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Growth, photosynthetic pigments and production of essential oil of long-pepper under different light conditions

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ABSTRACT

Piper hispidinervum C. DC. is popularly known as long-pepper and it owns a commercial value due to the essential oil it produces. Long-pepper oil is rich in safrole and eugenoln components that have insecticidal, fungicidal and bactericidal activity. It has been establish that to medicinal plants light influences not only growth but also essential oil production. The growth, the content of photosynthetic pigments and the essential oil production of *Piper hispidinervum* at greenhouses with different light conditions was evaluated. The treatments were characterized by cultivation of plants for 180 days under different light conditions, produced by shading greenhouses with 50% and 30% of natural incident irradiance, two colored shading nets red (RN) and blue (BN) both blocking 50% of the incident radiation and one treatment at full-sun (0% of shade). The results showed that the treatments of 50% shade and RN and BN were the ones which stimulated the greater growth. Blue and red light also had the best production of photosynthetic pigments. Essential oil yielded more under full sun therefore this is the most indicated condition to produce seedlings for the chemical and pharmaceutical industry.

Key words: colored nets, light quality, medicinal plants, safrole.

INTRODUCTION

Piper hispidinervum C. DC. is popularly known as long-pepper, belongs to the Piperaceae family, found naturally as secondary vegetative in regions of Amazon (Zacaroni et al. 2009). The species has commercial value in producing an essential oil, rich in safrole and eugenol, with insecticidal activity, fungicidal and bactericidal activity (Nascimento et al. 2008, Zacaroni et al. 2009, Giviziez 2010).

However, *P. hispidinervum* is still being domesticated and studies such as the influence of spacing, time and frequency of harvesting have been developed, however cultivation under shading nets for the species lacks of research (Bergo et al. 2005, Figueirêdo et al. 2005).

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Furthermore, it is also important the development of techniques to promote the cultivation (Pereira et al. 2008).

The light is essential to the growth and development of plants, once it is directly related to the photosynthesis biological, biochemical and morphological processes (Paez et al. 2000). Due to that, variations in the intensity and composition of incident light may affect plants. Plant processes of various scales such as cell growth rate, accumulation and composition of pigments, plastids differentiation and physiological processes can suffer alteration due to light, consequently affecting the yield (Almeida et al. 1998).

It has been establish that for medicinal plants light influences not only growth but also essential oil production. For instance *Mentha piperita* plants cultivated under high light density in environments enriched with red wavelength showed the greatest growth and essential oil production (Costa et al. 2012). *Ocimum gratissimum*, obtained similar results on environments enriched with blue wavelength (Martins et al. 2008). On the other hand, *Achillea collina* cultivated under low light density it was verified greater growth, but there was a drop at the production of essential oil (Giorgi et al. 2013).

Considering the countless variations of growth, essential oil production and the lack of agreement among researchers about light effects in the physiological processes, studies of this nature are crucial. Formerly a better comprehension of plant response to light might contribute to a better design of cultivation conditions that promote higher dry matter production (Lima et al. 2011). Moreover, it eases the development of cultivation techniques that advance the domestication of the species.

For instance, the objective of the present study was to evaluate the growth, the pigment content and the essential oil production of *Piper hispidinervum* in greenhouses with different light conditions.

MATERIALS AND METHODS

The experiment was conducted between December of 2012 and May of 2013 at the Gota de Esperança farm (21º14’07”S and 44°58’22”W, 879 m altitude) at the Agriculture Department of Universidade Federal de Lavras (UFLA). The total area of the experimental unit is approximately one hectare. The climate averages were provided by the Climatological station from the Agricultural-Engineering Department at UFLA. The maximum temperature was 32.8°C and the minimum was 9.6°C, 5.5 mm of precipitation per day and last the average relative humidity of 76%.

Seedlings of *Piper hispidinervum* were produced from seeds germinated in polypropylene trays containing the commercial substrate Tropstrato HA® (Vida Verde©, Brazil) and kept in a growth room for 4 months. After this period, the seedlings were transplanted to 6 litre plastic pots containing a substrate consisting of subsoil, sand and bovine manure, in proportion of 2:1:1, being arranged in different light treatments. Irrigation was performed daily, and the soil was kept under the condition of field capacity. The physicochemical characteristics of the soil were analyzed in laboratory the results were: pH: 5.4; P: 4.13 mg dm^{-3}; K: 73.32 mg dm^{-3}, Ca: 2.30 cmolc dm^{-3}, Mg: 0.30 cmolc dm^{-3}, Al: 0.10 cmolc dm^{-3}, H + Al: 2.90 cmolc dm^{-3}, V: 49.00%; organic matter: 2.10 dag kg^{-1}, Clay: 70.00 dag kg^{-1}; Silt: 16.00 dag kg^{-1} and Sand: 14.00 dag kg^{-1}.

The experimental design was completely randomised, with 25 plants for each treatment totalizing 125 plants analysed. The treatments were characterized by cultivation of plants for 180 days, under different light conditions, produced by shading greenhouses with 50% and 30% of natural incident irradiance, two colored shading nets red and blue (BN) both blocking 50% of the incident radiation and one treatment at full-sun (0% of shade). The radiation spectrum of different environments were evaluated, with a spectral resolution of 1 nm with the aid of a portable spectroradiometer (USB-650 Red Tide) coupled to a source of electromagnetic radiation DT-MINI (200 to 2000 nm) and a probe reflectance R400-7-VIS-NIR (US BioSolutions Ocean Optics®),. The normalized irradiances observed for the treatment at 50% were 6.54 W/m², 13.08 W/m² in the treatment at 70%, 15.42 W/m² at 100%, 8.86 W/m² in RN and 9.07 W/m² in BN. Environment presented the highest values in terms of amount and size of the spectrum in the 100% irradiance atmosphere, followed by the RN atmosphere, in which the value found was 70%, and 50% irradiance for the environment with BN. It was also noticed that the blue net provided irradiances, of approximately 450-550 nm, and the red net between 490 and 690 nm.

The growth was assessed by morphological variables: height, stem diameter, number of leaves, leaf dry mass (LDM), stem dry mass (SDM), root dry mass (RDM) and total dry mass (TDM). The dry mass was obtained by drying of the leaves; stem and roots, previously separated, were dried in a forced air oven, at 70°C, at constant weight. The height, stem diameter and number of leaves were measured every 15 days. The variables leaf dry mass, stem dry mass, root dry mass and total dry mass were obtained in the end of the experimental period (170 days).

The photosynthetic pigments chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were analyzed. The extraction was performed on fully expanded leaves located at the third node, collected at the end of the experimental period (180 days) according to the methodology reported by Lichtenthaler and Buschmann (2001), To extract the pigments it was weighed 200 mg of fresh leaves which were homogenized with 10 mL of 80% acetone (v/v). The mixture was then filtered through a glass wool and the volume was completed to 30 mL by adding 80% acetone. Immediately following this procedure, it was carried the reading of the absorbance at 663.2 nm, 646.8 nm and 470 nm. All procedures were done in the dark, to preserve the integrity of the pigments. The content of chlorophyll a, b and carotenoids were calculated using the equations: $[(12.25 \text{ X } A_{663.2}) - (2.79 \text{ X } A_{646.8})]$ (1); $[(21.5 \text{ X } A_{646.8}) - (5.1 \text{ X } A_{663.2})]$; $[(1000 \text{ X } A_{470}) - (1.82 \text{ X } A_{663.2}) - (82.02 \text{ X } A_{646.2})/198]$, respectively, and expressed in mg g^{-1} fresh weight.

The determinations of the content (%, w/w) and productivity (mg.plant^{-1}) of essential oil obtained from the hydrodestillation of 30 g of dry leaves during 120 min. This was effectuated in a modified Clevenger-type apparatus with 3 replicates for treatment. The oil extracted were weighed in a analytical balance. The essential oil content was determined by the equation TOC = (WO/WS) x 100, OC is the essential oil content; WO is the weight of oil (g) obtained in the extraction and WS is the sample weight (g) is the yield by the equation OC x LDM, In this equation OC is the essential oil content and LDM is the average dry matter of each plant.

The data obtained a long time (quantitative) were analyzed by polynomial regression ($p \leq 0.05$), while the qualitative data was submitted to an analysis of variance, and the measurements were compared by the Tukey's test ($p \leq 0.05$), using the SAEG program (SAEG-2007).

RESULTS AND DISCUSSION

The growth of *Piper hispidinervum* was affected by different light treatments (Figure 1). The plants cultivated under shading nets in red, blue and 50% presented the highest growth in height, stem diameter and number of leaves, throughout the experiment (Figures 1a, 1b). The treatment with 0% shading promoted a reduction of these variables. Other species showed a growth increase when cultivated under blue and red net such as *Ocimum selloi*, *Mikania glomerata* and *Mikania laevigata* (Souza et al. 2007, Costa et al. 2010).

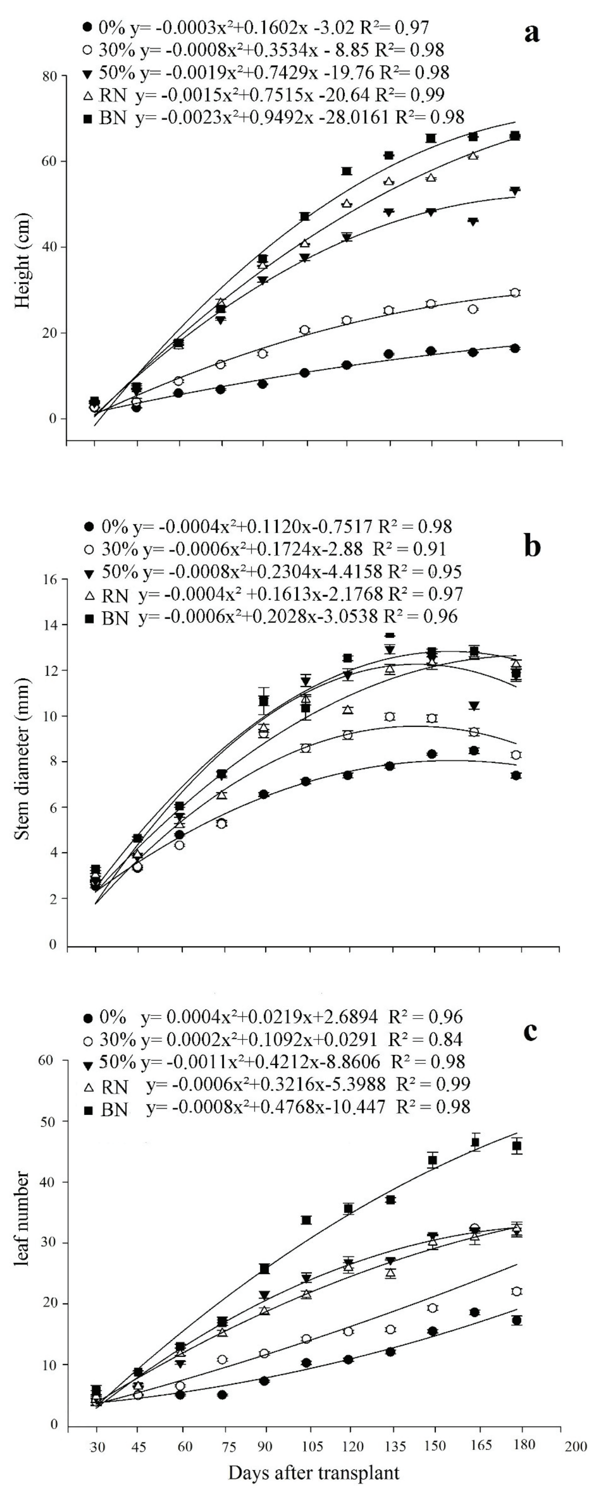

Figure 1 - Hight (**a**), diameter of stem (**b**) and number of leaves (**c**) of *P. hispidinervum* cultivated under different light conditions 0, 30 and 50%- Shading percentage; RN-red nets and BN-blue nets (both with 50% of shading).

The greatest growth in height and diameter observed during the treatment with blue and red net might be associated to the participation of photoreceptors which absorb light in this wavelength. A study done with *Arabidopsis* give evidence of the need of coordinated action between phytochromes (red light receptors) and blue light receptors (cytochromes and phototropins) to stimulate the growth of the aerial partial (Parks et al. 2001).

Leaf dry mass, stem dry mass, dry matter of roots and total dry matter were higher in the treatments with under shading nets in red and blue and 50% shading (Figure 2). While plants grew at 0% shading had the lowest average. The raise in the dry matter content under environments enriched with blue and red lights can be linked to a higher photosynthetic rate provided by this wavelength. A study using *Gossypium hirsutum* L. *in vitro* found that the use of red and blue monochromatic provided greater control of the stomata opening resulting in better photosynthetic efficiency and consequently greater dry matter production (Li et al. 2010).

According to Kinoshita et al. (2003) the blue light stimulates the photosynthetic activity for being responsible for the maintenance of the electric potential in the membranes of guard cells and for the stomata opening, resulting in the increase in the concentration of CO_2 inside the mesophyll. Furthermore, the balance between the wavelength in the red and blue spectrum might promote the increase in the net photosynthesis (Kong et al. 2012). Pacheco et al. (2013) discovered an increase in stomata conductance and in the internal concentration of CO_2 in *Piper aduncum* cultivated in environment enriched with red light.

Moreover it was ascertain that *Piper hispidinervum* shows greater growth in conditions of lower irradiance. The reduction in the production of dry matter observed in the treatments with high luminosities (0% and 30% of shadow) might be

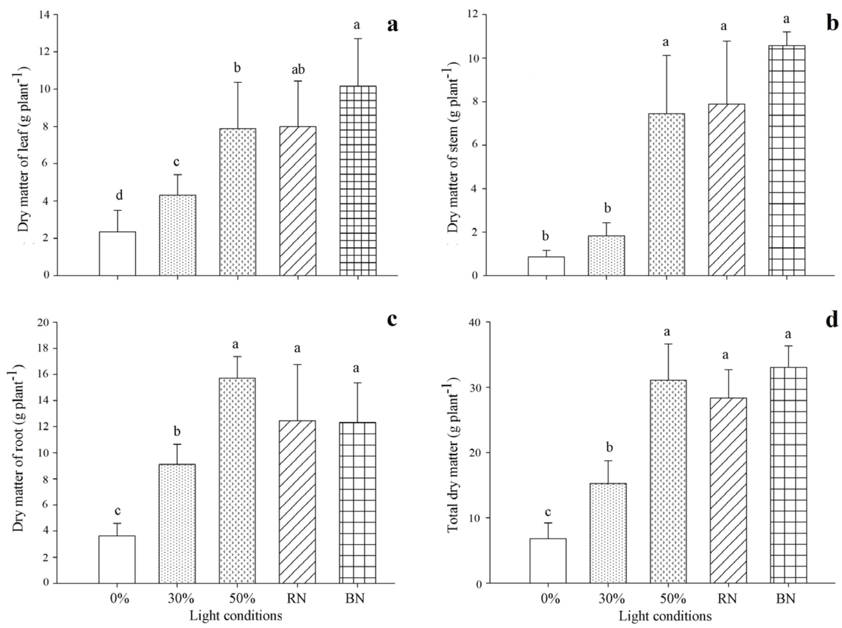

Figure 2 - Dry matter production of leaves (**a**); stem (**b**), roots (**c**) and total (**d**) of *P. hispidinervum*, cultivated under different light conditions with 180 days. 0, 30 and 50%- Shading percentage; RN-red nets and BN-blue nets (both with 50% of shading).

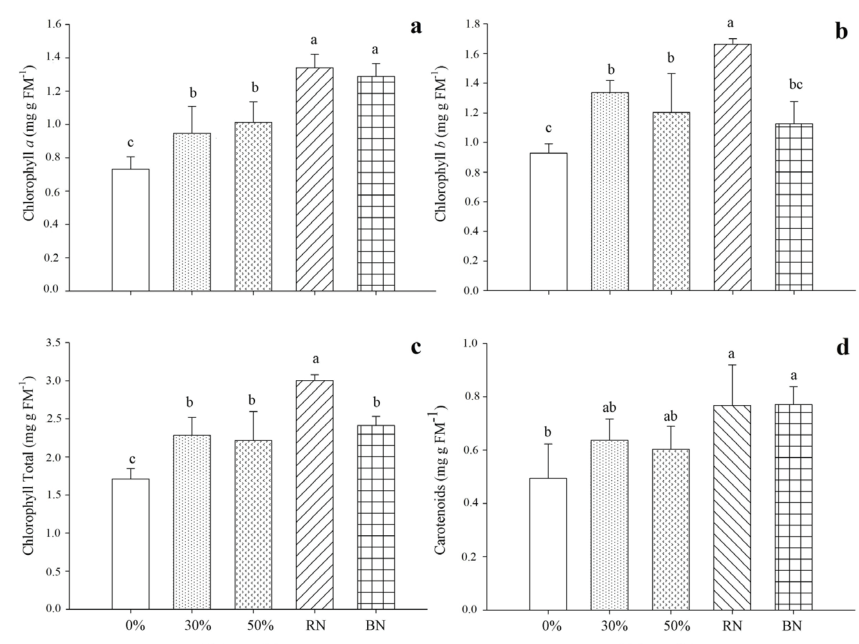

Figure 3 - Accumulation of chlorophyll *a* (**a**), chlorophyll *b* (**b**), total chlorophyll (**c**) and carotenoids (**d**) of *P. hispidinervum*, cultivated under different light conditions, with 180 days. 0, 30 and 50%- Shading percentage; RN-red nets and BN-blue nets (both with 50% of shading).

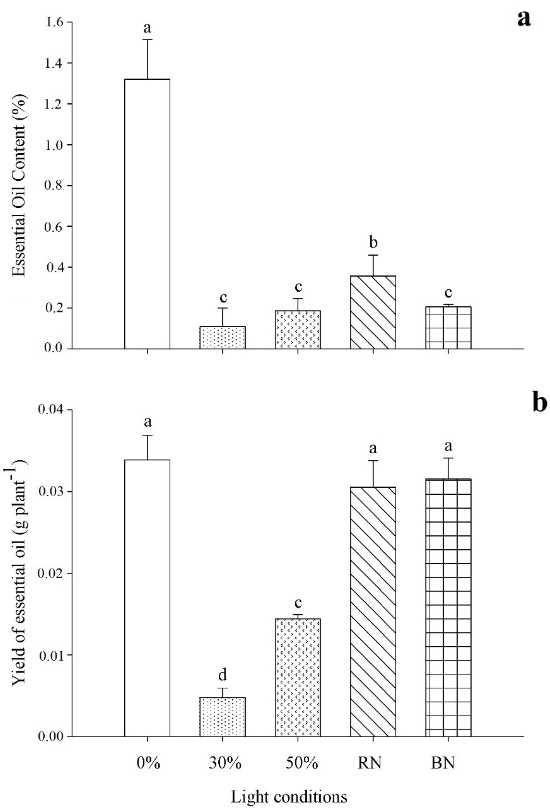

Figure 4 - Essential oil content (**a**) and yield of essental oil (**b**) of *P. hispidinervum*, cultivated under different light conditions, at 180 days of cultivation. 0, 30 and 50%- Shading percentage, RN-red nets and BN-blue nets (both with 50% of shading).

due to the prolonged exposition of the plant to this irradiances which end up being detrimental because it absorb more light photons than it is possible to use, inducing to the process of photoinhibition (Kitao et al. 2000). Results opposed to that were found at *Piper aduncum* which had greater production of dry matter under colored nets and on treatments with high irradiances (Pacheco et al. 2014).

The amount of photosynthetic also was altered by the light conditions. The plants grown under colored nets had the greater photosynthetic pigment yield (Figure 4). The chlorophyll and carotenes were more in plants grown under red and blue net. Whereas, the total amount of chlorophyll A and carotenes were statistically superior in the plants of the red net treatment. The lower amount of pigments was observed on the plants grew under full sun. This result demonstrates that the phenotypic plasticity is more closely related with the light quality than with the light intensity.

The synthesis and degradation of the photosynthetic pigments are associated with the plants adaptability to different environments. The chlorophylls are usually synthesized and photo-oxidized in the presence of light. Nonetheless, the excess of light can cause greater degradation and consequently, a reduction in the levels of total chlorophyll (Gonçalves and Santos Junior 2005). On the other hand, under deficit light conditions, the plants set a series of compensatory mechanisms into motion such as a substantial increment of the photosynthetic pigments. In environments with high solar radiation, the increase of photo-oxidation of chlorophylls depends upon the concentration of carotenoids, which can prevent chlorophylls photo-destruction (Gonçalves et al. 2005). Furthermore in low light environments carotenoids can play an important role in light absorption and its transfer to chlorophyll cells (Taiz and Zeiger 2009). A significative increase on chlorophylls and carotenoids (Figure 3) at color shading nets and a major production of dry matter, indicates that *P. hispidinervum* can properly regulate its metabolism toward the solar radiation conditions and goes better in environments with plenty of blue and red light.

The light incidence had significant effects in essential oil content, with the best content at full sun (Figure 4a). The essential oil has different functions in plants and one of these, is the protection against environmental stresses (Gershenzon and Dudareva 2007). High irradiances may have promoted damage to photosynthetic apparatus reducing production of dry matter (Gonzales-Burgos and Gomez-Serranillos 2012). This condition, however, may have promoted the activation of protection mechanisms promoting increased essential oil content. Similarly responses in essential oil content were observed in and *Mentha piperita*, and *Rosmarinus officinalis* with the major contents at full sun (Costa et al. 2012, Souza et al. 2013). Comparatively, different responses are observed when the plants growth in different light conditions. In *Ocimum gratissimum* and *Mikania glomerata*, full sun prejudiced the essential content (Martins et al. 2008, Souza et al. 2011). Thus, it is observed one specific response of adaptation to diverse conditions of light and production of essential oil.

The yield of essential oil was the greatest in the treatments of 0% of shading and the colored nets (Figure 4b). Although the greater production of dry mass of leaves in red and blue nets, their essential oil content were lower than the full sun, and this reflected in the yield of essential oil. It is possible to observe that, despite the low production of dry matter the treatment with 0% of shade had bigger essential oil content. This result demonstrates that under this conditions the yield compensates for the low dry matter production, it is indicated its cultivation in these conditions.

CONCLUSIONS

The different light conditions promoted important changes in growth, photosynthetic pigments production and essential oil of *Piper hispidinervum*. The plants cultivated under 50% shading in red and blue net showed the greatest growth. The superior production of chlorophyll a, b and carotenoids was observed in plants cultivated under the colored nets (red and blue). Therefore, the species *Piper hispidinervum* regulates its metabolism in relation to solar radiation conditions and also develops better in environments with plenty of blue and red light. Nevertheless, the greatest production of essential oil was observed in the 0% shading treatment, which provided the greatest content and yield of essential oil. Therefore, the cultivation under full sun is indicated when the aim is faster and more efficient production of seedlings, for the chemical and pharmaceutical industry.

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