

***In vitro* growth of *Sclerotium rolfsii* in response to the light quality of three types of fluorescent lamps**

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Abstract

The investigation was carried out to know the influence of the quality of light on the radial growth of the mycelium, production of sclerotia and mycelium biomass of *Sclerotium rolfsii* cultivated *in vitro*. The treatments consisted of cold, neutral and warm white light, emitted by fluorescent lamps, with a photosynthetic photon flux density (400 to 700 nm) of $\approx 300 \mu\text{mol m}^{-2} \text{s}^{-1}$, which were established under a completely randomly with 10 repetitions. The light spectrum emitted by fluorescent lamps influenced the growth of the fungus *in vitro*. Cold white light caused a reduction in radial growth, biomass and sclerotium production of *Sclerotium rolfsii* due to the higher spectral content of blue light (400-500 nm).

Keywords: *Sclerotium rolfsii*, biomass, light spectrum, mycelium, sclerotia.

Reception date: January 2021

Acceptance date: February 2021

Light is a fundamental source of energy and a signal of the surrounding environment in the life of fungi, which they use in the regulation and direction of growth (phototropism), asexual and sexual reproduction, and pigment production (Idnurm and Heitman, 2005). Fungi can respond to a wide range of wavelengths of light, from ultraviolet to far red (Purschwitz *et al.*, 2006; Fuller *et al.*, 2015; Dasgupta *et al.*, 2016). In addition, it has been found that infection by a range of pathogens can be affected by the host's light environment prior to inoculation (Meijer and Leuchtman, 2000; Koh *et al.*, 2003). There is evidence that the release of spores by these organisms is influenced by light in ecosystems (Su *et al.*, 2000).

Light can also directly inhibit spore germination or germ tube growth in various plant pathogenic fungi (Mueller and Buck, 2003; Beyer *et al.*, 2004). In this regard, plastic films that transmit more blue light are effective in suppressing the sporulation of mildew and *Botrytis cinerea* (Reuveni and Raviv, 1997). The objective of this research work was to determine the effect caused by the quality of light emitted by fluorescent lamps of cold white light (LBF), neutral white light (LBN) and warm white light (LBC) on the radial growth of the mycelium, biomass and production of sclerotia from *Sclerotium rolsii* grown *in vitro*.

The research was carried out in the Laboratory of Physiology and Plant Anatomy of the Faculty of Agronomy, Autonomous University of Sinaloa, in Culiacán, Sinaloa, Mexico, located at coordinates 24° 37' 29" north latitude and 107° 26' 36" longitude west, with an altitude of 38.5 meters above sea level. Growth chambers measuring 44 x 70 x 80 cm (length, width and height, respectively) were used, with walls of mesh woven with 16 x 16 crystalline monofilaments of high-density polyethylene per cm², sides covered with high-reflectance Mylar paper and lighting system with compact fluorescent lamps, spiral type (FLE23HLX, General Electric, USA) with cold white (LBF: 6500 K), neutral (LBN: 4000 K) and warm (LBC: 2700 K) and with a photoperiod 12 h (Figure 1).



Figure 1. *In vitro* culture of *Sclerotium rolsii* in a growth chamber with fluorescent lamps of cold white (left), neutral (center) and warm (right) light.

Photon flux spectral measurements were made, in the range 350 to 1050 nm at 1 nm intervals, with a spectroradiometer (Field SpecPro[®]VNIR, ASD Inc., USA). From the data obtained in said measurements, the absolute amounts of blue light (A: 400 to 500 nm), red light (R: 600 to 700 nm)

and far red light (RL: 700 to 800 nm) were determined; as well as, the proportional amounts of blue to red light (A: R), blue to far red (A: RL), red to blue (R: A) and red to far red (R: RL), in each camera of growth (Table 1).

Table 1. Spectral characteristics of the light emitted by cold white (LBF), neutral (LBN) and warm white (LBC) fluorescent lamps.

Parameter/lamp type	LBF	LBN	LBC
A (400-500 nm) ^x	84.19	64.87	44.24
R (600-700 nm) ^x	81.34	100.44	118.03
RL (700-800 nm) ^x	31.79	33.93	36.28
A: R (400-500/600-700 nm) ^y	1.04	0.65	0.37
A: RL (400-500/700-800 nm) ^y	2.65	1.91	1.22
R: A (600-700/400-500 nm) ^y	0.97	1.55	2.67
R: RL (600-700/700-800 nm) ^y	2.56	2.96	3.25

A= blue light; R= red light; RL= far red light; ^x=absolute quantities ($\mu\text{mol m}^{-2} \text{s}^{-1}$); ^y= proportional (dimensionless).

The temperatures recorded with data-logger thermohygrometers (CM-DT171, Twilight, Mexico), averaged 24.7, 24.6 and 24.2 °C in the LBF, LBN and LBC chambers, respectively. The *Sclerotium rolfsii* pathogen was obtained from the collection of phytopathogenic fungi of the Faculty of Agronomy of the Autonomous University of Sinaloa. The sowing and purification of the pathogen was carried out in potato dextrose agar (PDA) medium, at a temperature of 28 °C.

A completely randomized experimental design was used with three treatments (LBF, LBN and LBC) and ten repetitions (one Petri dish per repetition). The response variables evaluated were: radial growth of the fungus in PDA medium, which was measured every 8 h with a digital vernier (6MP, Truper Tools, Mexico), sclerotia production, which was obtained by visually counting the sclerotia in each Petri dish, after 10 days of exposure to light; as well as the biomass production of the mycelium, obtained after separating the mycelium from the culture medium, later they were placed on filter paper, to be deposited in the oven (FE293AD, Felisa, Mexico) at 70 °C drying until constant dry weight, determined with analytical balance (SA120, Scientech, USA). The data obtained were subjected to the analysis of variance, comparison of means with the Tukey test (5%) and simple linear regression analysis, using the statistical package Minitab 16CA version 7.0.

The quality of light emitted by fluorescent lamps caused significant effects ($p \leq 0.05$) on the radial growth of *Sclerotium rolfsii*. Thus, after 8 h of exposure to light (HEXL), the growth of the fungus, under the influence of LBN and LBC, exceeded the mentioned growth of the fungus that received LBF by the respective 9.01 and 6.56% (Table 2). Similar effects were found at 16 and 24 HEXL, in such a way that said growth of the fungus increased 14.02 and 7.48%, as well as 13.87 and 19.34%, respectively. At the end of 32 HEXL the increases were 11.69 and 19.08, at 40 HEXL at 13.12 and 18.32, at 48 HEXL at 13.03 and 29.06, at 56 HEXL at 14.02 and 32.34 and at 64 HEXL at 16.32 and 28.27%.

Table 2. Influence of the quality of light emitted by fluorescent lamps with cold white (LBF), neutral (LBN) and warm (LBC) light on the radial growth of *Sclerotium rolfssii*.

Treatments	HEXL							
	8	16	24	32	40	48	56	64
	Radial growth (mm)							
LBF	12.2 b*	21.4 b	27.4 b	32.5 b	44.2 b	49.9 c	53.5 c	61.9 c
LBN	13.3 a	24.4 a	31.2 a	36.3 a	50 a	56.4 b	61 b	72 b
LBC	13 ab	23 ab	32.7 a	38.7 a	52.3 a	64.4 a	70.8 a	79.4 a

HEXL= hours of exposure to light; * = averages with different letters in each column are significantly different (Tukey, $\alpha \leq 0.05$).

These results can be understood by considering that there is scientific evidence that describes how the quality of light in terms of wavelength plays an important role in the growth and reproduction of fungi. Blue light directly inhibits spore germination or germ tube growth in many plant pathogenic fungi (Mueller and Buck, 2003; Beyer *et al.*, 2004). Also, Yu *et al.* (2013) found that blue light affected the germination of *Oidium* conidia, but that this quality of light is essential to maintain the virulence of the pathogen. Furthermore, blue light irradiation inhibited the growth of the mycelium of *Aspergillus carbonarius* and *Aspergillus westerdijkiae* (Cheong *et al.*, 2016).

The sclerotia production of *S. rolfssii* also presented significant differences ($p \leq 0.05$) due to the quality of light emitted by the fluorescent lamps (Figure 2a), since the fungus cultivated under the effect of LBC produced the highest number of sclerotia, followed by LBN, which exceeded the fungus grown under the effect of LBF by 27.63 and 16.5%, respectively. This coincides with that reported by Schmidt-Heydt *et al.* (2011), since they point out that blue light irradiation inhibits the conidial formation of some *Aspergillus* strains. Regarding the production of mycelium biomass, the quality of light emitted by fluorescent lamps also caused significant effects ($p \leq 0.05$), since the fungus cultivated under LBF conditions produced 57.14% less quantity of biomass, than with the management in LBN and LBC (Figure 2b).

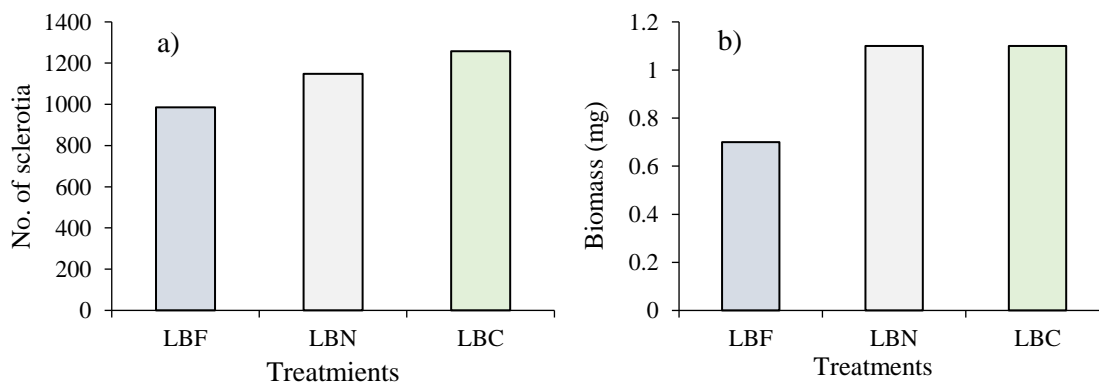


Figure 2. Influence of light quality, emitted by fluorescent lamps with cold white (LBF), neutral (LBN) and warm (LBC) light, on the formation of sclerotia (a) and mycelium biomass (b) from *Sclerotium rolfssii* grown *in vitro*.

Simple linear regression analysis between the amount of blue light (absolute and proportional) and radial growth, sclerotia production and biomass of *S. rolfssii* in the three treatments (LBF, LBN and LBC) indicated a negative relationship (Figure 3), in such a way that the progressive decreases in

the production of sclerotia, radial growth and biomass production of *S. rolfsii* are explained in the respective 98.3, 98.8 and 73.3%, due to the effect caused by the increases in absolute blue light (Figures 3a , 3d and 3g); in 100, 100 and 82.7%, due to increases in the proportion of blue light with respect to red light (Figures 3b, 3e and 3h) and in 99.2, 99.5 and 76.7%, due to increases in the proportion of light blue relative to far red light (Figures 3c, 3f and 3i), respectively.

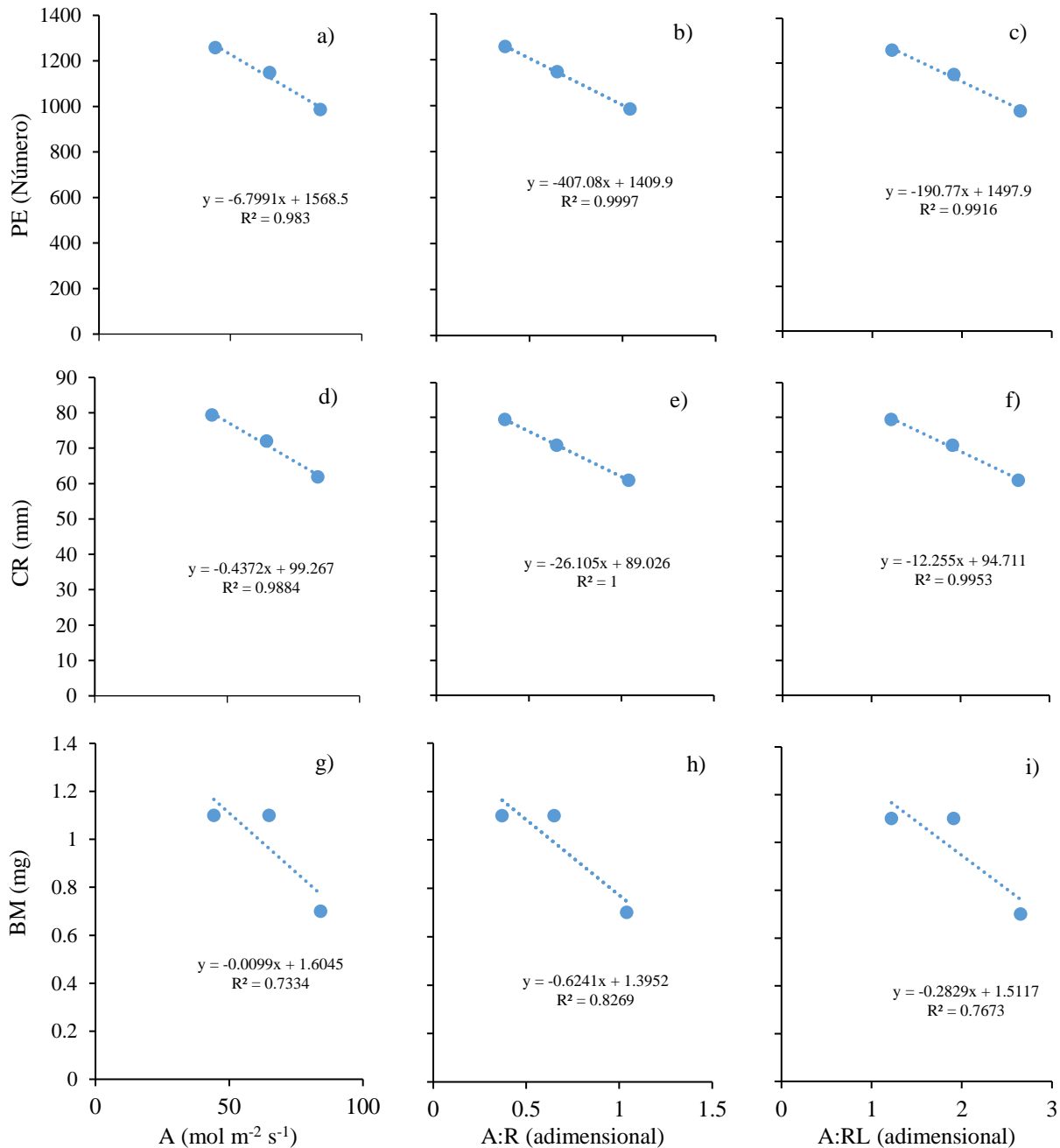


Figure 3. Interaction between sclerotia production (PE), radial mycelial growth (CR) and mycelium biomass (BM) of *Sclerotium rolfsii* with absolute amounts of blue light (A) and proportional amounts with respect to red light (A:R) and far red (A:RL).

In the present investigation, it was found that a greater amount of blue light, and less of red and far red, originated negative effects on radial growth, the production of sclerotia and biomass of *S. rolfssii*.

The absolute amount of blue light, as well as the proportional amounts of blue light with respect to red and far red light, varied with LBF, LBN or LBC (Table 1). It was found that the negative response on *S. rolfssii* was directly proportional to the amount of blue light in the environment and inversely proportional to the amount of red and far red, which suggests that it is the blue fraction of the light spectrum and not the red one. that caused negative effects on the pathogen, a response that agrees with that reported by Canessa *et al.* (2013) for *Botrytis cinerea* and Kim *et al.* (2011) for *Cercospora zea-maydis*.

The lower or higher production of sclerotia (Figures 3a, 3b and 3c), as well as the decrease or increase in radial growth (Figures 3d, 3e and 3f) and biomass of *S. rolfssii* (Figures 3g, 3h and 3i) are responses that they are not related to the temperatures presented in the three different chambers, because there was a maximum variation of 0.5 °C between them.

Conclusions

The light spectrum emitted by the fluorescent lamps influenced the growth of *Sclerotium rolfssii*, in such a way that with cold white light radial growth, the production of sclerotia and mycelium biomass of the phytopathogen decreased, while these variables increased with warm white light, compared to what was induced with neutral white light and cold white light.

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