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Aspects of photosynthesis and metabolic compounds in seedlings *Talisia esculenta* (A. St. Hil) Radlk under different shading nets. Aspectos da fotossíntese e compostos metabólicos em mudas de *Talisia esculenta* (A. St. Hil) Radlk sob diferentes redes de sombreamento.

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Abstract

The light directly affects the phytosanitary processes of plants, variations in plant length conditions and conditions directly affecting the production of environmental conditions and variations as well as pigments. In this study, this study aims to evaluate the changes that occur in the gas exchange process and in the production of metabolites in *Talisia esculenta* seedlings, through the use of colored shading screens. The seeds of *T. esculenta* were used, which were used in an alternative way, which were sown replacement in substrate (vermiculite: polypropylene), molded to the following treatments in the blue mesh, which were replaced by black and white treatments, which constituted the following treatments. The design was completely randomized with three of six seeds each, totaling eighteen seeds for treatment. After 180 days, plants already defined, the levels of pigments and phenolic compounds were defined. The results of increased energy and protection of wave modulation settings by secondary shadow networks of energy reserves and photoinhibition protection from secondary photoinhibition. Plants are white *Talisia esculenta*, similar to more identical interpretation shading, such as starch grains and also higher chlorophyll contents. The construction mesh provides the best development of *Talisia esculenta* (A. St. Hil) Radlk plants.

Keywords: Chloroplasts. Starch grains. Vesicles with oil.

Resumo

A luz afeta diretamente os processos fisiológicos das plantas, devido às variações de comprimento de onda e intensidade, modificando a resposta das plantas às condições ambientais e afetando diretamente a produção de compostos metabólicos, bem como de pigmentos. Nesse contexto, este estudo tem como objetivo avaliar as alterações que ocorrem no processo de troca gasosa e na produção de metabólitos em mudas de *Talisia esculenta*, por meio do uso de telas de sombreamento coloridas. Foram utilizadas sementes de *T. Esculenta*, que foram semeadas em bandejas de polipropileno contendo substrato inerte (vermiculita), submetidas aos seguintes tratamentos: 50% de sombreamento na malha azul, vermelha, preta e branca, que constituíram os tratamentos. O delineamento foi inteiramente casualizado com três repetições de seis sementes cada, totalizando dezoito sementes para cada tratamento. Após 180 dias, plantas já desenvolvidas, foram determinados os teores de pigmentos fotossintéticos e compostos fenólicos. Os resultados demonstram que a modulação de comprimentos de onda por redes de sombreamento promove o aumento de compostos secundários de reservas de energia e proteção de fotoinibição em plantas de *T. Esculenta*. As plantas de *Talisia esculenta* submetidas ao sombreamento de coloração branca, apresentaram compostos secundários mais elevados, como grãos de amido, e também teores mais elevados de clorofila. A malha de coloração branca proporciona melhor desenvolvimento fisiológico das plantas de *Talisia esculenta* (A. St. Hil) Radlk.

Palavras-chave: Cloroplastos. Grãos de amido. Vesículas com óleo.

Introduction

Talisia esculenta (A. St. Hil) Radlk, popularly known, is the species of the Sapindaceae family, original from the Amazon region and is much consumed in the northeast of Brazil. It's a perennial plant with annual production of fruits that are consumed in natura or used in the production of by-products of the foodstuff (LORENZI, 2002). The species has a hemprotein class of lectins, termed talisina, which has the ability to agglutinate other proteins. This property is capable of causing damage to pathogens in the digestive tract of ruminants (RIET-CORRÊA et al., 2014), inhibiting growth and development of insects (MACEDO et al., 2011). But although their qualities, there is no availability of pitomba plants as occurs for other species, improper for the commercial use of this native fruit in terms of consumption of fruits or use their metabolites.

In relation to the formation of new plants, light is the main factor interfering in their development, because different irradiation conditions tend to change the plant metabolism (LÜNING 1990; STEFANO; ROSARIO, 2003). Due to these changes, anatomical, morphological and physiological characters are affected by light availability (LI and KUBOTA, 2009). In response to radiation, the leaf is the main organ affected with changes in tissue constituent, production of energy reserves and plant protection compounds such as phenolic compounds (BRANT et al., 2009; MACEDO et al., 2011; SOUZA et al., 2007; SOUZA et al., 2011).

Research has been developed with the use of color shading nets relating to changes in the spectrum of light that reaches the plant, and many species of popular or commercial use (COSTA et al., 2007; MELO et al., 2009; BRANT et al., 2009; COSTA et al., 2010; SOUZA et al., 2011; BASTÍAS et al., 2012). So, changes occurring at the wavelength or intensity of these lengths affect the photosynthetically active radiation used by the plants and the efficiency of photosynthetic processes (PIRES et al., 2011; NERY et al., 2011; OREN-SHAMIR, 2001). The wavelengths absorbed interfere in the assimilation of CO₂, which consequently alter the net rate of photosynthesis, NADPH and ATP production in the photochemical phase, and thus all subsequent process, has changes that affect the biomass accumulation (NASCIMENTO et al., 2014), accumulation and distribution of reserve compounds and protection for plants, such as the photosynthetic pigment content and concentration of polyphenols, as oleosomos. These adaptive responses to radiation vary according to the plant species in question (LÜNING 1990; LIMA JUNIOR et al., 2005; MARTINS et al., 2010).

The objective of this work was to study the process of gas exchange and the production of secondary metabolites in plant *Talisia esculenta* (A. St. Hil) Radlk by using color shading nets.

Materials and methods

Pitomba fruit (*Talisia esculenta*) were collected in urban orchards, in Cascavel, Northeast Region of Brazil. Pericarp and aryl were manually removed, which surround the seeds. These were treated in water and commercial sodium hypochlorite solution diluted in 2% 30% distilled water in a flask and maintained under constant stirring for 15 minutes. Then they were planted in an inert substrate (expanded vermiculite) in polypropylene trays, which were arranged in structures individually covered with mesh 50% shading (Chromatinet[®]) in white, blue, red and black, for a period of 180 days. According to the manufacturer, the blue mesh reduces wave in the range of red and far-red and adds blue waves, while the red mesh reduces blue, green and yellow waves add waves on the red and far-red bands. The white mesh, enables passage of light in a diffuse manner, whereas only the black

mesh reduces the intensity of radiation, it does not interfere in the transmitted spectrum. Irrigation was performed daily and the fertilization solution MS medium (MURASHIGE; SKOOG, 1962) without the addition of agar and sucrose. The geographical coordinates of the experimental area are 21 ° 14 'S, 45 ° W and 918 meters above sea level. The site climate is type Cwb. The maximum temperature, minimum temperature and photoperiod were varied during the experiment according to the climatic conditions of the experiment place. The average intensity of radiation was measured with USB-850 spectroradiometer Red Tie coupled to a source of electromagnetic radiation DT-MINI (200 2000 nm) with reflectance probe R400-7-VIS-NIR (US Bio Solutions Ocean Optics) inside the nets shown in figure 1.

Two analyzes were performed by gas exchange and transpiration at 120 and 180 days after emergence of the plants by means of gas exchange analyzer infrared (IRGA), LI-6400XT Liquor model. For this step, fully expanded leaves from the top were selected (the plants had only one node) in total of ten plants per treatment, and the photon flux density photosynthetically active fixed to the device camera in $1000 \text{ mol m}^{-2} \text{ s}^{-1}$, this analysis were during morning, at 8:30 to 10:00.

Analysis of the contents of chlorophyll *a*, *b* and total were quantified by Arnon methodology (1949), performed after 180 days after emergence of seedlings, being collected three sheets of each treatment. At the time of collection, the leaves were placed in foil and chilled on ice until being transferred to the laboratory. The reading wavelength was realized in a spectrophotometer BioSystems - BTS-330 model 663 nm to 645 nm for chlorophyll *a* and chlorophyll *b*.

The analysis of the concentration of total polyphenols was carried out using the Folin-Ciocalteu method, according to the methodology modified by Denis (ANGELO; JORGE, 2007). Then the readings at 760 nm in spectrophotometer BioSystems - BTS-330 model were taken. The calibration curve was prepared with pyrogalllic acid, with linearity of 1g/ 100mL, with constant 92.61. The analyzes were performed in triplicate. The blank was prepared the same manner by replacing the sample in distilled water.

Ultrastructural analyzes were performed with leaf samples of dimension 0.5 to 1 mm immersed in a glutaraldehyde solution (2.5%) and paraformaldehyde (2.5%) in cacodylate buffer pH 7.0 0.05M + 0.001M CaCl₂ for four hours at room temperature, washed three times in cacodylate buffer for 10 min to remove the glutaraldehyde residues that can reduce the osmium tetroxide, post-fixed in 1% osmium tetroxide in buffer 0.1 M cacodylate for 1 hour (maximum 4 hours) at room temperature in a chapel. Then washed twice for 15 min in distilled water, transferred into 0.5% solution of uranyl acetate for 12h at 4°C and washed again in distilled water and dehydrated in acetone gradient increasing in series (30, 50, 70 and 90%) remaining about 10 min each, and the end remained in 100% solution for 3 times for 10 min each. The inclusion of resin was in increasing gradient Spurr / acetone 30% (8h), 70% (12h) to 100% twice each for 24 h. At the end of this process, the material included in Spurr resin 100%, with the specimens mounted on a mold and placed in an oven to polymerize the 70 °C for 48 h. From blocks obtained semi-thin sections were performed (1µm) and ultrafine (<100nm) through Reichrt-Jung ultramicrotome. The ultrafine sections were collected in copper grids covered with formvar. Sections were post-contrasted in uranyl acetate followed by lead acetate, and examined in a Zeiss transmission electron microscopy - EM 109. In ultrastructural analysis, the amount of the chloroplast starch granules, 3 leaf fragments in each fotoconversora net, with 10 different areas in each fragment were assessed.

The design was completely randomized with three of six seeds each, totaling eighteen seeds for treatment. All data were subjected to analysis of variance (ANOVA) and submitted to the mean test Scott-Knott ($p \leq 0.05$).

Results and discussion

The plants of *Talisia esculenta* who grew up under the color shading nets, white, black, blue and red, had similar averages for photosynthesis rate data, internal CO₂ concentration, leaf transpiration and stomatal conductance (Table 1). The modulation of wavelengths, the radiation intercepted by plants, did not affect the process of photosynthesis. The intensity of wavelengths intercepted by the netting, was different for each one, peaking at 4095 (uWatt) (Figure 1) and decay of the points intensities were different. The photosynthetic process requires wavelengths belonging to red light and blue light, in equilibrium, and these lengths intercepted by the photosystems therefore more efficient (YAMASAKI, 2010). Possibly under the mesh, this balance sheet lengths were established for the plant *Talisia esculenta* (ROCHA et al., 2008; WHITELAM; HALLIDAY, 2007), unlike the results of studies of Kong et al., (2012), who found better photosynthetic rate for the *Capsicum annuum* species under the white mesh. These results show that the species may respond differently, achieving an adaptation of their photosynthetic apparatus.

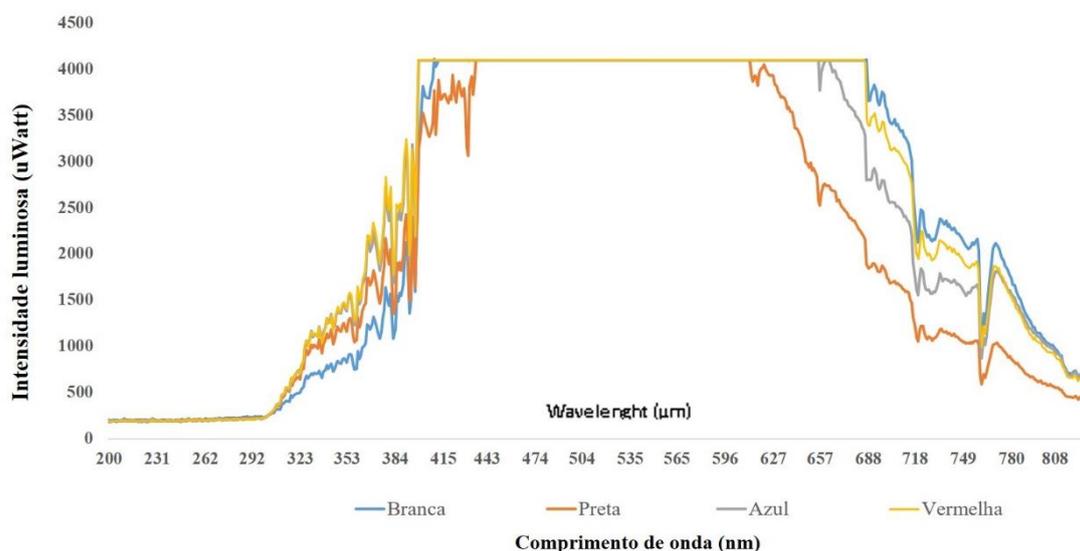


Figure 1 - Radiation intensity at the different wavelengths. *Radiation intensity at wavelengths in the fotoconversora nets.

Table 1 - Characteristics of gas exchange *Talisia esculenta* under shading nets.

| Shading nets | A | E | Ci | Gs |
|--------------|--|---|---------------------|--|
| | ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) | ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) | (μmol) | ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) |
| White | 7,907 a* | 5,032 a | 447,83 a | 5,576 a |
| Black | 7,238 a | 5,028 a | 472,65 a | 5,440 a |
| Blue | 7,285 a | 5,048 a | 568,46 a | 5,575 a |
| Red | 7,722 a | 5,032 a | 504,65 a | 5,559 a |
| CV% | 18,34 | 0,53 | 25,94 | 5,96 |

*Photosynthesis rate (A), leaf transpiration rate (E), CO₂ internal concentration (Ci) CO₂ $\mu\text{mol m}^{-2} \text{ s}^{-1}$ and stomatal conductance (Gs). Means followed by the same letter in the column do not differ by the Scott-Knott test at 5% significance level.

The process of gas exchange is influenced by several factors besides radiation, such as water availability, which partly controls the opening and stomatal closure, important to the assimilation of CO₂ (LAWSON et al., 2010). In this work, the water availability was maintained in equal conditions to all plants, as well as temperature and humidity conditions, so the similarity in the results of gas exchange may be due to the constancy of these conditions, similar to results found in studies of Nery et al. (2011) with *Talisia subalbans* (Mart.) Radlk. *Talisia esculenta* commonly occurs in regions with high irradiation, however in shade conditions, as in this work, the seeds germinated and developed vegetative parts, thus being, able to occupy regions with lower intensity of radiation, such as understory, without damage to photosynthetic process because of the attenuated radiation.

Radiation changes in growing environment such as *T. esculenta* exposure to different colored nets can provide the photosynthetic apparatus settings, including modifications to the content of photosynthetic pigments (ENGEL; POGGIANI, 1991; TAIZ; ZEIGER, 2009). The quantification of these pigments, it is a means of checking the plant modifications. In this study, *T. esculenta* plants under white mesh showed higher content of chlorophyll *a*, chlorophyll *b* and total. Thus the diffuse light favored the development of higher amount of pigment, while a lower content of these pigments in plants occurred in blue mesh. The absorption of Photosynthetically Active Radiation (PAR) is increased in a condition of higher pigment content and more light energy enters the photosynthetic system (TAIZ; ZEIGER, 2009). Thus, to protect this system, chlorophyll *b*, taken as an accessory, absorbs light at longer lengths (REGO; POSSAMAR, 2006), one way of adapting the plants to the shaded environment. This fact was observed by *T. esculenta*, in which the high content of chlorophyll *b* occurred in production of greater chlorophyll environment (Figure 2). Under blue mesh, should be greater production of photosynthetic pigments due to regulation by phytochrome activated by blue light (MELO et al., 2009; SOUZA et al., 2011). For this work, under the blue shading had lower production of both chlorophylls, possibly intensifying the blue light lengths causing a negative response of production (LARCHER, 2004).

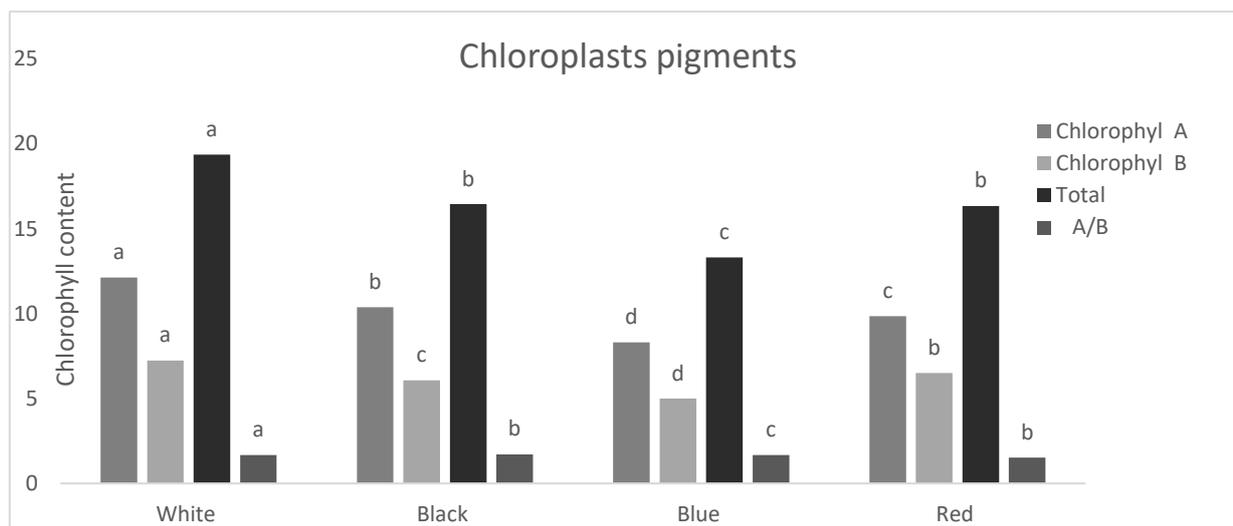


Figure 2 - The chlorophyll *a* content, chlorophyll *b*, and total chlorophyll ratio *a* / *b* in leaves of *Talisia esculenta* under shading nets. *Average amount of chlorophyll content. Means with the same letter on the line do not differ by the Scott-Knott test at 5 % significance level.

Some compounds in the leaves exert photoprotective function, for polyphenolic example that act as antioxidants. The leaves of plants under the red and blue loops were the most produced phenolic compounds with very similar values, while the leaves of plants grown under black net had the lowest

content of these compounds (Table 2). The presence and amount of phenolic compounds in plants is mainly influenced by light (GOBBO NETO; LOPES, 2007), precisely because the phenolics act as antioxidants in non-enzymatic protection against damage by excessive radiation (DAVEY et al., 2000).

Table 2 - Concentration of phenolic compounds for leaves of *Talisia esculenta* under different shadings.

| Concentration of phenolic | [mg/mL] |
|---------------------------|------------|
| White | 893,188 b* |
| Black | 781,950 c |
| Blue | 925,360 a |
| Red | 940,167 a |
| CV% | 1,40 |

*Means with the same letter in the column do not differ by the Scott-Knott test at 5% significance level.

Such compounds protect leaves against formation of free radicals, which can also be generated by the intense light according to Ma et al. (2010). It can be inferred that the transmission of light at wavelengths related to these meshes, while not altering the process of gas exchange, the wavelengths for the blue and far-red were intensified to generate damage to photosynthetic pigments and other cellular components that suffer photoinhibition, evidenced by the large production of phenolic compounds of these plants.

In addition, the production of phenolic compounds, the light interferes in the production of starch grains and chloroplasts, and it is possible to quantify them by electron microscopy. The chloroplasts were observed near the cell wall palisade parenchyma, or cells of spongy parenchyma (Figure 3). In studies of Costa et al. (2007) differences were observed between treatments with mesh in the area of starch grains *Ocimum selloi*. The diffuse light provided by the white net can cause a lower effect on amiloplastídeos and the starch grains compared to other, though here in the white mesh provided greater amount of chloroplasts and starch grains. The white net attenuates the light incidence, being considered the lowest temperature that provides the plants, however, it is unknown the relationship of this variable in the formation of reserve compounds, as observed in the work (SILVA et al., 2014). Starch grains result of higher carbon accumulation (TAIZ; ZEIGER, 2009), but for *T. esculenta* no significant differences between treatments, agreeing with Brant et al. (2009) that the plant behavior under color shading nets is different for each species.

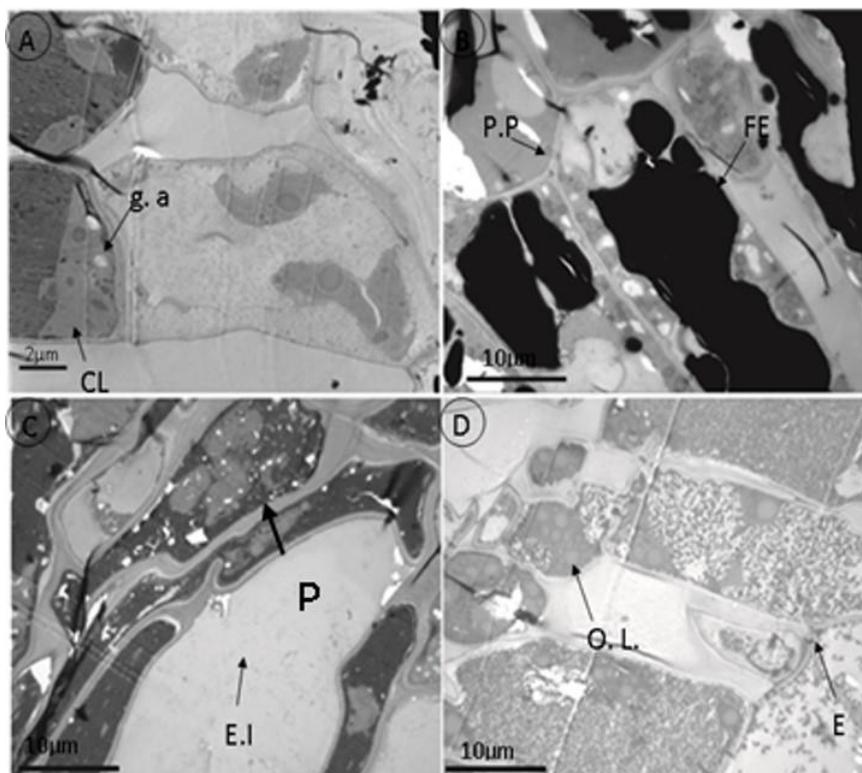


Figure 3 - Electromicrography transmission (A) white mesh, (B) black, (C) blue, (d) red, GA- grain starch, E-epiderme, PP - palisade parenchyma, FE - phenolic compound, OL- oil on vesicles, and CL - chloroplast, EI -intercellular space, Pa- cell after pathogen attack in *Talisia esculenta*.

The chloroplasts biosynthesis responds to the variation of the light spectrum (JIAO et al., 2007; RUCKLE et al., 2007). For plants under the red and blue nets decreased by 70% chloroplasts and starch grains (Figure 4). It was observed that under red and blue mesh plants produced more phenolic compounds, probably part of the energy for the production of reserves was directed to the production of phenolic compounds which act as sunscreens. In production of phenols, was found large number of vesicles with oil for all the nets (Figure 4). Plants under black net had higher amount of this reserve compared to the others. The greater attenuation of the radiation provided by the black net revealed influence the production of starch grains, decreasing it. The presence of phenolic compounds in large vacuole was observed as well as vesicle with oil (Figure 4). According to Lersten et al. (2006) it is common to find in vesicle with oil angiosperm leaves and lipid substances in trichomes. However, there are no studies assessing the presence of these compounds in sapindaceas leaves. In ultrastructural analysis visualized contamination of leaves of plants grown in blue nets by pathogens, thus preventing a quantitative analysis of starch grains (Figure 4), in addition, many cells under blue sheet net, are presented broken with cloroplastídeos present in small amounts.

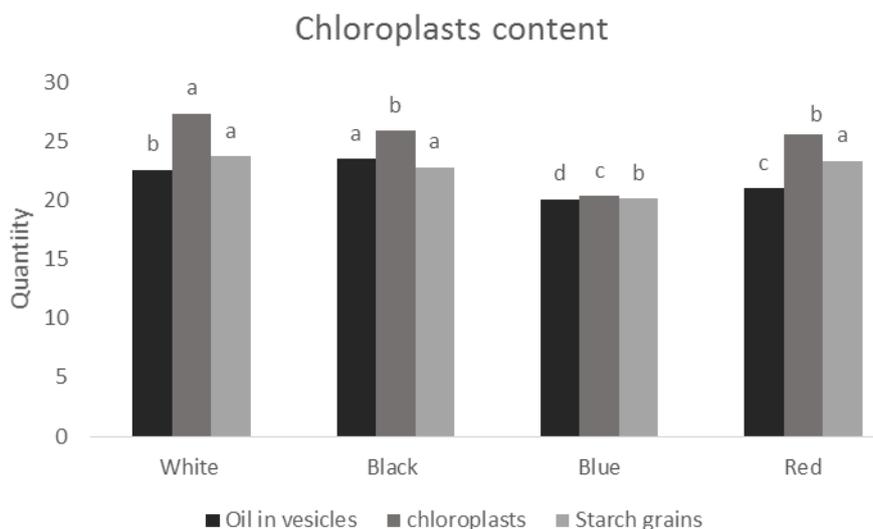


Figure 4 - Chloroplasts content and energy compounds stored in *Talisia esculenta* leaves under shading nets. *Average number of chloroplasts, oil in vesicles and starch grains. Means with the same letter do not differ by the Scott- Knott test at 5 % significance level.

Conclusion

The plants of *Talisia esculenta* submitted to white shading showed higher secondary compounds, such as starch grains, and also higher levels of chlorophyll.

The white colored mesh provides better physiological development of *Talisia esculenta* (A. St. Hil) Radlk plants.

Interest conflicts

There was no conflict of interest of the authors.

Responsible authorship statement

The authors declare that they all contributed equally to the preparation of this article.

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