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Growth of *Pleurotus ostreatus* in culture media based on formulated straw or grass

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Mycelial growth strains of POS 98/38, POS 09/100, POS 09/101, and POS 09/102 of *Pleurotus ostreatus* were evaluated in culture media with various compositions based on extracts of substrates formulated with sugar cane bagasse together with straws and grasses, and with or without nitrogen supplementation. The evaluation was performed during incubation regularly with a ruler graduated in millimeters until total colonization of the culture medium contained in Petri dishes. The statistical model explaining the kinetics of mycelial growth of mushroom strains of *P. ostreatus* as a deterministic component has an exponential Gompertz function. The results show that the culture medium with sugar cane straw and brizantha grass (supplemented) showed the highest rates of mycelial growth, regardless of strain used compared to wheat straw-based culture media with had the lowest velocities of growth, regardless of supplementation and strains studied.

Key words: Oyster mushroom, grass, straw, substrate, pasteurization.

INTRODUCTION

Cultivation and consumption of mushrooms of the *Pleurotus* genus, especially the species *Pleurotus ostreatus*, have always been at a lower level than the other below the species, *Agaricus bisporus* and *Lentinula edodes,* which are more competitive in the market. However, cultivation and consumption of mushrooms of this genus in the West have increased markedly (Royse et al., 2004; Lee et al., 2002).

In Brazil, the genus *Pleurotus* was introduced around 1980, being cultivated using mainly as a raw material base, the sugar cane bagasse supplemented with wheat bran or rice bran, due to the abundance of this material in power plants of sugar and ethanol (Maziero et al., 1992). Later, low productivity and competitiveness in the use of sugar cane bagasse for energy generation forced the producers to use wheat straw and rice straw in formulations of the substrates. Meanwhile, the straws of wheat and rice are produced only in Winter and south regions of Brazil that results an increase in the cost of raw material. As a result, producers have been perpetually trying alternative materials for the production of the substrate to achieve results, both biologically and economically satisfactory (Royse et al., 2004; Tisdale et al., 2006). Dias et al. (2011) highlighted that the method of substrate preparation mostly adopted by Brazilian producers by which short composting with posterior

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Table 1. Contents of moisture, nitrogen (N), carbon (C) and C/N ratio of the grasses.

Material	Moisture (%) [1]	N (g kg^{-1}) [2]	C (g kg^{-1}) [3]	C/N ratio
Sugar cane bagasse	34.85	3.6	517	144
Braquairia grass	12.80	5.9	483	81
Tobiatã grass	13.65	15	456	30
Brizantha grass	11.80	8.7	494	56
Sugar cane straw	11.85	6.5	511	78
Wheat straw	11.15	6.0	506	84
Wheat bran	11.00	20,7	509	18

[1]Moisture materials before assembly of the substrates, dried at 65°C until constant weight. [2]Determination by the method of perchloric acid oxidation (data on a dry basis). [3]Values expressed on a dry basis.

pasteurization and conditioning contributing to reduce production costs. The degradation ability of lignocellulolytic fungi in different types of waste is due to the secretion of enzymes to obtain nutrients such as carbon and nitrogen for growth (Elisashvili et al., 2008), and their actions depend directly on the chemical composition of the substrate used and environmental conditions (Membrillo et al., 2011; Sánchez, 2010).

Most edible mushrooms, including *P. ostreatus*, have good development rates in different types of raw material (Sales-Campos et al., 2010; Sánchez, 2010). However, due to the large number and variety of waste discarded in the environment, it is essential to study the viability of using those materials in the preparation of cultivation substrates for fungal culture of food interest.

The choice of strains more adapted to a particular type of substrate is an another important factor for success in the cultivation of *Pleurotus* spp. Mycelial growth rates may differ due to temperature and humidity optimum for incubation and fruiting, resistance to contaminant fungi, size and shape of the mushroom, and its productivity (Gaitán-Hernández and salmons, 2008; Houdeau et al., 1991; Stölzer and Grabbe, 1991).

In the study of optimum conditions for fungus growth, *in vitro* cultivation had been used (Montini et al., 2006; Sales-Campos et al., 2008), being that a typical sigmoidal curve can translate that growth during a period of time, with several stages with typical physiological properties (Montini et al., 2006).

The evaluation parameters that can be adopted are: Velocity, force, and mycelial mass. Thus, the use of solid culture medium is considered appropriate because fungi commonly grow on solid substrates in nature and the use of culture medium based on extracts of the compost itself has been recommended (Andrade et al., 2008; Montini et al., 2006).

Thus, this experiment evaluated the mycelial growth of commercial strains of *P. ostreatus* in culture medium based on extracts of alternative substrates cultivation formulated with sugar cane bagasse added straw or grass, with or without nitrogen supplementation.

MATERIALS AND METHODS

Pleurotus ostreatus strains

Four strains of *P. ostreatus* were used: POS 98/38, POS 09/100, POS 09/101, and POS 09/102, which are stored at the fungal collection Mushrooms Module - FCA/UNESP.

Substrate and preparation

The grasses sugar cane straw (*Saccharum officinarum* L.), wheat straw (*Triticum aestivum* L.), brizantha grass (*Brachiaria brizantha*), braquiária grass (*Brachiaria decumbes*), tobiatã grass (*Panicum maximum* var. Tobiatã) and sugar cane bagasse were harvested between February and April 2010, sun-dried, transported, stored, and analyzed in Mushrooms Module (Table 1). The materials were chopped to a particle size of between 5 and10 cm. The methodology proposed by Brasil (2007) was used to determine nitrogen (N), carbon(C) and C/N ratio.

The formulations of the substrates were based on the adjustment of the C/N ratio initial 60:1 and 90:1 (Table 2). The establishment of C/N ratio was based on the relations of the raw materials forming the substrates.

Composting in Phase I was performed in open shed with galvanized steel roof and concrete floor. The assembly of beds was in superimposed layers of bagasse plus grass or straw with or without wheat bran with dimensions 2 m (width) × 3 m (length) × 1.2 m (height) (Table 3).

In Phase II, the substrates were filled in truss boxes (56.5 cm length × 46.5 cm width × 28.5 cm height), and randomly placed within a controlled environment chamber model Dalsem Mushrooms Projects for pasteurization to 59.5°C for 8 h and conditioned to 46.5°C for 96 h, being monitored by software VEC 32 -Dalsem.

Preparation of culture media

The culture medium was prepared based on substrates extracts, immediately after Phase II of composting. Thus, three samples were collected (4 sub-samples) of each substrate and the dried in a stove at 65°C until constant weight. With the aid of scissors, the material was fragmented into pieces of 2 to 4 cm long. Then, the aqueous extracts were prepared from boiling 40 g (dry basis) of substrate in 500 ml of distilled water for 10 min. Then, it was filtered through a 60 mesh sieve. The filtrate volume was completed to 500 ml with distilled water. Then, this filtrate was placed inside Duran glass bottles sterilized under 121°C for 30 min. After 24 h, 7.5 g of agar

Table 2. Formulation of substrate used in the preparation of culture media.

Substrate [1,2]	C/N ratio	Bagasse	Braquiária grass	Sugar cane straw	Brizantha grass	Tobiatã grass	Wheat straw	Wheat bran
A1	60	13.03	47.96	-	-	-	-	8.9
B1	60	19.55	-	48.48	-	-	-	8.9
C1	60	39.09	-	-	48.51	-	-	8.9
D1	60	97.73	-	-	-	43.18	-	8.9
E1	60	13.03	-	-	-	-	48.87	8.9
A2	90	13.03	47.96	-	-	-	-	-
B2	90	19.55	-	48.48	-	-	-	-
C2	90	65.15	-	-	48.51	-	-	-
D2	90	169.39	-	-	-	43.19	-	-
E2	90	13.03	-	-	-	-	48.87	-

[1]Substrate (materialsdry base): A. braquiáriagrass + sugar cane bagasse; B. sugar cane straw + sugar cane bagasse; C. brizanthagrass + sugar cane bagasse; D. tobiatãgrass + sugar cane bagasse; E. wheat straw + sugar cane bagasse. [2]Supplementation of the substrate: 1. With wheat bran (A1, B1 C1, D1, and E1); 2. Without wheat bran (A2, B2, C2, D2, and E2).

Table 3. Procedures adopted in Phases I and II of composting.

Day	Procedure
	Phase I
01	Wetting of materials and installation of piles
03	1ª turned over and adding water
05	2ªturned over and adding water
07	3ª last turned over and addition water, followed by accommodation of the substrate boxed truss for pasteurization and conditioning
	Phase II
08	Pasteurization chamber in Dalsem to 59.5°C for 8 h
08	Conditioning in the chamber in Dalsem to 45.6°C for 96 h.

was added and it sterilized again, thus performing the process of tyndalization. Later, 20 ml of the medium was poured in sterile Petri dishes.

Adaptation of strains

Preceding the experiment itself, there was a prior cultivation of strains in culture medium based on all the substrates, thus facilitating their adaptation to the ingredients to be used in the cultivation. The proportion of the materials for the preparation of that medium was: 40% of sugar cane bagasse + 10% of wheat straw + 10% of sugar cane straw + 10% of Brachiaria grass + 10% of Brizantha grass + 10% of Tobiatã grass + 10% of wheat bran.

Inoculation and incubation of culture medium

5 mm discs from the secondary matrix were transferred to the center of the Petri dishes containing the culture media. They were incubated at 25±1°C, in the dark.

Determination of mycelial growth of *P. ostreatus*

The mycelial growth of *P. ostreatus* was observed by periodic measurements of the diameter of the colonies. The first measurement occurred after 48 h of inoculation and at that point, the Petri dishes received eight punctual marks diametrically opposed to each other on the outside surface of their base, on the edge of the colony. The subsequent measurements were taken every 24 h. Thus, four values corresponding to the colony diameter in millimeters were obtained for each reading period. After, those values were converted into averages, which were used to compare results between treatments.

The comparisons of the statistical parameters and the subsequent construction of instantaneous speeds were carried out to study the kinetics of the mycelial growth of *P. ostreatus* in the culture medium.

Experimental design

The experiment was randomized in a factorial 10 × 4 design (media culture × strains), with five replicates. The data were subjected to variance analysis. Means were compared by nonparametric Stundent-Newman-Kills test (5%). For that, SISVAR 4.2 software was used, which was developed by the Department of Exact Sciences, Federal University of Lavras, MG (UFLA).

RESULTS

Mycelial growth velocities were followed for nonlinear

Table 4. Statistical comparison of the alpha parameter of the model of nonlinear regression[1] on the kinetics of mycelial growth of *P. ostreatus*, the according culture medium and strain (by nonparametric test Student-Newman-kills).

Culture media[2]	Strains			
	98/38	09/100	09/101	09/102
	Parameter alpha			
A1	4.64[b(3)]	4.54[c]	4.42[b]	4.60[a]
B1	4.62[b]	4.67[b]	4.70[a]	4.60[a]
C1	4.69[a]	4.56[c]	4.51[b]	4.48[b]
D1	4.63[b]	4.81[a]	4.50[b]	4.58[a]
E1	4.52[c]	4.55[c]	4.53[b]	4.56[a]

[1] $Y_{ij} = \exp\{\alpha_j \exp[-\exp(\beta_j - \gamma_j X_{ij})]\} + e_{ij;}$ [2]Culture media extract of substrate: A. braquiáriagrass + sugar cane bagasse; B. sugar cane straw + sugar cane bagasse; C. brizanthagrass + sugar cane bagasse; D.tobiatãgrass + sugar cane bagasse; E. wheat straw + sugar cane bagasse. 2. Supplementation of substrate: 1. With wheat bran (A1, B1 C1, D1, E1); 2. Without wheat bran (A2, B2, C2, D2, E2). [3]By column: Medians followed by the same latter do not differ significantly at the 5% level of significance for this Student-Newman-Kills.

Table 5. Statistical comparison of the beta parameter of the model of nonlinear regression[1] on the kinetics of mycelial growth of *P. ostreatus*, the according culture medium and strain (by nonparametric test Student-Newman-kills).

Culture media[2]	Strains			
	98/38	09/100	09/101	09/102
	Parameter beta			
A1	0.922[bc(3)]	0.852[b]	1.237[a]	0.982[a]
B1	1.067[a]	0.833[b]	0.927[b]	0.910[ab]
C1	0.869[cd]	1.071[a]	1129[a]	0.987[a]
D1	0.971[b]	0.731[c]	1.197[a]	0.990[ab]
E1	0.814[d]	0.720[c]	0.903[b]	0.831[b]

[1] $Y_{ij} = \exp\{\alpha_j \exp[-\exp(\beta_j - \gamma_j X_{ij})]\} + e_{ij;}$ [2]Culture media extract of substrate: A. braquiária grass + sugar cane bagasse; B. sugar cane straw + sugar cane bagasse; C. brizantha grass + sugar cane bagasse; D. tobiatã grass + sugar cane bagasse; E. wheat straw + sugar cane bagasse. 2. Supplementation of substrate: 1. With wheat bran (A1, B1 C1, D1, E1); 2. Without wheat bran (A2, B2, C2, D2, E2). [3]By column: medians followed by the same latter do not differ significantly at the 5% level of significance for this Student-Newman-Kills.

regression models by using the methodology of Montini et al. (2006).

The model that best explained the growth kinetics of *P. ostreatus* strains in solid culture medium has the exponential of a Gompertz function as the deterministic component, according to Ratkoswsky (1983), cited by Montini et al. (2006).

$$Y_{ij} = \exp\{\alpha_j \exp[-\exp(\beta_j - \gamma_j X_{ij})]\} + e_{ij}$$

Where Y_{ij} stands for the growth diameter (mm. dia^{-1}), X_{ij} stands for the time (days), α, β, γ are the components of the parameters vector θ; e_{ij} = random component (noise). The indexes *i* and *j* are related to the observation to the treatment (crosses), respectively.

The comparisons of statistical parameters α, β, and γ (Tables 4 to 9) referring to the mycelial growth of *P. ostreatus* strains, according to the various nonsupplemented and supplemented culture medium used. In cases where two treatments present statistical equality between parameters α, β, and γ, it means that they have equal instantaneous velocities. Otherwise, it is enough that just one parameter is different to ensure statistical difference between the instantaneous velocities.

Mycelial growth was determined by the parameters α, β, and γ that interact during the colonization of the culture medium, triggering different behaviors between strains due to the culture medium (Tables 4 to 9).

DISCUSSION

Several authors have reported differences in mycelial growth of edible mushrooms in function of strains (Andrade et al., 2008; Gomes-da-Costa et al., 2008; Marino et al., 2008). According to Nyochembeng et al. (2008), biodegradation of waste can be optimized by the selection of efficient strains, since the growth of the

Table 6. Statistical comparison of the gamma parameter of the model of nonlinear regression[1] on the kinetics of mycelial growth of *P. ostreatus*, the according culture medium and strain (by nonparametric test Student-Newman-kills).

Culture media[2]	Strains			
	98/38	09/100	09/101	09/102
	Parameter gama			
A1	0.451[b(3)]	0.455[a]	0.590[a]	0.389[c]
B1	0.481[ab]	0.386[b]	0.382[c]	0.379[c]
C1	0.408[c]	0.497[a]	0.521[b]	0.508[a]
D1	0.424[a]	0.326[c]	0.565[ab]	0.438[b]
E1	0.332[d]	0.335[c]	0.320[d]	0.288[d]

[1] $Y_{ij} = exp\{\alpha_j exp[- exp(\beta_j - \gamma_j X_{ij})]\} + e_{ij}$; [2]Culture media extract of substrate: A. braquiária grass + sugar cane bagasse; B. sugar cane straw + sugar cane bagasse; C. brizantha grass + sugar cane bagasse; D. tobiatã grass + sugar cane bagasse; E. wheat straw + sugar cane bagasse. 2. Supplementation of substrate: 1. with wheat bran (A1, B1 C1, D1, and E1); 2. without wheat bran (A2, B2, C2, D2, and E2). [3] By column: medians followed by the same latter do not differ significantly at the 5% level of significance for these Student-Newman-Kills.

Table 7. Statistical comparison of the alpha parameter of the model of nonlinearregression[1] on the kinetics of mycelial growth of *P. ostreatus*, the according culture medium and strain (by nonparametric test Student-Newman-kills).

Culture media[2]	Strains			
	98/38	09/100	09/101	09/102
	Parameter alpha			
A2	4.64[b(3)]	4.51[b]	4.58 [ab]	4.50[c]
B2	4.62[b]	4.59[b]	4.51[c]	4.47[c]
C2	4.69[a]	4.53[b]	4.50[c]	4.58[b]
D2	4.63[b]	4.53[b]	4.61[a]	4.64[a]
E2	4.52[c]	5.04[a]	4.55[b]	4.55[b]

[1] $Y_{ij} = \exp\{\alpha_j \exp[- \exp(\beta_j - \gamma_j X_{ij})]\} + e_{ij}$; [2]Culture media extract of substrate:A. braquiária grass + sugar cane bagasse; B. sugar cane straw + sugar cane bagasse; C. brizantha grass + sugar cane bagasse; D. tobiatã grass + sugar cane bagasse; E. wheat straw + sugar cane bagasse. 2. Supplementation of substrate: 1. With wheat bran (A1, B1 C1, D1, and E1); 2. Without wheat bran (A2, B2, C2, D2, and E2). By column: medians followed by the same latter do not differ significantly at the 5% level of significance for this Student–Newman–Kills.

Table 8. Statistical comparison of the beta parameter of the model of nonlinear regression[1] on the kinetics of mycelial growth of *P. ostreatus*, the according culture medium and strain (by nonparametric test Stundent–Newman–kills).

Culture media[2]	Strains			
	98/38	09/100	09/101	09/102
	Parameter beta			
A2	0.923[a(3)]	0.965[a]	1.021[b]	0.907[b]
B2	0.967[a]	0.917[ab]	1.152[a]	1.042[a]
C2	0.960[a]	0.823[b]	1.078[ab]	0.927[b]
D2	1.013[a]	0.939[ab]	1.132[a]	0.957[b]
E2	0.748[b]	0.894[ab]	0.819[c]	0.811[c]

[1] $Y_{ij} = \exp\{\alpha_j exp[- exp(\beta_j - \gamma_j X_{ij})]\} + e_{ij}$; [2]Culture media extract of substrate:A. braquiária grass + sugar cane bagasse; B. sugar cane straw + sugar cane bagasse; C. brizantha grass + sugar cane bagasse; D. tobiatã grass + sugar cane bagasse; E. wheat straw + sugar cane bagasse. 2. Supplementation of substrate: 1. With wheat bran (A1, B1 C1, D1, and E1); 2. Without wheat bran (A2, B2, C2, D2, and E2). By column: medians followed by the same latter do not differ significantly at the 5% level of significance for this Student–Newman–Kills.

Table 9. Statistical comparison of the gamma parameter of the model of nonlinear regression[1] on the kinetics of mycelial growth of *P. ostreatus*, the according culture medium and strain (by nonparametric test Student-Newman-kills).

Culture media[2]	Strains			
	98/38	09/100	09/101	09/102
	Parameter gama			
A2	0.430$^{b(3)}$	0.467^{ab}	0.439^{c}	0.441^{b}
B2	0.485^{a}	0.429^{ab}	0.522^{a}	0.486^{a}
C2	0.427^{b}	0.405^{b}	0.465^{bc}	0.388^{c}
D2	0.497^{a}	0.486^{a}	0.491^{b}	0.427^{b}
E2	0.271^{c}	0.223^{c}	0.293^{d}	0.277^{d}

[1] $Y_{ij} = \exp\{\alpha_i \exp[-\exp(\beta_j - \gamma_j X_{ij})]\} + e_{ij;}$ [2] Culture media extract of substrate:A. braquiária grass + sugar cane bagasse; B. sugar cane straw + sugar cane bagasse; C. brizantha grass + sugar cane bagasse; D. tobiatã grass + sugar cane bagasse; E. wheat straw + sugar cane bagasse. 2. Supplementation of substrate: 1. With wheat bran (A1, B1 C1, D1, and E1); 2. Without wheat bran (A2, B2, C2, D2, and E2). By column: Medians followed by the same latter do not differ significantly at the 5% level of significance for this Student-Newman-Kills.

mycelium influences mushrooms production, as reported by Silva et al. (2005). In addition, Jonathan et al. (2008) reported that a rapid mycelial colonization of *P. tuber-regium* in the selective substrates, such as wood waste from *Holoptelia grandis* and *Milicia excelsa*, significantly reduces the growth of other competitive organisms.

The behavior of mycelial growth in the culture medium was supplemented by different media (Figure 1). These results are in agreement with Pedra and Marino (2006) that using sawdust, coconut shell to evaluate the growth of *P. ostreatus*, had better mycelial growth when the sawdust supplemented with wheat bran or rice bran. Sales-Campos et al. (2008) evaluated the mycelial growth of *P. ostreatus* on waste simaroubain which supplementation favored mycelial growth and provided good colonization by the fungus.

Strains such as POS 09/101 and POS 09/102 were tested and showed no statistical differences in some culture media supplemented. The strain POS 09/101 in culture media A1 and D1 showed statistically similar growth rate compared to the intermediate media B1 and C1 with a higher growth rate and culture medium E1 with lower growth rate. For the strain POS 09/102, mycelial growth rate for the culture media A1 and B1 had no significant differences, considering the higher velocities of growth along with the medium D1; intermediate growth velocities were obtained with medium C1 and the low growth velocities with medium E1. The strain POS 98/38 showed statistical differences for all media as follows: B1>D1>A1>C1>E1. The strain POS 09/100 showed statistical differences for all culture media tested, and the highest growth rates were observed for culture media A1 and B1, with intermediate growth of the media C1 and B1 and lower growth rate for the medium E1.

In the comparisons between culture media without supplementation (Figure 2), significant difference in mycelial growth of strain POS 98/38, except for the culture media B2>D2 which showed higher growth rate and the lower growth A2>C2>E2. Donini et al. (2006) using elephant grass found no statistical differences in some treatments. Andrade et al. (2008) evaluated the growth of L. *edodes*in culture media based on sawdust from different species of eucalyptus; also no differences in the growth of the fungus from the culture media, obtaining the best result based medium *Eucalyptus citriodora*.

For strain POS 09/100 significant differences occurred for all culture media without supplementation tested with a higher growth in culture medium D2 and in the culture media A2, B2, and C2 intermediate growth and finally with lower growth medium E1. Motato et al. (2006) assessed the mycelial growth of *Pleurotus djamor* on banana waste mixed with sawdust of Jequitibá observed differences in mycelial growth due to the culture medium, the treatments consist of banana leaves provide a mycelial growth significantly higher than other treatments.

The strain POS 09/101 grew statistically different for all culture medium tested; the culture media D2 and B2.The mycelial growth was high followed by intermediate values with the culture media A2 and C2 and finally the culture medium E2. The strain POS 09/102 was also observed growth rate statistically different for all tested media, following the order similar to that observed for strain POS 09/101, D2>B2>A2>C2>E2.

The culture media based on brizantha grass and tobiatã grass independent of the supplementation showed the highest rates of mycelial growth, regardless of strains used, suggesting the feasibility of using these materials for the production of *P. ostreatus.*

The culture media supplemented highest rates of mycelial growth, proving to be efficient for the vegetative stage.

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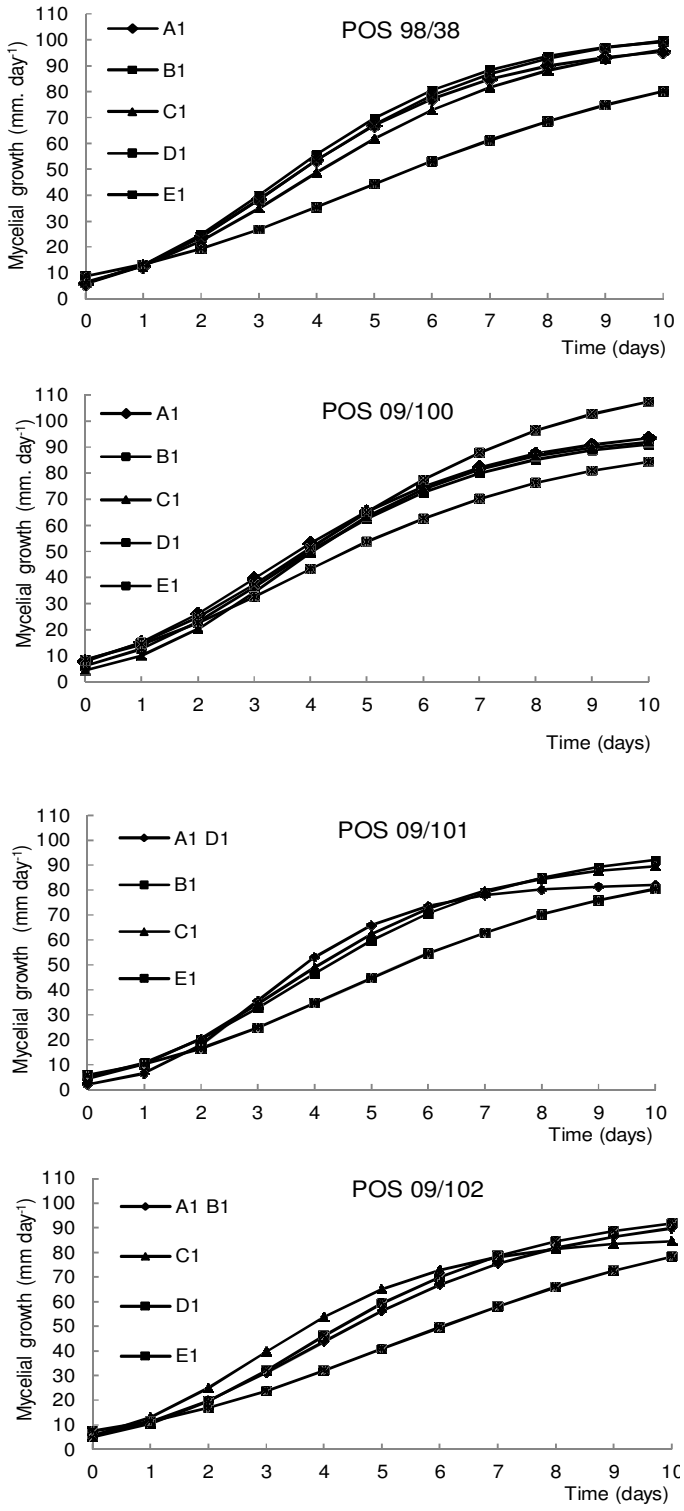

Figure 1. Estimated instantaneous velocity of mycelial growth of strains POS 98/38, POS 09/100, POS 09/101, and POS 09/102 of *P. ostreatus*, grown in culture media with supplementation: A1. braquiária grass + sugar cane bagasse; B1. Wheat straw + sugar cane bagasse; C1.brizantha grass + sugar cane bagasse; D1. tobiatã grass + sugar cane bagasse; E1. Wheat straw + sugar cane bagasse; Culture media with equal curves are not statistically different at 5% level of significance for this Student–Nnewman–Kills; mycelial growth millimeters per day.

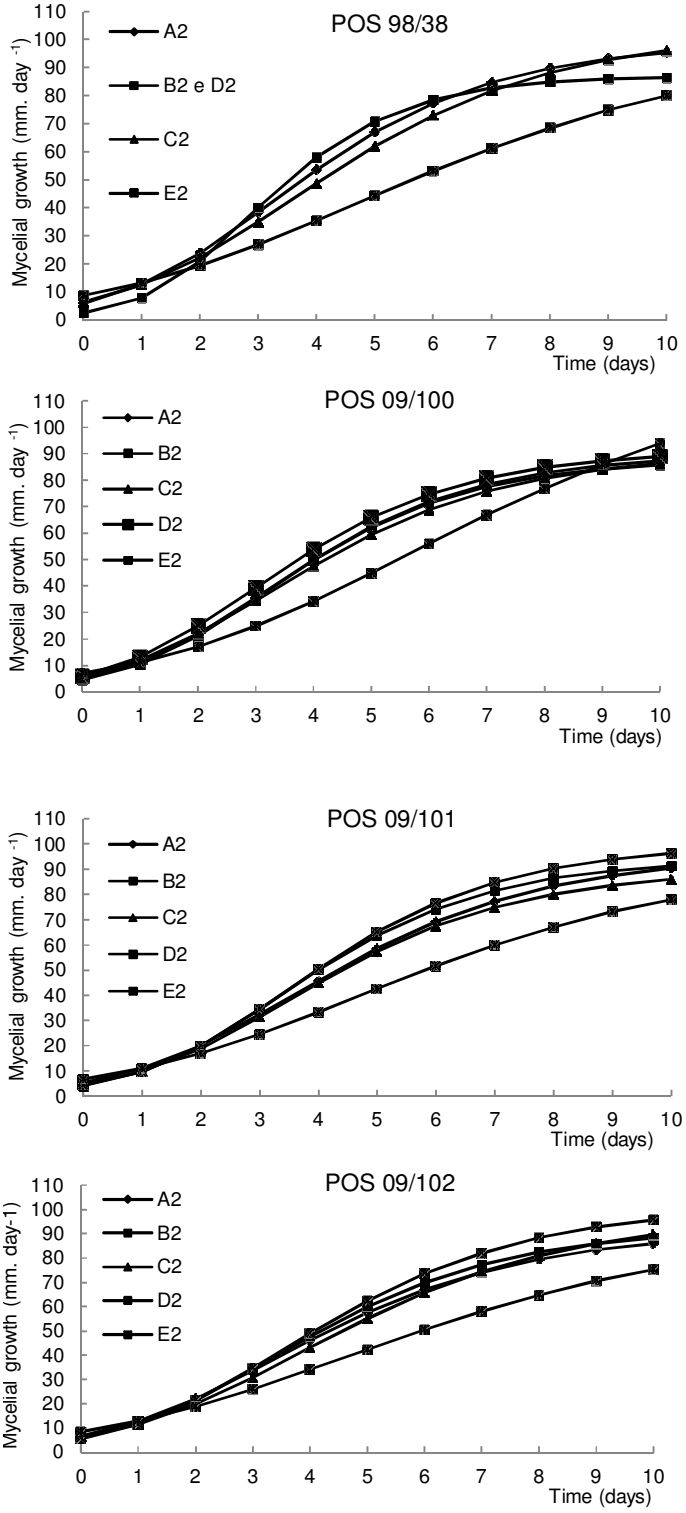

Figure 2. Estimated instantaneous velocity of mycelial growth of strains POS 98/38, 09/100, POS 09/101, and POS 09/102 of *P. ostreatus*, grown in culture media without supplementation: A2. braquiária grass + sugar cane bagasse; B2. Wheat straw + sugar cane bagasse; C2. brizantha grass + sugar cane bagasse; D2. tobiatã grass + sugar cane bagasse; E2. Wheat straw + sugar cane bagasse; Culture media with equal curves are not statistically different at 5% level of significance for this Student–Newman–Kills; mycelial growth millimeters per day.

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