

ALVES, AC; JESUS, FN; ALVES, PB; SANTOS, HV; SOUZA, GS; SANTOS, AR. 2018. Biomass production and essential oil of lemon balm cultivated under colored screens and nitrogen. *Horticultura Brasileira* 36: 094-099. DOI - <http://dx.doi.org/10.1590/S0102-053620180116>

Biomass production and essential oil of lemon balm cultivated under colored screens and nitrogen

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ABSTRACT

The aim of this study was to evaluate the effect of light quality and nitrogen availability on the biomass production, yield and composition of essential oil of lemon balm (*Lippia alba*). Five doses of N (0; 40; 80; 120 and 160 kg ha⁻¹) were studied, using urea and three light quality conditions obtained with the use of colored shading nets (blue and red), 50% irradiance and under full sun. The experiment was arranged in a 5x3 factorial scheme, in split plots, with seven replicates. After 60 days of cultivation under the shading nets, a higher dry leaf biomass (4.83 g per plant) was observed in the plants under blue net cultivation at a dose of 102 kg ha⁻¹ of N. The plants grown under full sun presented greater amount of neral and geraniol, major components of the essential oil of the lemon balm, which give commercial value to the species. The use of colored shading nets does not influence the composition of the essential oil of lemon balm, but increases the concentration of the compounds found in it. Nitrogen does not affect the quality of the essential oil of lemon balm, but it provides higher oil yield (0.27 g per plant) at the dose of 65 kg ha⁻¹ of N.

Keywords: *Lippia alba*, shading nets, nitrogen fertilization, medicinal plant, light quality.

RESUMO

Produção de fitomassa e óleo essencial em erva cidreira cultivada sob telas coloridas e nitrogênio

Objetivou-se avaliar o efeito da qualidade de luz e da disponibilidade de nitrogênio na produção de fitomassa, rendimento e composição do óleo essencial de erva cidreira (*Lippia alba*). Estudaram-se cinco doses de N (0; 40; 80; 120 e 160 kg ha⁻¹), tendo como fonte a ureia e três condições de qualidade de luz, obtidas com o uso de malhas fotoconversoras coloridas (azul e vermelha) com 50% de irradiância e sob pleno sol. O arranjo experimental foi em esquema fatorial 5x3, em delineamento em parcelas subdivididas, com sete repetições. Após 60 dias de cultivo sob as malhas, observou-se maior fitomassa seca das folhas (4,83 g por planta) nas plantas submetidas ao cultivo sob malha azul na dose de 102 kg ha⁻¹ de N. As plantas cultivadas sob pleno sol apresentaram maior quantidade de neral e geraniol, componentes majoritários do óleo essencial da erva cidreira, que atribuem valor comercial à espécie. O uso de malhas não exerce influência na qualidade do óleo essencial, mas aumenta a quantidade de compostos encontrados no mesmo. O nitrogênio não afeta a qualidade do óleo essencial, mas proporciona maior (0,27g por planta) rendimento de óleo, sob dose 65 kg ha⁻¹ de N.

Palavras-chave: *Lippia alba*, malhas fotoconversoras, adubação nitrogenada, planta medicinal, qualidade de luz.

Received on August 12, 2016; accepted on April 10, 2017

Lippia alba (Verbenaceae) is originated from South and Central Americas and it develops well in tropical, sub-tropical and temperate regions. The species is popularly known as lemon balm or Brazilian lemon balm. This herb has medicinal properties for stomach disorders, insomnia and anxiety (Carmona *et al.*, 2013); in Brazil, the species has pharmacological importance, since it is widely used in several herbal therapy programs (Neto *et al.*, 2012).

The essential oil of lemon balm has commercial importance, being an important raw material for

pharmaceutical, cosmetics and related industries (Soares & Tavares-Dias, 2013). Besides, in the essential oil, some compounds which inhibit the activity of protozoa, bacteria and fungi can be found (Tavares *et al.*, 2011).

Several studies have been carried out aiming to develop the productive chain of medicinal plant species, in order to obtain plants with high contents of active principles (Souza *et al.*, 2014). The mesh nets for selective ultraviolet radiation transmission can be an alternative to increase the production of secondary metabolites in medicinal plants (Corrêa *et al.*, 2012; Chagas *et*

al., 2013; Souza *et al.*, 2014).

Shading nets interfere with the quantity and quality of transmitted solar radiation, determining optical modifications of light scattering and reflectance. The spectrum of the blue mesh net shows a main transmission peak in the blue-green region (400-540 nm), whereas the red mesh net has higher transmittance at wavelengths exceeding 590 nm (Oren-Shamir *et al.*, 2001).

In experiments using colored shading nets, Souza *et al.* (2011) observed that conditions of light quality can show different desirable morphophysiological

responses and maximize the production of active principles. Corrêa *et al.* (2012) concluded that the quantity and quality of solar radiation can be modulated in order to obtain compounds which enhance oregano essential oil (*Origanum vulgare*).

In relation to the studies on the influence of luminosity on lemon balm (*Lippia alba*), Ventrela & Ming (2000) tested the production of dry biomass and essential oil in lemon balm leaves under different levels of shading and observed that higher sun exposure (unshaded treatment) increased essential oil contents in relation to the plants exposed to a lower irradiance level; in relation to light quality no information was found in literature so far, though.

The presence of nitrogen can also exert decisive action on the produced volume of active principles, since nitrogen fertilization can stimulate increases in biomass per area, leaf area and photosynthetic rate. Deschamps *et al.* (2012) observed that the nitrogen applied in the form of urea and ammonium sulfate can alter the quality and content of the essential oil of *Mentha x piperita*, and then ensure the productivity of the oil and high menthol content. However, in relation to lemon balm, little information on the amount of nitrogen to be applied for this aromatic species can be found.

Given the above, the aim of this study was to evaluate the light quality effect and nitrogen availability in biomass production, yield and composition of essential oil of lemon balm.

MATERIAL AND METHODS

The experiment was carried out in the experimental field at Centro de Ciências Agrárias, Ambientais e Biológicas, at Universidade Federal do Recôncavo da Bahia, in Cruz das Almas, Bahia State, Brazil (12°48'38"S and 39°06'26"W; 226 m altitude), from January to March, 2014.

The authors studied five doses of N (0, 40, 80, 120 and 160 kg ha⁻¹), using urea as nitrogen source and three conditions of light quality, obtained using red and blue shading

nets (Polysack Plastic Industries), 50% irradiance and under full-sun conditions (control). The experiment was arranged in a 5 x 3 factorial scheme, in split plots, where the mesh nets were considered the main treatment and the doses the secondary treatments, with seven replicates, totalizing 105 experimental units.

Lemon balm seedlings were produced from woody cuttings, 20-cm long and without leaves, in a nursery under shading net of 50% light transmission in the photosynthetically active region. The branches used for cutting were obtained from the mother plant, whose exsiccata is deposited in the Herbarium of Universidade Federal do Recôncavo Baiano, located in Cruz das Almas, inventory number HURB 8806.

After rooting, the seedlings were transplanted into 5-L plastic pots, containing a mixture of soil + sand + Plantmax (3:1.5:0.5). The soil used to compose the substrate is yellow latosol and it was collected from the 0 to 20 cm depth layer. Chemical attributes were: pH (water)= 6.54; P= 78 mg dm⁻³; K= 105 mg dm⁻³; Ca= 4.8 cmol dm⁻³; Mg= 1.7 cmol dm⁻³; Ca+Mg= 6.5 cmol dm⁻³; Al= 0.0 cmol dm⁻³; H+Al= 0.8 cmol dm⁻³; CTC= 7.8 cmol dm⁻³; S= 6.9 cmol dm⁻³; V= 89.9%; MO= 2.27%.

Doses of N-urea were fractionated, and the authors applied ½ of total N seven days after seedling transplanting and ½ twenty five days after the first fertilization.

Plants were grown on colored shading nets (red and blue), with an area of 32 m² and 2 m high and under full sun. After 60 days, the authors collected plants during the first hours in the morning. In order to determine the dry biomass yield, the plants were divided into root, stem and leaf, packed individually in Kraft paper bag and taken into a dryer with forced air circulation at 40°C where they were kept until reaching constant biomass. Then, the samples were weighed on a precision balance, being determined the dry biomass of each part of the plant which were submitted to different treatments. The obtained biomasses were used to determine root:shoot ratio.

The authors used a commonly

employed technique, the hydrodistillation method, in a modified Clevenger-type apparatus (LAPRON, Feira de Santana, BA). Three replicates were used per treatment. The dry leaves (20 grams per replicate) were transferred to a 500-mL capacity volumetric flask, containing 350 mL of distilled water. After boiling starts, the leaves were hydrodistilled for 3 h. The oil was decanted off for 5 min, and the supernatant was collected. Using these results, the authors determined the oil content, which was obtained directly as percentage; the essential oil yield was calculated by the multiplication of the oil content to the related dry leaf biomass.

For chemical composition analyses of the essential oil, the authors used three replicates per treatment. The oil was analyzed simultaneously by Gas Chromatography coupled to Mass Spectrometry (GC-MS) and Gas Chromatography with Flame Ionization Detector (GC-FID) using a GC-2010 Plus, GCMSQP2010 Ultra, Shimadzu Corporation, Japan, equipped with an autosampler AOC-20i (Shimadzu). Separation was carried out on fused-silica capillary columns RTx-5MS Restek (5% diphenyl-95% dimethyl polysiloxane) 30 m length × 0.25 mm internal diameter (d.i.); 0.25 µm film thickness, helium (99.999%) was used as carrier gas at a constant flow of 1.2 mL min⁻¹. The injection volume was 0.5 µL (5 mg mL⁻¹), at a split ratio of 1:10. The initial oven temperature was 50°C (isotherm for 1.5 min) at a rate of 4°C min⁻¹ at 200°C, then at 10°C min⁻¹ until 250°C, ending with 5 min isotherm at 250°C.

Data related to GC-MS and GC-FID were obtained using a detector separation system; the flow separation ratio was 4:1 (MS-FID). A restrictor tube of 0.62 m x 0.15 mm d.i. (capillary column) was used to connect the splitter to the MS detector; a tube restrictor 0.74 m × 0.22 mm id was used to connect the detector to FID. The injector temperature was 250°C and the ion source temperature was 200°C. The ions were generated at 70 eV; scan rate of 0.50 s⁻¹ fragments detected in the range from 40 to 350 Da. FID temperature was adjusted to 250°C, and the gas

supplies for the FID were synthetic air, hydrogen, helium at flow rates of 30, 300 and 30 mL min⁻¹, respectively. The quantification of each constituent was estimated by the peaks generated in the GC-FID (%). The concentrations of the compounds were calculated using the GC peak areas and were arranged in ascending order of GC elution.

The identification of the constituents was performed based on the comparison of the relative retention indices (RRI) using data found in literature (Adams, 2007). For retention index, the authors used the equation proposed by Van den Dool & Kratz (1963) in relation to a homologous series of *n*-alcanos (*n*C9-*n*C18). The authors also used three equipment libraries WILEY8, NIST107 and NIST21, which allow the comparison of spectra data with those from the libraries using a similarity index of 80%. Data processing was done using GC Postrun Analysis software (Labsolutions Shimadzu). The analyses were carried out in the Laboratório de Cromatografia at Universidade Federal de Sergipe.

The data obtained were submitted to the analysis of variance and when significant, the data were submitted to F test; in order to evaluate the simple effect of the mesh nets, the authors used Tukey test, whereas to evaluate the effect in relation to doses of N, the data were submitted to the regression analysis, all of them at 5%. The statistical program SISVAR 5.3 (Ferreira, 2008) was used.

RESULTS AND DISCUSSION

The dry biomasses of the leaf and root were not influenced by the interaction between the light quality and doses of N; however, the simple effect of both of them was significant. Root/shoot ratio was influenced only by the growing environment, whereas the dry biomass yield of the stem was the only trait in which the interaction was significant.

The plants grown under the blue shading net showed higher dry leaf biomass (4.83 g per plant), showing an increase of 14.69 and 10.97%, when comparing with the red shading net and full-sun treatments, respectively

(Table 1). The spectral quality of the blue shading net caused good radiation conditions for lemon balm, since greater supply of photo-assimilated for the leaves was noticed, which probably is due to greater cell elongation promoted by the blue light. In medicinal plants, this trait is favorable, since active principles are contained within plant biomass.

Similar results were found by Souza *et al.* (2014), who obtained greater dry leaf biomass in rosemary (*Rosmarinus officinalis*) grown under the blue shading net. In *Mikania glomerata*, the performance was similar; a reduction of plant dry biomass grown under full sun was observed (Souza *et al.*, 2011).

Dry biomass of lemon balm plants increased with an increase of N doses, up to estimated dose of 102 kg ha⁻¹ (5.43 g per plant). This dose provided an increase of 38.33%, when compared to absence of N (Figure 1A). The application of N doses close to 102 kg ha⁻¹, may contribute in lemon balm development, since it was enough to stimulate the highest dry leaf biomass. Lima *et al.* (2011) observed greater accumulation of biomass in the concentration of 75 kg ha⁻¹ of N in yarrow (*Achillea millefolium*), whereas Deschamps *et al.* (2012) observed that N doses did not influence leaf biomass production of *Mentha x piperita*. This divergence between the results of the studies is due to variations in the response of the plants, according to the species and environmental conditions.

The plants grown under full sun showed greater accumulation of root dry biomass (27.76 g) when compared to the plants grown under blue (18.82 g) and red shading nets (16.09 g). The highest root dry biomass in plants grown under full sun was determinant for the largest root/shoot ratio in plants under full sun (0.89 g g⁻¹), in relation to the

plants grown under blue (0.63 g g⁻¹) and red (0.66 g g⁻¹) shading nets (Table 2). No significant difference was observed between red and blue shading nets for root dry biomass and root/shoot ratio in lemon balm grass plants.

The preferential allocation of dry biomass to the root system is attributed to the effect of light intensity, since the plants grown under full sun show a greater investment of photoassimilates in roots, to detriment of leaves; this is an adaptative strategy in order to increase water and nutrient absorption capacity and to increase survival capacity of plants in environments with higher wind incidence and higher irradiance and, consequently, higher water requirement (Chagas *et al.*, 2013).

The root dry biomass of lemon balm roots increased approximately 39.52% with the increase of N doses, when comparing with plants which did not receive nitrogen fertilization (15.75 g per plant) (Figure 1B). Silva & Delatorre (2009), evaluating changes in root architecture of *Arabidopsis thaliana* Columbia ecotype in response to the availability of P and N, observed that the increases in root dry biomass were higher under higher doses of N. Freitas *et al.* (2012) observed that plants of *Passiflora alata* grown under different doses of N and lemon balm shading levels, showed an increase in root dry biomass with an increase of doses up to the maximum of 50 mg kg⁻¹ of N, which provided a biomass of 15.4 g.

The stem dry biomass of lemon balm plants under full sun increased with an increase of doses of N (Figure 1C), whereas under red and blue shading nets, the plants grew up to concentration 87.00 and 107.12 kg ha⁻¹ of N, respectively. This fact can be explained by the greater number of branches verified in plants under full sun (Figure 1C).

Table 1. Leaf dry biomass (BSF), root dry biomass (BSR), and root/shoot ratio of lemon balm plants grown under colored shading nets and full sun. Cruz das Almas, UFRB, 2014.

Treatments	Full sun	Blue shading net	Red shading net	CV (%)
BSF (g per plant)	4.30 b	4.83 a	4.12 b	13.11
BSR (g per plant)	27.76 a	18.62 b	16.09 b	26.88
Root/shoot ratio	0.89 a	0.63 b	0.66 b	27.01

Averages followed by same letter in line do not differ by Tukey test, 5%.

Table 2. Components of the essential oil of lemon balm leaves, grown under different qualities of light, blue shading net (MA) and red shading net (MV) and full sun (PS). Cruz das Almas, UFRB, 2014.

Compound	IRR exp*	IRR lit**	MA (%)	MV (%)	PS (%)	CV (%)
α -tujene	921	924	0.28 a	0.25 a	0.21 b	14.17
sabinene	968	969	0.76 a	0.71 a	0.71 a	16.73
myrcene	984	988	2.74 a	2.23 ab	1.58 b	34.00
p-cymene	1021	1020	6.01 b	6.41 b	9.18 a	25.23
y-terpinene	1055	1054	1.16 a	0.99 ab	0.85 b	35.05
linalool	1096	1095	1.18 a	1.11 a	1.17 a	16.56
neral	1237	1235	25.43 a	24.68 a	29.18 a	22.90
geranial	1267	1264	38.07 a	35.96 a	42.07 a	20.13
β -elemene	1390	1389	0.75 a	1.09 a	0.75 a	26.02
(E)-caryophyllene	1423	1417	1.67 a	1.94 a	1.67 a	18.87
y-elemene	1432	1434	1.24 ab	1.47 a	0.84 b	50.66
germacrene B	1565	1559	0.59 a	0.69 a	0.60 a	43.46
caryophyllene oxide	1593	1582	4.50 a	4.01a	4.94 a	38.56
humulene epoxide II	1618	1608	0.79 a	0.99 a	1.06 a	86.30
α -humulene	1457	1458	1.19 a	0.55 b	0.47 b	50.50
limonene	1025	1024	2.74 a	3.45 a	nd	40.82
carvone	1242	1239	10.12 a	11.67 a	0.10 b	30.47
β -bourbonene	1386	1387	0.07 a	0.09 a	nd	44.83
germacrene D	1487	1484	1.64 a	1.74 a	nd	65.88
piperitone	1253	1249	0.03	nd	nd	34.40
epi-cubebol	1497	1493	0.07	nd	nd	14.28
terpinen-4-ol	1458	1452	nd	0.39 a	0.42 a	17.49
allo- aromadendrene	1465	1458	0.15	nd	nd	22.94
Total	-	-	86.08	83.08	95.28	-

*Relative retention index (IRR exp) using a series of n-alkanes (C9-C18) using the equation proposed by Van den Dool & Kratz (1963), separation column Rtx-5MS (RESTEK). **Relative retention index (IRR lit). Averages followed by the same letter in line do not differ by the Tukey test ($p < 0.05$). nd: not detected.

Abreu *et al.* (2013) verified that basil (*Ocimum basilicum*) grown under colored shading nets accumulated higher stem biomass under full sun and Chagas *et al.* (2013), with Japanese mint (*Mentha arvensis*) also verified similar results, corroborating data shown in this study.

In relation to the yield of essential oil of lemon balm, the dose of 65 kg ha⁻¹ of N, provided higher yield (0.27 g per plant), representing increases of 15.65 and 33.43%, when compared with doses 0 and 160 kg ha⁻¹ of N, respectively (Figure 1D).

Studies on application of N have demonstrated the importance of N for yield of essential oil of medicinal plants, as it was noticed in patchouly (*Pogostemon cablin*) by Costa *et al.* (2014) observing the influence of the

nutrient on plant yield, and established that the application of 98 kg ha⁻¹ of N in the second cutting of the plant, promoted higher yield of essential oil. However, the yield of the essential oil varies according to the species and it depends on the growing conditions, considering that the yield of oil results from higher biomass accumulation generally obtained after fertilizer application (Pinto *et al.*, 2014).

The components of essential oil were not influenced by interaction between light quality and doses of N; however, the amounts of the chemical constituents of the essential oil of ginger grass varied in response to light quality. In chemical analysis of essential oil, 23 compounds were identified; among these 23 compounds, twelve are monoterperene and eleven sesquiterpenes

(Table 2).

Observing the amount of terpenoids in the essential oil of lemon balm, the authors verified that the blue shading net provided greater amount of compounds (22) in relation to the red shading net (21) and full sun (17). The major components are neral and geranial which, according to Budavari *et al.* (1989), are isomers which are mixed to produce citral; in this study, different conditions of light quality did not influence the production of major components of the essential oil.

With the sum of citral and geranial, concentrations of 63.50% (blue shading net); 60.64% (red shading net) and 71.25% (full sun) of citral were observed. These concentrations are close to the ones found by Silva *et al.* (2006) which found concentrations of citral ranging from 70.6 to 79.0% in

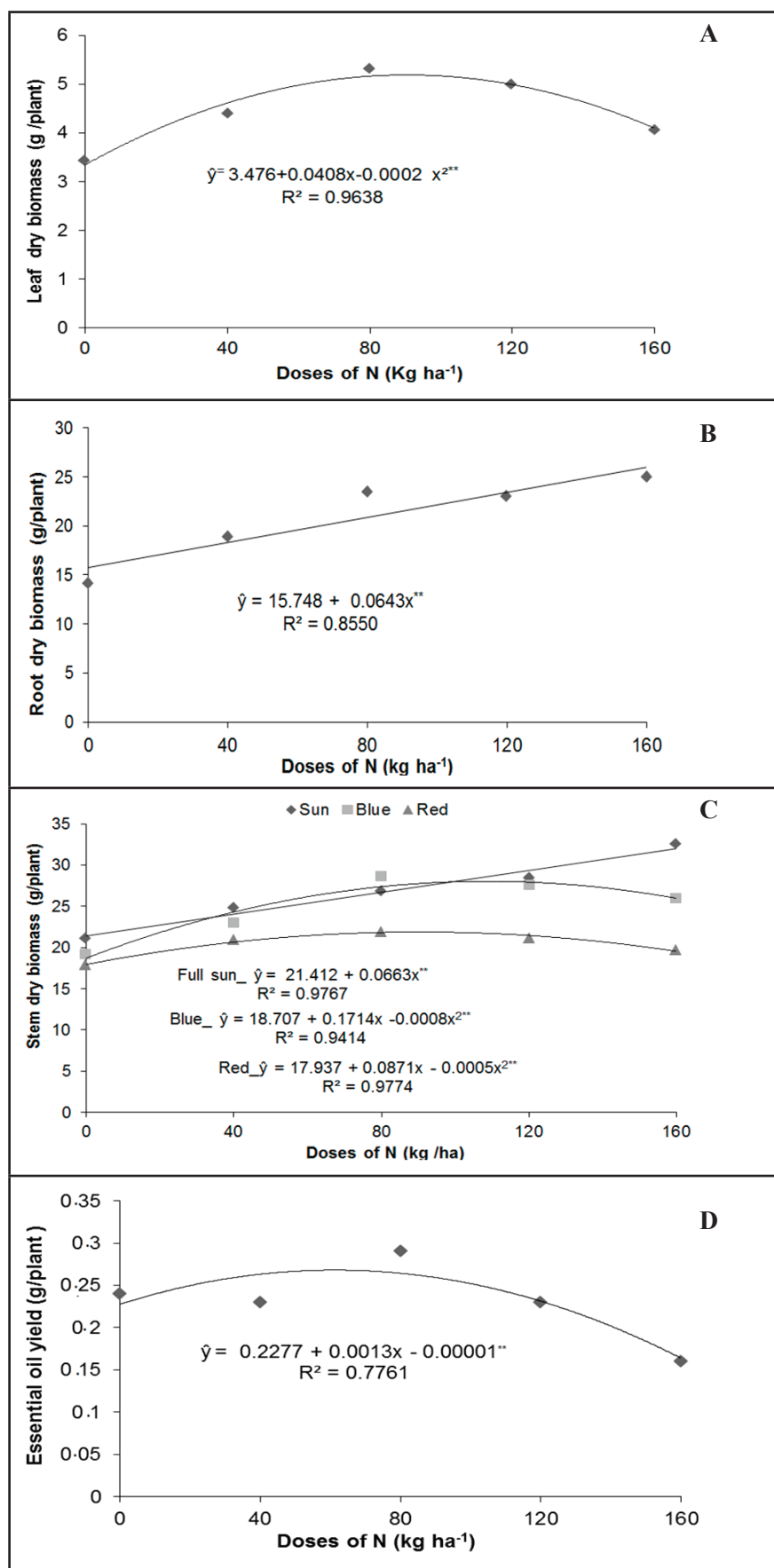


Figure 1. Leaf dry biomass (A), Root dry biomass (B) Stem dry biomass (C) and essential oil yield (D) of lemon balm plants. Cruz das Almas, UFRB, 2014.

lemon balm.

The α -tujene compounds were not influenced by the light quality but only by the light intensity. The α -tujeno contents were higher, 0.28% and 0.25%, respectively, for blue and red shading nets, when compared to the full-sun treatment (0.21%). Terpenoids, piperitone and epi-cubebol were only obtained when the plants were grown under the blue light, considering that monoterpene terpinen-4-ol was not identified in the essential oil of the plants under the blue shading net (Table 2).

Under full-sun cultivation, the authors verified higher p-cymene concentration (9.18%), increases of 34.53 and 30.17% in p-cymene concentration when the comparison between blue and red, respectively, was carried out. No significant difference between red and blue shading nets for this trait was noticed (Table 2).

In relation to concentrations of limonene, the results show that the light intensity affects the biosynthesis of two classes of terpenoids present in essential oil of lemon balm, β -bourbonene and germacrene, since these constituents were not observed in the essential oil of lemon balm extracted from the plants under full sun.

Terpenoids, in particular mono- and sesquiterpenoids, have several functions in plants, some of these functions are still little established for most isoprene derivatives, involving thermoprotection effects, oxidative damage protection, photorespiration at high temperatures and low concentrations of O₂, allelopathy and photoprotection (Machado & Junior Fernandes, 2011).

A relationship between the limonene and carvone compounds can be noticed, since both belong to the same biosynthetic pathway (via the mevalonic acid pathway) (Ehlert *et al.*, 2013). Light intensity affected the biosynthesis of limonene, as this compound was not noticed in the essential oil. Higher carvone concentrations (11.67 and 10.12%) were noticed when the oil was extracted from plants under shading nets (red and blue), representing increases of 99.01 and 99.14% in concentration, when compared to the full sun exposure (Table 2).

The major components (citral and linalool) which give commercial value to the species did not vary in relation to treatments, but the concentration of limonene and carvone was higher in oils of plants grown under shading nets (red and blue) when compared to full-sun cultivation.

Cultivation under the blue shading net can be an interesting alternative to grow *Lippia alba*, since it increases dry leaf biomass, which is the main responsible for the essential oil storage. The authors highlight that the application of appropriate concentration of N is essential, recommending the concentration of 102 kg ha⁻¹ in similar conditions to the ones used on this study.

ACKNOWLEDGEMENTS

The authors thank to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (Coordination of Improvement of higher education personnel) for financial support and for *Research Productivity Scholarship*. To Laboratório de Produtos Naturais (LAPRON), of Departamento de Ciências Exatas da Universidade Estadual de Feira de Santana (UEFS) and to Professor Angélica M Lucchese who allowed the quantification of the essential oil of the species used in this experiment.

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