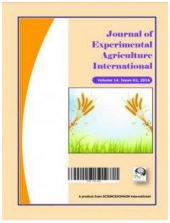

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Evaluation of Suitability of Various Agro-Wastes for Productivity of *Pleurotus djamor, Pleurotus citrinopileatus* and *Pleurotus eryngii* Mushrooms

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

The objective of this study was to evaluate the suitability of various lignocellulosic wastes for the cultivation of *Pleurotus djamor, Pleurotus citrinopileatus* and *Pleurotus eryngii* and to determine the correlations between lignocellulosic content of agricultural wastes and productivity of *these mushroom species*. In the study, *Pleurotus djamor, Pleurotus citrinopileatus* and *Pleurotus eryngii* were cultivated on oak sawdust (OS), bean straw (BS), safflower hay (SH) and sunflower head residue (SFH). Substrates were analysed for cellulose and lignin content using acid detergent fiber methods Neutral detergent fiber (NDF), Acid detergent fiber (ADF) and Acid detergent lignin (ADL), while N content was determined by Kjeldhal method. Several cultivation parameters (spawn running time, time to first primordia initiation, time to first harvest, yield, biological efficiency (BE%) and avarage mushroom weight) were evaluated during cultivation cycle. The most suitable substrates for mycelial growth showed to be SH and BS while mycelial growth was slower on OS substrate for all *Pleurotus* species tested. Biological efficiency of *P. djamor* and *P.eryngii* cultivated on SH (77.8% and 73.1, respectively) and BS (78.2% and 67.0%, respectively) were higher than OS (62.5% and 66.6%, respectively). The most suitable substrate for *P. citrinopileatus* was OS (73.9%). It was not found a correlation between chemical content of growing substrates and yield of *P. djamor* and *P. eryngii*. On the other hand, unlike *P. djamor* and *P. eryngii*, the positive relationship obtained between yield of *P. citrinopileatus* and cellulose (r^2 =0.973) and lignin contents (r^2 =0.991) of

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growing substrates. This result shows that cellulose and lignin contents have not got influence on fructification of *P. djamor* and *P. eryngii,* but are an important factor for fruit body formation of *P. citrinopiletus*. Based on the biological efficiency of the substrates tested, bean straw and safflower hay could be recommended as an alternative substrate to sawdust and wheat straw for *P. eryngii* and *P. djamor* cultivation. Morever, sunflower head residue may be used as an alternative for *P. citrinopileatus*.

Keywords: Pleurotus spp; safflower hay; bean straw; sunflower head residue; oak sawdust; cellulose; hemicellulose; lignin.

1. INTRODUCTION

Over the decades ago, mushrooms have been recognized as important food item because of their nutritional values and therapeutic properties. They are considered as a good source of protein and polysaccharides. It has been determined that more than 3000 mushroom species are edible, but only ten of those are on an industrial scale [1]. The most cultivated mushroom worldwide is *Agaricus bisporus*, followed by *Pleurotus* spp. that constitutes about 27% of the world's cultivated mushrooms, with 5 to 6 cultivated species. From 1997 to 2010, *Pleurotus* spp. production has increased at a rapid rate worldwide from 876 t to 6.288 t (618%) [2].

Pleurotus spp. contain various types of vitamins and amino acids, high content of fiber and protein and low fat content [1]. Besides nutritional attributes of *Pleurotus* spp, the health benefiting effects like anticancer [3], antihyperlipidemic [4], antioxidant [5,6] hepatoprotective [4,7], antiflammatory [8], antimicrobial activities [9] make them a health food.

Cultivation of mushrooms produces large amounts of protein on substrates consisting primarily agro-lignocellulosic wastes materials [10]. Preparation of growing substrate is considered the most critical stage in the mushroom cultivation [11,12,13]. Mycelial growth and fruit body formations of mushrooms are greatly affected by wood species and quality [14]. Growing substrate provides nutrients and physical support needed for mushrooms to complete their life cycle [1]. The mushroom growing substrates consist of three different groups: the basal material, the additive materials and other materials such as limestone and gypsum. The basal materials are generally rich in cellulose, lignin and hemicellulose and they constitute 60-85% of the growing substrate. The additive materials are rich in nitrogen, fat and carbohydrates and are added at a rate of 15-35% [15].

White rot fungi are the most efficient lignin degraders in nature. *Pleurotus* spp. is white rot fungus which has the most efficient ligninolytic activity. The ability of these fungi to degrade lignin varies greatly depending on the species and the growing media [16]. Several materials are used, including agricultural wastes, depending on the region to be produced in preparation of growing substrate of these mushrooms. Although sawdust and wheat straw are commonly used and preferred as basal medium for *Pleurotus* cultivation on commercial scale. It may not be possible to have sawdust in large quantities in all regions of world. On the other hand, wheat straw is often utilized as animal feed, which limits its availability. So, other sources of substrates need to be identified for mushroom cultivation in regions where sawdust and wheat are not available or expensive

A large volume of phaseolus (*Phaseolus vulgaris*), sunflower (*Helianthus annuus*) and sufflower (*Corthamus tinctorius*) are produced in arid area of Turkey. Aboveground of the biomass of these plants except seeds are stem but it is not used commercially. These by-products are left to rot in the field or are disposed off through burning. It is possible to find these agrowastes easily and at low cost. The objective of this study was to evaluate the suitability of various lignocellulosic wastes in abundant amounts in arid areas to replace sawdust for the cultivation of some *Pleurotus* spp. Morever, it was aimed to describe the correlations between lignocellulosic content of agricultural wastes used and mycelial growth and yield of mushroom.

2. MATERIALS AND METHODS

2.1 Materials

Strains of *Pleurotus djamor*, *Pleurotus citrinopileatus* used in this study were originally isolated from fruit bodies obtained from commercial sources (Agroma, Denizli, Turkey). The pure cultures of *Pleurotus eryngii* was

obtained from Ataturk Agricultural Research Instutue, Yalova, Turkey. The cultures were maintained on potato dextrose agar medium (PDA) (Merck) at 4ºC.

Stem of phaseolus (BS), sunflower head residue (SFH) and safflower hay (SH) obtained from local markets (Kırşehir, Turkey). Oak sawdust (OS) was purchased at Izmir, Turkey. The study was carried out at the Mushroom Production Unit of Agriculture Faculty of Ahi Evran University in Kırşehir, Turkey.

2.2 Spawn Preparation

Wheat grains were boiled in water bath for 10-15 min. at the ratio of 1:1 (wheat grains: water) and mixed with 2% (w/w) $CaCO_3$, filled to bottles and sterilized in an autoclave at 121ºC for 30 min [17]. After sterilization, the bottles were inoculated with actively growing mycelium of the *Pleurotus spp.* from Potato Dextrose Agar (PDA) and incubated at 25±2ºC for mycelial growth until the mycelium fully covered the grains.

2.3 Substrate Preparation

The material was ground in a Wiley mill and screened with sieves to obtain particle sizes that ranged from 20 to 48 mesh size.

Substrates was prepared using BS, SFH and SH (at the rate of 80%), wheat bran (%19) gypsum (1%) on the dry weight basis of the substrate and were mixed thoroughly with water. OS as a control substrate was supplemented with wheat bran (%19) and gypsium (1%) (Table 1). About 1 kg growing substrate (±10 g) was filled into polypropylene bags. The bags were plugged with a cotton plug, then autoclaved at 121ºC for 90 min. After cooling, substrates in bags were inoculated with approximately 30 g of spawn using surface spawning technique under laminar flow [18]. The inoculated substrates were incubated in the dark at 25°C for mycelial growth. After full colonization, the cotton plugs were removed, temperature and humidity were changed to 16-18°C and 85%, respectively. Cool white fluorescent bulbs provided 8 h of light daily, sufficient air changes were maintained for primordial initiation and fruiting body development.

Mushroom fruiting bodies were harvested when the mushroom cap surface was flat to slightly up-rolled at the cap margins. The yield of mushrooms and their different quality parameters were recorded regularly.

2.4 Evaluation of the Cultivation Parameters

Several cultivation parameters were evaluated during *Pleurotus* spp. cultivation on different substrates. The following data were recorded; spawn running time (day), time to first primordia initiation (day), time to first harvest (day) yield (g/kg), biological efficiency (BE%) and avarage mushroom weight (g).

Yield expressed as grams of fresh mushrooms harvested at maturity per gram of wet substrate (w/w), biological efficiency (BE%) defined as the percentage ratio of the fresh weight of harvested mushroom per gram of dry substrate [19].

2.5 Substrate Analysis

Substrates were oven-dried at 60 ^{o}C for 48 h and ground to pass through a 1 mm sieve. The ash and C were determined by standart procedure [20]. The Kjeldhal method was used to determine the total nitrogen content of each substrate. Then the carbon/nitrogen ratio of each substrate was calculated.

Substrates were analysed for cellulose and lignin content using acid detergent fiber methods Neutral detergent fiber (NDF), Acid detergent fiber (ADF) and Acid detergent lignin (ADL) were determined using the method described by Van Soest et al. [21]. Hemicellulose was calculated as the difference between NDF and ADF while cellulose is the difference between ADF and ADL [22].

Table 1. Content of substrates used in the study

	Ash (%)	N(%)	C(%)	C:N	Cellulose(%)	Hemicellulose(%)	Lignin(%)	Cel:Lig
OS	4.95**^{b}	0.63**^{d}	47.53**^{a}	75.33**^{a}	41.80**^{a}	10.77**^{d}	22.07**^{a}	1.89**^{d}
SH	5.60^{a}	1.16^{a}	47.20^{b}	40.81^{c}	29.60^{d}	19.47^{a}	6.57^{c}	4.51^{b}
BS	5.90^{a}	0.98^{b}	47.05^{b}	40.02^{bc}	31.90^{c}	18.30^{b}	7.20^{c}	4.43^{a}
SFH	5.22^{b}	0.81^{bc}	47.39^{a}	58.76^{b}	36.50^{b}	12.07^{c}	13.60^{b}	2.68^{c}

*Asterisks indicate significance at *P <0.05, **P <0.01; values within the same coloumn followed by the same letter are not significantly different. Mean values in the same coloumn followed by the same letters are not significantly different by Tukey's test.*

Table 2. Chemical composition of growing substrates tested in the study

Growing substrate code	Basal material (80%)	Addetive material (19%)	Other material (1%)
OS	Oak sawdust	Wheat bran	Gypsum
SH	Safflower hay	Wheat bran	Gypsum
BS	Bean straw	Wheat bran	Gypsum
SFH	Sunflower head residue	Wheat bran	Gypsum

2.6 Experimental Design and Statistical Analysis

In the experiments complete randomized design with ten replicates and three species of *Pleurotus* and four types of substrate were tested.

The data obtained from the experiment were subjected to variance and means analysis, and the statistical significance was compared employing Tukey's test, using the SPSS 16.0 for Windows statistical computer program at a significance level of 5%. Correlation analyses were carried out to determinate the relationship between chemical constituents of substrate and spawn running time, yield and BE (%).

3. RESULTS

The main physicochemical properties of the growing substrates are presented in Table 2. The differences in carbon, nitrogen and C:N ratios were statistically significant (*P <0.01*).

Nitrogen (N) concentration of SH (1.06%) was significantly richer than all other substrates in, followed by BS (0.87%). The OS and SFH had the lowest nitrogen contents (0.63% and 0.71%, respectively). The highest carbon content was in OS and SFH (47.53% and 47.39% respectively) while the lowest carbon content was in BS (41.05%), followed by SH (47.20%) . The highest C:N ratio (75.33) was obtained in OS while the lowest C/N ratio (45.06) was obtained in SH. As regards the individual fibre constituents, SH presented the highest values for hemicellulose (19.47%) and the lowest cellulose (29.6%) and in lignin (7.2%), followed by BS (18.3% hemicellulose, 31.9% cellulose and 6.57% lignin). In contrast, OS showed very low content in hemicelluloses and very high in cellulose (41.8%) and lignin (22.07%). The fibre content of SFH was similar with OS (12.07% hemicellulose, 36.5% cellulose and 13.66% lignin).

The spawn running time, time to first primordia initiation, time to first harvest of the three species grown on four tested substrates are presented in Table 3.

The periods of mycelium colonization of the four substrate were significant different for all of *Pleurotus* spp. *(P<0.01)*. Among the different agrowastes evaluated as a substrate for the cultivation of *Pleurotus* spp., SH supported fast colonization and produced the best earliness values for all species tested, followed by BS. *P. djamor* was the fastest colonizer among all *Pleurotus* spp tested. *P. djamor*, *P. citrinopileatus* and *P. eryngii* needed 16.4 days, 20.2 days and 20.4 day to colonize SH, respectively, while the colonization periods were 21.4, 24.2 and 24.2 days on OS for same *Pleurotus* spp., respectively. In *P. djamor, P. citrinopileatus* and *P. erygii*, spawn run time was found to be negatively correlated to N content (r^2= -0.955, r^2=- 0.962, r^2= - 0979), hemicellulose (r^2= 0.996 and r^2=0.976, r^2= 0.979), cellulose:lignin ratio (r^2= 0.988, r^2=0.996 and r^2= 0.997) and positively correlated to lignin (r^2= 0.957, r^2=0.952 and r=0.984) and cellulose (r^2=0.972, r^2=0.970 and r^2=0.987)

There was significant difference *(P<0.01*) between substrates on time to first primordia and time to first flush in *P. djamor*, *P. citrinopileatus* and *P. eryngii*. *P. djamor* were the most precocious of all species tested, forming their first primordia after 19.3- 25.2 days of incubation depending on substrate. OS promoted slower colonization than the other three substrates. fructification in this substrate was also induced later. The first flush started 3.5 days after the appearance of primordia on BS, while it started 6.2 days after the pinheading on SFH.

Time to the appearance of primordia for the substrates varried from 24.4 days to 29.8 days in *P. citrinopileatus*. Primordia initiation in *P. citrinopileatus* occurred earlier on SH substrate, followed by BS. OS and SFH substrates exhibited a later initiation. The first flush started 4.2 (OS) –6.8 days (SFH) after the appearance of primordia depending on substrate.

The substrate SH gave the fastest mycelial growth, however, this did not correspond with the appearance of primordia. The primordia started appearing 10.0 days after completion of mycelial growth in *P. eryngii* grown on BS, while primordia initiation on SFH occurred after 20.0 days. First flush started 37.6 days, 38.4 days, 45.8 days and 50.2 days of incubation on BS, SH, OS and SFH, respectively.

The yields and BEs of the four substrate were significant different for all of *Pleurotus* spp. (*P<0.01*). The results presented in Table 4 revealed that significantly better mushroom yields and BEs were obtained when *P. djamor* were cultivated on SH; followed by BS, while SFS performed less well. All of flushes were harvested within 60 days of cropping in all substrates, resulting in 248.84 gr/kg and 258.14 g/kg total yield and 77.8% and 78.2% average BE for P. djamor cultivated on SH and BS, respectively. Maximum yield was obtained in the first flush (over 60%); second flush followed by 26-30 %, the rest being harvested in the third flush on BS and SH. Morever, OS and SFS presented more similar yield distribution from all flushes. Similar was also the yield distribution on SFH and OS, particularly for the first flushes on SFH (52%) and on OS (54%). Although, mycelial growth was completed on SFH substrate in shorter time more than sawdust, yield was lowest on SFH for P. djamor.

Maximum yield (221.6 g/kg) and BE (73.9%) were obtained on OS substrate for *P. citrinopileatus*, which was distributed in two flushes with maximum yield in first flush. Although SFS appeared to be the worst performing substrate for *P. djamor*, this substrate appeared to be more suitable than BS and SH for *P. citrinopileatus* cultivation as a growing substrate especially as regards crop yield and avarage mushroom weight. On the other hand BS and SUS may be also sustained satisfactory productivity presenting overall range of BE (50.7% and 48.7%), respectively. The distribution of crop yield among individual flushes was similar on all substrates. More than 70% of the total yield was obtained in the first flush on all substrates.

Maximum yield (234.04 g/kg), BE (73.14%) and mushroom avarage weight (74.9 gr) were obtained on BS which was distributed in two flushes with maximum yield in the first flush for P. eryngii. It was followed by SH. Although SFS supported fructification, significantly poorer yields were obtained (140.23 g/kg yield and 46.7% BE). SH substrate presenting 2 flushes with in 60 days of cropping period, while OS and SFH produced 1 flush in same harvesting period. More than 75% of the total yield was obtained in the first break on SH and BS. But only 52% of the total yield corresponded to the first flush, with the second flushes contributing almost equally to the remaining 48% on SFH.

It was not found statitically significantly relation between N, hemicellulose, lignin, cellulose, C:N and cellulose:lignin ratio and mycelial growth and yield of *P. djamor* and *P. eryngii.*It was determined that yield of *P. citrinopileatus* is positively correlated to C:N ratio (r^2=0.980) ($p<0.05$) and lignin (r^2=0.991) (*P<0.01*) of growing substrates tested (Table 5).

Avarage mushroom weight of *Pleurotus* spp grown on different substrates was significantly different (*P < 0.01*). *Pleurotus djamor* gave the largest size of 17.56 g on the BS substrate, followed by 16.3 g on the substrate SH. Mushroom size was generally bigger for OS and SFH 4.84 gr and 4.54 g, respectively) as compared to SH and BS (3.36 gr and 3.46 g, respectively) in *P. citrinopileatus.* The highest avarage mushroom weight values were recorded on SH (74.88 g) while the lowest avarage mushroom weight was obtained on SFH (54.65 g) in *P. eryngii.*

4. DISCUSSION

Shorten time of spawn running period is of great importance in terms of reducing the risk of contamination. Evaluating OS, BS, SFS and SH agrowastes as growing media of *Pleurotus* spp consistently revealed faster growth of the tested species on BS and SH than other substrates, while the longest spawn running time was on OS. Yıldız et al. [23] reported that mycelial growth of *Pleurotus* on the fresh sawdust was slow and this entails a loss of time. Bean straw substrate was suggested for short colonization time and high mycelium density of oyster mushroom by Musieba et al. [24].

Optimum C:N ratio is different for any mushroom species. In previous studies, optimum C:N ratio was determined as 45-55 for *P. eryngii* [25], *P. sajor-caju* [26] and *P. ostreatus* [27]. In the study, the shortest spawn running time was obtained in all *Pleurotus* spp. grown on SH and BS (C:N ratios is 40.81 and 48.02, respectively). Our results are in line with Chang and Miles [25], Poppe [26] and Heltay et al. [27].

Table 3. Effect of different substrates on spawn running time, first primordia initiation and harvest start date of *Pleurotus* spp

	***Pleurotus* spp.**											
	P. djamor				***P. citrinopileatus***				***P. eryngii***			
	OS	**BS**	**SH**	**SFH**	**OS**	**BS**	**SH**	**SFH**	**OS**	**BS**	**SH**	**SFH**
SRT (day)	21.4**[a]	17.6[bc]	16.4[c]	19.2[b]	24.2**[a]	20.0[c]	20.2[c]	22.2[b]	24.2**[a]	20.6[c]	20.4[c]	22.8[b]
TFPI(day)	25.2**[a]	20.5[b]	19.3[b]	23.6[a]	29.8**[a]	24.4[b]	25.0[b]	28.6[a]	39.2**[a]	30.6[b]	31.0[b]	42.8[a]
TFH(day)	30.2**[a]	24.2[b]	23.8[b]	29.4[a]	34.0**[a]	29.8[b]	30.6[b]	35.4[a]	45.7**[b]	45.8[c]	38.4[c]	50.2[a]

SRT: Spawn run time (day); TFPI: Time to first primordia initiation (day); TFH: Time to first harvest (day)

*Asterisks indicate significance at *P <0.05, **P <0.01; values within the same row followed by the same letter are not significantly different. Mean values in the same row followed by the same letters are not significantly different by Tukey multiple range*

Table 4. Effect of different substrates on number of flushes. yield. BE and avarage mushroom weight of *Pleurotus* spp

***Pleurotus* spp**	**Substrates**	**No. Of flushes**	**Yield (g)**	**Yield distribution (g)**			**Biological efficiency (%)**	**Average mushroom weight (g)**
				Flush 1	**Flush 2**	**Flush 3**		
	OS	3	87.5**b	99.4	43.1	45.0	62.5**b	15.2**b
P. djamor	BS	3	258.1 a	162.6	77.4	18.5	78.2 a	17.6 ab
	SH	3	248.8 a	151.8	64.7	32.3	77.8 a	16.3 a
	SFH	3	136.4 c	70.9	39.6	25.9	45.5 [c]	11.5 [c]
	OS	2	221.6**a	155.1	66.5	-	73.9**a	4.8**a
P.citrinopileatus	BS	2	151.8 c	118.4	33.4	-	43.0 c	3.5 [b]
	SH	2	146.0 c	108.0	38.0	-	42.5 c	3.4 [b]
	SFH	2	162.2 c	115.2	47.0	-	54.1 [b]	4.5 a
	OS	1	199.9**b	199.9	-	-	66.6**a	70.6**b
P. eryngii	BS	2	221.2 a	168.1	53.1	-	67.0 a	73.3 a
	SH	2	234.0 a	182.5	51.5	-	73.1 a	74.9 ab
	SFH	1	140.2 c	140.2	-	-	46.8	54.7 [c]

*Asterisks indicate significance at *P <0.05. **P <0.01; values within the same coloumn followed by the same letter are not significantly different. Mean values in the same coloumn followed by the same letters are not significantly different by Tukey's test*

Table 5. Correlation between total yield values of *Pleurotus* spp. and constituents of the substrates

	Chemical content of growing substrates					
	N	**C:N**	**Hemicellulose**	**Cellulose**	**Lignin**	**Cellulose:Lignin**
Spawn running time						
P. djamor	(-) 0.957*	(-) 0.955*	(-) 0.996*	(+) 0.972*	(+) 0.957*	(-) 0.998**
P. citrinopileatus	(-) 0.962*	(+) 0.956*	(-) 0.996**	(+) 0.972*	(+) 0.952*	(-) 0.996**
P. eryngii	(-) 0.959*	(+) 0.976*	(-) 0.979*	(+) 0.987*	(+) 0.984*	(-) 0.997**
Yield						
P. djamor	(+) 0.685	(-) 0.630	(+) 0.864	(-) 0.676	(-) 0.636	(+) 0.807
P. citrinopileatus	(-) 0.929	(+) 0.980*	(-) 0.874	(+) 0.973*	(+) 0.991**	(-) 0.928
P. eryngii	(+)0.562	(-) 0.457	(+) 0.731	(-) 0.506	(-) 0.424	(+) 0.632
BE						
P. djamor	(+)0.646	(-) 0.574	(+) 0.826	(-) 0.622	(-) 0.568	(+) 0.754
P. citrinopileatus	(-) 0.929	(+) 0.980*	(-) 0.874	(+) 0.973*	(+) 0.991**	(-) 0.928
P. eryngii	(+) 0.440	(-) 0.310	(+) 0.602	(-) 0.359	(-) 0.259	(+) 0.482

*Asterisks indicate significance at *P <0.05. **P <0.01 (+) positive correlation (-) negative corelation*

Similar results were obtained from all *Pleurotus* species in terms of the effect of chemical content of growing substrate on spawn running time. There is a positive correlation between spawn running time of all *Pleurotus* spp. and C:N ratios of substrates, suggesting a low C:N ratio in the substrates led to short spawning time, thereby reducing the final maturity to harvest time. This result corrobrated with Philippoussis et al. [28] who reported that there is a possitive correlation between mycelial growth and low C:N ratio values of substrates used for the cultivation of *Pleurotus* spp. C:N ratio of growing substrate has also an effect on ligninolytic enzyme of white rot fungus [29,30].

Other efficient factor on mycelial growth could be hemicellulose content of substrates. BS and SH were found to possess significantly higher than the hemicellulose content of OS and SFH in the study. Although Obodai et al. [31] reported that a negative relation between yield and hemicellulose content of substrate, in the study it was determined a positive relation for all *Pleurotus* spp tested. Locci et al. [32] and Youri et al. [33] also reported that hemicellulose is mainly utilized during the active growth phase, prior to the breakdown of lignin and cellulose. Morever, Wang et al. [34] reported that *Pleurotus ostreatus* cannot immadiately benifit from lignin and cellulose content of substrate. SH presented a relatively higher nitrogen content. Bano et al. [35] reported that the nitrogen-rich materials increase the degradability of the ligniculosic material. In the study, mycelial growth may be accelerated by the increased utilization of ligniselulosic content such as hemicellulose, cellulose and lignin in parallel with the higher nitrogen content of BS and SH. It appears reasonable to assume that the high hemicellulose content associated with the high nitrogen concentration exerts a positive effect on mycelial growth. Morever, a positive correlation was obtained between spawn running time of *Pleurotus* spp. and cellulose and lignin content of the cultivation substrate. A possible explanation of the remarkable mycelial growth reduction recorded in *Pleurotus* spp is their sensitivity to the high lignin and cellulose levels of OS and SFH. It is well known that lignin is reduces the bioavailability of the other cell wall constituents [36] and cellulose may not be readily available as carbon source [37].

Yield of carpophores of *Pleurotus* spp. changed depending on the used cultivation substrate. Shah et al. [38] reported that different mushroom species need different substrate types. It was also noted in previous studies that the nutritional requirements of different species belonging to the genus *Pleurotus* may change [39,40]. Although mycelial growth of *P. citrinopileatus* grown on OS was slower than that on the SH and BS, however, the yield and biological efficiency were significantly higher. It could be evidence that fast mycelial spread need not necessarily lead to higher yields [41]. This may be due to the different nutrients needed for mycelial growth and fruitbody formation.

SH and BS and exhibited a better substrate conversion efficiency than other substrates in *P. djamor* and *P. eryngii*. This result is corroborated by Sanchez [42] who reported that ligninocellulosic enzymes easily degrade straw substrates to release nutrient for the basidiomycetes. In the past, successful use of SFH substrates for *Pleurotus* spp. cultivation has been reported [43]. But in the study, this substrate appeared to be the worst performing substrate for *P. djamor* and *P. eryngii* producing BE values of only 45.5% and 46.7%, respectively. Patra and Pani [44] reported that substrates for the cultivation of oyster mushrooms should produce BE values at least 50%. But SFH substrate could be promising for *P. citrinopileatus* cultivation.

It was not found a correlation between chemical content of growing substrates and yield of *P. djamor* and *P. eryngii*. Wang et al. [34] reported that there is not a correlation between yield of mushroom and chemical content of substrates. Lo et al [45] and Youri et al. [33] reported that lignin has an inhibitory effect on the disintegration of cellulose and hemicellulose, so it may restrict availability of the needed nutrients for the growth of fruitbodies. Contrary to Lo et al [45] and Youri et al. [33], a positive correlation was obtained between yield of *P. citrinopileatus* and cellulose and lignin content of the cultivation substrate. Thomas et al. [46] showed also a positive correlation between yield of *Pleurotus sajor-caju* and cellulose content of substrates. On the other hand, Sivaprakasam et al. [47] was reported that yield of *P. sajor-caju* are effected positively by cellulose content and negatively by lignin content. Salmones et al. [48] reported also that *Pleurotus* species and strains digest hemicellulose, cellulose and lignin selectively and distinctly. Differences among *Pleurotus djamor, P. citrinopileatus* and *P. eryngii* could be identified with potential of lignocellulolytic enzyme production of *Pleurotus* spp. [49].

5. CONCLUSION

The increase in nitrogen, hemicellulose content and cellulose: lignin ratio of growing substrate leads shortening the spawn running time of *Pleurotus* spp tested. On the other hand, the high cellulose and lignin content and high C:N ratio slow the mycelial growth down. According to results of the present study, high hemicellulose content and cellulose: lignin ratio of substrates are the most important factors for shorten spawn running time. Unlike mycelial growth, the species have a species-specific preference for mushroom yield. *P. eryngii* and *P. djamor* need that the low lignin and cellulose content and the high nitrogen concentration and cellulose:lignin ratio , while *P. citrinopileatus* prefers high cellulose and lignin, low N and cellulose:lignin ratio for higher yield and biological efficiency.

In conclusion, *P. eryngii* and *P. djamor* colonized on the SH and BS substrate in shorter period with higher mushroom productivity and quality. So bean straw and safflower hay could be recommended as an alternative substrate to sawdust and wheat straw for *P. eryngii* and *P. djamor* cultivation. Using SH as a growing substrate substantially support the diversification of the mushroom industry in arid areas. Morever, SFS may be used as an alternative for *P. citrinopileatus*. Neverthelless, this results should be supported with additional experiments to test SH and SFS in combinaton with other wastes as cultivation substrates.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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