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Using Vegetation Spectral Indices from UV-VIS-NIR Spectroscopy to Evaluate Okra Plant Growing under Different Artificial LED Light Source

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Abstract

We use several spectral vegetation indices obtained from UV-VIS-NIR spectroscopy to non-destructively evaluate chlorophyll, anthocyanin and flavonoid content in okra plants irradiated with 3 different artificial light spectra in the blue, green and red regions of the electromagnetic spectrum; thus leading us to assess the effects of specific wavelength on the plants' biochemical compounds and physiological state. The results show that blue light gives the highest anthocyanin and chlorophyll content, whereas the highest flavonoid content is found under red light. Therefore, these biochemical compounds with a well-known impact on human health, may be adjusted by selecting specific wavelengths to improve the quality of plants.

Keywords

UV-VIS-NIR Spectroscopy, Horticulture, Light Spectrum, LED, Vegetation Spectral Indices, Biochemical, Okra

1. Introduction

Spectroscopy techniques have rapidly developed in recent years and are now well-known and well-proven techniques in the field of analyzing plant nutrients, soil properties and biochemical compounds [1]. Besides, growing plants under controlled

environments with artificial light sources have been of great interest in recent years. It allows the control of the climate environment of plants, overcomes the negative effects of climate change and reduces food insecurity in many regions of the world [2]-[6]. The Okra plant, known in several regions of the world, and used both in food and traditional medicine makes it a plant of great interest [7]. It is therefore important to carry out research activities on improving the quality and production of okra plants using artificial lighting in a controlled environment and above all, to be able to evaluate the impact of different types of light sources on the okra plants; to finally allow producers to make appropriate choice on their lighting systems. The evaluation of plants during the growth process is carried out preferably with non-destructive methods such as UV-VIS-NIR spectroscopy, which uses spectral vegetation indices at the agricultural level to evaluate certain physiological processes of the plant. Here, our objective, over a period of 60 days of okra growth, is to evaluate with spectral vegetation indices, the effect of different sources of artificial lighting using LEDs irradiating respectively in the blue, red and green regions of the electromagnetic spectrum on the chlorophyll, anthocyanin, flavonoid content.

2. Material and Methods

2.1. General Experimental Design

The experiment was carried out in mounted wood boxes covered with black plastic to inhibit daylight. The light treatment was obtained with locally purchased LED spots. One light treatment was used within each box resulting in 3 boxes for the blue, red and green light treatment. Table 1 illustrates the different light treatment characteristics.

Table 1. Light treatment characteristics: PW = Peak Wavelength, FWHM = Full Width at Half Maximum, PPFD = Photosynthetic Photon Flux Density, DLI = Daily Light Integral.

	Region of emission	PW	FWHM	PPFD	Light/Dark photoperiod	DLI
Blue treatment	Blue	455.45 ± 1.80 (n = 3)	33.35 ± 2.36 (n = 3)	200 $\mu mol \cdot m^{-2} \cdot s^{-1}$	18 h/6h	12.96 $mol \cdot m^{-2} \cdot d^{-1}$
Green treatment	green	522.27 ± 1.46 (n = 3)	39.10 ± 0.55 (n = 3)	200 $\mu mol \cdot m^{-2} \cdot s^{-1}$	18 h/6h	12.96 $mol \cdot m^{-2} \cdot d^{-1}$
Red treatment	red	635.03 ± 1.33 (n = 3)	22.11 ± 0.47 (n = 3)	200 $\mu mol \cdot m^{-2} \cdot s^{-1}$	18 h/6h	12.96 $mol \cdot m^{-2} \cdot d^{-1}$

Inside each box, we have 3 okra crops growing under the corresponding light treatment resulting in 3 replicates per light treatment; this gives a total of 9 okra crops for the 3 light treatments. Seeds of Okra from the species Abelmoschus esculentus locally available, were collected from the Center of National Agricultural Research of Côte d'Ivoire (CNRA) [8]. And the same soil with the following characteristics was used for all crops: Na^+, 0.2 ± 0.06 $cmol \cdot kg^{-1}$; Ca^{2+}, 2.1 ± 0.14 $cmol \cdot kg^{-1}$; K^+, 2.5 ± 0.14 $cmol \cdot kg^{-1}$; Mg^{2+}, 1.0 ± 0.09 $cmol \cdot kg^{-1}$; CEC, 17 ± 2 $cmol \cdot kg^{-1}$; assimilable Phosphorus, 69.9 ± 2.57 ppm; nitrogen, 0.3% ± 0.3%; carbon, 2.9% ± 0.15%; soil PH, 7.0 ± 0.06. These properties show a fertile soil rich in

organic matter with a correct ph that guarantees an optimal absorption of nutrients [7] [9]-[11].

Materials used during this experiment are listed in Table 2 with their different function. These experiments were carried out with an average temperature of 27.96˚C ± 0.59˚C and an average humidity of 75.41% ± 4.22% in the room. The temperature for better growth and normal development of okra is between 20˚C and 30˚C [12]. According to Lamont (1999) [7], temperatures above 20˚C are necessary for normal okra development; but it is unable to tolerate temperatures below 15˚C and above 42˚C.

Table 2. Material and equipment used for experimentation.

Materials	Function
Seeds of Okra, *Abelmoschus esculentus*	Collected from Center of National Agricultural Research (CNRA Côte d'Ivoire).
Ocean Optics USB4000	Optical characterization of light sources
Lightscout 3415 FXSE	PPFD over the plants canopy measurements and adjustments
Programmable electrical socket	Photoperiod and DLI adjustment
AcuRite Model 01036	Temperature and humidity measurements

The process to evaluate the different vegetation spectral indices obtained from Okra crop growing under different light treatments is described in Figure 1.

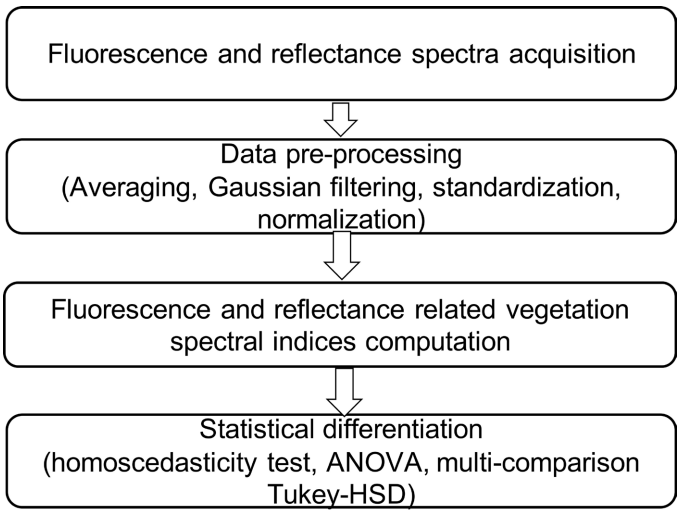

Figure 1. Vegetation spectral indices evaluation process.

2.2. Spectra Acquisition

Acquisition material was based on the excitation of leaf samples using different LEDs in specific wavelengths; and resulting light was collected with an optical spectrometer Ocean optics USB4000 coupled with his embedded software SPECTRA SUITE to acquire, collect and store the different spectra. Figure 2 illustrates

this material already well described in [13]-[15]. Note that the excitation Leds were driven by a ATMEGA328 based microcontroller. For chlorophyll excitation, three Leds were used: green (peak = 519.9 nm, fwhm = 37.438); UV (peak = 380.4 nm, fwhm = 17.195) and red (peak = 632.2 nm, fwhm = 20.88). The white LED was used for reflectance measurements, calibrated with a 99% Edmunds Optics reflectance standard. Table 3 gives a description of the acquisition parameters and used equipment.

Table 3. Material and equipment used for experimentation.

Spectrometer	USB4000 from Ocean Optics
Acquisition software	SpectraSuite installed on MS Windows OS
Fluorescence filter	650 nm red long-pass filter to block excitation light
Reflectance standard reference	99% white reflectance standard Edmund Optics
Wavelength range for recorded spectra	345 nm - 1042 nm
Sampling interval	0.2 nm (result in 3134 values for each spectrum)
Reflectance acquisition period	100 ms
Fluorescence acquisition period	3 s
Number of scans for one measurement	3 scans (averaged)
Frequency of acquisition session	4 days-interval
Duration of acquisition campaign	60 days
Total acquisition sessions	15

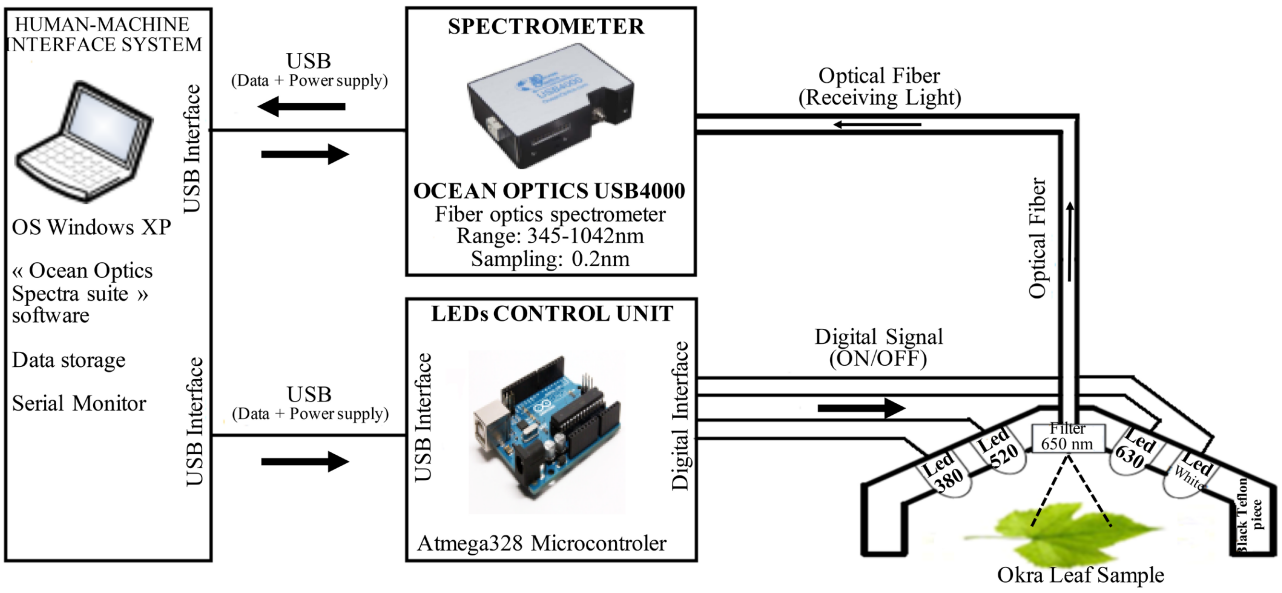

Figure 2. Acquisition system.

2.3. Preprocessing of Acquired Spectra

Unnecessary wavelengths have been removed to keep the fluorescence spectrum

from 672 nm to 810 nm; because the well-known peaks of chlorophyll fluorescence are in the red (680 to 690 nm) and in the far-red (720 to 755 nm) [16]. Regarding reflectance spectra, wavelengths from 400 nm to 1000 nm, useful for spectral vegetation indices were kept.

Furthermore, the application of spectral preprocessing techniques to extract relevant and useful information from recorded spectra is necessary and essential; because it makes it possible to eliminate undesirable effects linked to several factors such as the noise of measuring devices, harmonics in the near infrared, and ambient effects. Therefore, we used the following techniques:

The average of several scans (3 *scans*) *is used to reduce fluctuations and noise linked to acquisition manipulations and the light excitation source while also minimizing* the effects of thermal noise.

Forward-backward Gaussian filter: to reduce noise in the spectral data.

Standard Normal Variate technique: to normalize each recorded spectrum.

Min-max normalization: to resize our spectrum with a Y-max of 1 and Ymin of 0.

These preprocessing operations were carried out using scripts developed on Matlab Software.

2.4. Vegetation Spectral Indices Computation

A developed MATLAB program was used for the calculation of the reflectance and fluorescence indices. For each phase, we extracted, from each spectral curve, 109 wavelength parameters, reflectance and fluorescence indices. 5 indices were determined to be the most pertinent for this study and were selected to be examined in greater detail. These 5 reflectance and fluorescence indices were related to plant physiology and allowed us to evaluate the flavonoid content, anthocyanin content, chlorophyll content and plant stress/senescence among the different light treatments. Table 4 gives a description of each spectral index with its computation formula and the linked references for further clarification.

Table 4. List of fluorescence and reflectance vegetation indices used in this study. FRF_U: far-red Fluorescence emission excited by UV light; FRF_R: far-red fluorescence emission excited by red light; FRF_G: far-red fluorescence emission excited by green light; R_λ: reflectance at wavelength λ.

Spectral indices	Formula	Link with	References
FLAV_735	FRF_U(735)/FRF_R(735)	Flavonoid content	[17] [18]
ANTH_730	Log(FRF_R(730)/FRF_G(730))	Anthocyanin content	[19]
CARTER4	R_{710}/R_{760}	Stress	[20]
mNDVI	$(R_{800} - R_{680})/(R_{800} + R_{680} - 2R_{445})$	Chlorophyll content	[21]
PSRI	$(R_{680} - R_{500})/R_{750}$	Senescence/carotenoid content	[22]

2.5. Statistical Analysis

Excel, R, Tanagra and Matlab software tools were used to carry out statistical analysis. First, a homoscedasticity test Brown-Forsythe has been used to verify

variance homogeneity among acquired data for each spectral index. Then, One-Way ANOVA was used to differentiate each light treatment for each spectral index. Finally, a turkey-HSD post hoc test allows us to compare differences among light treatments.

3. Results and Discussions

Fluorescence and reflectance spectra

Normalized fluorescence spectra during the full period of growth are presented below in Figures 3-6.

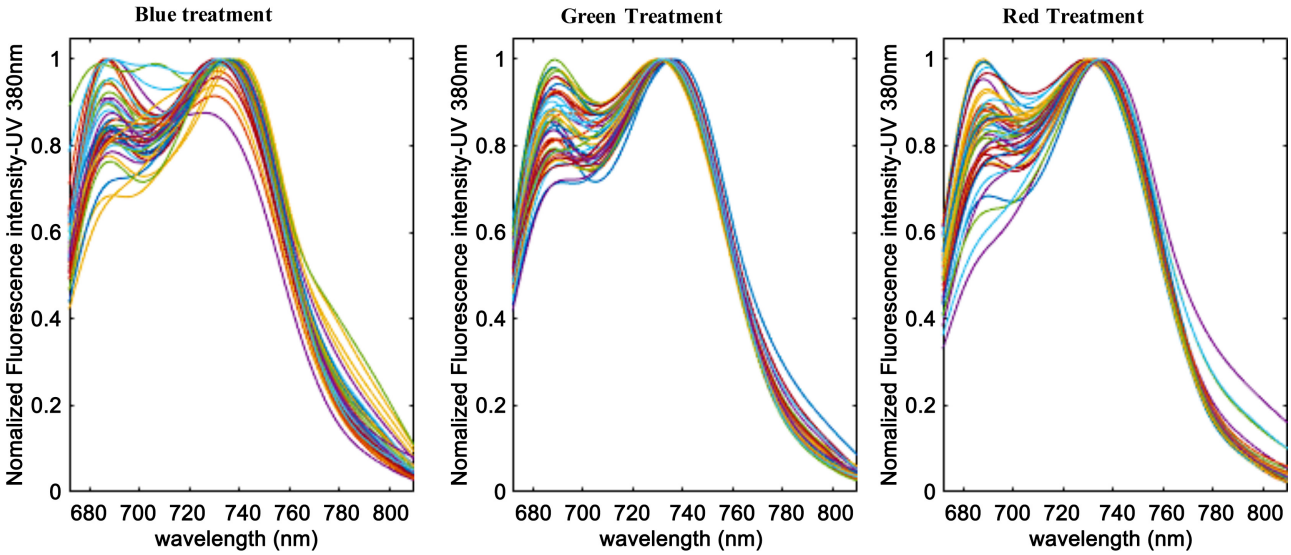

Figure 3. Normalized fluorescence spectra resulting from UV 380 nm LED excitation are presented per light treatment: total of 45 spectra per light treatment during 60 days of growth period.

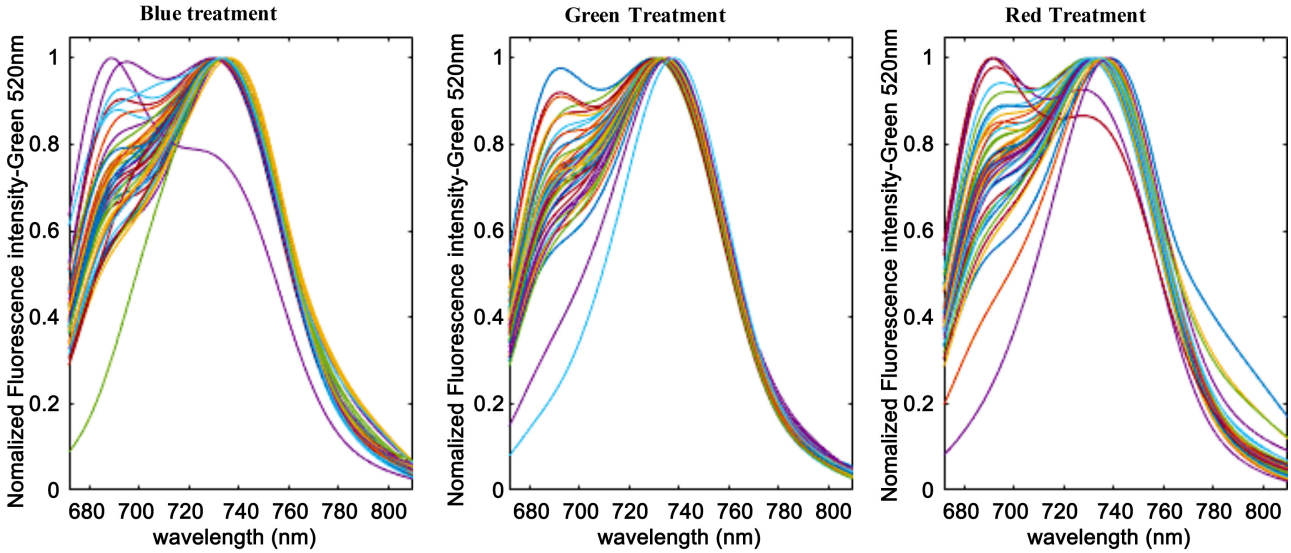

Figure 4. Normalized fluorescence spectra resulting from green 520 nm LED excitation are presented per light treatment: total of 45 spectra per light treatment during 60 days of growth period.

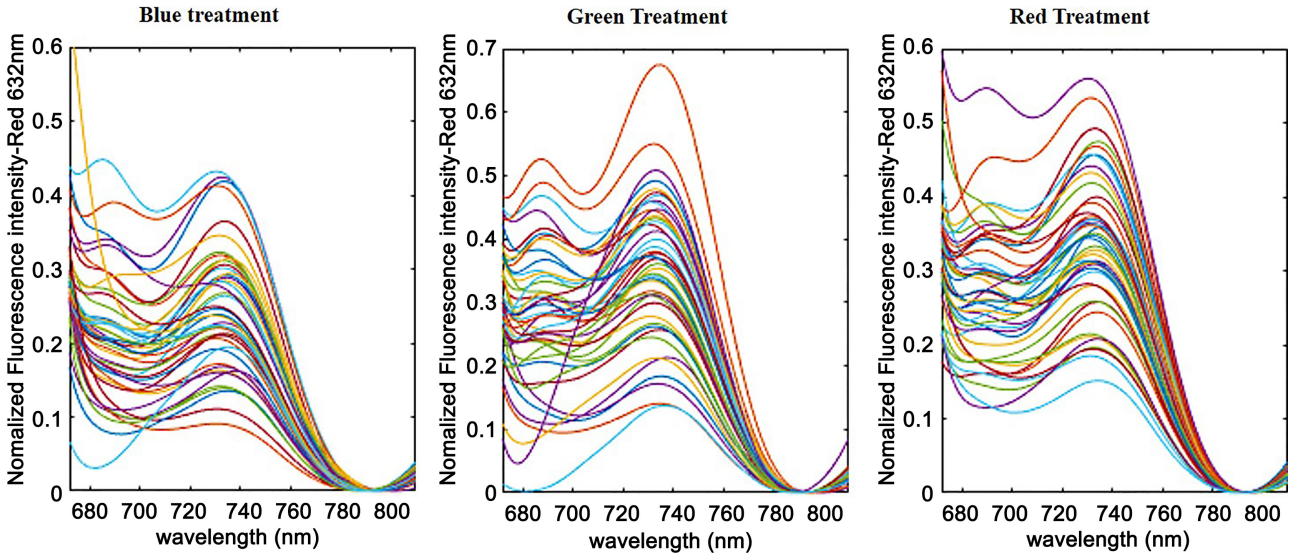

Figure 5. Normalized fluorescence spectra resulting from red 632 nm LED excitation are presented per light treatment: total of 45 spectra per light treatment during 60 days of growth period.

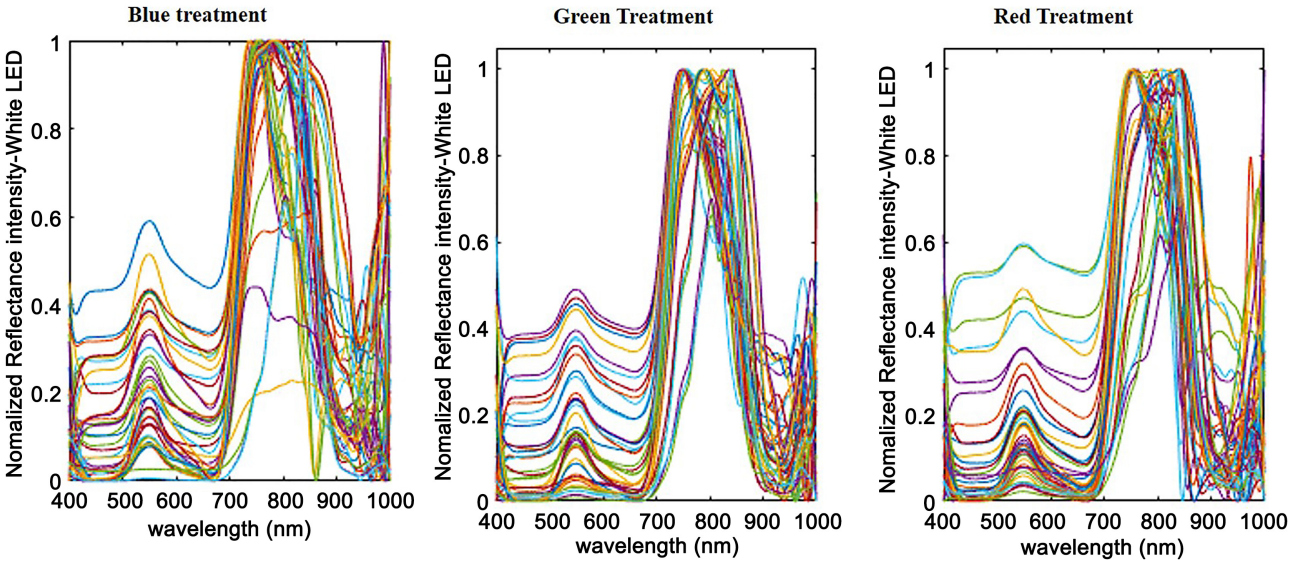

Figure 6. Normalized reflectance spectra resulting from white (400 - 700 nm) LED excitation are presented per light treatment: total of 45 spectra per light treatment during 60 days of growth period.

The results of the study over the 60-day growth period are summarized in Table 5 and illustrated in Figure 7.

ANTH_730 index related to anthocyanin content: the anthocyanin content of plants treated under blue light is significantly different from those treated with red light. The highest anthocyanin content is obtained with blue; and the lowest content is obtained with red. Green light is not significantly different from blue and red for anthocyanin content. Anthocyanins have anti-cancer, neuroprotective, anti-inflammatory, antimicrobial and antiviral properties; furthermore they

play a significant role in protecting plants against external environmental threats. In this study, plants grown with blue light show the highest anthocyanin levels. Results obtained by references [23]-[25] on respectively tomatoes, red leaf lettuce and lettuce have also revealed that blue light treatment increases anthocyanin concentration in plant leaves. In fact, the presence of cryptochrome in plants, and more specifically Cry1 which is involved in the control of anthocyanin accumulation may explain this result; as cryptochromes are photoreceptors sensitive to blue light.

Table 5. Mean (±SE) of spectral indices measured on okra leaves irradiated under different light spectrum treatments during phase 1.

Light spectrum		Spectral indices				
		FLAV_735	***ANTH_730***	***Carter4***	***mNDVI***	***PSRI***
BLUE		1.07 ± 0.04**b**	−0.05 ± 0.03**a**	0.45 ± 0.02**b**	0.97 ± 0**b**	0.01 ± 0**a**
GREEN		1.01 ± 0**ab**	−0.01 ± 0**ab**	0.39 ± 0.02**a**	0.95 ± 0**a**	0.02 ± 0**b**
RED		1 ± 0**a**	0.01 ± 0**b**	0.4 ± 0.02**ab**	0.96 ± 0**ab**	0.02 ± 0**b**
Brown-Forsythe test	W	3.268	2.8227	0.9284	2.1594	0.1587
	P	0.0412	0.063	0.3977	0.1194	0.8534
ANOVA test	F(2; 6)	2.6739	4.2658	2.5289	5.1421	3.0075
	P	0.0727	0.016	0.0836	0.007	0.0528

For each parameter, different letters indicate significant differences among treatments at $p < 0.05$ and $p < 0.1$ by Tukey's HSD test. (W: Statistics of the Brown-Forsythe Test; P: probability).

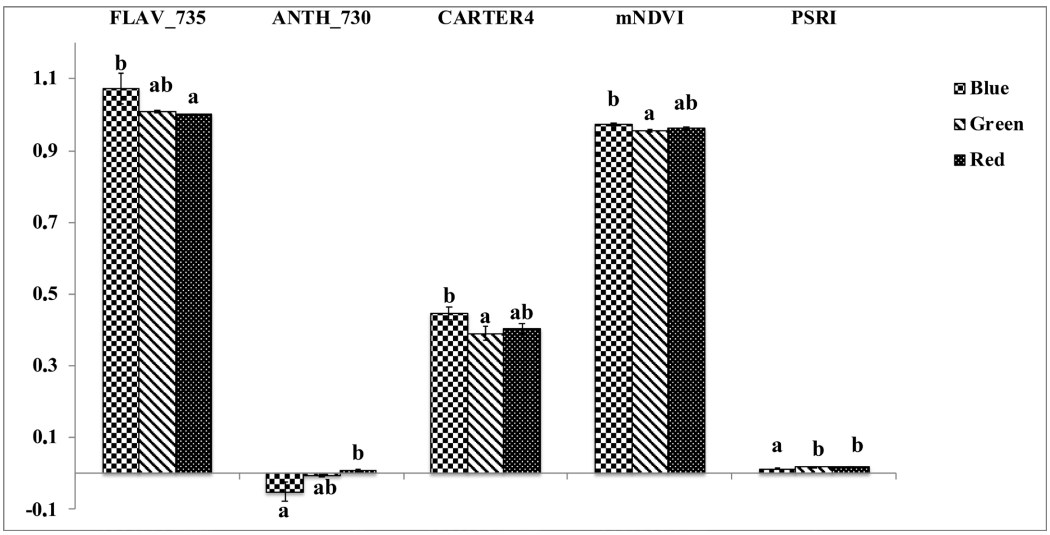

Figure 7. Spectral indices calculated for okra, *Abelmoschus Esculentus* plants grown under different light treatments. Different letters indicate significant differences among treatment at $p < 0.05$ and $p < 0.1$ by Tukey's HSD test. The bars represent standard errors.

FLAV_735 index related to flavonoid content: the lowest flavonoid contents are obtained under blue light, and the highest, under red. Red and blue are significantly

different, while green light gives flavonoid content similar to red and blue. Flavonoids have significant antioxidant activity and help prevent neurodegradation, cancer and heart disease [26]. The data from our experiment, which was analyzed globally over the 60 days of the growth period, demonstrated that red light has the highest flavonoid content.

mNDVI index related to chlorophyll content: mNDVI analysis shows that blue light results in higher chlorophyll content than green light in okra leaves. Chlorophyll levels in plants treated with green and red are not significantly different. Throughout the entire growth period, the highest chlorophyll content is obtained with plants irradiated under blue light. This result is confirmed by studies carried out by Ouzounis *et al.* (2014) [27] and Snowden *et al.* (2016) [28] on species like Phalaenopsis "Vivien" et "Purple Star", tomatoes, pepper, cucumber and radish; in their studies, Blue light increase significantly chlorophyll content and this turns out to be beneficial for human health because chlorophyll has healthy functions such as antimutagenic, antioxidant and anticancer.

CARTER4 index related to stress: The CARTER4 stress index tells us that plants grown under blue light are significantly different from those grown with the other two treatments; blue gives more stressed plants than green and red. On the other hand, red and green are not significantly different. According to Seigler (1998) [29], plants react to various stresses by raising their levels of anthocyanin. And this could explain why plants growing under blue light which contains the highest level of anthocyanin are the most stressed in this study. Indeed, results show that blue light gives the highest level of stress; however the type of stress is not specified because the stress indicators used here are linked to several stress agents such as dehydration, herbicides, pathogens, ozone, mycorrhizae and competition.

PSRI index related to senescence: The analysis of the PSRI senescence index (carotenoid/chlorophyll ratio) shows that plants under blue light irradiation show a low progression of senescence compared to plants grown under red and green light.

4. Conclusion

The results observed during this study, using vegetation spectral indices, confirm that artificial lighting in a controlled environment makes it possible to act on biochemical compound contents in plants. Also this study made it possible to show the influence of different light spectra on the physiological and metabolic processes and, furthermore, identify the optimal light spectrum for the regulation of these processes inside okra crops; thus leading to the production of vegetables with high nutritional qualities for human health. Ultimately, this study guides us toward a better understanding of the light spectrum on plants and supports vegetable producers in the choice of lighting systems according to their needs.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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