AGRO-FOOD INDUSTRY WASTES AND AGRICULTURAL RESIDUES CONVERSION INTO HIGH VALUE PRODUCTS BY MUSHROOM CULTIVATION

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ABSTRACT

Several aspects of our research on the valorization of various agro-industrial residues through mushroom cultivation for the production of added-value products (mycelial biomass, mushrooms, enzymes and medicinal compounds) are presented. These comprise: (a) evaluation of lignocellulosic wastes for the production of the important edible and medicinal mushrooms genera *Pleurotus* and *Lentinula*, through examination of their growth rates and conversion efficacy to fruiting bodies, and (b) production of mycelial biomass and extracellular enzymes during solid-state fermentation (SSF) of agricultural residues by *L. edodes* and the impact of agro-residues properties on the bioconversion process. Firstly, mycelial growth rates of several *Pleurotus* and *Lentinula* strains cultivated on agro-food industry wastes and their bioconversion to fruiting bodies (monitored by biological efficiencies - BEs) are presented. *Pleurotus* species demonstrated high colonization rates on wheat straw (WS) and cotton waste (CW), while peanut shells (PS) furnished the poorest results. Recorded BEs on the former two substrates averaged between 75% and 100%. Regarding *L. edodes*, oak-wood sawdust (OS) and WS supported faster growth than corncobs (CC) and CW, while the highest average BEs ($\leq$ 100) were achieved with CC and WS. Secondly, experiments investigating the ability of several *L. edodes* strains to grow on reed grass (RG), bean stalks (BS) and WS residues are presented. Results showed the importance of simultaneous evaluation of mycelium growth rate, biomass yield and activities of hydrolytic and oxidative enzymes, along with analysis of constituents of the substrates. Data obtained support the potential effectiveness of RG and BS residues as *L. edodes* cultivation substrates.

Keywords: *Pleurotus* spp.; *Lentinula edodes*; Growth rate; Biomass; Enzymes; Fruiting bodies

INTRODUCTION

Agricultural production and the agro-food industry furnish large volumes of solid wastes, residues and by-products, produced either in the primary agro-forestry sector (crop-based) or by secondary processing industries (processing-based), the major part being lignocellulosic biomass. Recently, Zhang [1], reviewing the global world information about lignocellulose availability, estimated the production of lignocellulosic biomass to be more than 200×10^9 tons per year. Especially, the amount of crop residues produced annually in the world from 27 food crops is estimated at about 4×10^9 tons, from which 3 billion tons account per annum for lignocellulosic residues of cereals [2]. The majority of this organic matter becomes a source of environmental

problems. However, if residues are utilized, such as to enhance food production, they are not considered as wastes but new resources.

Residues such as cereals straw, corn cobs, cotton stalks, various grasses and reed stems, maize and sorghum stover, vine pruning, sugarcane and tequila bagasse, coconut and banana residues, corn husks, coffee pulp and coffee husk, cottonseed and sunflower seed hulls, peanut shells, rice husks, sunflower seed hulls, waste paper, wood sawdust and chips, are some examples of residues and by-products that can be recovered and upgraded to higher value and useful products. Current literature shows that lignocellulose degrading mushroom species are used in various SSF applications such as biodegradation of hazardous compounds and biological detoxification of toxic agro-industrial wastes [3-7], biotransformation of agro-industrial residues to mushroom food and animal feed [8-11], compost and product developments such as biologically active metabolites, enzymes, food flavour compounds and other added value compounds [12-14]. Moreover, recent research work indicates medicinal attributes in several species, such as antiviral, antibacterial, antiparasitic, antitumor, antihypertension, antiatherosclerosis, hepatoprotective, antidiabetic, anti-inflammatory, and immune modulating effects [15-17].

Commercial mushroom production, carried out in large or small scale, is an efficient and relatively short biological process of food protein recovery (regarded also as functional food) from low value lignocellulosic materials utilizing the degrading capabilities of mushroom fungi [11]. Among edible mushroom fungi, *L. edodes* and *Pleurotus* species have received considerable attention for their nutritional value, medicinal properties and biodegradation abilities [15, 17, 18]. They both are efficient colonizers and bioconverters of lignocellulosic agro-industrial residues into palatable human food with medicinal properties, with the productivity of the conversion being expressed by biological efficiency [11, 12, 18]. Their mycelium can produce significant quantities of a plethora of enzymes, which can degrade lignocellulosic residues and use them as nutrients for their growth and fructification [19]. However, the nature and the nutrient composition of the substrate affect mycelium growth, mushroom quality and crop yield of this value-added biotransformation process [20, 21].

The present paper addresses aspects of (a) lignocellulose and nitrogen composition of agro-residues and their effect on *L. edodes* and *Pleurotus* spp. growth and fructification, (b) evaluation of different residues for cultivation of *Pleurotus* mushrooms, emphasizing on their colonization and efficiency of conversion to fruiting bodies, (c) evaluation of selected residues for cultivation of *L. edodes* through monitoring mycelium growth rate, biomass yield and endoglucanase and laccase activities.

MATERIALS AND METHODS

Culture media and substrate analysis. The culture medium used for routine culture and storage purposes was Potato Dextrose Agar (PDA, Merck). Grain spawn was prepared as previously described [9]. All substrates (e.g. wheat straw-WS, mixture of reed grasses-RG or bean stalks-BS) were prepared in a ratio of 80% residue to 20% supplements (d.w), i.e. 12% wheat bran, 7% soybean flour and 1% $CaCO_3$ [20]. The residues mixture was left to soak in water for 12-24 h, and after the surplus water had been drained off, supplements were added and mixed. The moisture content of the sterilized substrates was 60-65% and the C:N ratio 50-55:1

For substrate analysis, samples were dried to constant weight in a 60 °C oven and milled to size <0.3 mm. Carbon and nitrogen concentrations were determined using a combustion-gas

chromatography technique, while cellulose, hemicellulose and lignin were determined by, ^{13}C cross-polarization CP magic angle spinning MAS NMR spectroscopy as previously described [21].

SSF in tubes, extension rate and biomass determination. Solid state fermentation was performed as previously described by Philippoussis et al. [9] in glass-tubes (200 x 30 mm) uniformly filled with the substrates to a 80 ml volume and sterilized twice for 1 h at 121 °C. The moisture content of the sterilized substrates was 62-65%. In a completely randomized design, fifteen replicate tubes per strain and substrate were inoculated with two agar plugs (6 mm diam.) cut from the periphery of actively growing mycelium on PDA, transferred onto the top of the substrate and incubated at 26 °C in the dark.

The growth rate of mycelium (mm day^{-1}), was recorded daily in a set of three test tubes by measuring the visible penetration of mycelia into the substrate in two perpendicular directions and the extension rate K_r (mm day^{-1}) was calculated after the mycelium front has reached more than 30 mm.

Twelve replicate tubes per substrate and strain were used for biomass estimation and enzymes assays. At predetermined percentages of substrate volume colonization (i.e. intervals of 4-7 days, depending on the strain and substrate used) two replicate tubes per substrate and strain were withdrawn for biomass and enzymes activity determinations. Samples, comprising the entire solid fermented medium (substrate and mycelium), were frozen (-20 °C, 48 h) and dried by a Heto LyoLab 3000 freeze-dryer (Heto-Holten Als, Denmark), milled and sieved.

The glucosamine content of the fungal cell wall was used to monitor mycelial biomass. At the beginning, a glucosamine standard curve was obtained. Moreover, the glucosamine content of mycelium for each individual strain were determined through liquid state fermentation (LSF) for 35 d, in 100 ml Erlenmeyer flasks with 50 ml Malt Extract Broth (MEB; Merck, Germany), at 26 °C under static conditions. The biomass was determined by the method of the fungal chitin hydrolysis into N-acetylglucosamine, as described by Rigas et al. [6], glucosamine was quantified spectrophotometrically (Jasco V-530 UV/VIS) and results were expressed as mg fungal biomass per g of dry substrate.

Determination of enzymes activities. For enzymes extraction, frozen colonized substrate of each replicate (equivalent to 2 g of dry sample) and 20 ml 0.05 M sodium acetate buffer (pH 5.0) were transferred to 100 ml Erlenmeyer flasks and extracellular enzymes were extracted after agitation at 100 rpm for 1 h at room temperature in an orbital shaker [11]. Endoglucanase (cellulase, EC 3.2.1.4) activity was determined using as substrate 0.5 ml of 1% (w/v) carboxymethylcellulose sodium salt (CMC; Sigma, Germany), in 0.05 M sodium citrate buffer (Merck, Germany), pH 4.8, incubated with 0.5 ml crude enzyme extract at 50 °C for 30 minutes. Laccase (EC 1.10.3.2) activity was measured spectrophotometrically using syringaldazine (4-hydroxy-3,5-dimethoxybenzaldehydeazine, Sigma, Germany) as substrate at 525 nm (extinction coefficient$^{TM}_{525}$ of 5,000 M^{-1} cm^{-1}) for about 10 min. Reaction was carried out in 3-ml cuvettes containing 0.2 ml of crude enzyme extract, 1.7 ml of 0.1 M sodium phosphate buffer at pH 6.8 and 0.1 ml of 1mM syringaldazine dissolved in absolute ethanol. Absorbance measurements were conducted by the Jasco V-530 UV-VIS spectophotometer and enzyme activities, representing the mean of 6 replicates (3 sample replicates x 2 assay replicates), were expressed as U g^{-1} (units g^{-1} of dry substrate).

Fructification in tube and bag cultivation. To induce sporophore production in tubes, sets of three replicate tubes from each one of the substrates tested (fully colonized) were subjected to a low temperature shock at 5 °C for three days, they were later placed into a 24h/day illuminated fruiting chamber (200-300 lux) at 17.5 ± 1.0 °C and the number of basidiomata was scored.

For bag-log cultivation, polypropylene-autoclavable bags were filled with 2 kg of substrate, closed with a cotton plug and sterilized twice for 1 h at 121 °C. After cooling, inoculation was carried out by adding about 60 g of grain spawn along the central vertical axis of the bag. Substrate colonisation took place in growth chambers, at 25 ± 1 °C.

For fructification and productivity evaluation, bags with fully colonized substrate were transferred to the fruiting room (used for all stages of fruiting) and subjected to a cold shock for 3 days to induce primordia formation at 15 °C, 90% relative humidity and 24 h/day illumination. During harvesting, the light intensity was set at 300 lux (24 h/day, fluorescent lamps), air exchange rates were controlled to maintain low CO_2 level (<1200 ppm), relative air humidity was adjusted between 75 and 90% and temperature was set at 17.5±1.0 °C. Mature basidiomata were harvested, counted and weighted. For all replicates, the time needed for complete substrate colonization and appearance of primordia was measured, in addition to earliness (i.e. time elapsed between the day of inoculation and the day of the first harvest). Yield (total and in individual flushes) and biological efficiency (BE: percentage ratio of fresh mushrooms harvested per dry substrate weight) were recorded.

Experimental design and statistical analysis. In all experiments, a completely randomized design was applied, using fifteen replicates per strain and substrate. Variance analysis was performed by Statgraphics Plus version 5.1 statistical package, using the Least Significant Difference (LSD) test at 5% level of probability to compare mean values.

RESULTS AND DISCUSSION

Evaluation of lignocellulosic residues for mushroom cultivation by growth characters (mycelium growth rate and biomass production). During the vegetative phase of mushroom life cycle, mycelium grows through the substrate, biodegrades its components and supports the formation of fruiting bodies. Both *L. edodes* and *Pleurotus* species reveal high efficiency in degradation of a wide range of lignocellulosic residues, due to their capability to synthesize relevant hydrolytic (cellulases and hemicellulases) and unique oxidative (ligninolytic) extracellular enzymes, responsible for the degradation of residue components into low-molecular-weight compounds that can be further assimilated to ensure fungal growth. [19].

Mycelial growth measurements of *L. edodes* and *Pleurotus* species conducted in 'race tubes' [9, 22] revealed that the type of wastes, as well as the strain used, exercised a considerable influence on the colonization rates and therefore on the time needed to complete incubation and cropping. As there is a high contamination risk at the beginning of the cultivation process, reduction of the duration of the colonization phase is of prime importance. Our experimental data suggest that both lag-phase time and mycelium extension rates play a decisive role on the efficacy of substrate colonization, with the 'race tube' method proving to be helpful for a quick evaluation of the suitability of lignocellulosic residues to serve as cultivation substrates.

Regarding *Pleurotus* spp., in our previous studies, CW (including stem-leaf residues and gin trash), WS and PS were evaluated as substrates of *P. ostreatus* and *P. pulmonarius* through

measurements of their linear growth rates in tubes. Initially, extension rates assessed on non-composted WS, CW and PS indicated that the type of substrate used had a pronounced effect on mycelium growth, with WS supporting high extension rates (8.1 – 9.6 mm/day) for all *Pleurotus* strains examined, while PS furnished the poorest results (Fig. 1). In general, WS and CW supported fast colonization and produced the best earliness values for all strains tested. Comparison of growth rates on composted and non-composted WS and CW substrates (data not shown) revealed that in the latter case faster colonization was achieved. However, a strain and substrate dependent behaviour was detected as two wild strains, one *P. ostreatus* and one *P. pulmonarius*, presented 30% reduction of colonization speed on composted WS while the growth rate of commercial strains of both species were marginally affected on composted CW as compared to non-composted CW, but it was always significantly suppressed (about 40%) in comparison to non-composted WS. However, non-composted CW promoted earlier basidiomata induction, presenting the shortest pre-harvest period of *P. pulmonarius* and *P. ostreatus* commercial strains (26 and 27 days respectively).

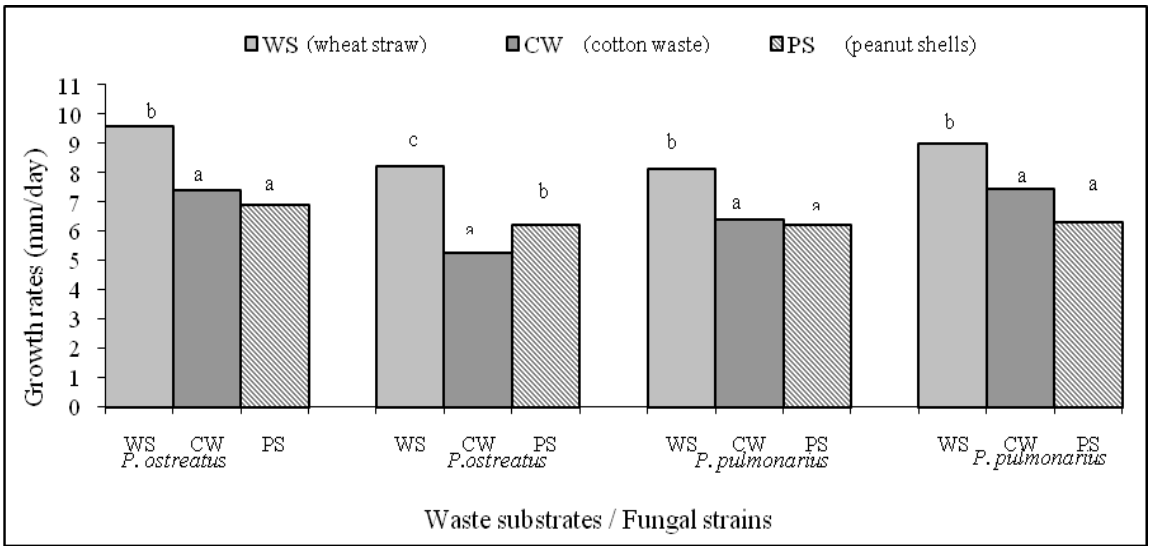

Figure 1: Effect of WS, CW and PS substrates (non-composted) on mycelial growth rates of *Pleurotus* species. (Columns within the same strain not sharing the same letters are significant different at P=0.05).

Our previous studies, evaluating several commercial and wild *L. edodes* strains as regards their efficacy of mycelium growth on WS, OS and CC, have demonstrated that OS and WS supported faster growth than CC [22]. In addition, a strain-dependent behaviour was detected since three strains performed much better on OS and WS, while one commercial strain performed satisfactorily on CC. In general, significantly lower linear growth rates were recorded for CC (Table 1). Results were verified by the fruiting technique conducted in glass tubes that furnishes a remarkable reduction in the time necessary for the first fructification (≥2 months), conducing to a quick evaluation of the production potential of tested substrates.

Recently [21], mycelium growth rates, biomass concentration (estimated as glucosamine content) and laccase and endoglucanase secretion were monitored during solid state fermentation (SSF) of wheat straw (WS), reed grass (RG) and bean stalk (BS) residues by *Lentinula edodes*

strains 119, 121 and 122. In a first experiment, these strains were subjected to screening regarding their growth rates and biomass yield, where strain 121 proved to be the fastest colonizer. However, the greatest biomass yield at the end of colonization was demonstrated by strain 122 on BS (465.93 mg g^{-1} d.w.).

Table 1: Lag time t (r=30mm), full colonization period and linear extension rates *Kr* during growth of two *L. edodes* strains on OS, WS and CC lignocellulosic substrates in 'race tubes'.

Strains	Substrates	Lag time t (r=30mm) (days)	Full colonization period (days)	Extension rate *Kr* (mm/ day)
S4080	OS	9	29	4.8 b*
	WS	12	36	4.3 b
	CC	17	45	3.4 a
SIEF 0231	OS	14	34	4.4 b
	WS	22	51	3.2 a
	CC	20	56	2.6 a

*Values within the same column followed by the same letter are not significant different (P=0.05)

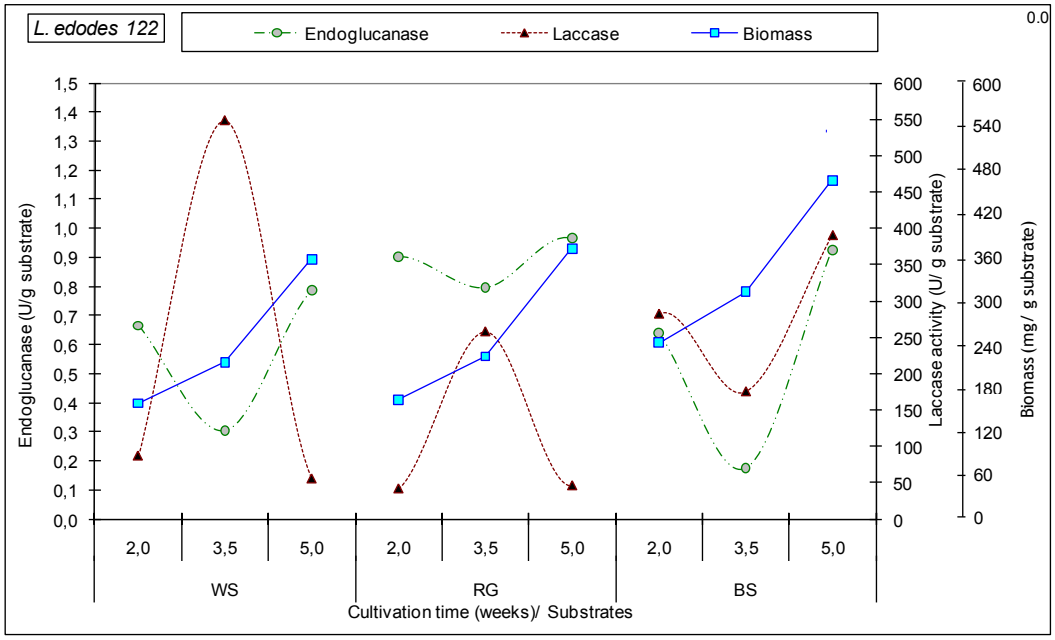

Figure 2. Patterns of biomass, endoglucanase and laccase production, monitored at 33%, 66% and 100% of WS, RG and BS colonization, during SSF by *L. edodes* strain AMRL 122.

In a second experiment, biomass, as well as endoglucanase and laccase production patterns of the selected strains 121 and 122 were monitored at three intervals i.e. at 33%, 66% and 100% of substrate colonization. BS furnished the highest endoglucanase production for strain 121, while RG for strain 122. A strain and substrate-dependent behaviour of the enzyme secretion was detected, with strain 122 presenting maximal endoglucanase activity in all substrates at the initial (33%) and final (100%) stages of colonization (0.64-0.90 and 0.79-0.97 U g^{-1} respectively)

(Fig 2). However, in strain 121 the peak of endoglucanase production was detected in the early stages of colonization (at 33% on WS and at 66% on RG and BS). Laccase activity showed increased values (maxima on WS, 353.68 and 548.67 U g^{-1} by strains 121 and 122 respectively) at 66% of colonization. Correlation analysis of growth data demonstrated negative relations between growth rate and biomass yield and between laccase and endoglucanase activities on WS and RG substrates fermented by strain 122. Correlation analysis between growth parameters and nutritional constituents of the substrates showed that biomass production rate is significantly ($p<0.05$) positively correlated with nitrogen content of the substrates ($R^2=0.99$), and with their hemicellulose content ($R^2=0.78$).

Evaluation of residues by mushroom crop yield and bioconversion efficiency (BE). The efficacy of residues bioconversion process and the productivity of the mushroom crop are assessed by the biological efficiency (BE). Apart from the type of substrate and stain used, yield response is determined by the duration of the cropping period and cultivation practice applied e.g. high spawn levels enhance mushroom yields [23].

A significant number of agro-industrial lignocellulosic materials are used as substrates for the production of *Pleurotus* species [11]. The overall BE values obtained on these residue-substrates for *P. ostreatus* and *P. pulmonarius* strains indicated that among all residues cardboard, coffee pulp, paper wastes and softwood residues, presented the highest (≥100%) biological efficiencies [24, 25, 26] BEs between 75% and 100% were recorded on cotton wastes and wheat straw [21, 27] while satisfactory productivity (BEs 50-75%) is demonstrated by most of agro-industrial residues, namely corncobs, various grasses and reed stems, vine shoots, cottonseed hulls and sugarcane bagasse [11].

In our experiments *P. ostreatus* and *P. pulmonarius* strains presented significantly higher yields and size of basidiomata on WS and CW in comparison to PS (Fig. 3). *P. pulmonarius* furnished significantly better yields than *P. ostreatus* with BE values on WS and CW, varing respectively for *P. ostreatus* between 87.37-94.39 and 70.61-116.70 and for *P. pulmonarius* between 81.39-123.07 and 92.87-97.87. Regarding straw pre-treatment, data demonstrated an approximate 20% reduction of overall BE when *P. ostreatus* is cultivated on non fermented wheat straw, as compared to fermented substrate (mean values 70.5% and 85.5% respectively). On pretreated wheat straw, supplementation with cotton seed cake and soybean cake proved to enhance productivity of *P. ostreatus* [27, 28].

Regarding *L. edodes*, studies were conducted for evaluating WS, CC and OS bio-transformation efficiency in respect of substrate colonization, earliness of sporophore production, biological efficiency and mushroom nitrogen content as well as basidiocarps number and size. WS and CC substrates furnished significantly higher crop yields (BEs) than OS, with the highest BE (80.64%), and the heavier fruit bodies (21.40 g) being recorded on CC substrate. WS appeared to promote earliness and furnished good crop productivity and quality results. However, the higher mushroom protein content was detected from OS substrate. Apart from higher N content of CC and WS which contributed to higher productivity [29], their high yields may also be attributed to their high water soluble sugar contents, particularly hemicelluloses [30], which could have exercised a positive effect during the active growth phase, prior to the breakdown of lignin and cellulose. Also, Moyson and Verachtert [31] have demonstrated that substrate decomposition by *L. edodes* is initially associated to its hemicellulose content.

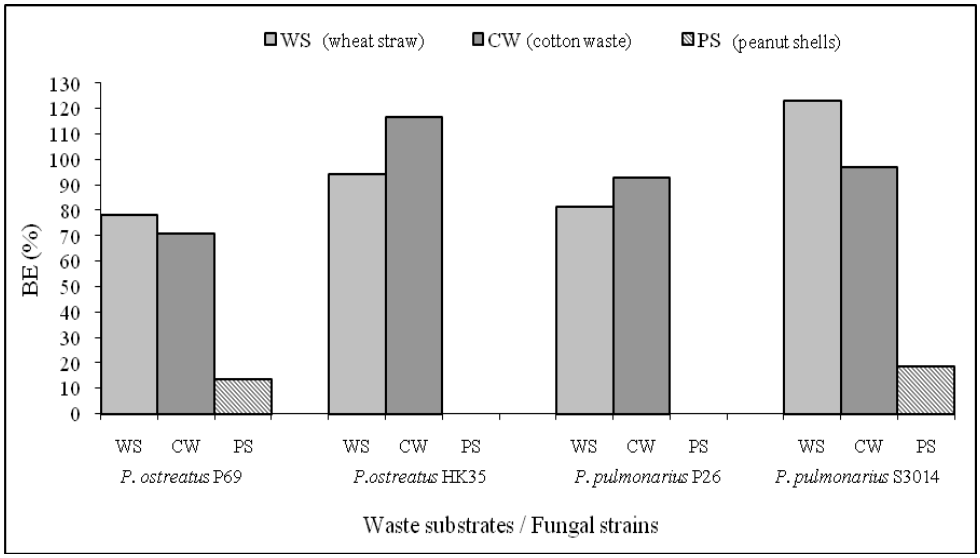

Figure 3: Productivity evaluation (BEs) of *P. ostreatus* and *P. pulmonarius* strains on WS, CW and PS waste-substrates.

Impact of lignocellulose and nitrogen composition of agro-residues on *Pleurotus* and *Lentinula* species cultivation. Solid agro-industrial residues are heterogeneous water insoluble materials having as common feature their basic macromolecular structure being cellulose, hemicellulose and lignin. Cellulose and hemicellulose (the main sources of carbohydrates) are often incrusted with lignin, which forms a physical seal around these two components. The proportions of the three structural components along with nitrogen content of residues affect mycelium growth, mushroom quality and crop yield [9, 21, 22, 38]. In fact, the general strategy of white rot mushroom-forming fungi, the most efficient degraders comprising popular cultivated species like *L. edodes* and *Pleurotus* species, is to decompose the lignin in wood, so that they can gain access to the cellulose and hemicelluloses embedded in the lignin matrix.

Understanding the impact of substrate on mushroom productivity and quality is valuable to determine the combination of suitable substrate composition and fungal strain that bioconvert effectively the agricultural residues into nutritional and medicinal food. Since the carbon sources utilized by basidiomycetes are usually of a lignocellulosic character, fungi during vegetative growth produce a wide range of enzymes to degrade the lignocellulosic substrates. Data obtained in various studies demonstrate that the type and composition of lignocellulosic substrate appear to determine the type and amount of enzyme produced by basidiomycetous fungi during vegetative growth [19, 20, 32, 38]. Finally, the nature and the nutrient composition of the substrate affect mushroom quality and crop yield of this value-added biotransformation process [9, 21, 22]. Investigations of the impact of substrate constituents on mycelium growth and mushroom production demonstrated that both are affected by cellulose, hemicellulose and lignin proportions along with nitrogen content of the cultivation substrate [22, 33].

The edible mushrooms belonging to the genus *Pleurotus* are commercially grown on pasteurized straw-based substrates or hardwood sawdust, with added supplements. However, as these fast-growing mushrooms display a complete lignocellulolytic enzyme system [34], they can use a wide spectrum of agricultural and industrial wastes that contain lignin and cellulose for growth and fruiting [35]. In our previous studies [9], CW, WS and PS were evaluated as

substrates of *P. ostreatus* and *P. pulmonarius* cultivation. *Pleurotus species* demonstrated high colonization rates and BE's on WS and CW, while PS furnished the poorest results. Chemical analysis of the substrates demonstrated that nitrogen level in WS was approximately half of that determined in CW and C/N ratio almost double of the values measured for the PS. CW presented the higher cellulose content while the cellulose: lignin ratio was found to be 5-fold more in the WS and CW than in the PS. The high lignin content of the latter was probably the cause of the slow mycelium growth, as cellulose may not be readily available as carbon source. Cellulose/lignin ratios of WS and CW substrates were positively correlated to mycelial growth rates and mushroom yields of *P. ostreatus* and *P. pulmonarius*. Regarding the influence of nitrogen availability, our studies revealed a positive correlation between the C/N ratio and mycelium growth rates of *Pleurotus* species [8]. In fact, the highly positive relation of mycelium growth with C/N and cellulose: lignin ratios were more significant for *P. pulmonarius* than for *P. ostreatus*.

Additional data furnished by the 'race-tube'method experiments provided an estimate of the potential of WS, CW, PS, poplar sawdust and CC to serve as alternative mushroom cultivation substrates [36]. Furthermore, in recent studies conducted to evaluate different grass and reed stalks as cultivation substrates of *Pleurotus* species [10, 37] bean plant residues (BS) and a mixture of reed-grass maces (RG) supported fast colonization rates for both genera strains, while BS enhanced laccase and endoglucanase activities [37].

As far as *L. edodes* cultivation is concerned, this efficient wood degrader can be grown on a variety of agro-industrial residues such as oak, ash, poplar, alder, eucalypt, beech, pine, maple and birch sawdust, cereal straws (mainly barley and wheat), corn cobs, sugarcane bagasse, sunflower seed hulls, peanut shells, cotton straw and seed hulls, vine shoots, coffee husk and pulp etc [11]. Substrate bioconversion efficacy and successful cultivation of shiitake mainly depends on the development of mycelia and complete colonization of the substrate in the first cultivation stage. As there is a high risk of contamination at the beginning of the cultivation process, reduction of the duration of the colonization phase is of prime importance.

Table 2: Correlation (R^2 values) between substrate chemical properties and mushroom production characteristics of *L. edodes* AMRL 121 grown with the 'bag-log'method.

	Chemical constituents		
Parameters	C%	N%	C/N
Earliness [a]	NS	(-) 0.8138*	(+) 0.8179*
Mushroom number	NS	(+) 0.9735**	(-) 0.9800**
BE% [b]	NS	(+) 0.9663**	(-) 0.9652**
Mushroom average weight	NS	NS	NS

[a] Period (days) from inoculation to the first sporophore harvest

[b] Biological efficiency: percentage ratio of fresh mushrooms harvested per dry substrate weight

[c]NS: Not significant. * Significant at P=0.05, ** Significant at P=0.01

Correlation analysis of carbon and nitrogen content of WS, CC and OS substrates influence on shiitake mushroom yield and quality (Table 2) revealed that nitrogen content of the substrates affected earliness of fructification and productivity [10]. Earliness and crop cycle duration were found to be related to the nitrogen content and consequently to C/N ratio values of substrates. Actually, the positive relation found between pre-harvest period and C/N ratio, which explains earliness of fructification on the substrate mixtures with lower C/N ratio, is consistent with

previous reports indicating the positive effect of nitrogen in ligninolytic activity during *L. edodes* cultivation [29, 37]. Also, Philippoussis et al. [22] have demonstrated that mycelium extension rate is related to bioavailability of nitrogen and that substrate formulation affects nutritional and porosity levels (O_2 availability). Moreover, data obtained in this investigation showed that there is a strong negative correlation between mushroom yield (mushroom number and BE%) and C/N ratio of the substrates.

In our recent work [21], the rate of lignocellulosic residues colonization, through monitoring and comparing *L. edodes* mycelium growth, biomass yield and enzyme activities in SSF of WS, RG and BS substrates was investigated, as well as possible relations of the tested growth parameters with composition of substrates regarding cellulose, hemicellulose and lignin percentages, as well as carbon and nitrogen contents (Table 4). Correlation studies revealed that biomass production rate is significantly positively correlated with nitrogen content of the substrates (R^2=0.99) and with their hemicellulose content (R^2=0.78). However, substrates as RG, with higher lignin and lower cellulose content appeared to support higher levels of endoglucanase and lower values of laccase. Correlation analyses demonstrated significant positive relation of endoglucanase production with initial hemicellulose content of the substrates (R^2=0.76) and negative relation with cellulose per hemicellulose ratio (R^2=0.90) and of laccase activity with lignin per hemicellulose ratio (R^2=0.86).

Table 4: Analysis of the main constituents of WS, RG and BS substrates at the beginning of SSF

	Carbon %	Nitrogen %	C N^{-1}	Cellulose %	Hemicellulose %	Lignin %	Cellulose $lignin^{-1}$	Cellulose $hemi\text{-}cellulose^{-1}$
Residues[1]								
WS	37.70	0.64	58.81	80.03	6.32	8.51	9.40	12.66
RG	45.30	0.63	72.02	74.68	6.78	13.13	5.69	11.01
BS	45.60	0.80	57.07	80.39	5.73	8.41	9.56	14.03
Substrates[2]								
WS	40.0	1.26	31.82	68.93	11.16	7.58	9.10	6.18
RG	46.1	1.25	36.94	64.65	11.53	11.27	5.73	5.61
BS	46.34	1.38	33.48	69.22	10.69	7.50	9.23	6.48

[1]Raw residues (before mixing with supplements)
[2]Cultivation substrates (after mixing with 12% wheat bran, 7% soybean flour and 1% $CaCO_3$)

CONCLUSION

This paper addresses evaluation of several lignocellulosic wastes for the cultivation of two important mushroom genera, *Pleurotus* and *Lentinula* that have nutritional or pharmacological value. The ability of the different mushroom species to utilize various substrates depends on both mushroom- and substrate-associated factors. Understanding the impact of residue-substrates on bioconversion process and the use of their nutritional elements by the mycelium for growth and fruiting is a prerequisite in order to determine the combination of suitable substrate composition

and fungal strain towards developing an efficient method of fermentation or cultivation to obtain palatable nutritional and medicinal food and other high value compounds.

Data concerning analysis of residue-substrate components, their colonization and bioconversion efficiencies to fruiting bodies demonstrated CW as a promising alternative substrate for *P. ostreatus* and *P. pulmonatius* cultivation, commercially grown on pasteurized straw-based substrates or hardwood sawdust. Cellulose/lignin ratios of WS and CW substrates were found to be positively correlated to mycelial growth rates and mushroom yields of *P. ostreatus* and *P. pulmonarius*, while a positive correlation between the C/N ratio and mycelium growth rates of *Pleurotus* species was detected.

Regarding *L. edodes*, the presented results support its efficient production on a mixture of WS (supports earliness and quality) and CC (supports high yield) in the framework of utilizing cereal residues as an alternative to wood. Moreover, results demonstrated the importance of simultaneous evaluation of mycelium growth rate, biomass yield and activities of hydrolytic and oxidative enzymes during residues bioconversion, along with analysis of their constituents. The results obtained showed that high mycelium growth rate and biomass yield, as well as high endoglucanase and laccase production during vegetative growth of *L. edodes* are usually negatively related variables. Their desirable coincidence depends on the fungal strain and is strongly influenced by the nature and composition of the lignocellulosic substrate.

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