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Light intensity on growth, leaf micromorphology and essential oil production of *Ocimum gratissimum*

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Abstract: Light conditions can promote the growth and development of plants and contribute to increase the essential oil production of commercially cultivated medicinal and aromatic species. In view of the great importance of *Ocimum gratissimum* L., Lamiaceae, as an aromatic plant, the objective of this work was to determine the effect of light intensities (approximately 4, 7, 11 and 20 mol m^{-2} d^{-1}) on growth, foliar micromorphology, essential oil content, yield and chemical composition of *O. gratissimum*. Biomass production of different organs, root:shoot ratio and leaf mass per area were found to linearly increase with increased light availability, whereas stem dry matter fraction, number of leaves, leaf area and plant height have increased up to 10 mol m^{-2} d^{-1} and decreased from this value. The tector trichomes density increased with increased light availability, but there was no effect of light treatments on the glandular trichomes density and essential oil content. Regardless of the light level, the major component of the essential oil was eugenol. The essential oil yield per plant increased linearly with light intensity as a direct effect of increased leaf biomass under similar conditions.

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Introduction

Ocimum gratissimum L., Lamiaceae, popularly known as 'alfavaca', 'alfavacão' or 'alfavaca-cravo', is an aromatic shrub, up to 1m in height, originated from Africa and sub-spontaneous in Brazil. The species belonging to the genus *Ocimum* are generally characterized as being rich in essential oils for pharmaceuticals, fragrances and cosmetics whose major component in *O. gratissimum* is eugenol. Several studies have proven the antibacterial (Matasyoh et al., 2007) antifungal (Faria et al., 2006), antioxidant and hypoglycemic properties of its extract and essential oil (Aguiyi et al., 2000; Trevisan et al., 2006). Despite the potential importance of *O. gratissimum* to generate products of medicinal and pharmaceutical interest, there is little information about cultivation practices for this species.

Light radiation is essential for growth and development of plants, since it is directly related to photosynthesis and other physiological, biochemical and morphological processes (Paez et al., 2000). Plants grown under low-light environments exhibit a significant reduction in biomass with changes in the biomass allocation to different organs (Claussen, 1996; Silva et al., 2006). Besides being the primary photosynthetic organ, the leaf shows great phenotypic plasticity due changes in the light radiation available to ensure a positive carbon balance (Valladares & Niinemets, 2008). Changes in leaf morphology could give rise to variations in leaf micromorphology and therefore interfere with the essential oil content.

The study of leaf micromorphology is an important tool to identify the secretory structures responsible for the biosynthesis and storage of a variety of bioactive compounds. Essential oils of the mint-family species (Lamiaceae) are synthesized and stored in peltate and capitate glandular trichomes. Capitate glandular trichomes secrete a small amount of essential oil and some polysaccharides whereas peltate glandular trichomes have a greater number of secretory cells in the head and are the most important for the essential oils production (Werker, 1993). The study of the density of these structures may be indicative of the production capacity of essential oils in aromatic species, such as *Lippia citriodora*, which showed a substantial reduction in glandular density when subjected to low-intensity light radiation (Gomes et al., 2009).

The production of secondary metabolites by medicinal and aromatic plants, including essential oils, may be affected by shading, since the carbon fixed in photosynthesis is the fundamental component of organic compounds (Paez et al., 2000; Sangwan et al., 2001). Plants of *Ocimum basilicum* had significant reductions in essential oil content and changes in chemical composition when cultivated under low light availability (Chang et al., 2008).

The objective of this work was to determine the effect of light intensity on growth, foliar micromorphology, essential oil content, yield and chemical composition of *Ocimum gratissimum*.

Materials and Methods

The experiment was carried out at Universidade Estadual de Santa Cruz, Ilhéus, Bahia, Brazil. A voucher herbarium specimen is deposited at the HUESC herbarium under number 14427. The plants were propagated by basal stem cuttings and after rooting, they were transferred to pots containing 10 L of substrate with a 3:1:1 ratio (soil:organic matter:sand) and subjected to four light environments. Throughout the experiment, photosynthetic photon flux density (PPFD) was monitored in full sun, at each light environment, using S LIA-M003 light radiation sensors coupled to a Hobo Data Logging Micro Weather Station (Onset Computer, Massachusetts, USA). The datalogger was programmed to perform readings at one-minute intervals and store readings every ten minutes.

Dry biomass of roots (RDB), stems (SDB), leaves (LDB), leaf area (LA) and total plant dry biomass (TDB) were assessed on ten plants per treatment at the beginning and at the end of the experiment. In flourished plants it was also assessed the dry biomass of inflorescences. Plant material was dried in a circulating air oven at 75 ^{o}C until a constant dry biomass was reached. Leaf area was estimated using a LI-3100 Area Meter (Li-Cor, Lincoln, NE, USA). Leaf mass per area (LMA) was calculated by the quotient between LDM and LA.

Fully expanded leaves were collected from the third node from the apex to the base of the plant. Segments of the the median portion leaf were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 6.9, dehydrated in crescent ethanol series and dried at critical point (CPD 030, Bal-Tec, Balzers, Liechtenstein), and golden coated with a sputter coater apparatus (SCD 050, Bal-tec, Balzers, Liechtenstein). After that, the samples were examined using a scanning electron microscope (Leo 1430 VP, Cambridge, UK). Four replicates were carried out and five observation fields were randomly selected, totaling twenty fields per treatment.

The extraction of essential oil was performed through hydrodistillation, using a Clevenger apparatus, with 50 g of leaf dry biomass, in quadruplicate, for 1 h. The essential oil content was determined based on the volume extracted per 100 g of leaf dry biomass (% w/v). The essential oil yield was determined by multiplying the content by the average value of leaf dry biomass (g plant-1).

The essential oil was analyzed by gas chromatography, using Varian Saturn 3800 apparatus (Varian Inc., Palo Alto, USA) equipped with a flame ionization detector (GC-FID), using a VF-5ms capillary column (30 mm x 0.25 mm x 0.25 µm film thickness) and helium as carrier gas, at a flow of 1.2 mL min^{-1}. The injector and detector temperatures were at 250 and 280 °C, respectively. The column temperature programming began at 70 °C, followed by an increase of 8 °C min^{-1} until reaching 200 °C, and 10 °C min^{-1} until reaching 260 °C; keeping this temperature during 5 min. A volume of 1 µL of a 10% solution of oil in chloroform was injected in the 1:10 split mode. The concentration of volatile constituents was calculated based on the full area of their respective peaks, related to the total area of all the constituents of the samples. The qualitative analysis of the essential oil was carried out using mass spectrometer Chromopack 2000/MS/MS mass spectrometer (Varian Inc., Palo Alto, USA). The same VF-5ms column and the same column programming were used. The transferline temperature was 250 °C and the trap temperature was 220 °C. The chemical constituents were identified through computer comparison with the apparatus library and literature. Linear retention indices were calculated injecting a series of n-alkanes (C8-C26), under the same chromatographic conditions of the samples.

The experiment was a completely randomized design. Effects of four light levels on growth parameters, trichomes density and essential oil production were submitted to analysis of variance and regression at the level of 5% of probability.

Results

Changes in light availability affected significantly all growth variables (Table 1). There has been increased dry matter content in the root, leaves, inflorescences, total root:shoot ratio and LMA with increased light intensity. Stem dry biomass had a quadratic fit in relation to light availability, with a production that did not exceed 53 g in 15 mol m^{-2}d^{-1}. Plant height had a quadratic response, reaching a maximum value of approximately 112 cm in 10 mol m^{-2}d^{-1}. Likewise, leaf number and leaf area have exhibited quadratic fits with the limit points estimated at 1090 and 9264 cm^2, in 17 and 12 mol m^{-2} d^{-1}, respectively (Table 1).

The leaves in all light environments exhibited multicellular tector trichomes, capitate trichomes with a bicellular head and peltate glandular trichomes with four secretory cells in the head, on both leaf sides (Figure 1). The density of capitate and peltate glandular trichomes was not influenced by light environments, with mean values of 60.51 and 23.69 trichomes mm², respectively in adaxial surface and 43.11 and 39.21 trichomes mm², respectively in abaxial surface of the leaf. However, there have been changes in tector trichomes density. The adaxial side showed a growing linear effect reaching a maximum value of 81 trichomes mm² at 20 mol m^{-2} d^{-1}, whereas the abaxial surface showed a maximum quadratic adjustment of 142 trichomes in approximately 15 mol m^{-2} d^{-1} (Figures 2A and 2B).

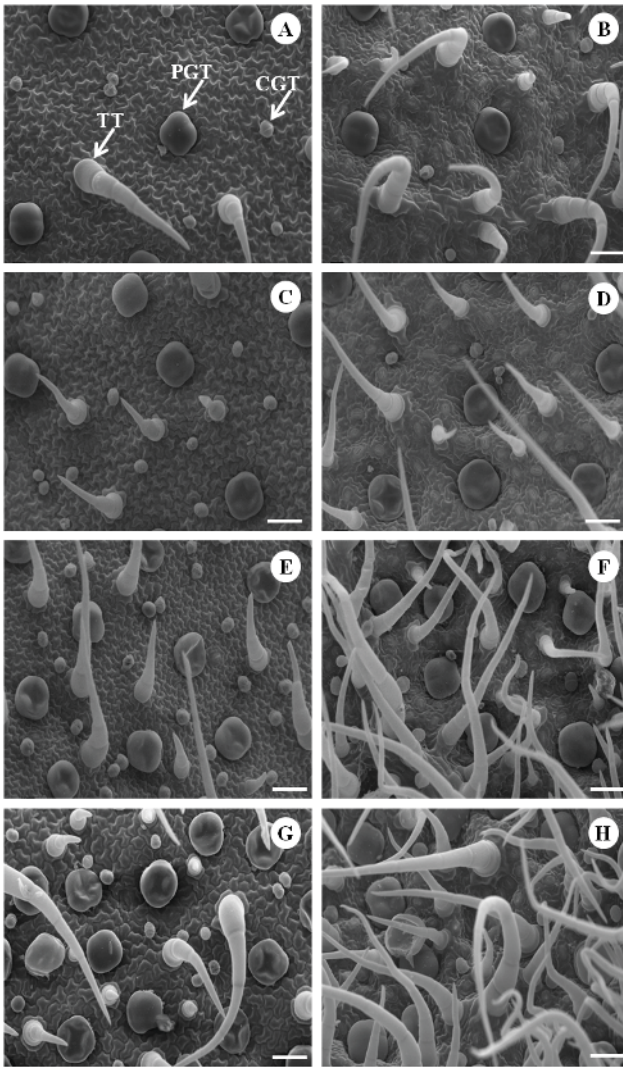

Figure 1. Scanning electron micrographs of the adaxial (A, C, E, G) and abaxial (B, D, F, H) surfaces of *Ocimum gratissimum* leaves as a function of different light intensities: A and B = 4; C and D = 7; E and F = 11; G and H = 20 (mol m^{-2} d^{-1}). TT: tector trichome; PGT: peltate glandular trichome; CGT: capitate glandular trichome. Bar: 40 µm.

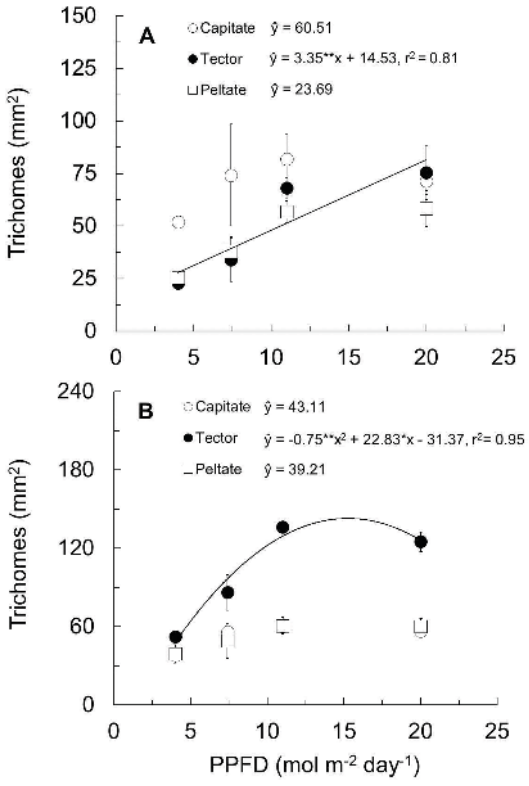

Figure 2. Trichome density on the adaxial (A) and abaxial (B) surfaces of *Ocimum gratissimum* leaves in different light intensities. $^{**}p \leq 0.01$, $^{*}p \leq 0.05$. The bars represent the mean standard error, n=4.

There was no effect of light radiation on the essential oil content, whose average value was 1.2%, but the essential oil yield per plant was significantly changed and showed a linear fit with increasing light intensity (Figure 3).

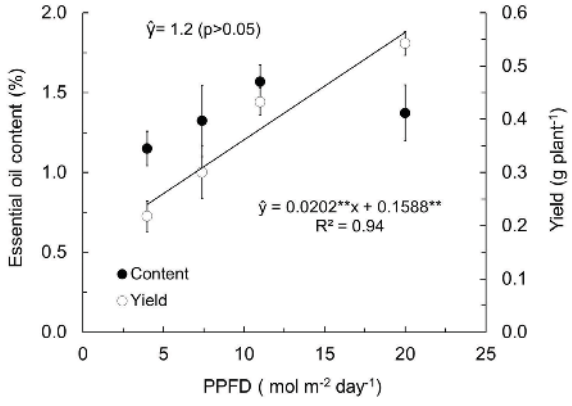

Figure 3. Essential oil content (%) and yield (g $plant^{-1}$) of in dry leaves of *Ocimum gratissimum* plants grown in different light intensities; $^{**}p \leq 0.01$, t test. Bars denote the mean standard error, n=10.

Table 1. Plant growth variables of *Ocimum gratissimum* as a function of different light intensities.

Variables	PPFD (mol m^{-2} d^{-1})				Equation	r^2
	4	7	11	20		
PH	102.80±4.30	109.90±4.91	111.30±3.16	80.40±1.43	ŷ = - 0.28**x2+5.51**x+85.19**	0.99
RDB	5.38±0.57	8.45±1.39	20.05±3.40	37.34±2.89	ŷ = 2.08** x - 4.27*	0.98
SDB	22.74± 2.02	32.92±3.89	52.05±1.43	44.92±1.36	ŷ = - 0.27**x2+8.10**x - 7.24*	0.92
LDB	18.50±1.52	24.40±2.46	36.14±1.32	44.73±1.60	ŷ = 1.64**x+13.46**	0.93
IDB	0.46±0.19	2.23±0.74	9.32±0.84	14.73±1.19	ŷ = 0.92**x - 3.14**	0.94
TDB	47.09±4.03	68.00±8.10	117.56±5.22	141.72±5.13	ŷ = 5.97**x + 30.22**	0.89
R:S	0.13±0.005	0.14±0.011	0.20±0.030	0.36±0.025	ŷ = 0.01*x + 0.04*	0.97
LN	573.7±39.60	746.6±64.46	1018.6±33.31	1049.90±39.23	ŷ = - 3.33**x2+111.37**x+ 159.37*	0.96
LA	7374.2±447.23	8101.4±563.43	9521.3±297.96	7398.08±310.74	ŷ = - 30.44**x2+745.77**x+ 697.00**	0.89
LMA	24.90±0.97	29.61±1.27	38.10±1.34	60.71±1.13	ŷ = 2.29**x + 13.98**	0.99

PH: plant height (cm), RDB (root dry biomass, g), SDB (stem dry biomass, g), LDB (leaf dry biomass, g), IDB (inflorescence dry biomass, g), TDB (total dry biomass, g), R:S (root: shoot ratio), LN (number of leaves), LA (leaf area, cm^2), LMA (leaf mass per area, g m^{-2}).±mean standard error (n=10). Significant *p<0.05; **p<0.01.

Eight components were identified, totaling 99.0% of the essential oil's chemical composition (Table 2). The major component was eugenol, with an average content of 91%, and remained stable regardless of the light environment. Essential oil plants subjected to higher light radiation intensities (11 and 20 mol m^{-2} d^{-1}) presented small variations in chemical composition, with the presence of three components (terpin-4-ol, β-bourbounene and caryophyllene oxide) that have not been identified in other treatments.

Discussion

The acclimation of plants in response to environmental conditions may reflect changes on physiological and morphological parameters and on the production of secondary metabolites (Chang et al., 2008). The highest production of total dry biomass obtained under higher light radiation indicate that low light intensities may be limiting for the growth of *O. gratissimum* plants. The decrease in stem dry biomass observed in environments with higher light radiation suggests a carbon allocation shift to produce new reproductive organs. At the same time, low light radiation was a limiting factor for the production of inflorescences in environments with lower PPFD levels. Changes in dry biomass partitioning between root and shoot are a common response of plants to changes in the light radiation intensity during growth (Valladares & Niinemets, 2008). The increase of the root:shoot ratio in response to the increase in light radiation is an acclimation response that enables greater absorption of water and nutrient; this strategy probably allows plants to achieve higher photosynthesis and transpiration rates (Claussen, 1996). According to Morelli & Ruberti (2000), plant responses to light radiation are diverse, and height growth stimulation is one of the fastest reactions to shading environments. The medicinal species *Baccharis trimera* (Silva et al., 2006) and *Aloe vera* (Paez et al., 2000) have also shown greater height growth when grown under shaded environments. Increments in plant height as a consequence of the decreases in light availability are related to the apical dominance induced by the increase in auxin levels in the stem apex (Vanneste & Friml, 2009).

The increment in LMA with increasing the light availability was possibly the result of greater thickening of leaves in these treatments. Structural leaf changes such as in the specific leaf mass are described as major mechanisms of acclimation to different environmental conditions (Aranda et al., 2004) and their increase is directly related to leaf thickness (Castro-Díez et al., 2000); moreover, sun leaves are thicker than shade leaves (Lambers et al., 2008).

There was an increasing trend in the number of leaves, whereas the leaf area decreased with increased light radiation. The increased leaf area of plants grown under low light radiation provides an increase in the photosynthetic surface and contributes to a more efficient absorption of light radiation (Lambers et al., 2008). The significative leaf area reduction found in *Gallesia integrifolia* and *Schinnus terebinthifolius* subjected to dense shading were related with insufficient carbon assimilation rates for the production of new photosynthetic tissues (Feijó et al., 2009).

The increased density of tector trichomes of *O. gratissimum* with increasing light radiation may be an adaptive strategy for reducing transpiration rate, increasing the sunlight reflection and decreasing the leaf temperature (Evert, 2006). A similar behavior was observed in *Elaeagnus angustifolia* leaves subjected to different light radiation conditions at the canopies, with reduced density of stellate trichomes on leaves collected

Table 2. Percentage of the essential oil constituents of *Ocimum gratissimum* plants cultivated in different light intensities.

		PPFD (mol m^{-2} d^{-1})			
		4	7	11	20
Components	LRI*	Relative percentages (%)			
cis-ocimene	1034	1.4	3.8	4.1	3.4
trans-4-thujanol	1073	0.5	0.6	0.5	0.5
terpin-4-ol	1185	-	-	0.4	0.6
eugenol	1358	91.9	91.6	89.7	90.7
β-bourbounene	1388	-	-	0.4	0.4
(*E*)-caryophyllene	1429	1.3	1.2	1.4	1.4
germancrene D	1486	3.9	2.5	2.8	2.2
caryophyllene oxide	1589	-	-	0.4	0.4
Total identified (%)		99.0	99.7	99.7	99.6

LRI*:linear retention index; - unidentified.

in shading conditions (Klich, 2000).

The fact that light intensity has not altered the essential oil content in our study is associated with the density of glandular trichomes which also remained constant. The significant increase in essential oil yield in face of increased light radiation is directly related to the increased leaf biomass production under these conditions, as previously observed in *Baccharis trimera* (Silva et al., 2006).

Small changes in the chemical composition of the essential oil may be related both to the light radiation availability and to the growth stage of the plants, since those grown in environments with higher PPFD levels were already at reproductive phase and completely bloomy. The chemical composition of essential oils is determined by genetic factors, but environmental factors may bring about significant changes in the production of secondary metabolites (Lima et al., 2003; Gobbo-Neto & Lopes, 2007). *O. basilicum* plants exposed to higher radiation levels had higher linalool and eugenol rates, whereas the more shaded treatment presented higher methyl eugenol content (Chang et al., 2008). The chemical composition of essential oils may also be influenced by the plant physiology and depends upon their stage of development (Sangwan et al., 2001).

Thus we can conclude that the light intensity interferes on growth of *Ocimum gratissimum* without affecting the essential oil content and chemical composition. The essential oil content remains constant due to the density of glandular trichomes that was not affected by light. The significant increase in essential oil yield with the light intensity is related to the increased leaf biomass production under these same conditions.

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Authors' contributions

VFF and EVRSF (Masters students) contributed in running the field and laboratory work, collection and analysis of the data and drafting the paper. LBA (undergraduate student) contributed in collecting plant sample and identification, herbarium confection, essential oil quantification and chromatographic analysis. DCS, RAO, MSM and LCBC designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

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