mesh	Thermo-reflective mesh	Black mesh
PHe (cm)	49.42 d	71.71 ab	56.14 cd	72.43 a	61.21 bc
SD (mm)	5.74 ab	6.21 a	5.10 bc	5.05 c	5.78 a
NL	78.00 ab	71.86 b	65.28 b	86.50 a	77.78 ab
LDM (g)	7.85 a	5.54 bc	6.63 b	5.49 c	5.43 c
TDM (g)	17.19 a	12.13 bc	13.82 b	11.27 c	13.06 b
SDM/RDM	4.97 d	7.65 a	6.05 bc	6.68 ab	5.36 cd
SLA ($cm^2\ g^{-1}$)	325.36 c	515.88 a	441.31 b	505.07 a	447.62 b
LAR ($cm^2\ g^{-1}$)	148.48 d	235.52 ab	209.99 bc	246.27 a	186.92 c
LMR ($g\ g^{-1}$)	0.46 ab	0.46 ab	0.48 a	0.48 a	0.42 b
CLO A (FCI)	30.46 a	28.39 b	30.89 a	28.74 b	28.30 b
CLO B (FCI)	10.57 a	8.98 b	11.33 a	9.58 b	9.34 b
CLO T (FCI)	41.02 a	37.37 b	42.22 a	38.33 b	37.64 b
CLO A/B	2.90 bc	3.20 a	2.75 c	3.01 ab	3.06 ab

* Means followed by different letters in lines differ statistically by Tukey test ($p \leq 0.05$)

The number of leaves in basil plants was higher when cultivated under thermo-reflective and black meshes and under full sun. There was no direct relation with leaf dry matter and total dry matter since these variables did not differentiate among themselves in the light environments evaluated.

The evaluated characteristics for leaf dry matter and total dry matter presented increases with the increase in light intensity. This behavior can be explained because plants increase photosynthesis according to a greater light availability, which culminates in an increase in the carbohydrate content in leaves, stem and root, which in turn directly influences the increase in dry matter (Larcher, 2000).

For the variable ratio between shoot dry matter and root dry matter of basil plants, the highest values were observed when the plants grew under red and thermo-reflective meshes, and lower values were obtained in the absence of meshes (full sun) and the black mesh. The smaller the shoot/root ratio in plants under high light intensity, the greater the photoassimilate allocation to the root system. This strategy is responsible for allowing the plant to perform a greater absorption of water and nutrients, supporting the high rates of photosynthesis and transpiration under a high light intensity.

The red mesh and the thermo-reflective mesh provided the highest values for the variable specific leaf area. This variable relates the surface and the weight of the leaf, representing its thickness. According to Larcher (2004), plants grown at a high intensity of radiation develop thick leaves. Therefore, they present a more active metabolism, providing a greater production of dry matter and, consequently, a higher energetic content.

Regarding the leaf area ratio, the highest values were observed in plants grown under the thermo-reflective mesh. According to Freitas (2013), the leaf area ratio represents the useful leaf area, that is, the photosynthetically active area. The cultivation of basil under the thermo-reflective and blue meshes obtained the highest values for the variable leaf matter ratio, and plants grown under the black mesh obtained the lowest value for the variable in question. This physiological variable expresses the fraction of dry matter not exported from leaves to the rest of the plant.

Melo & Alvarenga (2009), studying the effects of blue and red meshes on the development of *Catharanthus roseus* (L.) G. Don, observed that, when cultivated under full sun, it presented a significantly reduced leaf area and lower leaf area ratio and leaf weight ratio when compared to shaded environments.

The values of chlorophyll A, B and total chlorophyll showed that there was a significant difference between the evaluated light environments, with no difference between plants grown under full sun and under the blue mesh. Paulus et al. (2016) obtained contrary results. No significant difference was observed between photo-converting meshes for chlorophyll A, chlorophyll B and total chlorophyll. Results contrary to this were obtained by Souza et al. (2011), who, working with guaco plants (*Mikania laevigata*), observed that, when cultivated in full sunlight, plants obtained lower levels of chlorophyll A and B. For plants grown under a blue mesh, there was a higher production of chlorophyll A and B, corroborating with the data of this work.

For the chlorophyll A/B ratio, the highest values were obtained when plants were cultivated under the red mesh. The lowest values were found by using the blue mesh: it did not differentiate from the full sun cultivation. The increase in the proportion of chlorophyll B in shaded plants can be seen as an important characteristic of plant adaptation to these environments, since chlorophyll B absorbs energy at wavelengths differently from chlorophyll A, transferring it to the reaction center, which maximizes the energy capture that effectively acts on the photochemical reactions (Taiz & Zeiger, 2013).

The isolated effect of soil correction (absence and presence of limestone) is presented in Table 4. For the variable root length, the highest values were obtained in the presence of liming. The increase in root length caused by the increase in base saturation from liming has a direct relationship with the increase of Ca and Mg contents in the soil, since these elements participate in the synthesis of the cell wall, forming pectates of Ca and Mg (Epstein & Bloom, 2006).

Table 4. Mean values for root length (RL), leaf area (LA), leaf area ratio (LAR) and leaf matter ratio (LMR) of basil plants grown in the presence and absence of liming.

Variables	Limestone	
	Without	With
RL (cm)	26.53 b	32.03 a
LA (cm²)	2,843.37 a	2,565.38 b
LAR ($cm^2\ g^{-1}$)	213.88 a	196.99 b
LMR ($g\ g^{-1}$)	0.47 a	0.45 b

* Means followed by different letters in lines differ statistically by Tukey test (p ≤ 0.05)

Leaf area, together with physiological indexes represented by leaf area ratio and leaf matter ratio, showed a similar statistical behavior. The highest values were reached when basil plants were cultivated in the absence of limestone. Souza et al. (2014) emphasized the importance of leaves for the biological production of the plant, considering leaf area a productivity index.

The contents and yields of essential oil of basil plants grown under different soil and environment conditions are presented in Figures 1 and 2.

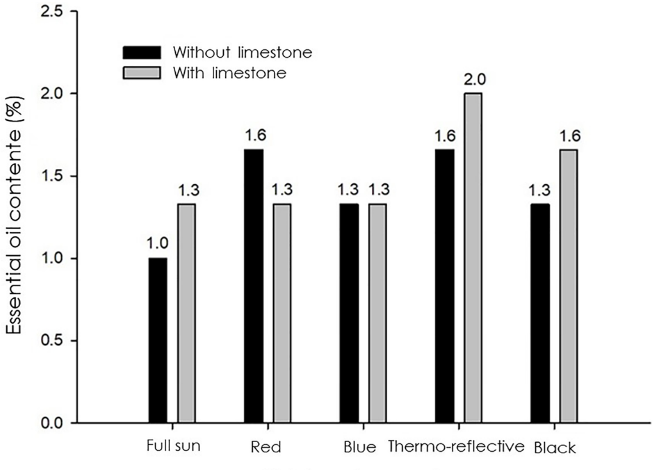

Figure 1. Essential oil content of basil plants grown in the presence and absence of limestone and different light environments.

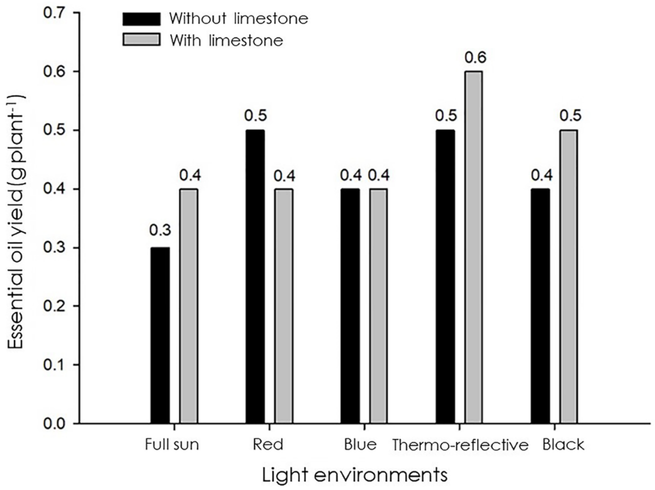

Figure 2. Essential oil yield of basil plants grown in the presence and absence of limestone and different light environments.

The highest yield and oil content were obtained in the presence of limestone in the environment under the thermo-reflective mesh. Plants grown in full sunlight obtained the lowest content and yield of essential oil compared to the other environments. This is probably a consequence of the basil plant presenting a better growth in this environment since it needs a greater amount of sunlight. It can be concluded that in shaded environments, the metabolism is secondary and activated in plants as a reaction to some kind of stress.

According to Barbosa et al. (2017), the essential oil comes from the secondary metabolism, and it is influenced by environmental factors. Chagas et al. (2013) describe that, under adverse conditions, there is a greater production of essential oil. Thus, the results allow inferring that a 50% shading favors the production of basil essential oil and that there are diverse physiological responses depending on the species, such as for elixir-paregoric plants (*Ocimum selloi*), for which a partial shading of 50% did not cause variations due to the reduction of light intensity (Gonçalves et al., 2003).

Martins et al. (2008) observed that *O. gratissimum* plants grown under full sunlight produced a lower essential oil content than plants cultivated under blue, red and black meshes. On the other hand, Paulus et al. (2016) observed that the content and yield of essential oil of basil plants under full sun were higher than in plants grown under photo-converting meshes. Oliveira Júnior et al. (2005), working with fertilization and liming on arnica (*Lychnophora ericoides*), observed that the amount of essential oil produced in plant shoots was negatively influenced by liming.

Conclusions

Different light environments and soil fertility conditions influence the growth and production of essential oil by basil plants.

The environment under full sun, with or without limestone, causes increases in the stem volume and stem dry matter of basil plants.

For the production of essential oil of basil plants, the highest content and yield are obtained by plants grown under thermo-reflective mesh together with liming.

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