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Evaluation of growth and phenolic compounds profile of purple lettuce under *indoor* cultivation

ABSTRACT

The objective of this study was to observe indoor cultivation system with LED lights (white and infrared) to purple lettuce cv. Mimosa in a micro-growing environment for home cultivation. A conventional cultivation with white lamps was used as a Control. The quality of lettuce was evaluated by measuring several parameters, including the number of commercial leaves (intact)/plant, stem diameter, height of the aereal portion, leaf area, absolute growth rate and productivity. Also, individual phenolics contents and photosynthesis parameters were evaluated. The results obtained showed that indoor cultivation growing results around 60% higher number of leaves per plant and 4.75 x more leaf area in comparison to Control treatment (28 days after transplant). In addition, productivity and absolute growth rate of purple lettuce were positively affected by indoor cultivation system (a leaf area difference of 300 g.cm⁻² more than the Control). The light intensity provided by the LED's resulted in increased biomass and yield, mainly due to the elevated photosynthetic activity of plants. At the same time, individual phenolic compounds like chlorogenic acid, caffeic acid, chicoric acid, luteoline-7-o-glucoronide, quercetin-3malonylglucoside, quercetin acetyl hexoside and cyanidine 3-o-malonylglucoside were identified in purple lettuce cv. Mimosa. Therefore, indoor cultivation system is a viable option to provide the consumer a more natural, practical and healthy food.

KEYWORDS: *Lactuca sativa*; light emitting diodes; productivity; photosynthesis; individual phenolic compounds.

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INTRODUCTION

The advent of local food systems has emerged as a multifaceted movement that comprises distinct causes such as health, nutrition and lifestyle, social justice, food security, environmental conservation, and community and economic development (KREMER; DELIBERTY, 2011). According to Pölling, Mergenthaler and Lorleber (2016), urban agriculture is a very relevant topic in societies around the world and should be methodically studied in several areas. In addition, food produced within the concept of local food allows consumers to know how and where their food was grown and, as such, might impact their decision. Within that context, in the last decade, with the development of light-emitting diodes (LEDs) the cultivation of *indoor* food has rapidly evolved into a commercially viable, sustainable, and large-scale production system (LOCONSOLE *et al.*, 2019).

Plants use light as an energy source in the process of photosynthesis and respond to this luminous energy according to light quality, light intensity and photoperiod. Plants perceive light through photoreceptors, such as phytochromes and cryptochromes, and respond to these receptors by generating a series of specific physiological responses (MUNEER *et al.*, 2014). Red and blue lights have the greatest impact on plant growth because they are the major energy sources for photosynthetic CO₂ assimilation in plants, however, other wavelengths (green, white, UV) could also affect plant growth and development, as well as phytochemical accumulation (ZHANG and FOLTA, 2012; HYTÖNEN *et al.*, 2017; VIRŠILĖ *et al.*, 2020; HE *et al.*, 2021). The use of red and blue lights plays an important role in physiological plant responses, chlorophyll synthesis, photomorphogenesis and biomass production (LI and KUNOTA, 2009; SHAO *et al.*, 2020; PENNISI *et al.*, 2020).

LED's were suggested as a light source for facilities of agriculture in controlled environments. Plant growth chambers equipped with LED's have several advantages in comparison to traditional and artificial light sources. Amongst those: constant light output over the years, low electricity (up to 70%) consumption (SINGH *et al.*, 2015), and low heat radiation yield while emitting high light intensities (YEH and CHUNG, 2009). In addition, the possibility of modulating the spectrum of LED illumination might improve the accumulation of important bioactive compounds, as shown in lettuce by Lee, Son and Oh (2016). Furthermore, SHAO *et al.* (2020) observed that the use of high light irradiation provided by red and blue LEDs enhanced biomass production and secondary metabolite accumulation in hydroponic purple leaf lettuce cultivated in an environmentally controlled plant factory.

Lettuce is a vegetable with high production and consumption rates worldwide. The species is a good source of fibers, magnesium, potassium, vitamin C, as well as being rich in health-promoting nutrients, such as phytochemicals (PÉREZ-LÓPEZ *et al.*, 2014; USDA, 2015). In the state of Rio Grande do Sul, lettuce is produced throughout the year, though two periods with more unfavorable climatic conditions occur. The summer period when high air temperatures and solar radiation come about and the winter period with the occurrence of low temperatures and prolonged precipitation slowing down growth and also damaging the plants (SEGOVIA *et al.*, 1997).

LED lighting systems have been shown to increase the growth rate of plants (MUNEER *et al.,* 2014; HYTÖNEN *et al.,* 2017). The use of red and blue LED lights



was proven to be effective light sources for development of an array of plant species in controlled environments, including lettuce (LEE *et al.*, 2016). Additionally, lettuce seedlings grown under blue and red diodes supplemented with white fluorescent light presented higher production of chlorophyll, carotenoids, and sugar contents than plants grown either only under blue and red LED light or under white fluorescent light (LIN *et al.*, 2013).

Conversely, the combinations of white and infrared LED's could be an alternative to the use of red and blue lights and has not been studied yet in vegetables. Besides, no reports on the effects of that combination of lights in photosynthetic performance of lettuce leaves are available. Therefore, the objective of the present study was to observe growth, development and bioactive compounds profile of purple lettuce (cv. *Mimosa*) produced in an *indoor* cultivation system with white and infrared light-emitting diodes.

MATERIALS AND METHODS

PLANT MATERIAL AND GROWING CONDITIONS

Purple lettuce seedlings of the cv. Mimosa (*Lactuca sativa L*.) were purchased from a plantlets dealer at Porto Alegre (Rio Grande do Sul, Brazil). The seedlings were transported to the Postharvest Laboratory of Universidade Federal do Rio Grande do Sul (UFRGS) and immediately after transplanted into clean 1.7 L-plastic pots filled with perlite as substrate.

Lettuce samples were cultivated by the indoor method in two different treatments:

a) Indoor cultivation chamber equipped with white and infrared LED light, as prototype provided by Plantário[®] company (Figure 1). Six plants per chamber were kept in a room at a constant temperature of 18 °C, with a photoperiod of 18:6 of light and dark conditions.

b) Control: conventional cultivation in pots under four white lamps. Six plants per chamber were maintained in a photoperiod of 12 hours of light and 12 hours in dark conditions.

The nutrient solution, substrates, and irrigation were the same for both treatments. The nutrient solution supplied to the plants presented the following composition: $Ca(NO_3)_2 - 42.32\%$; $KNO_3 - 26.45\%$; $NH_4H_2PO_4 - 7.93\%$; $MgSO_4 - 21.16\%$; $KH_2PO_4 - 0.53\%$; Fe-EDTA - 1.58\%. The pH and the initial electrical conductivity of nutrient solutions used were 5.45 and 2.016 mS, respectively. The volume of the solution of each experimental unit was maintained by replacing the consumed volume with water. That replacement was carried out when a reduction of 20% in the volume of each pot was determined. That percentage corresponded to a decrease of 2.5 cm in the height of the solution. Irrigation was automated. The pump was set off by a timer three times a day for 15 minutes at 6 AM, 12 PM and 6 PM. No agrochemicals were applied along the vegetative cycle of the lettuce plants.

The experiment was conducted in two entirely randomized blocks. Lettuce samples were harvested and analyzed destructively to determine the production parameters, such as growth and development on the day of installation of the experiment (Day 0), 20 days after transplant (Day 20), and 28 days after transplant



(Day 28). Each retrieval for every sampling period and treatment consisted of 3 whole plants (collecting randomly one plant from each pot). During the evaluations, the relative air humidity was 80% \pm 5%, the ambient temperature varied from 18 to 20 °C, and the irradiance saturation was 400 µmol m⁻² s⁻¹ photons in the Plantário[®] unit and 200 µmol m⁻² s⁻¹ photons in the control cultivation method.



Figure 1. Prototype provided by the company Plantário®

PLANT MEASUREMENTS

At day 0 and after 20 and 28 days the samples were evaluated for number of leaves per plant. The leaf area was determined using an area integrator (LI3100, Liqueur), which all the leaves from each collected plant were used. The height of the aerial part was determined immediately after the separation of the root system. The weight of the fresh mass of the aerial part of the plants was determined using a precision digital scale (0.01 g). After initial evaluations, the plants were packed in previously identified paper bags and placed for drying in an oven with forced air circulation at 65 °C \pm 1 °C until constant weight. The absolute growth rate (TAC) was calculated by the following expression: TCA = (P2-P1)/(T2-T1), where P2 - P1 is the difference in dry matter mass (g) of a determined area, and T2 - T1 is the time interval (days) of the two samples. The values were expressed in g.day⁻¹. Productivity was calculated by dividing the total dry mass by cultivation area. The values are expressed in g.cm⁻².

DETERMINATION OF PHENOLIC COMPOUNDS BY HPLC-DAD-MS

Phenolic compounds were determined by HPCL-DAD-MS. Samples of 0.5 g tissue were frozen and freeze-dried and then homogenized in liquid nitrogen and extracted with 80% (v/v) methanol by ultrasound and subsequent centrifugation according to method described by Becatti *et al.* (2010). The supernatants were filtered with 0.22 μ m cellulose acetate membranes (Millipore[®], Massachusetts, USA) and 20 μ L injected into an Ultra High-Performance Liquid Chromatograph



(Shimadzu, Kyoto, Japan) equipped with two pumps, degasser, column oven, connected in series to a diode arrangement detector and a mass spectrometer with Q-TOF analyzer and an electrospray ionization source (Bruker Daltonics, micrOTOF-QII model, Bremen, Germany) according to the methodology described in Rodrigues *et al.* (2013). Chromatographic analyses were carried out on an Atlantis C18 RP-T3 column (5 μ m, 250 × 4.6 mm) and Synergi Hydro-RP C18 column (4 μ m, 250 × 4.6 mm, Phenomenex). The mobile phase solutions were: (A) Ultrapure water solution (Milli-Q[®], Millipore[®]) acidified with formic acid (99.5:0.5 v/v) and (B) acetonitrile solution acidified with formic acid (99.5:0.5 v/v). The flow rate was 0.7 mL min⁻¹ in a linear gradient starting with a ratio of 99:1 for A/B, linearly increasing the proportion of mobile phase B until reaching 50:50 (A/B) in 50 min. Subsequently, the ratio of B to 1:99 (A/B) was increased again in 5 minutes. This previous relationship (1:99) was maintained for another 5 minutes, and after this time the system returned to the initial condition of 99:1 for A/B. The chromatograms were recorded at 320, 360 and 520 nm.

The column eluate was divided to allow entry 0.35 mL.min⁻¹ in the electrospray ionization source (ESI) interface of the mass spectrometer. The mass spectra were acquired with a scanning range of 100 to 700 *m/z*. The MS parameters were as follows: ESI source in the positive and negative ionization modes; capillary voltage: 2000 V (positive) or -3000 V (negative); end plate offset: -500 V; drying gas: N₂; temperature: 310 °C; nitrogen flow: 8 L.min⁻¹; nebulizer gas: 2 bar. Fragmentation (MS2) was obtained in automatic mode, applying fragmentation energy of 34 eV. The phenolic compounds were identified based on elution order and retention time in the reverse phase column, UV-vis and characteristics of the mass spectra (MS and MS2) compared with standards analyzed under the same conditions and with data available in the literature.

PHOTOSYNTHESIS PARAMETERS

Carbon dioxide (CO₂) assimilation and stomatal conductance were evaluated with an infrared gas analyzer (6400 XTLicor), equipped with an LED light source camera. The measurements were performed with a light density of 1,000 μ mol photons m⁻² s⁻¹ at room temperature on three fully developed leaves per plot, in 6 plots, adding up to 18 measurements per cultivation system. The photosynthetic active radiation was also determined in each system by means of the LI 190R meter.

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STATISTICAL ANALYSIS

The data were analyzed for variance (ANOVA) using the software Statistica 12.0 (STATSOFT Inc.). The averages were compared by Tukey's multiple range test at a significance of p < 0.05.

RESULTS AND DISCUSSION

QUALITY AND PRODUCTIVITY PARAMETERS

Quality is a crucial factor affecting the consumers' choices and for lettuce, quality might be described by several parameters, which include the visual appearance. For that reason, the number of leaves of each plant is of critical commercial value for its acceptability. The number of commercial leaves per plant had the propensity to increase faster inside the *indoor* cultivation chamber in comparison to the Control treatment (Figure 2A). Conversely, concerning the height of the plants (Figure 2C), with the occurrence of the phenomenon of etiolation of the plants cultivated in the control treatment it was not possible to determine differences between the growing systems.

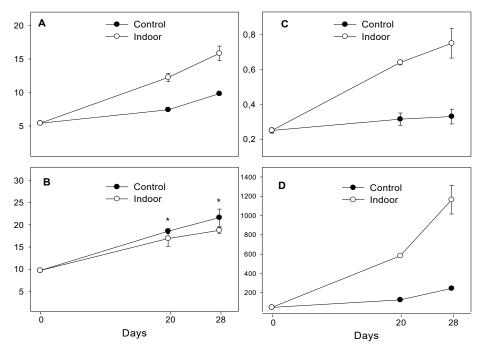


Figure 2. Number of commercial leaves (intact)/plant (A), stem diameter (mm) (B), height of the aerial portion (cm) (C), and leaf area (cm²) (D) of purple lettuce cv. *Mimosa* was grown in an *indoor* chamber and a simulating domestic cultivation (Control) at the days 0, 20, and 28 after seedling transplantation. * Indicates that no significant difference (p < 0.05) were determined.

The leaf area index (Figure 2D) had a similar tendency as the values of number of commercial leaves/plant and stem diameter (Figure 2B), though the amplitude of differences is larger. Segovia *et al.* (1997) presented similar results when comparing the growth and development of three lettuce cultivars inside polyethylene-covered greenhouses.

Radin *et al.* (2004) concluded that the parameters of leaf mass and leaf area index indicates that the specific mass of the leaves is most likely smaller inside greenhouses, suggesting that in the interior of a greenhouse the leaves expand more rapidly due to higher levels of relative air humidity. That characteristic contributes positively to attributes such as the appearance of the leafy vegetables, but has a negative effect from the point of view of resistance to transport and



postharvest shelf life. Yet the lettuce of the present work is grown at home for immediate consumption, *i.e.*, there is no need of shipping.

According to Despommier (2013), *indoor* cultivation linked to this style of home systems offers many advantages in comparison to the traditional soil-based agriculture. The most relevant is in detail control of the requirements to achieve optimal development, growth, and ripening of any crop, consequently ensuring maximum yields. For that reason, this system represents one of the few opportunities for progress in the coming decades.

The results indicate that the purple lettuce plants grown inside the *indoor* cultivation chamber had a higher growth rate (Figure 3A), indicating that the chamber imparts better efficiency in mass accumulation per day in relation to the Control treatment. In addition, the growing chamber also has higher efficiency in mass accumulation per area (Figure 3B) and productivity. The *indoor* cultivation chamber resulted in a leaf area of 300 g.cm⁻² beyond that of the control plants. Therefore it is possible to conclude that the *indoor* cultivation growing unit results in early harvesting, higher number of leaves per plant, and per area in comparison the control treatment.

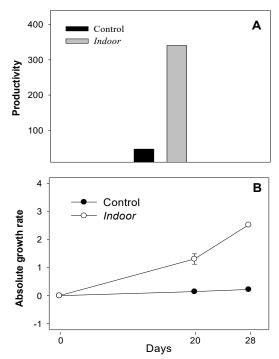


Figure 3. Productivity (g.cm⁻²) (A) and absolute growth rate (g.day⁻¹) (B) of purple lettuce cv. *Mimosa* grew in an *indoor* chamber and a simulation of domestic cultivation (Control treatment) at the days 0, 20 and 28 after seedling transplantation.

INDIVIDUAL PHENOLIC CONTENT

Indoor and Control-treatment samples showed similar profiles of individual phenolic compounds (Figure 4). The following compounds were identified: chlorogenic acid, caffeic acid, chicoric acid, luteolin-7-*o*-glucoronide, quercetin-3-malonyl-glucoside, quercetin acetyl hexoside, and cyanidin 3-*o*-malonylglucoside



(Table 1). The characteristic fragments of each chemical structure allowed the confirmation of the groups.

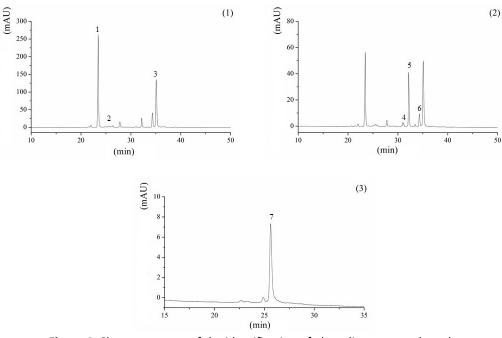


Figure 4. Chromatograms of the identification of phenolic compounds under different wavelengths (1: 320 nm; 2: 360 nm; 3: 520 nm) in purple lettuce cv. *Mimosa* grew in *indoor* cultivation chamber and a simulation of domestic cultivation (Control treatment).

eak number	Phenolic compound	Retention time (min)	۸ (nm)	Aduto
1	Chlorogenic acid	23.3	320	[M-H] ⁻
2	Caffeic acid (3,4-dihydroxycinnamic acid)	24.7	320	[M-H]⁻
3	Chicoric acid	35.2	320	[M-H]⁻
4	Luteolin-7- <i>o</i> -glucoronide	31.3	360	[M-H]⁻
5	Quercetin-3-malonyl-glucoside	31.7	360	[M-H]⁻
6	Quercetin acetyl hexoside	33.3	360	[M-H] ⁻
7	Cyanidin 3- <i>o</i> -malonyl-glucoside	25.8	520	[M+H]⁺

Table 1. Phenolic compounds identified in purple lettuce cv. Mimosa by HPLC-DAD-MS.
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The profile of bioactive compounds from different lettuce cultivars has been studied by several authors such as Pérez-López *et al.* (2014) and Marin *et al.* (2015). The identification of luteolin, cyanidin, chicoric acid, chlorogenic acid, and quercetin isomers is supported by the results of Becker *et al.* (2014) in a study also with purple lettuce. In the present study, the major anthocyanin compound identified was the cyanidin.

Phenolic compounds are considered important components once that they assist in the maintenance of the antioxidant cell status. The aptitude of flavonoids to reduce the risk of various cardiovascular diseases, cancers and atherosclerosis are already well known. According to Pérez-López *et al.* (2014), the antioxidant capacity of purple lettuce is mostly attributed to quercetins and anthocyanins

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suggesting that the consumption of this type of compound is potentially beneficial to human health, and indoor production could be a factor to bring the consumer closer to a healthier diet, with products free of pesticides and environmental contaminants.

The sysnthesis of secondary metabolites in food such as those identified in the present work depends on several aspects, including the availability of light (GARCÍA-MACÍAS *et al.*, 2007) light quality, light intensity, and photoperiod. Accordingly, Loconsole *et al.* (2019) observed highest values of phenolic compounds in Romaine lettuce cultivated in an *indoor* system.

PHOTOSYNTHESIS PARAMETERS

The highest CO₂ assimilation rates and stomatal conductances were determined in lettuce leaves grown in the *indoor* cultivation chamber, with a 2.7 and 2-fold significantly increase in the CO₂ assimilation rates and stomatal conductances, respectively, compared to the Control plants (Figure 5). The curves of CO₂ assimilation in response to photosynthetic active radiation can be seen in Figure 6. The highest results were found in samples treated in *indoor* cultivation, where the maximum photosynthetic activity was achieved with the photon flux density of 500 μ mol.m⁻².s⁻¹ (Figure 6). LED's present the maximum photosynthetically active radiation (PAR) efficiency and have the potential to cover fluence and wavelength by supplying the light quantity and quality essential for growth of lettuce plants.

The most distinctive result is the relationship between the leaf photosynthetic rates and the growth of plants. The high light intensity provided by the LED's resulted in increased biomass and yield, mainly due to the high photosynthetic activity of plants. Similarly, Kelly *et al.* (2020) cultivated green butterhead lettuce ('Rex') and red oakleaf lettuce ('Rouxai') under LED lights (50 % warm-white and 50 % red) with a photoperiod of 16 h d⁻¹, and was observed an increased in photosynthetic photon flux density of fresh (dry) mass by 47 % (30 %) and 52 % (41 %) for 'Rex' and 'Rouxai', respectively. On the other hand, the lower values of photosynthetic rates observed in lettuce plants grown in the control treatment may be due to etiolation and possibly changes in photosystems to increase light uptake. It is likely a response to long-term adjustments of photosynthetic efficiency (CHOW; MELIS; ANDERSON, 1990).

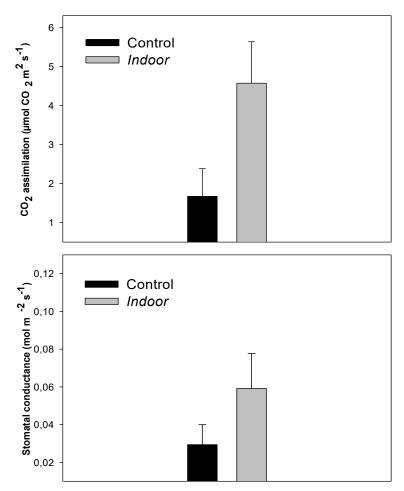
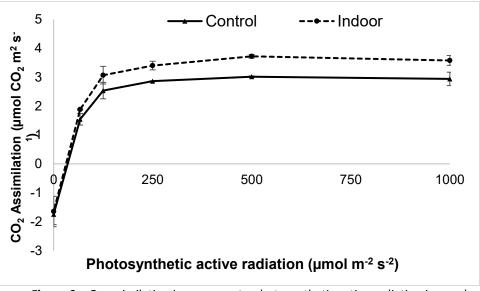
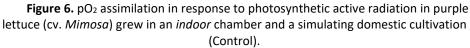


Figure 5. CO₂ assimilation (μ mol CO₂ m² s⁻¹) and stomatal conductance (mol m⁻² s⁻¹) of purple lettuce cv. *Mimosa* grew in *indoor* chamber and a simulating domestic cultivation (Control).







CONCLUSIONS

Indoor cultivation using the Plantário[®] chamber provided higher growth and development of purple lettuce plants of the cv. *Mimosa* in comparison to a Control treatment. The results indicate that the *indoor* growing chamber is an efficient alternative to obtain higher productivity and overall quality of purple lettuce plants besides being a viable option to provide the consumer with a more natural, healthy and local food. However, more detailed investigations need to conduct to evaluate luminous efficiency, light quality together with the exact energetic, economic, and environmental impact of this production system. Further studies on the effect of other combinations of LED lights to enhance purple lettuce bioactive compounds and sensory quality are also necessary.

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Avaliação do crescimento, desenvolvimento e perfil dos compostos fenólicos de alface roxa em cultivo *indoor*

RESUMO

O objetivo deste estudo foi observar o sistema de cultivo indoor com luzes LED (branco e infravermelho) para alface roxa cv. Mimosa em um ambiente de microcrescimento para cultivo caseiro. Um cultivo convencional com lâmpadas brancas foi usado como Controle. A gualidade da alface foi avaliada por meio da determinação de vários parâmetros, tais como, número de folhas comerciais (intactas)/planta, diâmetro do caule, altura da porção aérea, área foliar, taxa de crescimento absoluto e produtividade. Também foram avaliados os compostos fenólicos individuais e parâmetros de fotossíntese. Os resultados obtidos mostraram que o cultivo indoor resulta em um aumento em torno de 60% de folhas por planta e 4,75 x mais área foliar em comparação com o tratamento Controle (28 dias após o transplantio). Além disso, a produtividade e a taxa de crescimento absoluta da alface roxa foram positivamente afetadas pelo sistema de cultivo indoor (uma diferença de área foliar de 300 g.cm⁻² a mais do que o Controle). A intensidade luminosa proporcionada pelos LEDs resultou em aumento da biomassa e do rendimento, principalmente devido à elevada atividade fotossintética das plantas. Ao mesmo tempo foram identificados em alface roxa cv. Mimosa os seguintes compostos fenólicos individuais: ácido clorogênico, ácido cafeico, ácido chicórico, luteolina-7-o-glucoronida, quercetina-3malonilenoglicósido, acetil hexósido cianidina quercetina е 3-0malonilenoglicósido. Portanto, o sistema de cultivo indoor é uma opção viável para fornecer ao consumidor um alimento mais natural, prático e saudável.

PALAVRAS-CHAVE: *Lactuca sativa*; diodos emissores de luz; produtividade; fotossíntese; compostos fenólicos individuais.



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