







## Mycelial Growth of *Pleurotus* spp. Isolates in the Light and the Dark


### Crescimento Micelial de Isolados de *Pleurotus* spp. na Luz e no Escuro

Tainara Reis Santos: Universidade Federal de Sergipe, Departamento de Engenharia Agrônômica, Laboratório de Microbiologia Agrícola, Campus de São Cristóvão. SE, Brazil. 

Ester da Silva Murta: Universidade Federal de Sergipe, Departamento de Engenharia Agrônômica, Laboratório de Microbiologia Agrícola, Campus de São Cristóvão. SE, Brazil. 

Andréa Verônica Gobbi Barbosa: Universidade Federal de Sergipe, Departamento de Engenharia Agrônômica, Laboratório de Microbiologia Agrícola, Campus de São Cristóvão. SE, Brazil. 

Marcos Cabral de Vasconcellos Barretto: Universidade Federal de Sergipe, Departamento de Engenharia Agrônômica, Laboratório de Remediação de solos. SE, Brazil. 

Regina Helena Marino: Universidade Federal de Sergipe, Departamento de Engenharia Agrônômica, Laboratório de Microbiologia Agrícola, Campus de São Cristóvão. SE, Brazil. E-mail: rehmarino@hotmail.com 

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### Abstract

The white rot fungus *Pleurotus* spp. traditionally cultivated on lignocellulolytic waste and used as food for a healthy diet, has also been exploited in the production of biocomposites. However, little is known about the effect of light on the mycelial growth of *Pleurotus* spp. in the culture medium. The aim of this study was to evaluate the influence of room light and continuous darkness on the mycelial growth of *Pleurotus* spp. isolates *in vitro*. The design used was entirely randomized in a 6 x 2 x 3 factorial scheme, corresponding to the cultivation of six fungal isolates (*Pleurotus ostreatus*: DF39, EF39, EF60, POS AJU1 and POS W; and *Pleurotus ostreatoroseus*: POS SP1), in two lighting conditions (room light and continuous dark) and three evaluation periods (3rd, 4th and 5th day after inoculation, with four replicates per treatment). For fungal isolate, luminosity did not influence mycelial diameter, growth rate and mycelial density. The increase in the cultivation period, regardless of light and fungal isolates, favored mycelial density. Compared to cultivation in room light, darkness favored an increase in mycelial diameter and mycelial growth rate, depending on the *Pleurotus* spp. isolate.

**Keywords:** Basidiomycetes. Luminosity. Oyster Mushroom.

## Resumo

O fungo de podridão branca *Pleurotus* spp. cultivado tradicionalmente em resíduos lignocelulolíticos e utilizado como um alimento para uma dieta saudável, também tem sido explorado na produção de biocompósitos. Entretanto, pouco se conhece sobre o efeito da luminosidade no crescimento micelial das espécies de *Pleurotus* spp. em meio de cultura. O objetivo deste trabalho foi avaliar a influência da luz ambiente e do escuro contínuo no crescimento micelial de isolados de *Pleurotus* spp. *in vitro*. O delineamento utilizado foi o inteiramente casualizado no esquema fatorial de 6 x 2 x 3, correspondente ao cultivo de seis isolados fúngicos (*Pleurotus ostreatus*: DF39, EF39, EF60, POS AJU1 e POS W; e *Pleurotus ostreatoroseus*: POS SP1), em duas condições de luminosidade (luz ambiente e escuro contínuo) e três períodos de avaliação (3º, 4º e 5º dia após a inoculação) com quatro repetições por tratamento. Por isolado fúngico, a luminosidade não influenciou no diâmetro micelial, na velocidade de crescimento e na densidade micelial. O aumento do período de cultivo, independentemente da luminosidade e dos isolados fúngicos, favoreceu o adensamento micelial. Em comparação ao cultivo na luz ambiente, o escuro favoreceu o aumento do diâmetro micelial e da velocidade de crescimento micelial, a depender do isolado de *Pleurotus* spp.

**Palavras-chave:** Basidiomicetos. Luminosidade. Cogumelo Ostra.

## 1 Introduction

Edible fungi belonging to the genus *Pleurotus* (Agaricales, Agaricaceae) are white rot basidiomycetes known as oyster mushrooms. They are cultivated on lignocellulosic residues and have been used as food and in the production of medicines due to their antioxidant, antitumor, and bacteriostatic activities (Steffen, Saccol, Steffen, 2023; Zhao *et al.*, 2024). These fungi can also be used in environmental remediation through bioremediation processes (Ramamurthy *et al.*, 2024) and in the production of biocomposites (Teixeira *et al.*, 2018).

In biocomposite production, basidiomycetes produce hyphae capable of binding the particles of the cultivation substrate, thereby promoting the biomaterials mechanical strength (Deepika *et al.*, 2024; Yang, Park, Qin, 2021; Matos *et al.*, 2019). Furthermore, after complete substrate colonization, the biocomposite can be used for the production of vegetable and/or fruit seedlings due to the increased availability of nutrients (Jesus *et al.*, 2024; Santana *et al.*, 2024). During the substrate colonization phase, basidiomycetes may also secrete secondary metabolites, and the culture medium has shown nematicidal and fungicidal activity (Awad; Hassan, 2023 ; Pereira *et al.*, 2024; Silva *et al.*, 2024;).

Among the factors that may affect mycelial growth is the synthesis of oxidative enzymes such as laccases and manganese peroxidase, which are important for the cultivation substrate degradation and for releasing nutrients and energy sources essential for fungal development (Araújo *et al.*, 2021; Brito *et al.*, 2021; Malhotra; Suman, 2021). In addition, light can influence microbial metabolism (Kim *et al.*, 2020; Belletini *et al.*, 2019; Seo; Koo, 2019), as well as the formation and pigmentation of edible mushrooms (Kim *et al.*, 2020; Kuforiji; Fasidi, 2009; Marino *et al.*, 2003).

In India, Khandakar *et al.* (2009) reported that the mycelial density of the mushroom *Grifola frondosa* was favored in the absence of light. Rout *et al.* (2015) and Nidhi and Sud (2023) also observed that low light intensity or darkness favored the mycelial growth of *Pleurotus spp.* isolates. Moreover, Nidhi *et al.* (2023) found that diffuse light stimulated the mycelial growth of *Pleurotus ostreatus* compared to cultivation in darkness or light. However, there is little discussion about the influence of light on the mycelial growth of *Pleurotus spp.* isolates cultivated under Brazilian environmental conditions.

Therefore, the objective of this study was to evaluate the influence of room light and darkness on the in vitro mycelial growth of *Pleurotus ostreatus* and *Pleurotus ostreatoroseus* isolates originally cultivated in the states of São Paulo and Sergipe, aiming at the production of multifunctional biocomposites of agronomic importance.

## 2 Material and Methods

The experiment was conducted at the Agricultural Microbiology Laboratory of the Department of Agronomic Engineering, São Cristóvão Campus, Sergipe, Universidade Federal do Sergipe, Brazil.

### 2.1 Fungal inoculant

The tested fungal isolates included *Pleurotus ostreatus* (DF39, EF39, EF60, POS AJU1, and POS W) and *Pleurotus ostreatoroseus* (POS SP1), obtained from the Microbial Collection of the Agricultural Microbiology Laboratory, Department of Agronomic Engineering (DEA), São Cristóvão Campus, Universidade Federal do Segipe (UFS), Brazil.

Isolates DF39, EF39, and EF60 were donated by the Mushroom Module of the Faculty of Agronomic Sciences (FCA) of Universidade do Estado de São Paulo (UNESP), Botucatu, São Paulo. The isolates POS W and POS SP1 were purchased from a supermarket in São Paulo State, and POS AJU1 was collected in Aracaju.

Multiplication of fungal isolates was carried out by aseptically removing basidioma fragments and transferring them to Potato Dextrose Agar (PDA, 39 g·L<sup>-1</sup>) in Petri dishes. Incubation was carried out at room temperature.

The fungal inoculant was prepared by transferring a 6 mm mycelial disc to Petri dishes containing PDA medium. After seven days of cultivation at room temperature, the colonized medium was used as the inoculant.

### 2.2 Mycelial growth of fungal isolates under ambient light and darkness

The experimental design used was completely randomized (CRD) in a 6 × 2 × 3 factorial scheme, corresponding to the cultivation of six fungal isolates (DF39, EF39, EF60, POS AJU1, POS

SP1, and POS W) under two environmental conditions (room light and darkness) and three evaluation times (3rd, 4th, and 5th day after inoculation), with four replicates each.

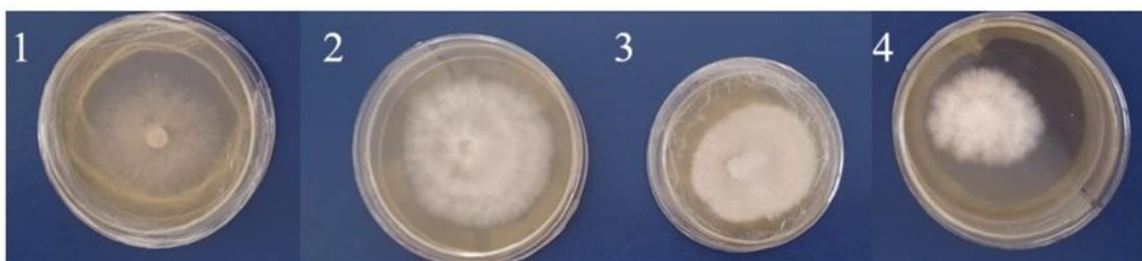
A 6 mm diameter mycelial disc from the fungal inoculant, with the mycelial side facing the culture medium, was placed at the center of PDA plates. Incubation was carried out at room temperature under two lighting conditions: room light (daytime) and continuous darkness (day and night) for five days. Plates maintained in the darkness were covered with black crepe paper.

The variables analyzed included: mycelial diameter, mycelial density, mycelial growth rate, and the percentage of inhibition of mycelial diameter and growth rate under continuous darkness relative to room light, and vice versa.

Mycelial growth was monitored daily from the third to the fifth day of cultivation. Mycelial diameter (MD, cm) was measured in two perpendicular directions using a millimeter ruler, and the mean was calculated.

Density was assessed based on the growth at the colony margin using a subjective scoring system: 1 = slightly dense, 2 = moderately dense, 3 = highly dense, and 4 = cottony (“fluffy”) (Figure 1).

**Figure 1** - Classification of mycelial density of *Pleurotus spp.* isolates based on a subjective scoring system\*



Notes: (1) slightly dense, (2) moderately dense, (3) highly dense, and (4) cottony (“fluffy”)

Source: research data.

Mycelial growth rate (GR,  $\text{cm} \cdot \text{day}^{-1}$ ) was determined using the equation  $\text{GR} = (\text{Df} - \text{Di})/\text{I}$ , where Df = final mycelial diameter, Di = initial mycelial diameter, and I = incubation period in days.

The percentage of inhibition (PI, %) of mycelial diameter and mycelial growth rate under light (day) conditions was calculated using the equation:  $\text{PI} = ((\text{Vc} - \text{Vt})/\text{Vc}) \times 100$ , where Vc = value of the variable in the control treatment (light) and Vt = value of the variable in the dark treatment. The darkness influence on the PI variable was determined by setting Vc = value in the control (darkness) and Vt = value in the light treatment. Positive values indicate inhibition of mycelial growth, while negative values indicate stimulation of mycelial growth.

## 2.3 Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA), and when statistical differences were detected, Tukey's test at 5% probability and the t-test at 1% and 5% probability were applied for regression analysis using SISVAR software version 5.8 (Ferreira, 2019).

## 3 Results and Discussion

Nguyen and Ranamukhaarachchi (2020) observed that darkness inhibited the growth of *P. eryngii* and *P. ostreatus*. On the other hand, the fungi *P. ostreatoroseus* and *Grifola frondosa* showed increased mycelial density during cultivation in the darkness (Coelho *et al.*, 2021; Khandakar *et al.*, 2009). Fonseca *et al.* (2015), in turn, observed that darkness did not affect the mycelial density of *P. ostreatoroseus* DPUA 1720, although it increased the growth rate. In the present study, the tested light conditions did not influence the mycelial diameter, growth rate, or mycelial density of the *Pleurotus* spp. isolates evaluated (Table 1).

**Table 1** - Mycelial density, mycelial diameter, and growth rate of fungal isolates of *Pleurotus ostreatus* (DF39, EF39, EF60, POS AJU1, and POS W) and *P. ostreatoroseus* (POS SP1) grown on PDA medium under two light conditions (ambient light and darkness) after five days of incubation

Treatments	Mycelial Density (score) <sup>1</sup>	Mycelial Diameter (cm)	Growth Rate (cm·day <sup>-1</sup> )
Darkness	2.38a <sup>2</sup>	5.82a	1.53a
Ambient light	2.35a	5.80a	1.50a
CV (%) <sup>3</sup>	32.54	8.36	19.17

<sup>1</sup>Subjective scoring criteria: 1 = slightly dense, 2 = moderately dense, 3 = highly dense, and 4 = dense ("fluffy");<sup>2</sup>Means followed by the same letter in the column do not differ from each other by Tukey's test at 5% probability, per treatment;<sup>3</sup>CV = coefficient of variation.

**Source:** research data.

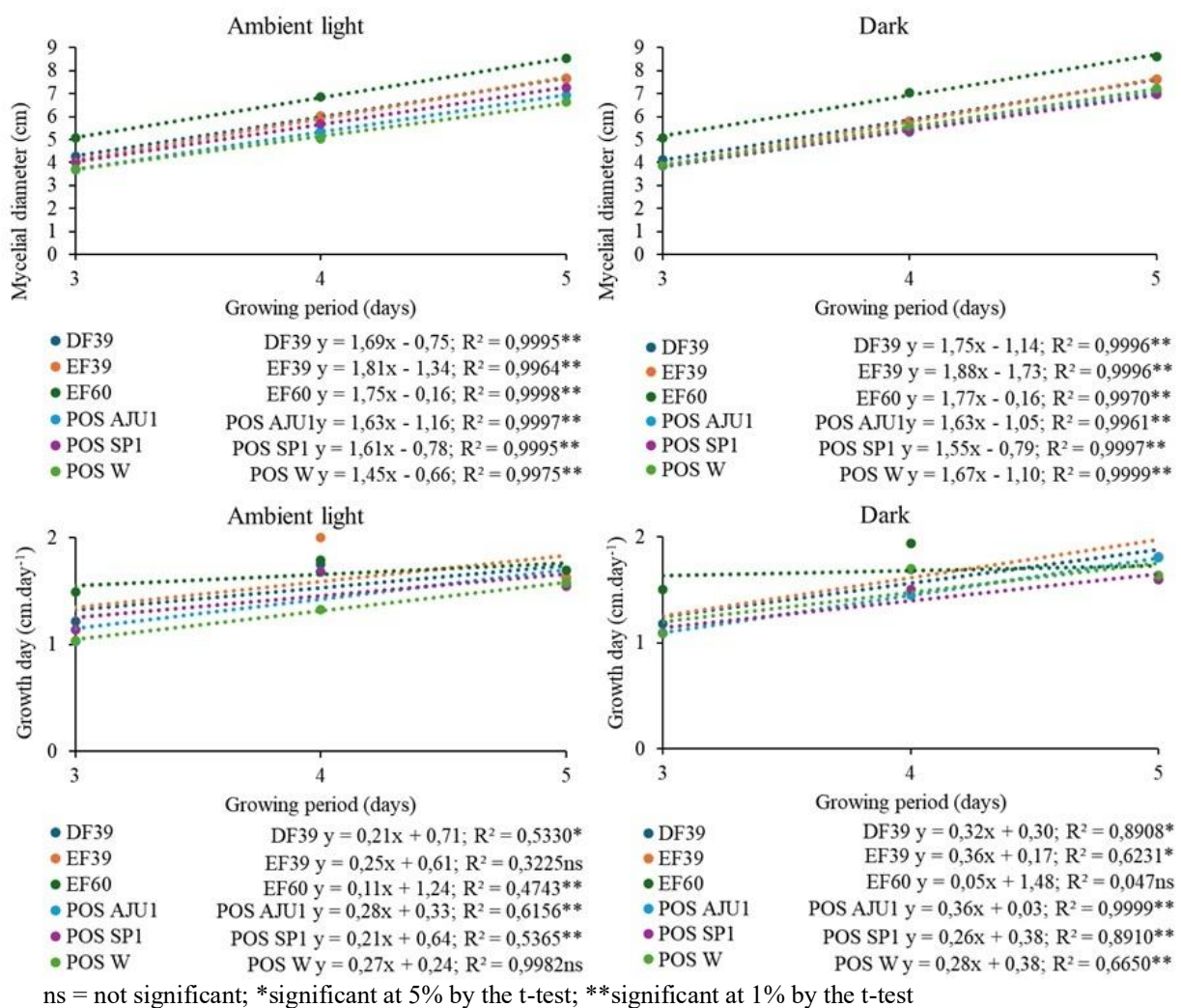
In this result, it should be considered that the culture medium used may have minimized the effect of darkness on the mycelial growth of the tested *Pleurotus* spp. isolates. This is because PDA medium is composed of starch and dextrose, which are carbon and energy sources that are easily metabolized by fungi, similar to the media used for cultivating *P. ostreatoroseus* (Coelho *et al.*, 2021) and *P. eryngii* and *P. ostreatus* (Nguyen; Ranamukhaarachchi, 2020).

Another important factor to consider is that increasing the cultivation period from three to five days for *Pleurotus* spp. isolates under ambient light and darkness favored the growth in mycelial diameter of all the fungal isolates. The data fitted a linear regression model in all the treatments (Figure 2), probably due to the physiological adaptation of the fungal isolates and the enzymes

synthesis that promote substrate degradation and release of nutrients essential to mycelial development, as reported by Brito *et al.* (2021) for *P. djamor*.

Regarding mycelial growth during the incubation period, Sales-Campos *et al.* (2022) observed that on the fifth day there was a reduction in the growth rate of isolate 474 of *P. ostreatus* grown on a lignocellulosic waste-based substrate. In this study, conducted on potato dextrose agar (PDA) medium, the growth rate increased with the cultivation period in all the treatments (fungal isolates and light conditions), except for isolates *P. ostreatus* EF39 and POS W under ambient light and EF60 in darkness, whose data did not fit any regression model (Figure 2).

**Figure 2** - Mycelial diameter and growth rate of *Pleurotus ostreatus* isolates (DF39, EF39, EF60, POS AJU1, POS W) and *P. ostreatoroseus* (POS SP1) grown on potato dextrose agar (PDA) medium under two environmental conditions (room light and continuous darkness) during five days of incubation



Source: research data.

On the 5th day of cultivation and for each fungal isolate, light conditions did not influence mycelial diameter, growth rate, or mycelial density (Table 3; Figure 3).

**Table 3** - Mycelial diameter, mycelial growth rate, and mycelial density of fungal isolates of *Pleurotus ostreatus* (DF39, EF39, EF60, POS W) and *P. ostreatoroseus* (POS SP1) grown on PDA medium under room light and darkness conditions, after five days of incubation

Treatments		Mycelial Diameter (cm)	Mycelial growth Rate (cm·day <sup>-1</sup> )	Mycelial Density (score) <sup>2</sup>
Fungus	Light condition			
DF39	Darkness	7.63a <sup>1</sup>	1.81a	2.50a
	Room light	7.65a	1.63a	2.50a
EF39	Darkness	7.63a	1.81a	2.25a
	Room light	7.65a	1.63a	2.00a
EF60	Darkness	8.63a	1.60a	1.50a
	Room light	8.55a	1.70a	1.75a
POS AJU1	Darkness	7.13a	1.80a	2.25a
	Room light	6.95a	1.58a	1.25a
POS SP1	Darkness	6.98a	1.60a	1.75a
	Room light	7.25 a	1.55a	1.25a
POS W	Darkness	7.21a	1.58a	2.25a
	Room light	6.63a	1.64a	1.25a
CV (%) <sup>3</sup>		6.80	15.53	38.75

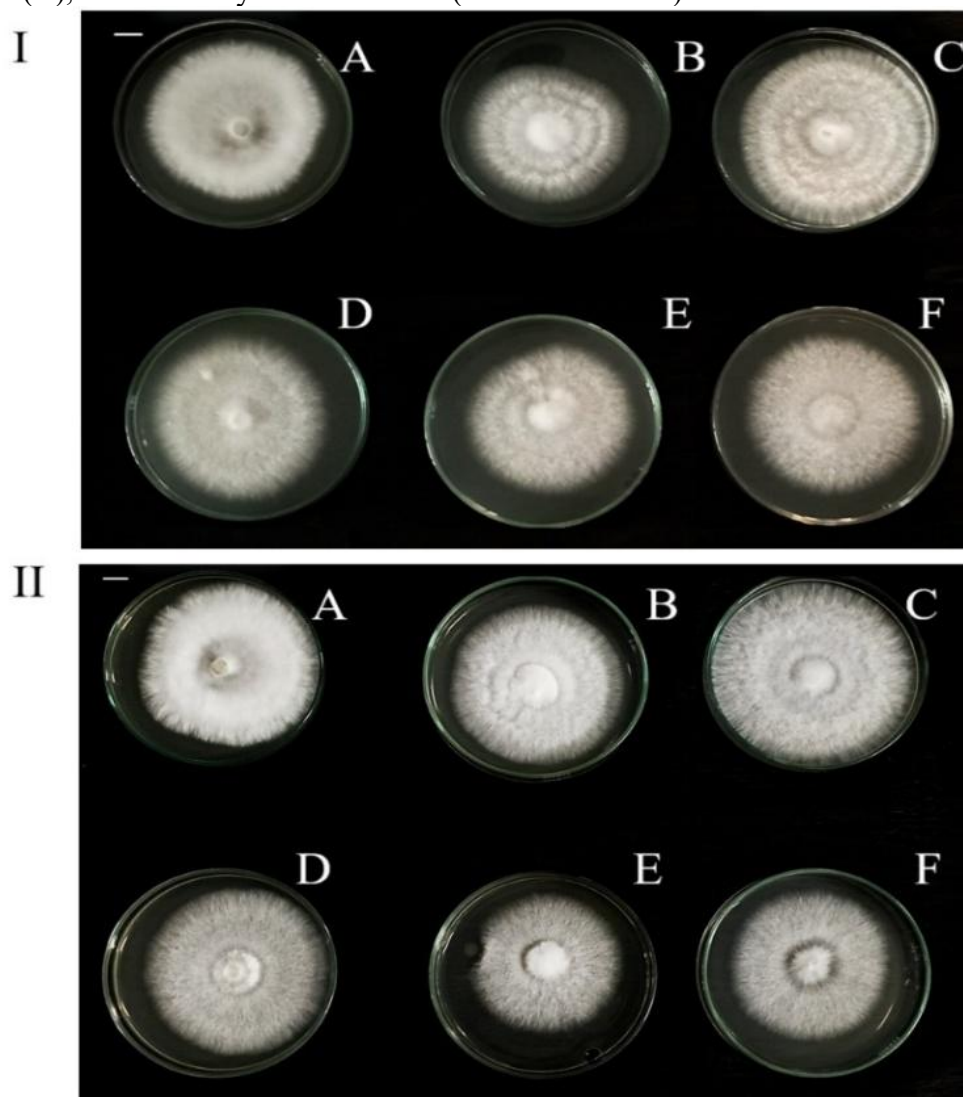
<sup>1</sup>Means followed by the same letter in the column do not differ significantly from each other by Tukey's test at 5% probability, within each treatment;<sup>2</sup>Subjective scoring criterion: 1 = weakly dense, 2 = moderately dense, 3 = strongly dense, and 4 = fluffy;<sup>3</sup> CV = coefficient of variation.

**Source:** research data.

It is noteworthy that during the cultivation phase, an increase in mycelial density was observed in all the treatments (fungal isolates and light conditions) with the extension of the incubation period, as also reported by Santana-Santos *et al.* (2023) and Correia *et al.* (2021) for the PS isolate of *Pycnoporus sanguineus* grown on coconut powder supplemented with bran.



**Figure 3** - Mycelial growth of *Pleurotus ostreatus* isolates A (DF39), B (EF39), C (EF60), D (POS AJU1), E (POS W) and *P. ostreatoroseus* F (POS SP1) cultivated on PDA medium in the dark (I) and under room light (II), after five days of cultivation (scale bar = 1 cm)

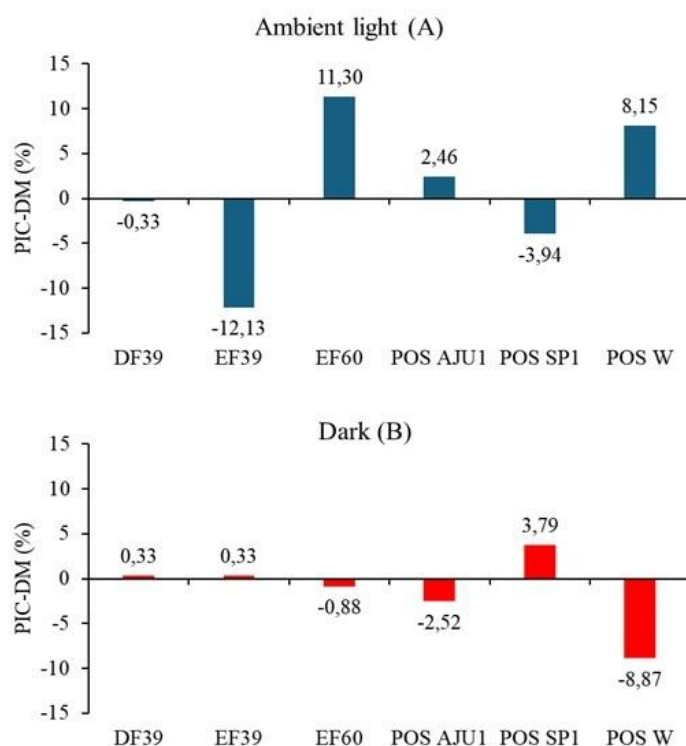


**Source:** research data.

Compared to darkness, room light favored the mycelial diameter growth of DF39 (-0.33%), EF39 (-12.13%), and POS SP1 (-3.94%), but reduced this variable in EF60 (11.30%), POS AJU1 (2.46%), and POS W (8.15%) (Figure 4A).



**Figure 4** - Percentage of inhibition\* of mycelial diameter (PIC-DM) of fungal isolates of *Pleurotus ostreatus* (DF39, EF39, EF60, POS AJU1, POS W) and *P. ostreatoroseus* (POS SP1) grown on PDA medium under room light compared to cultivation in the dark (A) and in the dark compared to ambient light (B), after five days of incubation



\*positive values = growth inhibition; negative values = growth stimulation

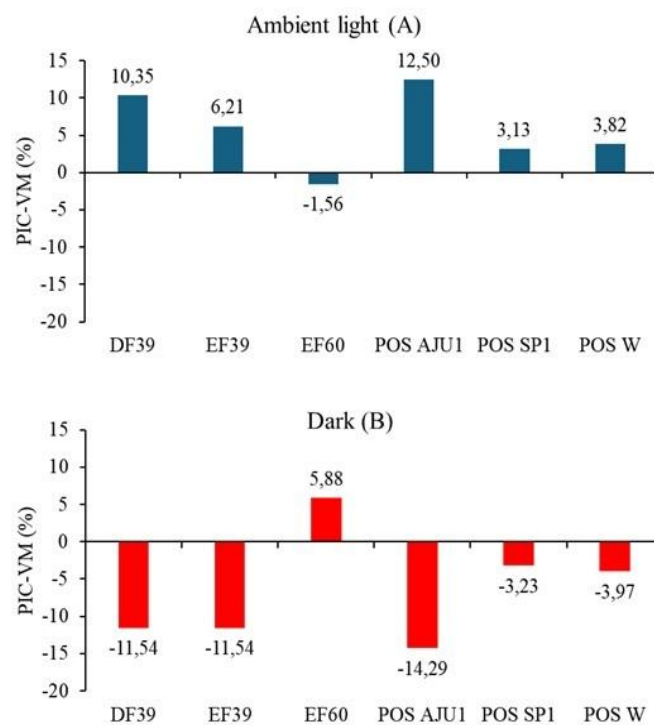
**Source:** research data.

When comparing cultivation in the dark to room light, the mycelial diameter of *P. ostreatus* (DF39 and EF39) and *P. ostreatoroseus* (POS SP1) isolates was inhibited by 0.33 to 3.79%, but growth was stimulated in *P. ostreatus* isolates EF60 (-0.88%), POS AJU1 (-2.52%), and POS W (-8.87%) (Figure 4B), as also reported by Nidhi *et al.* (2023).

According to Araújo *et al.* (2021), the increased growth of *Pleurotus citrinopileatus* U16-23, *P. djamor* U16-28, *P. eryngii* U16-30, *P. ostreatus* U16-22, and *P. pulmonarius* U16-21 isolates during cultivation in the dark occurred due to higher synthesis of the oxidative enzyme laccase, which is responsible for substrate degradation and the release of important nutrients for fungal development (Brito *et al.*, 2021; Malhota; Suman, 2021). This may also have occurred in both dark and light conditions for the tested fungal isolates, due to the use of a culture medium that is easily metabolized, as previously discussed.

In terms of growth rate, room light reduced the growth by 3.13% to 12.50% in *P. ostreatus* (DF39, EF39, POS AJU1, and POS W) and *P. ostreatoroseus* (POS SP1) isolates when compared to dark conditions (Figure 5A). In contrast, the basidiomycete *Grifola frondosa* cultivation under constant light inhibited growth by 54.83% compared to incubation in the dark (Khandakar *et al.*, 2009).

**Figure 5** - Percentage of inhibition\* of mycelial growth rate (PIC-VM) of fungal isolates of *Pleurotus ostreatus* (DF39, EF39, EF60, POS AJU1, POS W) and *P. ostreatoroseus* (POS SP1) grown on PDA medium under room light compared to dark cultivation (A), and in the dark compared to room light (B), after five days of incubation



\*positive values = growth inhibition; negative values = growth stimulation.

**Source:** research data.

In the dark, the growth rate of isolates DF39, EF39, POS AJU1, POS SP1, and POS W increased by 3.23 to 14.29%, but growth of EF60 was inhibited by 5.88% compared to incubation under room light (Figure 5B). The increased growth rate of isolates DF39, EF39, POS AJU1, POS SP1, and POS W in the dark compared to cultivation in room light may be a positive aspect for biocomposite

production, since the faster the fungal isolate colonizes the substrate, the lower the contamination by saprophytic fungi and the smaller the loss during the production phase.

Overall, light influenced the mycelial growth of *Pleurotus* spp. isolates depending on the fungal isolate, but specific knowledge of the requirement for room light or continuous darkness by the *Pleurotus* spp. isolate could also contribute to improving mushroom quality, as reported by Marino *et al.* (2003) with *P. ostreatus*, as well as reducing costs in mushroom and biocomposite production.

Furthermore, light during the cultivation of *P. ostreatus*, *P. eryngii*, *Lentinula edodes*, and *P. sanguineus* also influenced the synthesis of secondary metabolites (Aguiar *et al.*, 2023; Zawadzka *et al.*, 2022; Kim *et al.*, 2020; Wu *et al.*, 2013), which may be important in the manufacture of multifunctional biocomposites of medicinal and/or agronomic interest and will be the subject of future study with the tested isolates.

#### 4 Conclusion

For each fungal isolate, light did not influence mycelial diameter, growth rate, or mycelial density. Increasing the cultivation period, regardless of light conditions and fungal isolates, favored mycelial densification. Compared to cultivation under room light, darkness favored an increase in mycelial diameter and growth rate, depending on the *Pleurotus* spp. isolate.

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