

Production of biocomposites from the reuse of coconut powder colonized by Shiitake mushroom

Produção de biocompósitos a partir do reaproveitamento de pó de coco colonizado pelo cogumelo shiitake

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ABSTRACT

The demand for biodegradable composite has grown worldwide in recent years, mainly in order to reduce environmental contamination by structural materials produced from the oil industry. The objective of this study was to evaluate the growth of isolates from the edible mushroom "Shiitake" (*Lentinula edodes*) in substrate coconut powder-based supplemented with wheat bran, as well as to analyze the influence of fungi growing period and drying time of the colonized substrate on the mechanical properties of the composite, in order to produce a biodegradable composite. The mycelial density is not influenced by the type of hyphae of *L. edodes*. Drying of the composite does not influence the residual odor, depending on the isolate. The compressive strength and foam type of the fungal composite may be influenced by the culture period and type of hyphae, depending on the fungal isolate. The composites colonized by the *L. edodes* isolates presented higher mechanical resistance at 30 days of complete colonization. The coconut powder supplemented with wheat bran colonized by isolated fungi LED 96/18 is an ecological alternative in the packaging production considering its mechanical properties.

Index terms: Biotechnology; fungiculture; biodegradable composite.

RESUMO

A procura por compósito biodegradável vem crescendo mundialmente nos últimos anos, principalmente com o intuito de reduzir a contaminação ambiental por materiais estruturais produzidos a partir de derivados de petróleo. O objetivo deste estudo foi avaliar o crescimento de isolados do cogumelo comestível "Shiitake" (*Lentinula edodes*) em substrato à base de pó de coco suplementado com farelos, bem como analisar a influência do período de cultivo dos fungos e tempo de secagem do substrato colonizado nas propriedades mecânicas do compósito, visando a produção de compósito biodegradável. A densidade micelial não é influenciada pelo tipo de hifa dos isolados de *L. edodes*. A secagem do compósito não influencia no odor residual do compósito, a depender do isolado. A tensão compressiva e o tipo de espuma de compósitos fúngicos podem ser influenciados pelo período de cultivo e pelo tipo de hifa, a depender do isolado. Os compósitos colonizados pelos isolados de *L. edodes* apresentam maior resistência mecânica aos 30 dias da completa colonização. O pó de coco suplementado com farelo de trigo e colonizado pelo isolado fúngico LED 96/18 é uma alternativa ecológica na produção de embalagens considerando as propriedades mecânicas.

Termos para indexação: Biotecnologia; fungicultura; compósito biodegradável.

INTRODUCTION

The composites can be defined as any multiphase material, which results in the improvement of the quality of its isolated properties, such as the plastic composite, the green composite and / or the fungal origin. The plastic composites such as those produced from expanded polystyrene (EPS), which stands out as a light impermeable, non-flammable, high strength

material and can be used in the production of: trays for growing seedlings, thermal and/or sound insulation (Borges; Gonçalves Júnior; Almeida, 2017). However, in the United States, only 5% of the total produced polymers is recycled (Pathak; Navneet, 2017), then resulting in terrestrial and aquatic pollution. In nature, EPS is not decomposed by saprophytic microorganisms, because this composite does not provide nutrients and/or

energy sources for the soil microbiota contributing to its permanence in the environment for more than 150 years (Balbo; Tosta, 2012).

In order to reduce environmental contamination, the green composite is produced from the mixture of vegetable fibers with EPS (Fernandes et al., 2017; Zhang et al., 2016). Alternatively, edible fungi and/or white rot fungi as: *Agaricus bisporus* (Román-Ramos; Luna-Molina; Bailón-Pérez, 2014), *Pleurotus eryngii* (Hemmati; Garmabi, 2012), *P. ostreatus* (Teixeira et al., 2018), *Phaenerochaete chrysosporium* (Ramirez-Chan et al., 2014) and *Pycnoporus sanguineus* (Teixeira et al., 2018) were used at production of composites through its reutilization as agricultural residues.

The fungal composites have physical and mechanical properties as equal as higher than EPS, biodegradable (Zeller; Zocher, 2012) and not toxic as well (Arifin; Yusuf, 2013). These composites have been used as: packaging (Román-Ramos; Luna-Molina; Bailón-Pérez, 2014), sound insulation (Pelletier et al., 2013) and as thermal insulation (Haneef et al., 2017). After use, the fungal composite can be used: as organic fertilizer or soil conditioner (Fontalvo et al., 2013); in the control of phytopathogens (Ishihara et al., 2018), reducing the consumption of mineral fertilizers and pesticides.

The company named Ecovative also produces bio-packages colonized by filamentous fungi, but uses composting and pasteurization in the substrate preparation (Zeller; Zocher, 2012), which increases the required time to obtain the bio-packages in relation to the axenic process performed in which it is used autoclaving as mentioned in the patent developed by the following authors (Marino et al., 2017a; Teixeira et al., 2018) with the cultivation of *Pleurotus ostreatus*, *P. eryngii* and *Pycnoporus sanguineus* in coconut powder supplemented with wheat bran.

The use of the edible mushroom “Shiitake” (*Lentinula edodes* (Berk.) Pegler) was cited only in the production of composites based on the mixture of polypropylene and sugarcane bagasse (Hemmati; Garmabi, 2012). However, the mushroom “Shiitake” can be produced in the mixture coconut powder with bran (Marino; Abreu, 2009), which represents an alternative of reutilization of residues in coconut water industry of and reduction of the contamination of the environment, because only a portion of these residues has been reused in the production of pots or substrate for seedling production. Thus, the objective of this study was to evaluate the growth of isolates from the edible mushroom “Shiitake” (*Lentinula edodes*) in substrate coconut powder-based supplemented with wheat bran, as well as to analyze the influence of fungi growing

period and drying time of the colonized substrate on the mechanical properties of the composite, in order to produce a biodegradable composite.

MATERIAL AND METHODS

Mycelial growth of *Lentinula edodes*

The experimental design was completely randomized (DIC) composed of three fungal isolates of *Lentinula edodes* (LED AJU1, LED CHI and LED 96/18) on a coconut powder-based substrate supplemented with 40% wheat bran, with three repetitions.

The fungal isolates were multiplied in BDA culture media (39 g L⁻¹ commercial product) at 25 ± 1 °C without photoperiod for seven days, to obtain the primary matrix. The culture substrate of the fungal isolates was produced in the mixture coconut powder-based with particles smaller than 1 mm, supplemented with 40% wheat bran and moistened at 60 to 70% with distilled water. The composition (25 g, wet mass) was transferred to Petri dishes (80 mm diameter) and autoclaved at 120 °C and 1 atm for 1 h. After cooling, a 6 mm diameter mycelial disc of the primary matrix was transferred for autoclaved substrate. The fungal isolates were cultivated in an incubator at 25 ± 1 °C without photoperiod (without light) until complete colonization.

The evaluated variables were: hyphae growth (branching, thickness, length), mycelial diameter, growth rate, mycelial density and residual odor of the substrate colonized by isolates of *L. edodes*.

The hyphae growth was analyzed under optical microscopy after the cultivation of the fungal isolates in microculture. The fungal isolates were cultivated in commercial BDA culture media in incubator at 25 ± 1 °C without photoperiod for seven days. After this cultivation period, a 6 mm diameter mycelial disc of the primary matrix was transferred to an autoclaved slide disposed on a filter paper moistened with autoclaved distilled water and suspended by clips. The fungal isolates were cultivated in an incubator at 25 ± 1 °C, without photoperiod for three days. After this period, the mycelial discs were removed, the hyphae stained with 0.1% methylene blue and a cover slip was added per slide. The hyphae characterization was as follows: length (short or long), thickness (thin or thick), presence of branches or hyphae grouped. Microscopic photographs were obtained with a 13 megapixel camera coupled to an optical microscope with magnification of 400x.

The mycelial diameter was evaluated by two cross-measurements of the colony diameter using a millimeter

ruler after seven days of culture. The growth rate (GR) was calculated by the equation: $GR (cm^{-1}) = (A-B)/C$, where: A is mycelial diameter at the last evaluation day (cm), B refers mycelial diameter at the first evaluation (cm) and C is evaluation days interval. The mycelial density was evaluated after complete colonization of the substrate at 23 days of culture of the fungal isolates. For this, a subjective scale of notes was adopted, as follow: 0 to 1 - little density; 1.1 to 2.0 - medium density and 2.1 to 3.0 - strongly density.

The residual odor was evaluated on substrate colonized by fresh and dry of *L. edodes* isolates after 23 days of culture. The drying of the colonized substrate was in a forced air circulation oven at 50 °C during 24 h. In the evaluation of the residual odor of the colonized substrate, a subjective notes scale was used, as follow: 0 to 1 = bad; 1.1 to 2.0 = regular and 2.1 to 3.0 = good. This was evaluated by 13 random people from the academic community of the Universidade Federal de Sergipe.

Data were submitted to Anova and Tukey test was applied at 5% probability for comparison of the means.

Mechanical strength of Shiitake mushroom composite

The experimental design was completely randomized in a 3 x 3 factorial method, with three fungal isolates of *Lentinula edodes* (LED AJU1, LED CHI and LED 96/18) and three cultivation periods (15, 30 and 45 days) after complete substrate colonization, with three repetitions.

The methodology used in this bioassay was described in the patent deposited by the authors of this work at Instituto Nacional de Propriedade Industrial (INPI) (Marino et al., 2017a).

The fungal composites were produced in a mixture of coconut powder-based substrate with particles smaller than 1 mm, supplemented with 40% wheat bran and moistened at 60-70% with distilled water. The mixture (250 g; wet mass) was packed in 1000 ml cylindrical plastic containers. The assembly was autoclaved at 120 °C and 1 atm for one hour and repeated after 24 h. After cooling, 10 grams of the fungal isolates previously produced in the same substrate (coconut powder and wheat bran) were transferred in an aseptic chamber. The fungal isolates were grown in an incubator at 25 ± 1 °C, without photoperiod until complete colonization.

The analyzed variables were: composite mass, composite volume, mass loss of the composite, volume loss of the composite, compressive strength and tenacity, after 15, 30 and 45 days of total substrate colonization of growing fungal isolates.

The mass composite determination was carried out after 0, 24 and 72 h of oven drying with air-forced circulation at 60 °C, using a semi-analytical balance. The volume (V) was calculated by the equation: $V (cm^3) = \pi \cdot r^2 \cdot h$, where: $\pi = 3.1416$, r = radius (cm) and h = height (cm). The measurements were performed with digital caliper after drying of the composite. The mass loss (ML) was calculated by the equation: $ML (\%) = 100 - (\text{final dry mass} \times 100) / \text{initial fresh mass}$. The volume loss (VL) was calculated by the equation: $VL (\%) = 100 - (\text{final volume} \times 100) / \text{initial volume}$.

The compressive strength tests were performed on the universal Instron model 3367 machine with a compression speed of 10 mm/min until crack formation. The tenacity was calculated as the area under the stress-strain curves, to estimate the energy absorption capacity of the composite until the beginning of cracks.

The mechanical data were processed by the Bluehill 2 software and submitted to analysis of variance. When a data significant difference was found, the Tukey test was applied at 5% probability. Data of mass loss, volume loss and compressive strength were submitted to regression analysis and the F test was applied at 1 and 5% probability.

RESULTS AND DISCUSSION

Mycelial growth of *Lentinula edodes*

The mean mycelial diameter of *L. edodes* was 4.3 ± 0.31 cm grown on coconut powder-based substrate supplemented with 40% wheat bran, showing no significant difference between the tested isolates, except for LED 96/18, which reduced mycelial diameter by 13.0% in relation to the LED CHI (Table 1).

In eucalyptus sawdust supplemented with rice bran, the mycelial diameter of the LED 96/18 was 4.5 cm (Andrade et al., 2007), higher than 4.0 cm observed in this study with coconut powder and wheat bran (Table 1), but supplementation and the type of culture substrate may have influenced the growth of this isolate.

The mean mycelial growth rate of *L. edodes* isolates was 0.67 ± 0.11 cm day⁻¹, without difference between the treatments (Table 1), which demonstrates that the smaller diameter growth of LED 96/18 did not influence this variable. However, the values obtained by the isolates of *L. edodes* were higher than those cited by Marino e Abreu (2009), but these authors evaluated other Shiitake isolates despite working with the same type of substrate.

The mycelial density of the *L. edodes* isolates also did not present a significant difference between the

treatments. Mycelial growth was characterized by the presence of low density mycelium (note 1), even after 23 days of cultivation (Table 1), which differs from the results cited by Sakamoto et al. (2018) with *L. edodes* with increased of the cultivation period.

Thus, the variations in mycelial growth of *L. edodes* isolates evaluated by diameter, growth rate and mycelial density may be due to the type of used agricultural residue as the culture substrate (Kuijk et al., 2017); size of the agricultural waste particles used as substrate for the production of the biocomposite (Islam et al., 2018); and type of supplementation (Kirsch; Macedo; Teixeira, 2016; Andrade et al., 2013).

Edible fungi species exhibit distinct mycelial growth depending on the type of hyphae morphology (Islam et al., 2017). In the mycelial growth of the three isolates of *L. edodes* the formation of three types of hyphae was observed. The hyphae of the LED AJU1 were long, thick and without branching, but entangled, which can generate a compact and more plasticity composite as cited by Haneef et al. (2017) who worked with the fungi *G. lucidum*, also presenting entangled hyphae, but shorter.

In the LED CHI, the hyphae were presented long, thin with short branches and non-entangled. And the hyphae of the LED 96/18 were long, thick, without branching and not entangled until the third day of cultivation (Figure 1).

In a similar way to LED 96/18, Haneef et al. (2017) observed hyphae of *Pleurotus ostreatus* characterized by a single filament resulted in a rigid foam. No reports were found on the morphology of hyphae of *L. edodes*, but the presence of morphologically distinct hyphae may influence the mechanical properties of the fungal composite. However, hyphae type variation is not restricted only among fungal species as mentioned by Haneef et al. (2017), but among isolates of the same species, as observed among the isolates of *L. edodes*.

In the selection of fungal isolates for the production of composites, it is important to consider the release of volatile substances by the cultivated fungi, since the residual odor of the colonized material can compromise the acceptance of the composites by consumers. In the literature, reports on residual odor have been described only for related to the odor of the dried mushroom (Özdemir et al., 2017) and not of the culture substrate were found.

Table 1: Mycelial diameter (MDIA), growth rate (GR), mycelial density (MD) and fresh residual odor (FRO) and dry residual odor (DRO) of the coconut powder-based substrate supplemented with wheat bran and colonized by isolates of *Lentinula edodes*.

Fungi isolates	MDIA (cm)	GR (cm day ⁻¹)	MD (notes)	FRO (notes)	DRO (notes)
LED AJU1	4.3 ± 0.23ab ¹	0.7 ± 0.14a	1.0 ± 0.0a ²	2.7 ± 0.25Aa ³	2.3 ± 0.05Ba ³
LED CHI	4.6 ± 0.23a	0.8 ± 0.06a	1.0 ± 0.0a	2.5 ± 0.05Aa	2.5 ± 0.29Aa
LED 96/18	4.0 ± 0.08b	0.6 ± 0.08a	1.0 ± 0.0a	2.4 ± 0.22Aa	2.7 ± 0.15Aa
CV (%)	4.5	14.7	0.0	7.7	8.7

¹ Means followed by the same letter, lower case (column) and upper case (line), do not differ by Tukey test at 5% probability;

² Subjective scale of notes was adopted, as follow: 0 to 1 - little density; 1.1 to 2.0 - medium density and 2.1 to 3.0 - strongly density; ³ Subjective notes scale: 0 to 1 = bad; 1.1 to 2.0 = regular and 2.1 to 3.0 = good.

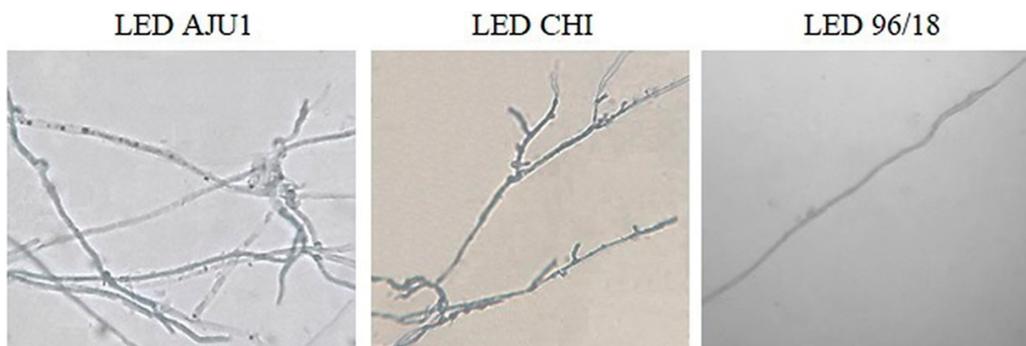


Figure 1: Hyphae of the LED AJU1, LED CHI and LED 96/18 of the *Lentinula edodes* isolates after three days of culture.

The fresh and dry coconut powder-based substrate and wheat bran colonized by *L. edodes* isolates presented residual odor classified as good (note 2) on the acceptance scale, without significant difference between isolates, except for LED AJU1 that showed a significant reduction of this variable after the drying of the colonized substrate, but residual odor kept good according to the evaluation (Table 1). Thus, coconut powder supplemented with wheat bran colonized by *L. edodes* allowed the mycelial growth of the tested isolates and did not leave unpleasant residual odor after drying the material, which shows a potential to be used as a biocomposite.

Mechanical strength of Shiitake mushroom composite

The *L. edodes* tested isolates colonized the culture substrate and formed compact blocks (Figure 2), similar to that observed by Islam et al. (2017, 2018), and Yang et al. (2017) with mycelium, but without specifying the fungi species.

The formation of the compact composites by *L. edodes* can be correlated with the aggregation of the culture particles substrate by the chitin (Islam et al., 2017; Yang et al., 2017) or by the increase in β -1,3-1,6-glucan present on the fungal cell wall (Sakamoto et al., 2018), which can be considered as a natural polymer and responsible for the

resistance of the fungal composite (Haneef et al., 2017; Yang et al., 2017; Tudryn et al., 2018)

Moreover, *L. edodes* fungi releases oxidative enzymes, such as laccases, to promote the decomposition of the substrate culture favoring mycelial growth (Sakamoto et al., 2018), which reduces the biomass of the vegetal residue (Bento; Casaril, 2012; Ramirez-Chan et al., 2014). Sales-Campos and Andrade (2011) mentioned that the *Lentinus strigosus* fungi resulted in loss of mass of the substrate culture between 42.2% and 58.6% of wood residues. And Teixeira et al. (2018) mentioned that the cultivation period may influence in mass loss of fungal composites with white rot fungi.

In the composites with *L. edodes* isolates, mass loss varied from 22.2% to 32.5% between 15 and 45 days after complete colonization, respectively. In composite LED AJU1, the mean mass loss was 23.5%, without influence of the increase of the culture period of 15 to 45 days, since the data were not adjusted to any regression model. In the treatments LED CHI and LED 96/18, the greatest composite mass loss was observed at 30 days of culture, with values of 32.5% and 26.3%, respectively. After 45 days of cultivation, the mass loss was 24.8% and 23.1% for LED CHI and LED 96/18, respectively, whose data were adjusted to the quadratic regression (Figure 3a).

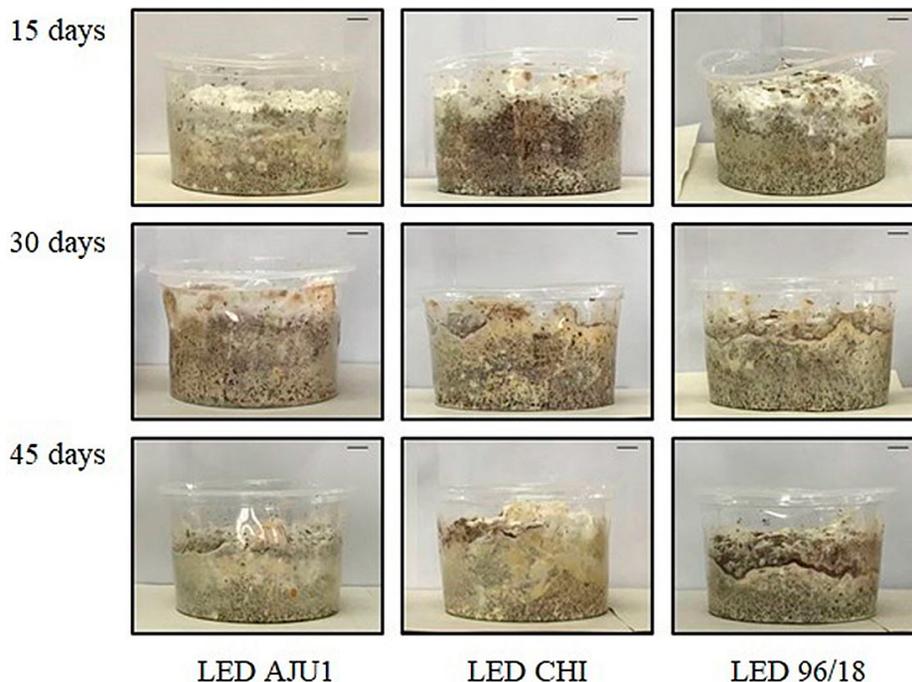


Figure 2: Composites of the *Lentinula edodes* isolates after 15, 30 and 45 days of complete colonization and dried for 0, 24 and 72 h.

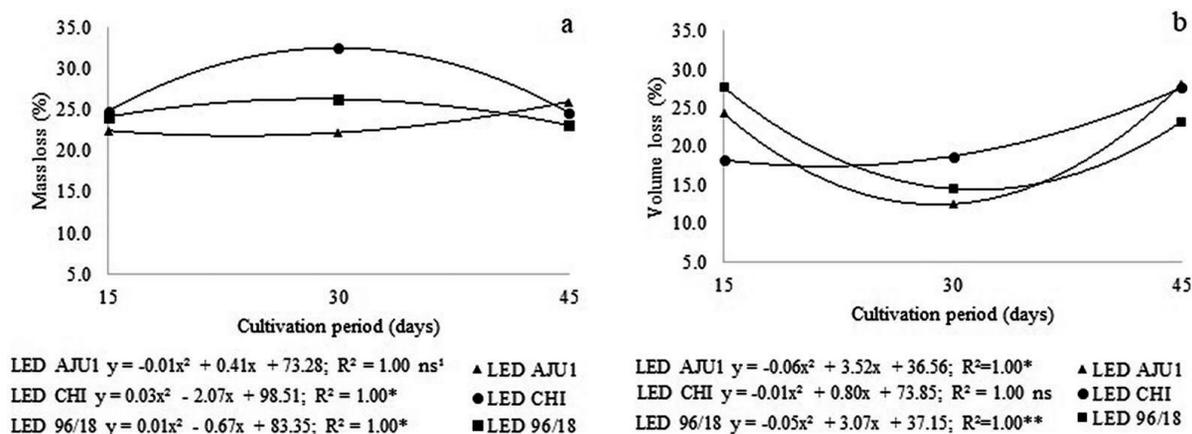


Figure 3: Composites mass loss (a) and volume loss (b) colonized by *Lentinula edodes* isolates after 15, 30 and 45 days of complete colonization.

ns = not significant ($p > 0.05$); * = significant at 5% ($0.01 \leq p < 0.05$) and ** = significant at 1% ($p < 0.01$).

The greater mass loss of composites LED CHI and LED 96/18 occurred between 15 and 30 days after complete colonization of the substrate, and may be correlated with the higher consumption of the substrate based on coconut powder and wheat bran by these fungal isolates. However, the increase in the cultivation period from 30 to 45 days of complete colonization may have resulted in reduced nutrient availability for microbial metabolism, and in the lower mass loss of the composite colonized by LED CHI and LED 96/18, when compared to 30 days.

Teixeira et al. (2018) observed a composite mass loss of 72.4%, on average with the isolates of *P. sanguineus*, *Pleurotus* spp. cultivated also in coconut powder-based substrate with wheat bran, which represents greater mass loss than that of composites colonized by *L. edodes*, probably due to the higher rate of degradation of the composite by the isolates tested by Teixeira et al. (2018).

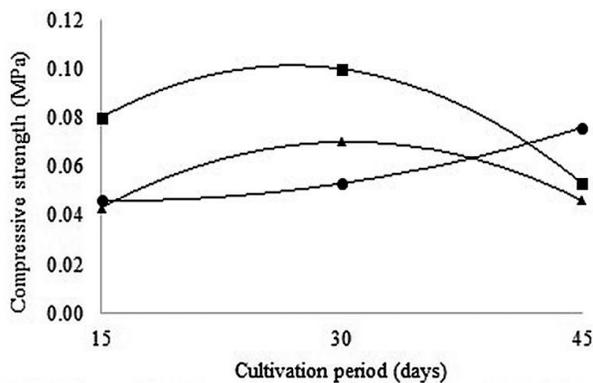
On the other hand, the composites colonized by *L. edodes* presented greater mass loss, when compared to 0.3 to 3.3% of the vegetal composites in mixture with plastics colonized by *P. ostreatus* (Faria; Wisbeck; Dias, 2015) and also compared to the 1.2 and 2.4% mass loss of the composite colonized by *Phanerochaete chrysosporium* coconut fiber and recycled plastic (Ramirez-Chan et al., 2014). The higher mass loss of the composites produced by *L. edodes* can result in a lightweight material similar to the EPS. On the other hand, the composite obtained from the mixture of coconut powder and wheat bran colonized by the fungi has the characteristic of being fully biodegradable material in the environment, different from the EPS, which takes more than 100 years to be decomposed. The decomposition was paralyzed with the drying process, which allows the conservation of the

fungal composites under environmental conditions (Haneef et al. 2017), but with rehydration it can be used as source of nutrients and energy to the soil saprophytic microorganisms, which will contribute to the cycling of nutrients reducing the time of bio-packaging in the environment.

Another aspect to be considered is that drying of the colonized substrate by filamentous fungi may also promote the collapse of hyphae (Haneef et al., 2017), which may influence in the composite volume. In this context, the cultivation period influenced the composite volume loss depending on the *L. edodes* isolate.

In the treatment with LED AJU1 and LED 96/18, the increase in the culture period from 15 to 30 days after complete colonization significantly reduced the composite volume loss, whose data were adjusted to the quadratic regression (Figure 3b). In this behavior it should be considered that the mycelial density increased with the increase of the culture period, which results in the filling of the voids existing between the particles of the coconut powder and the wheat bran, contributing to reduce the loss of volume of the composite. On the other hand, increasing the cultivation period from 30 to 45 days after complete colonization of the LED AJU1 and LED 96/18 composite resulted in a significant increase in volume loss, probably due to the higher consumption of nutrients available in the composite by these fungal isolates and followed by mycelial collapse as suggested by Haneef et al. (2017). In the LED CHI composite, there was no influence of the culture period on the volume loss, whose average value was 21.5% (Figure 3b), what differs from the results obtained by Teixeira et al. (2018) with *P. sanguineus* and *Pleurotus* spp. cultivated on the same type of substrate used in this work.

The fungal composites presented a foam-like behavior, open and elastic with compressive strength of 0.06 to 2.00 MPa (Islam et al., 2017; Yang et al., 2017). Carvalho and Fronllini (1999) mentioned that the compressive strength above 0.08 MPa characterizes a rigid foam and values between 0.015 and 0.080 MPa a semirigid foam. On average, *L. edodes* composites presented compressive strength of 0.06 ± 0.02 MPa, which characterizes a semi-rigid foam type material (Figure 4).



LED AJU1 $y = -0.0001x^2 + 0.01x - 0.03$; $R^2 = 1.00$ ns¹ ▲ LED AJU1
 LED CHI $y = 4E-05x^2 - 0.0011x + 0.05$; $R^2 = 1.00$ ns ● LED CHI
 LED 96/18 $y = -0.0001x^2 + 0.008x - 0.007$; $R^2 = 1.00$ * ■ LED 96/18

Figure 4: Compressive strength of composites cultivated with *Lentinula edodes* after 15, 30 and 45 days of complete colonization of the substrate. ns = non-significant ($p > 0.05$) and * = significant at 5% probability ($0.01 \leq p < 0.05$).

The compressive strength of the composites colonized by LED AJU1 and LED CHI was not influenced by the increase of the isolates culture period, whose data did not adjust to any regression model. The average values of the compressive strength of the composite LED AJU1 and LED CHI were 0.05 ± 0.02 MPa and 0.06 ± 0.02 MPa, respectively (Figure 4), which characterizes a semi-rigid foam (Carvalho; Fronllini, 1999) and differs from the results mentioned by Yang et al. (2017) with other fungal isolates. This behavior may have been due to the type of observed hyphae in LED AJU1 and LED CHI (Figure 1). Once, Haneef et al. (2017) observed that the entangled hyphae of *G. lucidum* resulted in a compact composite with greater plasticity of the material as observed with LED AJU1 with long and unbranched hyphae and with LED CHI with long hyphae and short branches, what may better absorb the impact and possible breakages of materials to be packed by this type of material.

The increase of the cultivation period of the LED 96/18 significantly influenced the compressive strength of the composites, whose mean values of this variable were adjusted to quadratic regression, with values of 0.08 ± 0.03 MPa, 0.10 ± 0.01 MPa and 0.05 ± 0.02 MPa after 15, 30 and 45 days of complete colonization of the substrate, respectively (Figure 4). The composite with LED 96/18 characterizes a rigid foam at 30 days and semirigid at 15 and 45 days (Carvalho; Fronllini, 1999). So, the compressive strength and foam type classification can be influenced by the culture period depending on the fungal isolate.

The commercial use of composites should be evaluated with the compressive strength at 10% maximum deformation (Horvath, 1997), whose EPS values may range from 0.033 to 0.165 MPa depending on the type of isopor® (Borges; Gonçalves Júnior; Almeida, 2017). In the classification of Román-Ramos, Luna-Molina and Bailón-Pérez (2014), the compressive strength at 10% deformation of 0.05 MPa should be used in the production of EPS packages and values lower than 0.05 MPa for acoustic insulation.

In the composites of LED AJU1 and LED CHI, the compressive strength versus deformation at 10% was less than 0.05 MPa and without influence of the increase of the fungi culture period (Figure 5), which results in an ideal material for acoustic insulation, as also mentioned by Teixeira et al. (2018) with *P. sanguineus* and by Román-Ramos, Luna-Molina and Bailón-Pérez (2014) with *P. ostreatus*.

The cultivation period of the LED 96/18 isolate influenced the mechanical strength of the composite. At 15 days after the complete colonization a mechanical resistance of 0.05 MPa was observed, similar to EPS recommended for packaging by Román-Ramos, Luna-Molina and Bailón-Pérez (2014). And at 30 and 45 days of complete colonization, the compressive strength of the LED 96/18 composite was lower than 0.05 MPa, similar to that observed for the other *L. edodes* tested isolates, which could be used for acoustic insulation (Figure 5), as cited by Pelletier et al. (2013) with *Phanerochaete chrysosporium* and plastic composite.

In the compressive strength versus deformation of the LED AJU1 composite an elastic behavior was observed, without influence of the fungi culture period as stated by Islam et al. (2018) with other fungi species. The composite LED CHI presented plastic behavior at 15 and 45 days, and elastic at 30 days of complete colonization. While, the LED 96/18 composite presented plastic behavior in the three culture analyzed periods (Figure 5).

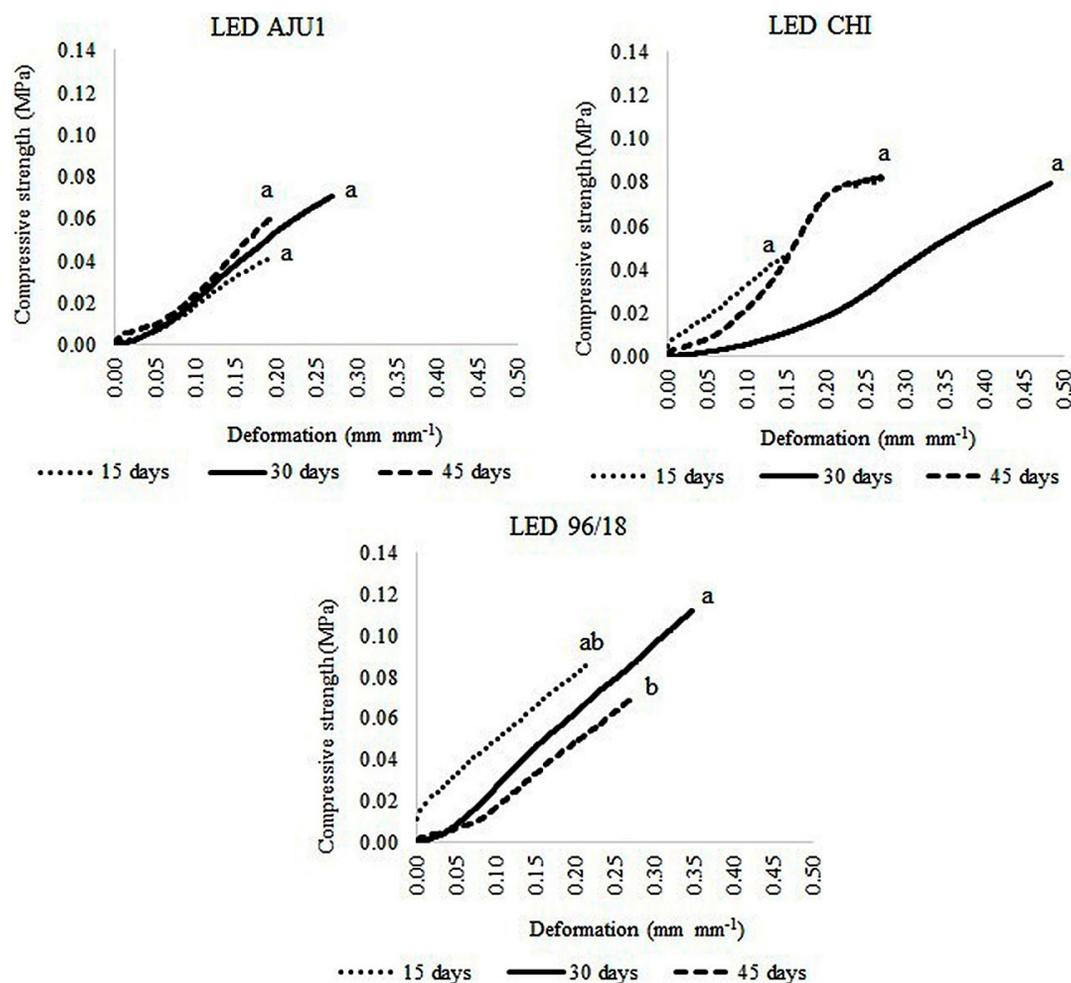


Figure 5: Compressive strength versus deformation of the composites of the *Lentinula edodes* after 15, 30 and 45 days of complete colonization. Means followed by the same letter do not differ by the Tukey test at 5% probability.

The elastic behavior of fungal composites was also reported by Islam et al. (2017). Similarly, Yang et al. (2017) also observed this same behavior of fungal composites, but these authors reported that increasing the culture period reduced elastic behavior, as an observer on LED CHI. The elastic or plastic behavior may be associated with the morphological differences of the observed hyphae among *L. edodes* isolates, as previously discussed and cited by Haneef et al. (2017) with *P. ostreatus* and *G. lucidum*; and Tudryn et al. (2018) without specifying the fungal studied species.

The materials with plastic behavior tend to absorb more energy and have greater durability than elastic materials (Horvath, 1997). The energy absorption capacity of a composite can be evaluated by the area below the compression strength versus deformation until cracks appear

in the composite and this energy reflects the tenacity of the material. The increase of the fungi cultivation period from 15 to 30 days resulted in the increase of the tenacity of 130.1%, 336.0% and 68.9% with the cultivation of the isolates LED AJU1, LED CHI and LED 96/18, respectively (Table 2), which characterizes a more tenacious material. However, the increase of the culture period from 30 to 45 days reduced in 40.1%, 26.2% and 55.4% the tenacity of the colonized composites by LED AJU1, LED CHI and LED 96/18, respectively, which characterizes that a more fragile material behavior (Table 2), also observed by Teixeira et al. (2018) with *P. sanguineus*. Thus, for composite production that require higher strength and higher tenacity, the culture *L. edodes* tested isolates should be performed preferentially for up to 30 days after complete colonization.

Table 2: Mean tenacity (MPa x mm mm⁻¹) of the composites from the area of the stress-strain curve.

Isolates fungi	Tenacity (MPa x mm mm ⁻¹), by culture period		
	15 days	30 days	45 days
LED AJU1	3.06	7.04	4.22
LED CHI	2.78	12.12	8.95
LED 96/18	8.30	14.02	6.26

The isolates of *L. edodes* cultivated in coconut powder and wheat bran present the potential for composites production in order to replace isopor[®], with the advantage of being biodegradable composites. Also it can be reused in several areas of the agricultural sector, such as in the production of seedlings, soil conditioner and organic fertilizer (Marino et al., 2017b; Fontalvo et al., 2013), and in the control of phytopathogens (Ishihara et al., 2018), which can reduce the consumption of mineral fertilizers and pesticides. Moreover, Airifin e Yusuf (2013) observed that the cultivation of edible fungi represents an ecological alternative of production of composites, when compared to the process used to obtain the EPS, because in addition to reuse the agricultural waste, releases about 10 times less carbon dioxide, which reduces the greenhouse effect on the planet and does not contaminate the environment with toxic substances.

CONCLUSIONS

The mycelial density is not influenced by the type of hyphae of *L. edodes*. Drying of the composite does not influence the residual odor of the composite, depending on the isolate. The compressive strength and foam type of the fungal composite may be influenced by the culture period and type of hypha, depending on the fungal isolate. The composites colonized by the *L. edodes* isolates presented higher mechanical resistance at 30 days of complete colonization. The coconut powder and wheat bran colonized by LED 96/18 is an ecological alternative in the production of the packaging considering its mechanical properties.

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