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Growth and production of secondary compounds in monkey-pepper (*Piper aduncum* L.) leaves cultivated under altered ambient light

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Abstract

The species *Piper aduncum* L., native to the Americas, has important biological benefits for the production of secondary compounds such as phenolics and flavonoids. Among the factors that influence the metabolism of the plant, is considered as one of the most important for promoting changes in plant physiology that can directly interfere in growth and production of secondary compounds. This study aimed to evaluate the growth and production of different secondary compounds (total phenolic acids, flavonoids, lignin and activity of phenylalanine ammonia lyase - PAL) of *Piper aduncum* L. The treatments were: four shading greenhouses (modified ambient lights), with 50%, 70% of natural irradiance, red and blue nets, and one at full sun (100% of natural irradiance), with 20 replicates per treatment. The growth of *Piper aduncum* was affected by the different altered ambient light, showing a higher growth under blue net. However, the species shows responses which allow its survival at full sun, such as more production of roots. The production of secondary compound was also affected by light, and the production of phenolic compounds and lignin higher (respectively 0.18 and 11.7 µg g DM^{-1}) in treatment of blue net shade greenhouse. Moreover, PAL can be considered the key enzyme for the production of phenolic compounds in this species. The concentration of flavonoids in treatment with 100% irradiance was the lowest. This shows that the species has other mechanism of protection for high irradiance.

Keywords: flavonoids; light; lignin; medicinal plant; monkey-pepper; phenolic compounds; phenylalanine ammonia liase.
Abbreviations: DAT_days after transplant; PAL_ phenylalanine ammonia liase; LDM_leaf dry mass; SDM_ stem dry mass; RDM_ roots dry mass; TDM_total dry mass, SLA_specifc leaf area; LAR_ leaf area ratio; RWR_ root weight ratio; RN_red net; BN_blue net.

Introduction

Piper aduncum (Piperaceae) is a shrub native to the Americas, and that has a potential for commercial use (Rocha et al., 2008). It produces essential oils in a high yield (2.5 to 3.5%), has low toxicity and is rich in dilapiolle (31.5 to 91.1%) (Maia et al., 1998; Sousa et al., 2008). This compound presents antimicrobial properties, and acts as an insecticide and molluscicide (Orjala et al., 1994; Fazolin et al., 2005; Lara-Junior et al., 2012; Misni et al, 2011). However, according to Bernard et al. (1995) the genus *Piper* can still produce many types of secondary metabolites, such as phenylpropanoids, flavonoids and lignoids, which can be exploited by the chemical and pharmaceutical industry. The production and quality of secondary compounds, however, is modified by several environmental factors (Petkovsek, 2009). The intensity and quality of light are important environmental factors that alter the synthesis of these compounds because it affects the morphology and physiological processes of plants. The flavonoids biosynthesis and other phenolic acids, for example, need larger amounts of irradiance, or are increased in these conditions (Ghasemzadeh and Ghasemzadeh, 2011). Previous studies show that changes in intensity and light quality result in an increase in the production of flavonoids and total phenolics in medicinal plants (Ghasemzadeh et al., 2010; Karimi et al., 2013). Therefore, in medicinal plants cultivation, handling the microclimate could be a promising alternative to control of phytochemical levels and quality of plant material. The control and the uniformity of light can result in an increase in growth, better planning of the production and larger control of contamination or attack of insects (Karimi et al., 2013). However, the existence of interspecific differences in micro-environment may influence plant accumulation and distribution of total phenolics and flavonoids (Jaafar and Rahmat, 2008). Flavonoids and phenolic compounds are important for plants, against to UV light and protect to reactive oxygen species. A phenylalanine ammonia lyase (PAL) is an enzyme involved in their biosynthesis. The activity of PAL is regulated from many factors, such as age, herbivory and mostly light conditions (KUMARI et al., 2009; NAWKAR et al., 2013). Studies performed with *Kalanchoe pinnata Lam.* showed an increase in production, when exposed to a suplementation with blue light (Nascimento et al., 2013). In *Larix gmelinii (Rupr.)*, an increase in the production of secondary compounds was observed when they were exposed to low irradiances (Yan et al., 2013). In *Zingiber officinale* Roscoe, high irradiances caused an increase in the production of flavonoids, while low

irradiances increased the production of total phenolic compounds (Ghasemzadeh and Ghasemzadeh, 2011). In this context, the objective of the present study was to evaluate the growth and production of different secondary compounds (total phenolic acids, flavonoids, and lignin) and activity of phenylalanine ammonia lyase (PAL) in *Piper aduncum* L., grown under different irradiance.

Results and Discussion

Relations of growth

The growth of *Piper aduncum* was affected by different irradiance treatments (Fig 1). The plants cultivated under red and blue nets presented the highest growth in height and stem diameter, throughout the experiment (Fig 1A, 1B). Similar results were observed for *Ocimum selloi*, *Mikania glomerata* and *Mikania laevigata*, which showed the best height in treatments with red and blue nets (Souza et al., 2007; Costa et al., 2010). The total foliar area was larger in the plants that grew under blue and red nets (Fig 1D). The increase in leaf area under photoselective nets can be considered a means for plant to increase the photosynthetic surface, with greater use of low light intensities and thus compensating photosynthesis rates per unit area, which is a feature of adapted leaves to shading (Jones and McLeod, 1991). Similar results were obtained by Oliveira (2009), with the species *Artemisia vulgaris L.* which presented a larger foliar area under red and blue nets, when compared to 50% and 100% irradiances. Plants grown under red and blue nets, where the proportions of red and blue range of the spectrum are altered and radiation intensity is blocked, presented the largest averages for foliar area. The smallest foliar area was observed in the treatment with 50% and 70% irradiance. This result indicates that light quality is a decisive factor for the expansion of the leaf blade in monkey-pepper. The number of leaves was higher in plants grown under red and blue nets, 70% and 100% irradiance. In the treatments with 100% and 70%, a reduction in foliar area was observed, but they increased the number of leaves. Similar responses were observed in *Ocimum gratissimum*, where the high levels of irradiances provided a decrease in the foliar area and an increase in the number of leaves (Fernandes et al., 2013). Leaf dry mass and stem dry mass were higher in the treatments with 50% and 100% irradiance and, both RN and BN (Fig 2). It was possible to observe a tendency for a higher amount of root dry mass in the plants grown under 100% irradiance and red nets.The production of total dry mass was higher in treatments with 100% irradiance, both for RN and BN. This result shows the importance of the intensity and quality of radiation for plant growth, not only for the supply of energy for photosynthesis, but for generating signs that regulate their development. LWR was higher in the treatment with 100% and 50% irradiance and smaller under red nets. RWR was higher in the plants cultivated under 100% and 70% irradiance and under red nets (Table 1). The largest leaf area and total dry mass found in the plants cultivated under blue nets evidences that the increase in leaf area allowed a higher light interception, favoring the production of photoassimilates (Li and Kubota, 2009). The R/AP ratio was higher in plants grown under 100%, 70% irradiance and under red nets, which was smaller in treatments with blue nets and 50% irradiance (Table 1). The largest R/AP ratio found in plants grown under 100% irradiance confirms the high allocation of dry matter for the roots. Plants with root systems are usually more developed, have a higher capacity to support high photosynthetic rates under a high transpiration in that environment type, because they can absorb more water and nutrients (Claussen, 1996). Besides, the low R/AP ratio and the highest height in the plants cultivated under blue nets, confirms the highest investment, partly aerial, under these conditions.The occurrence of a higher LWR and higher R/AP ratio in plants under 100% irradiance can be related to the plasticity growth induced by the different conditions of lightness, resulting in the adaptability of the species to the regime of larger irradiances. A different result was observed in *Catharanthus roseus* (L.) G. that did not demonstrate adaptability to 100% irradiance (Melo and Alvarenga, 2009). The specific leaf area (SLA) was smaller in the treatment with 70% and 100% irradiance and larger with blue nets (Table 1). The decrease in SLA observed for the treatments with the increase in irradiance is, perhaps, a result of a thickening of the leaves in these treatments. Changes in leaf thickness are a response to acclimatization under different environmental conditions (Aranda et al., 2004). Furthermore, the reduction in leaf thickness in shaded plants is due to the difference in the distribution and in the consumption of photoassimilates for leaf expansion, especially in plants cultivated under blue nets, which presented leaves with a larger foliar area and a larger LAR. This response is an adaptive strategy that can make the increase in light capture possible and allow a larger photosynthetic efficiency for larger carbon earnings (Taiz and Zeiger, 2009). In *Ocimum selloi Benth.* a reduction in leaf thickness was also verified, when the plant was cultivated under blue nets (Costa et al., 2010). Such results allow us to implement the cultivation of medicinal plants, with greater control of production, since there are few studies with cultivation in protected environments. Knowing growth responses, as we direct the growing environment for increased production of secondary compounds and biomass.

Phenolics and PAL activity

Irradiance also presented an effect on the production of total phenolics (Fig 3). The content of phenolic acids and lignin was larger in the treatment with blue nets and smaller in the treatment with RN and 100% irradiance (Figs 4A, 4B). Such a result indicates that secondary compounds are influenced by quality and quantity of irradiance. Studies conducted with *Protea cynaroides* L., cultivated *in vitro*, showed that it also presented a higher concentration of total phenolics when cultivated under blue light (LED) and a smaller amount under red light (Wu and Lin, 2012). However, the ginger (*Zingiber officinale* Roscoe) cultivated under different intensities of natural light presented different results, where high irradiances caused an increase in these compounds (Ghasemzadeh et al., 2010). The increase of lignin content in plants cultivated under blue nets can be a response of anatomical changes, usually induced in adverse conditions that provide the protection of the cells (Ghasemzadeh et al., 2010). Therefore, the major production of total phenolics and lignin can be related to a higher resistance in plants (Martti et al., 2004). The increase in total phenolic compounds for the treatment with blue nets can be beneficial for plants for fighting diseases or attack of herbivores in *Piper aduncum.* Additionally, the synthesis of these compounds is seemingly influenced by the quality of light. The content of flavonoids was smaller in the treatment with 100% irradiance, and significant differences in the other treatments were not observed (Fig 4C). This result indicates the high influence of irradiance intensities on the production of flavonoids. Ghasemzadeh and Ghasemzadeh (2011) observed that *Zingiber officinale* Roscoe leaves presented a higher content

Table 1. Roots/aerial parts ratio (R/AP), specific leaf area (SLA), leaf area ratio (LAR), leaf weight ratio (LWR) and root weight ratio (RWR) of *Piper aduncum* grown under different conditions of irradiance.

TRAT	R/AP (g)	SLA ($cm^2. g^{-1}$)	LAR ($cm^2. g^{-1}$)	LWR ($g.g^{-1}$)	RWR ($g.g^{-1}$)
50%	0.47b	108.70a	32.63a	0.30a	0.18b
70%	0.63a	100.52b	28.04b	0.28b	0.23a
100%	0.60a	90.78b	27.18b	0.30a	0.22a
RN	0.60a	116.43a	30.43a	0.26b	0.23a
BN	0.46b	118.81a	33.08a	0.28ab	0.18b
CV(%)	22.09	16.69	17.39	11.18	18.89

*Means followed by the same letter in columns do not differ by the Tukey's test ($p \leq 0.05$). 50, 70 100% of irradiance, red nets – RN and blue nets –BN. Lavras, Brazil.

of flavonoids, when exposed to shade. Flavonoids are known as protectors substances against damage induced by excess light, and as antioxidants (Jaakola et al., 2004; Nascimento et al., 2013). The growth of *Piper aduncum* at 100% irradiance was not beneficial for the stimulation of these defenses, or this species still presents other protection mechanisms against high irradiances. Pacheco et al. (2013) observed under conditions of full sun, an increase of carotenoids. Thus, this can pigments associated with photoprotection mechanism for this species (Lu and Li, 2008). The activity of PAL increased in the treatments with 50% and 70% irradiance, and blue nets (Fig 4D). PAL is a key enzyme in the secondary metabolic pathway, and it demonstrates that the high contents of total phenolics can be related to the highest activity of this enzyme (Ghasemzadeh and Ghasemzadeh, 2011). Moreover, the blue light causes the induction of genes that regulate the expression of PAL (Meng et al., 2004). Different results were observed in *Labisia pumila* Benth leaves, grown in greenhouses, where the highest activity of PAL was observed in the treatment with a higher light intensity - 630 µmol $m^{-2}s^{-1}$ (Karimi et al., 2013). The production of photoassimilates of the primary metabolism can cause an increase in the concentrations of phenolic compounds at high irradiances (Warren et al., 2003). However, when light, water and nutrients are enough; the growth and the adaptation of the plant are prioritized. Therefore, a high quantity of phenylalanine is used for the synthesis of proteins, and consequently decreased the production of phenolic compounds (Gonçalves et al. 2008). The lowest synthesis of secondary compounds observed in the plants cultivated under 100% irradiance, is related to the induction of a stress that forces its adaptation under these condition. However, more studies are necessary to determine the increase in the production of secondary metabolites, due to the decrease in the production of primary metabolites through the photosynthesis or the stress induced by irradiance conditions (Ghasemzadeh and Ghasemzadeh, 2011).

Materials and methods

Locality and plant material

The experiment was conducted in Federal University of Lavras, Brazil. Seedlings of *Piper aduncum* were produced in the Department of Biology, from seeds. The seeds were pre-germinated in petri dishes, on three filter paper leaves and kept in a Mangesdorf germination chamber, at 25°C and through a 12-hour photoperiod, for 30 days. After this period, the seedlings were transferred to polypropylene trays containing the commercial substrate Tropstrato HA® (Vida Verde©, Brazil) and kept in greenhouses with 50% shading until they reached 2.5 cm height. The plants destined to full-sun cultivation (100% irradiance) were previously acclimatized for 7 days in 70% irradiance and later for 7 days in full sun before they were transplanted to the definitive substrate. Irrigation was performed daily, and the soil was kept under the condition of field capacity. After acclimatization, the seedlings were transplanted to plastic pots, with a capacity of 6 liters, containing a substrate comprised of subsoil, sand and bovine manure, in proportion of 2:1:1, being disposed in the different irradiance treatments. The physicochemical characteristics of the soil were analyzed in the Laboratory Analysis of Soil, and 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 experiment was conducted between April and July of 2012, at Gota da Esperança Farm, belonging to the Department of Agriculture, with the following geographical coordinates: 21°14'07"S and 44°58'22"W, at 879 m altitude. The average climatic conditions observed during the experiment were provided by Climatological Station of the Department of Agricultural Engineering, had maximum temperature of 30.2°C and a minimum of 6.3°C, precipitation of 1.28 mm and a relative humidity of 72.7%.

Altered ambient light

The treatments were characterized by cultivation of plants for 120 days, under four altered ambient light, produced by shading greenhouses with 70% and 50% of natural incident irradiance, two colored shading nets (Chromatinet® Green.tek©, USA) in red (RN) and blue (BN) colors, blocking 50% of the incident radiation and one treatment at full-sun. 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 radiation spectrum of different environments was evaluated, with a spectral resolution of 1 nm. The normalized irradiances observed for the treatment at 50% was 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. Each environment presented the highest values in terms of amount and size of the spectrum for the atmosphere with 100% irradiance, 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.

Growth analyzes

Growth was evaluated in twenty plants of each treatment, and the following morphologic variables were taken as a base: height, stem diameter, leaf area (LA), number of leaves, leaf dry mass (LDM), stem dry mass (SDM), root dry masss (RDM) and total dry mass (TDM). The following ratios were also calculated: root/aerial part (R/AP=RDM/ (LDM+SDM)),

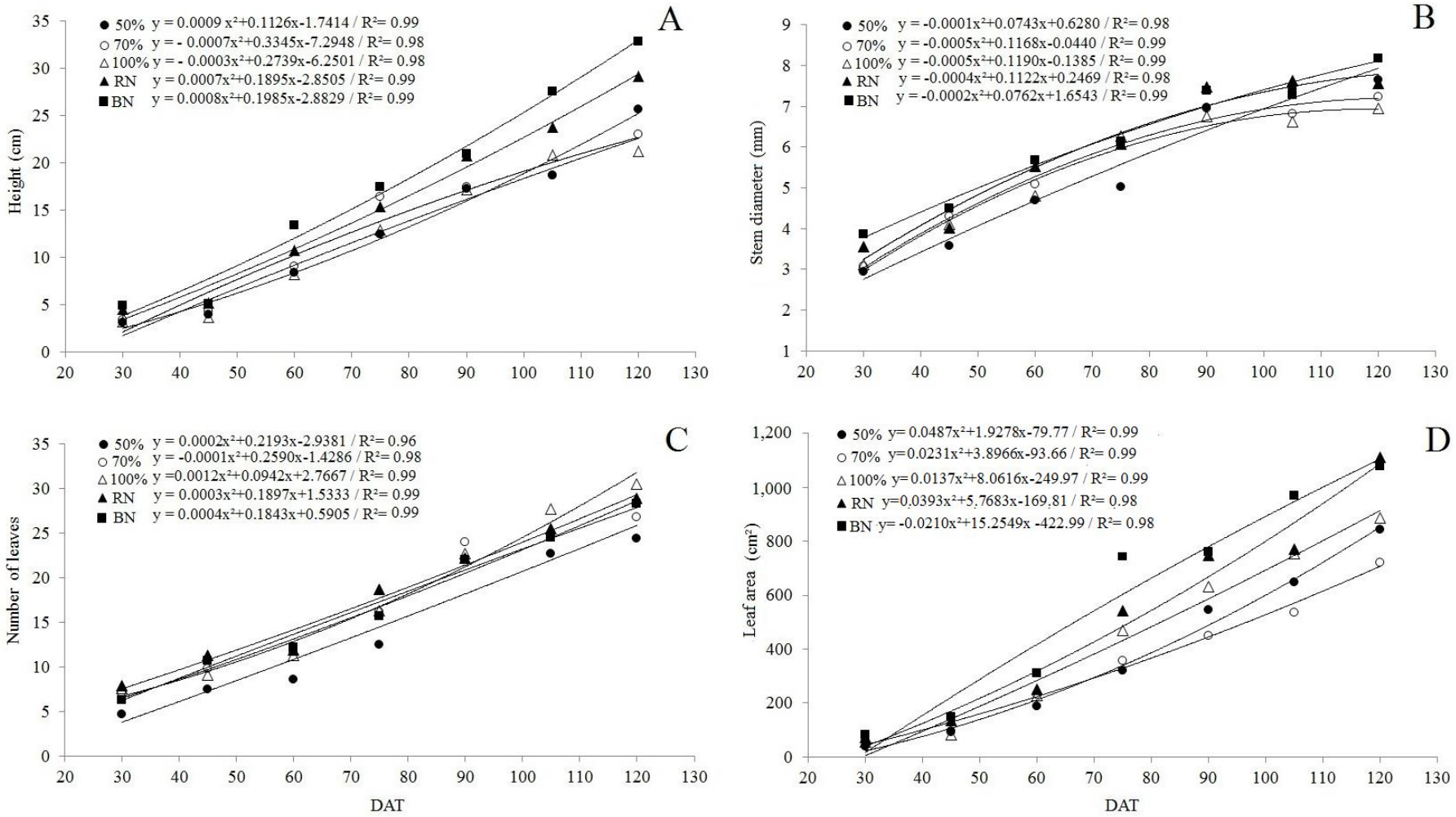

Fig 1. Height (A), stem diameter (B), number of leaves (C) and leaf area (D) of *Piper aduncum* grown under different irradiances (50, 70 100% of irradiance and red nets – RN and blue nets – BN), at 120 DAT (days after transplant). Lavras, Brazil.

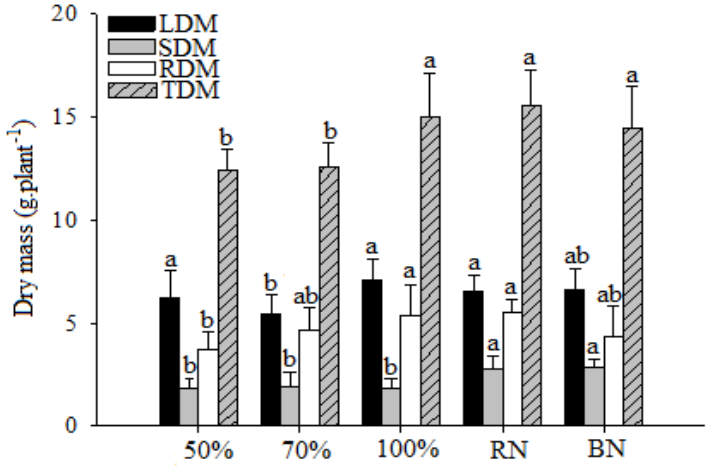

Fig 2. Leaf dry mass (LDM), stem dry mass (SDM), root dry mass (RDM) and total dry mass (TDM) of *Piper aduncum* grown under different conditions of irradiance (50, 70 100% of irradiance and red nets – RN and blue nets –BN). Means followed by the same letter in columns do not differ by the Tukey test ($p \leq 0.05$). Lavras, Brazil.

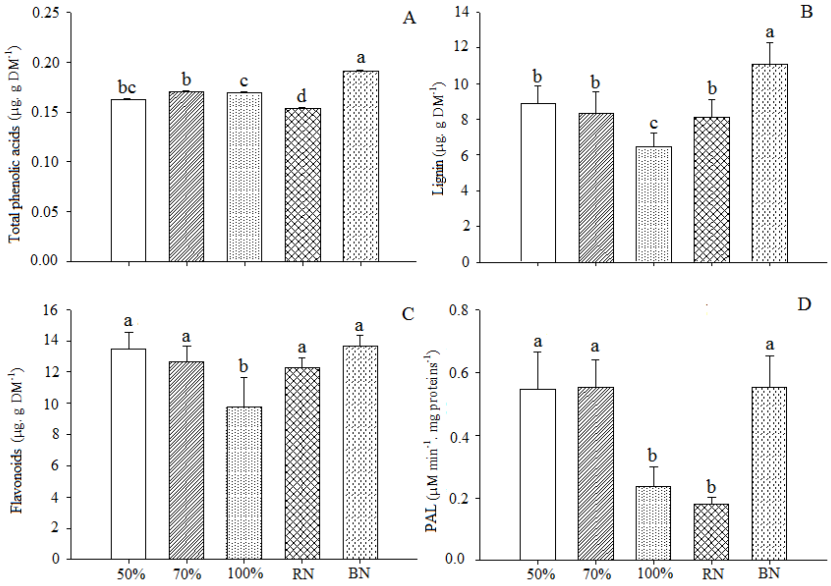

Fig 3. Total phenolic acids (A), lignin (B), flavonoids (C) and PAL activity (D) in leaves of *Piper aduncum* L. grown under different irradiance conditions (50, 70 100% of irradiance and red nets – RN and blue nets –BN) . *Means followed by the same letter in columns do not differ by the Tukey test ($p \leq 0.05$).Lavras, Brazil.

specific leaf area (SLA = LA/LDM), leaf area ratio (LAR =LA/TDM), leaf weight ratio (LWR=LDM/TDM) and root weight ratio (RWR=RDM/TDM), using equations in agreement with Benincasa (2003). The height, stem diameter, number of leaves and leaf area were measured in 15 days. The height was measured with a graduate scale, precision of 1.0 mm, and the distances from the surface of the soil to the top were measured. The stem diameter was measured accurately with 0,01mm digital caliper on the surface of the soil. The number of leaves was obtained through the direct count of the leaves. The leaf area was determined by measuring all expanded leaves of the plant, and the width and length of the leaf blade were measured with a scale and, in the last measurement, the leaf area was adjusted with the aid of a LI 3100 - LICOR® area meter, from which a correction factor (0.62) was determined for the previous measurements.
The variables leaf dry mass, stem dry mass, root dry mass and total dry mass were obtained in the end of the experimental period (120 days). 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.

Secondary metabolic

The secondary compounds were analyzed 150 days after the transplant, and the contents of total phenolic compounds, lignin and flavonoids were quantified. Furthermore, the activity of phenylalanine ammonium liase (PAL) was also verified. Two leaves were collected, completely expanded, located between the second and third node of five plants for each treatment; composition analyses were performed in triplicate. The contents of total phenolic acids and lignin were determined in accordance with Zieslin and Ben-Zaken (1993) with modifications. For the extraction, the tissues were triturated with liquid nitrogen and then lyophilized for six hours. The lyophilized material was weighed (30mg) and

transferred to a 2-mL tube, added 1.5 mL of 80% methanol, and kept under agitation for 15 h at room temperature and in the dark. The extract was centrifuged at 12.000 x g for 15 min; the supernatant was used for the quantification of total phenolics and the precipitate for lignin extraction. For the quantification of total phenolic acids, 30µL of Folin-Ciocalteu reagent were mixed with methanol extract (0.25 N) in ELISA plate, and rested for 5 minutes. Then, 30 µL of 1M sodium carbonate were added and homogenized for 10 minutes. It completed 160 µL of distilled water, resting for 1 hour and centrifuged at 2.250 x g for 5 minutes. Then, 180 µL of this mixture were collected and deposited in another ELISA plate. The whole procedure was conducted at room temperature. Readings were performed at 725 nm. The content of total phenolic acids was calculated based on the standard curve of catechol and the value was expressed in µg catechol per gram of dry matter. Lignin was extracted from the precipitate resuspended in 1.5 mL of 80% methanol and centrifuged at 12.000 x g for 10 minutes. The supernatant was then discarded and the precipitate was dried at 65°C for four hours. The dry precipitate was resuspended in 1.5 mL of a 1:10 solution of thioglycolic acid and 2N hydrochloric acid and homogenized in a water bath at 100°C for four hours, cooled on ice for 10 minutes and centrifuged at 12.000 x g for 10 minutes. Again, the supernatant was discarded and the precipitate resuspended in 1.5 mL distilled water and submitted to centrifugation at12.000 x g for 10 minutes under 4°C. The precipitate resuspended in 1.5 mL of 0.5 M sodium hydroxide, kept under agitation for 15 h at room temperature and submitted to centrifugation at 12.000g for 10 minutes under 4°C. The supernatant was transferred to a new 2-mL tube and 200 µL of concentrated hydrochloric acid were added; it was then kept on ice for four hours and centrifuged at 12.000 x g for 10 minutes under 4°C. The supernatant was discarded and the precipitate resuspended in 2 mL of 0.5 M sodium hydroxide, and homogenized. 200 µL of the extract were collected and deposited in an ELISA plate. Readings were performed at 280 nm. The lignin content was calculated based on the standard curves of lignin and the value was expressed in µg lignin per gram of dry matter. The total flavonoids were extracted from leaves according to a method adapted from Santos and Blatt (1998). Approximately 250 mg of dry matter were kept in 70% methanol (v/v) for 24 hours. An aliquot of 50 µL of the supernatant was transferred to test tubes containing 1.8 mL of 70% methanol. Then, 130µL of an aluminum chloride solution were added (5g aluminum chloride in 100 mL of 70% methanol) and 6.7 mL of 70% methanol, which were shaken vigorously. Readings were performed at 452 nm. The standard curve was prepared with increasing concentrations of rutin ((Sigma-Aldrich, 95%), and expressed in $\mu g.g^{-1}$ of dry mass. The activity of PAL was evaluated according to Mori et al. (2001) with modifications. A 50µL aliquot of the enzymatic extract was added to 150 µL of an incubation medium. The incubation medium was comprised of 100 µL of 100 mM Tris-HCL buffer, pH 8.8 and 50 µL of 40 mM phenylalanine. Incubation was performed on acrylic plates at 37°C for 2 hours in an ELISA spectrophotometer (Power Wave XS of Biotek®) at 280 nm; readings were performed every 10 minutes. The activity of PAL was expressed in $\mu M\ min^{-1}$ .protein mg^{-1}; the coefficient of molar extinction used was 104 $mM^{-1}\ cm^{1}$ (Zucker, 1965).

Statistical analysis

The experimental design was completely randomized, in which 20 replicates by treatment were used for growth evaluations, and 5 replicates for biochemical analyses. 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).

Conclusions

The growth and the synthesis of the secondary compounds (total phenolics acids, lignin and flavonoids) of *Piper aduncum* was influenced by the different irradiance conditions. The plants cultivated under blue photoselective shade nets presented a higher growth and production of dry mass. The highest amount of secondary compounds was obtained in the plants grown in blue shade nets.
Thus, for it is the producer species with commercial potential of bioactive compounds, their cultivation in environments supplemented with blue light is recommended.

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References

Aranda I, Pardo F, Gil L, Pardos JA (2004) Anatomical basis of the change in leaf mass per area and nitrogen investment with relative irradiance within the canopy of eight temperate tree species. Acta Oecol. 25:187-195.

Benincasa MMP (2003) Análise de crescimento de plantas (noções básicas). FUNEP. Jaboticabal-SP. 41 p.

Bernard CB, Krishnamurty HG, Chauret D, Durst BJR, Philogene BJR, Sánchez-Vindas, P, Hasbun C, Poveda L, San Román L, Arnason, JT (1995) Insecticidal defenses of Piperaceae from the Neotropics. J Chem Ecol. 21:801-814.

Claussen JW (1996) Acclimation abilities of three tropical rainforest seedlings to an increase in light intensity. For Ecol Manag. 80: 245-255.

Costa LCB, Pinto JEBP, Castro EM, Alves E, Bertolucci SKV, Rosal LF (2010) Effects of coloured shade netting on the vegetative development and leaf structure of *Ocimum selloi*. Bragantia. 69:349-359.

Fazolin M, Estrela JLV, Catani V, Lima MS, Alécio MR (2005) Toxicidade do óleo de *Piper aduncum* L. a adultos de *Cerotoma tingomarianus* Bechyné (Coleoptera: Chrysomelidae). Neotrop Entomol. 34: 485-489.

Fernandes VF, Almeida LB, Feijó EVRS, Silva DC, Oliveira RA, Mielke MS, Costa L CB (2013) Light intensity on growth, leaf micromorphology and essential oil production of *Ocimum gratissimum*. Rev Bras Farmacog. 23:419-42.

Ghasemzadeh A, Ghasemzadeh, N (2011) Flavonoids and phenolic acids: Role and biochemical activity in plants and human. J Med Plants Res. 31: 6697-6703.

Ghasemzadeh A, Hawa ZE, Jaafar HZE, Rahmat A. Synthesis of phenolics and flavonoids in ginger (*Zingiber officinale* Roscoe) and their effects on photosynthesis rate (2010). Int J Molr Sci. 11:4539-4555.

Gonçalves B, Correia CM, Silva AP, Bacelar EA, Santos A, Moutinho-Pereira JM (2008) Leaf structure and function of sweet cherry tree (*Prunus avium* L.) cultivars with open and dense canopies. Sci Hortic. 116:381–387.

Jaafar H, Rahmat A. Accumulation of partitioning of total phenols in two varieties of *Labisia pumila* Benth. under manipulation of greenhouse irradiance (2008). Acta Hortic. 797:387–392.

Jaakola L, Määttä-Riihinen K, Kärenlamp IS, Hohtola A. Activation of flavonoid biosynthesis by solar radiation in bilberry (*Vaccinium myrtillus* L.) leaves (2004). Planta. 218: 721–728.

Jones RH, McLeod KW.Responses to a range light environments in chinese tallowtree and carolina ash seedlings (1991). Forest Sci. 36:851-862.

Karimi E, Hawa ZEJ, Ghasemzadeh A, Ibrahim MH (2013) Light intensity effects on production and antioxidant activity of flavonoids and phenolic compounds in leaves, stems and roots of three varieties of *Labisia pumila* Benth. Aust J Crop Sci. 7:1016-1023.

Kumari R, Singh S, Agrawal SB (2009) Effects of supplemental ultraviolet-B radiation on growth and physiology of *Acorus calamus* L. (sweet flag). Acta Biol Cracoviensia Ser Bot. 51: 19-27.

Lara-Júnior CR, Oliveira GL, Mota BCF, Fernandes MFG, Figueiredo LS, Martins E R, Moreira DL, Kaplan MAC (2012) Antimicrobial activity of essential oil of *Piper aduncum* L. (Piperaceae). J Med Plants Res. 21:3800-3805.

Li Q, Kubota C (2009) Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. Enviro and Exper Bot. 67:59-64.

Lu S, Li L(2008). Carotenoid metabolism: biosynthesis, regulation, and beyond. J of Inter Plant Biol. 50:778-785.

Maia JGS, Zoghbi MGB, Andrade EHA, Santos AS, Silva MHL, Luz AIR, Bastos CN (1998) Constituents of the essential oil of *Piper aduncum* L. growing wild in the Amazon region. Flavour Frag J. 13:269-272.

Martti V, Anni MH, Pekka S, Pirjo K, Markku T, Pirkko V (2004) The concentration of phenolics in brown-rot decay resistant and susceptible scots pine heartwood. Wood Sci Technol. 38:109-118.

Melo AAM, Alvarenga AA (2009) Sombreamento de plantas de *Catharanthus roseus* (L.) g. don 'Pacifica White' por malhas coloridas: Desenvolvimento vegetativo. Cienc Agrotec. 33: 514-520.

Meng X, Xing T, Wang X (2004) The role of light in the regulation of anthocyanin accumulation in *Gerbera hybrida*. Plant Growth Regul. 44:243–250.

Misni N, Othman H, Sulaiman S (2011) The effect of *Piper aduncum* Linn. (Family: Piperaceae) essential oil as aerosol spray against *Aedes aegypti* (L.) and Aedes albopictus Skuse. Trop Med. 28: 249-258.

Mori T, Sakurai M, Sakuta M (2001) Effects of conditioned medium on activities of PAL, CHS, DAHP synthase (DS-Co and DS-Mn) and anthocyanin production in suspension cultures of *Fragaria ananassa*. Plant Sci. 160:355–360.

Nascimento LBS, Leal-Costa MV, Coutinho MAS, Moreira NS, Lage CLS, Barbi NS, Costa SS, Tavares ES (2013) Increased antioxidant activity and changes in phenolic profile of *Kalanchoe pinnata* (Lamarck) Persoon (Crassulaceae) specimens grown under supplemental blue light. Photochem Photobiol. 89:391–399.

Nawkar GM, Maibam P, Park JH, Sahi, VP, Lee, SY, Kang, CH (2013) UV-Induced cell death in plants. Int J Mol Sci. 14:1608-1628.

Oliveira MI, Castro EM, Costa LCB, Oliveira C (2009) Características biométricas, anatômicas e fisiológicas de *Artemisia vulgaris* L. cultivada sob telas coloridas. Rev Bras Plant Med. 11:56-62.

Orjala J, Wrighth AD, Behrends H, Folkers G, Sticher O (1994) Cytotoxic and antibacterial dihydrochalcones from *Piper aduncum*. J Nat Prod. 57:18-26.

Pacheco FV, Silveira HRO, Alvarenga AA, Alvarenga ICA, Pinto JEBP, Lira JMS (2013) Gas exchange and production of photosynthetic pigments of *Piper aduncum* L. grown at different irradiances. Am J Plant Sci. 4:114:121.

Petkovsek MM, Stampar F, Veberic R (2009) Accumulation of phenolic compounds in apple in response to infection by the scab pathogen, *Venturia inaequalis*. Physiol Mol Plant Pat. 74:60-67.

Rocha SFR, Ming LC, Chaves FCM, Scarda FM (2008) Role of light and phytochrome on *Piper aduncum* L. germination: an adaptive and environmental approach. J Herbs Spices Med Plants. 11:85-96.

Santos MD, Blatt CTT (1998). Teor de flavonóides e fenóis totais em folhas de *Pyrostegia venusta* Miers. de mata e de cerrado. Rev Bra de Bot. 21 135:140.

Sousa PJC, Barros CAL, Rocha JCS, Lira DS, Monteiro GM, Maia JGS (2008) Avaliação toxicológica do óleo essencial de *Piper aduncum* L. Rev Bra Farmacog. 18:217-221.

Souza GS, Castro EM, Pinto JEBP, Alves E, Biagiotti G, Deuner, S (2007). Estrutura foliar e de cloroplastídeos de *Mikania laevigata* Schultz Bip. ex Baker em diferentes condições de qualidade de luz. Rev Bra Bioc. 5:78-80.

Taiz L, Zeiger E (2009) Plant physiology. 5ed. Artmed. 719 p.

Warren MJ, Bassman J, Fellman JK, Mattinson DS, Eigenbrode S (2003) Ultraviolet-B radiation of *Populus trichocarpata* leaves. Tree Physiol. 23:527-535.

Wu H, Lin C (2012) Red light-emitting diode light irradiation improves root and leaf formation in difficult-to-propagate *Protea cynaroides* L. plantlets *in vitro*. HortScience. 47:1490-1494.

Yan JX, Lu YF, Yan SC (2013) The effects of irradiance on the production of phenolic compounds and condensed tannins in *Larix gmelinii* Needles. Biol Plantarum. 20:1-5.

Zieslin N, Ben-Zaken R (1993) Peroxidase activity and presence of phenolic substances in peduncles of rose flowers. Plant Physiol Bioch. 31:333-339.

Zucher M. (1965) Induction of phenylalanina deaminase by light and its relation to chlorogenic acid synthesis in potato tuber tissue. Plant Physiol. 40:779-784.