Comparison of growth rate of four *Pleurotus* fungi species on wheat straw and date palm leaf substrates

A. Kabirifard[1], A., Fazaeli[2*] H. and Kafilzadeh[3] F.

[1]Agricultural and Natural Resources Research Center, Bousher, Iran
[2]Animal Science Research Institute, P.O. Box 31585-1483, Karaj, Iran
[3]Department of Animal Science, Faculty of Agriculture, Razi University, Kermanshah, Iran
*Corresponding author: hfazaeli@gmail.com

Abstract

In a 4×3 factorial completely randomized experiment, four *Pleurotus* species of cultures, namely *P. florida, P. ostreatus* A., *P. ostreatus* M. and *P. ostreatus* T., were cultured on wheat straw (WS), date palm leaves (DPL) and potato dextrose agar (PDA) substrates. Growth rate of the mycelium was measured for two weeks of incubation. At the end of second week, all plates were removed from the incubator and the biomass was dried and analyzed. During the first week of fermentation, growth rate of *P. ostreatus* A. and *P. ostreatus* M. were significantly higher ($P<0.05$) than *P. florida* and *P. ostreatus* T., whereas it was inverse during the second week of incubation. A significant interaction effect ($P<0.05$) was found between cultures with substrates. During the first week of incubation, the highest amount of mycelial growth rate was shown by *P. ostreatus* A. and *P. ostreatus* T. on PDA followed by *P. florida*. In addition, *P. ostreatus* A. and *P. ostreatus* T. had significantly higher growth rate ($p<0.05$) on DPL than WS. However, at the end of second week, the cumulative growth rate was not significantly different among the cultures or the substrates. Regarding the dry mater (DM) and organic mater (OM) losses, significant differences ($P<0.05$) were found between the substrates after two weeks of fermentation but no significant variation was shown among the cultures. However, there was a significant interaction effect ($P<0.05$) of cultures with substrates for DM and OM losses. Crude protein (CP) content was significantly different ($p<0.05$) among the cultures on the different substrates.

Keywords: *Pleurorus* fungi, mycelial running, wheat stubble, date palm leaf.

Introduction

Lignocellulosic materials represent a major quantity of biomass from cereal production and some other sources such as tree leaves and branches. These biomasses are mostly made up of carbohydrates but most parts of their carbohydrates are cellulose and hemicellulose that are bonded with lignin (Adamovic *et al.*, 1998). This bond reduces digestibility and limits the availability of its nutrients (Zadrazil *et al.*, 1995). Biological delignification of straws by white-rot fungi especially *Pleurotus* species seems to be a promising way for improving of nutritive value (Jalc *et al.*, 1999b). Several authors have explored possibility of using wheat straw as a substrate for growth of *Pleurotus* species (Valmaseda *et al.*, 1991; Tripathi and Yadav, 1992; Zadrazil, 1997; Fazaeli *et al.*, 2004). Some fungi can grow on straws and utilize from its carbon compounds as energy source (Burla *et al.*, 1992).

The white-rot fungi have different ability to grow on straws and decompose

their structural carbohydrates because of the variation in culture behaviour and culturing conditions (Jalc *et al.,* 1996b; Fazaeli *et al.,* 2006). Many species of white-rot fungi have been screened on a variety of lignocellulosic materials to improve the nutritional value of poor quality cereal residues for use as ruminant feeds (Pelaez *et al.,* 1995; Jalc *et al.,* 1997). However it cannot be expected that solid state fermentation of straw through higher fungi always improves nutritive value of substrate (Yamakawa *et al.,* 1992; Adamovic *et al.,* 1998). The most important and effective factors on solid state fermentation process are cultures and substrates (Zadrazil *et al.,* 1996). Access to suitable cultures is a major factor in all biological processes. Potential of fast growth and competitive ability to other microorganisms present in culture medium are characteristics of a suitable microorganism (Zadrazil *et al.,* 1995).

In Iran, annually about 15 million metric tonnes of cereal straw are available that is a potential feed for ruminant. Date palm leaf is another by product obtained from tree trimmings often become a problem in garden management if left unattended. This research was conducted to study the growth rate of four *Pleurotus* fungi on the wheat stubble and date palm leaf as substrates and to determine the effect of fungal treatment on the dry mater, organic mater losses and crude protein content of these substrates.

Materials and Methods

Cultures

Four species of *Pleurotus* (*P. florida, P. ostreatus* A., *P. ostreatus* M. and *P. ostreatus* T.) grown on potato dextrose agar were obtained from Plant Pests and Diseases Research Institute of Iran.

Preparation and inoculation of substrates

Wheat straw (WS) and date palm leaves (DPL) were ground through a laboratory mill to pass a 1mm sieve and used as substrates. The laboratory plates were prepared and five grams of milled substrates were placed in each plate, then they were sprayed with water in a ratio of 3 to 1 (water/substrate) to obtain 75% moisture and left for 24 h at room temperature to let the substrate absorbed enough water. All plates were autoclaved the next day for 20 min at 121˚C in 1.5 bar pressure and were inoculated with the cultures, in laboratory sterile conditions. In addition to the WS and DPL, separate plates contained basal media, potato dextrose agar (PDA), were used as control (Fazaeli *et al.,* 1999).

Measurement of mycelium growth

After first and second week of incubation, all plates were monitored visually for growth rate and contamination. The mycelial growth rate was measured through an attachment of a plastic sheet on the top of packed plates and the borderline of surface area covered by mycelium was copied. The surface area on plastic sheet was determined by a handle plan meter (Fazaeli, 2001). The values of mycelium growth rate for each period of measurement were determined by calculation of the differences between two consequent measurements and presented as PW1 and PW2. The cumulative growth rate of mycelium was measured at the end of second week.

Proximate analysis

After the end of second week, samples were removed from the incubator, dried and used for determination of DM, OM and CP values. The DM of samples was determined in 65˚C for 48h. The OM was measured by

ashing the samples at 500˚C for 4h. The CP was determined by Kjeltek auto 1030 analyzer method (AOAC, 1990).

Statistical analysis

A complete randomized design with 4x3 factorial arrangement of 12 treatments with four replicates was used. The factors were four species of fungi which were *P. florida, P. ostreatus A., P. ostreatus M* and *P. ostreatus T* and three substrates which were PDA, WS and PDL. The data were analyzed as a two- factorial treatments by using the general linear model procedure of SAS (1992). The major sources of variation were species of fungi, substrates and their interaction. The treatments means were compared using Duncan multiple range test at the 5% level of probability.

Results and Discussion

Influence of cultures on growth rate of fungi

The mycelial growth rate of the fungi used in the present study is shown in Table 1. The periodic growth of the mycelium was significantly different (P<0.05) among the species. During the first week of incubation, the highest and the lowest growth rate was shown by *P. ostreatus* A. and *P. ostreatus* M., respectively. During the second period (PW2), *P. ostreatus* M. showed the highest growth followed by *P. florida*, while species *P. ostreatus* A and *P. ostreatus* T. showed the lowest amount of mycelial growth rate (P<0.05). However, at the end of the second week, cumulative growth rate was not significantly (P>0.05) different among the cultures. The differences of mycelial running speed might be due to the specific characteristics of the fungi as in accordance with the findings of Fazaeli *et al*. (1999). Chahal and Khan (1991) who studied the ability of various species of *Pleurotus* fungi on the rice straw reported that *P. sajor-caju* was found to have a higher ability for growing on straw and production of biomass. Moyson and Verachtert (1991) reported that *P. sajor-caju* and *P. pulmunarius* grew rapidly on wheat straw and after nine days of incubation, the plates were totally filled with the mycelium. Jalc *et al*. (1997), who studied the effect of six species of basidiomycetes on wheat straw reported that *P. ostreatus* and *P. ostreatus* mutant showed better growth rate than the other cultures.

Table 1. Average (±se) surface area (cm^2) covered by mycelium of different cultures

Culture	Periodic growth		Cumulative growth
	PW1	PW2	CW2
P. florida	$49.4^{b} \pm 5.6$	$45.3^{a} \pm 5.7$	$94.7^{a} \pm 0.1$
P. ostreatus A.	$69.5^{a} \pm 3.0$	$25.4^{b} \pm 3.1$	$95.0^{a} \pm 0.0$
P. ostreatus M.	$33.1^{c} \pm 0.9$	$55.3^{a} \pm 3.9$	$88.4^{a} \pm 1.8$
P. ostreatus T.	$64.3^{a} \pm 4.4$	$28.7^{b} \pm 5.0$	$93.0^{a} \pm 0.5$

Means with the different superscripts within column are significantly different (p<0.05), PW1= first week of incubation, PW2 = second week of incubation, CW2 = cumulative growth of mycelium after two weeks of fermentation.

Influence of substrate on growth rate of fungi

Irrespective of the fungal species, the growth rate of mycelium was significantly (P<0.05) different on various substrates (Table 2). At the end of first week, PDA and WS showed the highest and the lowest amount of mycelial running rate, respectively (P<0.05). This could be due to the soluble nutrients and more available energy source in the PDA that was required for starting growth and primary metabolism of the fungi (Quimino *et al.,* 1990). Kiani (1999) who studied the growth rate of five *Pleurotus* fungi on wheat straw and PDA reported that all fungi showed higher mycelial running on PDA in comparison with wheat straw. During the second week of fermentation, in the present study, WS indicated the highest amount of mycelial running in comparison to the other substrates, whereas the lowest growth rate was shown in PDA (P<0.05). Meanwhile, at the end of fermentation (after two weeks), cumulative growth rate of mycelium was not significantly (P>0.05) different among the substrates. Ragunathan *et al*. (1996) studied growth ability of three species of *Pleurotus* on rice straw, maize straw and sugar cane bagasse. They reported that species responses were different in various substrates.

Interaction of cultures with substrates on mycelial running

The interaction of cultures with substrates on the growth rate of mycelium showed that there was a significant (P<0.05) variation among the treatments (Table 3). During the first week of fermentation (PW2), the *P. ostreatus* T. (82.6 cm^2) and *P. ostreatus* A. (82.2 cm^2) cultured on PDA had the highest amount, but *P. florida* cultured on wheat straw had the lowest (27.9 cm^2) growth rate followed by the *P. ostreatus* M. cultured on WS (29.1 cm^2). In general, *P. ostreatus* M. had significantly (p<0.05) the lowest mycelial running on all substrates at the end of first week of fermentation. The interaction effect of cultures with substrates was statistically significant (p<0.05) on the growth rate during the second week (PW2) of incubation. *P. florida* showed the highest but *P. ostreatus* A. had the lowest growth rate on wheat straw. With the exception to the *P. ostreatus* M., all cultures had the lowest (P<0.05) value of mycelial running on PDA during the second week due to higher rate of growth during the first week. This variation may be due to the growth ability of the fungi as well as the chemical composition and physical structure of the substrate. The growth ability of three species of *Pleurotus* on rice straw, maize straw, sugar cane bagasse and their responses on these various substrates were different (Ragunathan *et al.,* 1996). The enzymes produced by these fungi could affect their growing ability. Pelaez *et al.* (1995) reported that the ability of enzyme production varied among the white-rot fungi. They concluded that *Pleurotus eryngi* could show simultaneous production of lacase, aryl-alcohol oxidase and Mn-peroxidase.

However, at the end of second week, those treatments that retarded mycelial running during first week compensated the growth rate and reached to the growth rate of the other treatments so that no significant differences were observed among the treatments at the end of second week when the surface area of all plates were covered by the mycelia.

Table 2. Average (±se) surface area (cm^2) covered by mycelium of different cultures

Substrate	Periodic growth		Cumulative growth
	PW1	PW2	CW2
Wheat straw	40.9^{c} ± 2.5	51.8^{a} ± 3.3	92.8^{a} ± 0.7
Date palm leaves	53.4^{b} ± 3.6	37.3^{b} ± 2.7	90.7^{a} ± 1.7
Potato dextrose agar	67.9^{a} ± 3.2	27.0^{c} ± 4.3	95.0^{a} ± 0.0

Means with the different superscripts within column are significantly different ($p<0.05$), PW1= first week of incubation, PW2 = second week of incubation, CW2 = cumulative growth of mycelium after two weeks of fermentation.

Table 3. Mean (±se) cumulative growth (cm^2) of fungi grown on different substrates

Substrate	Growth phase	Culture			
		P. florida	*P. ostreatus* A.	*P. ostreatus* M.	*P. ostreatus* T.
WS	PW_1	27.9^{d} ± 4.5	59.0^{c} ± 1.1	29.1^{d} ± 1.9	47.8^{dc} ± 0.3
DPL	PW_1	47.5^{dc} ± 2.5	67.0^{abc} ± 1.7	36.1^{d} ± 1.5	62.5^{abc} ± 1.6
PDA	PW_1	72.8^{ab} ± 4.4	82.2^{a} ± 1.4	34.2^{d} ± 5.7	82.6^{a} ± 1.1
WS	PW_2	67.1^{a} ±2.3	36^{cd} ±0.8	60.9^{ab} ±1.4	43.4^{c} ±0.7
DPL	PW_2	46.7^{bc} ±3.3	28^{d} ±1.2	44.5^{bc} ±2.1	30.5^{cd} ±1.4
PDA	PW_2	22.2^{de} ±4.1	12.8^{e} ±0.6	60.8^{ab} ±2.8	12.4^{e} ±0.3
WS	CW_2	95.0^{a} ± 0.0	95.0^{a} ± 0.0	90.0^{a} ± 0.6	91.2^{a} ± 1.9
DPL	CW_2	94.2^{a} ± 0.4	95.0^{a} ± 0.0	80.6^{a} ± 7.0	93.0^{a} ± 1.0
PDA	CW_2	95.0^{a} ± 0.0	95.0^{a} ± 0.0	95.0^{a} ± 0.0	95.0^{a} ± 0.0

Means with the different superscripts within column are significantly different ($p<0.05$), PW_1= first week of incubation, PW_2 = second week of incubation, CW_2 = cumulative growth of mycelium after two weeks of fermentation.
WS = wheat straw, DPL = date palm leaves, PDA = potato dextrose agar

Table 4. Average growth rate (cm^2/ day) ±se of cultures on different substrates

Substrate	Culture			
	P. florida	*P. ostreatus* A.	*P. ostreatus* M.	*P. ostreatus* T.
WS	8.1^{ab} ±0.4	9.5^{a} ± 0.01	6.6^{c} ± 0.45	7.5^{bc} ± 0.25
DPL	6.8^{b} ± 0.3	8.1^{ab} ±0.4	6.2^{c} ±0.9	8.0^{ab} ±0.25
PDA	9.1^{ab} ±0.2	9.5^{a} ± 0.01	9.5^{a} ±0.01	9.5^{a} ±0.01

Means with the different superscripts are significantly different ($p<0.05$)
WS = wheat straw, DPL = date palm leaves, PDA = potato dextrose agar

As presented in Table 4, the amount of mycelial growth rate per day was significantly ($P<0.05$) affected by the cultures on the different substrates. It might be due to the higher initial rate of mycelial running of the fungi on PDA (Fazaeli *et al.*, 1999).

Table 5. Average DM and OM losses (g/kg) ±se of the cultures grown in different substrates

	DM loss	OM loss
Substrate		
WS	100.0 [a] ± 12.0	93.0 [a] ± 11.0
DPL	56.0 [b] ± 11.0	49.0 [b] ± 9.0
Culture		
P. florida	76.0 [a] ± 28.0	82.0 [a] ± 28.0
P. ostreatus A.	66.0 [b] ± 23.0	73.0 [a] ± 32.0
P. ostreatus M.	76.0 [a] ± 28.0	83.0 [a] ± 28.0
P. ostreatus T.	67.0 [ab] ± 21.0	75.0 [a] ± 21.0

Means with the different superscripts within column either for substrate or for cultures are significantly different ($p<0.05$)
WS = wheat straw, DPL = date palm leaves, PDA= potato dextrose agar

Table 6. Average DM and OM loss (g/kg) ±se of cultures on different substrates after fermentation

Parameter	Substrate	Culture			
		P. florida	*P. ostreatus* A.	*P. ostreatus* M.	*P. ostreatus* T.
DM loss	WS	102.0[a] ± 4.0	86.0[a] ± 7.0	100.0[a] ± 10.0	84.0[a] ± 11.0
	DPL	50.0[b] ± 8.0	45.0[b] ± 9.0	53.0[b] ± 12.0	50.0[b] ± 12.0
OM loss	WS	108.0[a] ± 5.0	95.0[a] ± 8.0	107.0[a] ± 10.0	90.0[a] ± 14.0
	DPL	55.0[b] ± 8.0	50.0[b] ± 12.0	58.0[b] ± 13.0	59.0[b] ± 14.0

Means with the different superscripts are significantly different ($p<0.05$)
WS = wheat straw, DPL = date palm leaves, PDA = potato dextrose agar

DM and OM loss

As shown in Table 5, DM and OM loss were significantly ($P<0.05$) higher in the fungal treated WS in comparison with the solid state fermented DPL. The effect of cultures was significantly ($p<0.05$) different for DM loss, but no differences were shown between the various cultures for OM loss in the substrates. Such a decrease in DM and OM was due to the saprophytic ability of fungus to provide their energy requirements from the substrate components (Zadrazil *et al.*, 1995). However, the amount of depletion of DM and OM may be different between the substrate that is related to the composition and structural carbohydrates.

There was no interaction effect on the treatments for DM and OM loss of the substrates (Table 6). The DM and OM loss were the highest (102 g/kg and 108 g/kg) for *P. florida* on WS but the lowest DM and OM loss were found in DPL fermented with *P. ostreatus* T. and *P. florida.* The DM loss was the lowest (45 g/kg) for the case of WS cultured with *P. ostreatus* ***A***. but the lowest OM loss (50 g/kg) was found in PDL when cultured with *P. ostreatus* A. It was found that growth rate and saprophytic ability of fungal species were higher in WS than DPL, during the first and second week of incubation causing greater DM and OM losses. The differences in DM and OM losses among the different substrates were

due to the specific characteristics of cultures that could have different responses to the substrates. According to Fazaeli *et al.* (1999) the ability of *Pleurotus* species was different in depleting the OM when wheat straw and PDA were inoculated with four cultures of *Pleurotus* fungi. Additionally, DM and OM loss might also be affected by the duration of solid state fermentation. According to Zadrazil (1997), OM loss of 12 strains of *Pleurotus ostreatus* and *Pleurotus eryngii* varied from 15.3 to 27.5% during 60 days of incubation. Yoshida *et al.* (1993) reported that DM loss of wheat straw, after four weeks of incubation with *Pleurotus ostreatus*, was 22.1%. Fazeli *et al.* (2004) reported a 15% DM loss, when they had cultured *Pleurotus florida* on wheat straw.

Crude protein (CP) content

The CP value increased significantly ($P<0.05$) in the fungal treated WS (45 g/kg) and DPL (47 g/kg) in comparison to the untreated WS (37 g/kg) and initial DPL (38 g/kg). However, no significant difference was observed between the fermented substrates. Regarding the cultures, CP content was the highest (51 g/kg) in substrates inoculated with *P. ostreatus* T. and the lowest (41 g/kg) for *P. florida* and *P. ostreatus* M. ($P<0.05$). There was also significant ($P<0.05$) interaction between cultures and substrates for the CP values. It was the highest for *P. ostreatrus* T. on WS (51 g/kg) and DPL (51 g/kg), but the lowest for *P. ostreatus* M. on DPL (41 g/kg). Jalc *et al.* (1997) treated wheat straw with *P. ostreatus and P. ostreatus* mutant for 30 days and reported that the CP values of wheat straw with *P. ostreatus and P. ostreatus M.* were 5.9 and 5%, respectively, while CP value of the untreated wheat straw was 4.5%. Gupta and Langar (1988) reported that the CP value of wheat straw increased significantly ($P<0.05$) when fermented by *P. florida* in order to improve the nutritive value of the straw.

The protein content of the mycelium was reported to be relatively high (Ragunathan *et al.*, 1996), so it was expected that the treated WS as well as the DPL, that contained the fungal mycelium to have a higher concentration of CP. An increase of CP content in wheat straw incubated with *Pleurotus* species had also been reported by Ardon *et al*. (1996) and Zadrazil *et al*. (1996). An increase of CP in wheat straw up to 2-folds was reported by Kundu (1994), when it was treated with *Poecilomyces voriotii* and *Aspergilus niger*.

Conclusion

There is a potential application of *Pleurotus* fungi growing on wheat stubble and date palm leaf. The fungi grew faster on date palm leaf than wheat stubble, however the mycelial running speed of fungi was the highest on potato dextrose agar as a basal medium. Among the four species, *P. ostreatus* A. and *P. ostreatus* T. showed a higher rate of mycelial running than the other species. Therefore they seemed to be more suitable due to their faster growth to prevent contamination by other microorganisms, lower OM loss and higher crude protein.

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