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Comparing the Growth Rate of Four *Pleurotus* Fungi on Wheat Stubble and Date Palm Leaf

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ABSTRACT

In a 4×3 factorial completely randomized experiment, four *Pleurotus* strains including *P. florida, P. ostreatus A., P. ostreatus M* and *P. ostreatus T*, were cultured on wheat stubble (WS), date palm leaf (DPL) and potato dextrose agar (PDA) as well. Growth rate of the mycelium was measured for two weeks of incubation. At the first week of incubation, growth rate of *P. ostreatus A.* And *P. ostreatusM.* were higher (P<0.05) than *P. florida* and *P. ostreatus T.*, whereas it was inverse during the second week of incubation. An interaction effect (P<0.05) was found between strains with substrates. During the first week of incubation, the highest amount of mycelial running 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 (p<0.05) more growth rate on DPL than WS. However, the final cumulative growth rate was not statistically different among the strains or the substrates. The dry mater (DM) and organic matter (OM) losses were different (P<0.05) between the substrates but no significant variation was shown among the strains. The crude protein (CP) was increased in the biomass obtained from fungal treatment but it was significantly (p<0.05) different among the strains on the different substrates. In conclusion, *P. ostreatus A.* and *P. ostreatus T.* showed a substantial mycelium running ability in wheat straw and date palm leaf to producing higher crude protein.

Keywords: *Pleurorus* fungi, Mycelial running, Wheat stubble, Ddate palm leaf

INTRODUCTION

Lignocellulose materials represent a major quantity of biomass from cereal production and other sources such as tree leaves and branches. These biomasses are mostly made up of carbohydrates including cellulose and hemicelluloses, bonded with lignin (Adamovic *et al.*, 1998) that limits digestibility and nutrients availability when fed to animals (Zadrazil *et al.*,1995; Tuyen *et al.*, 2012). Biological delignification by white-rot fungi seems to be a promising way for improving of nutritive value of Lignocellulosics (Jalck *et al.*, 1996b, Akinfemi *et al.*, 2012). 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,

Akinfemi *et al.,* 2010). White-rot fungi can grow on straw and utilize its carbon compounds as energy sources (Burla *et al.,* 1992), but they have different ability to grow and decompose the structural carbohydrates (Jalc *et al.,* 1996b; Fazaeli *et al.,* 2006). Many species of white-rot fungi have been screened on a variety of lignocellulosic and improve the nutritional value of poor quality cereal residues for use as ruminant feeds (Pelaez *et al.,* 1995; Jalc *et al.,* 1997, Fazaeli, 2008). However, it could not be expected that treated straw by these fungi always improves nutritive value of substrates (Yamakawa *et al.,* 1992; Adamovic *et al.,* 1998). The most effective factors in this process are type of fungi and substrates (Zadrazil *et al.,* 1996).

In Iran, annually about 15 million metric tons of cereal straw are available that is a potential feed resources for ruminant. Date palm leaf is another by-product product obtains from tree branching that left in gardens and makes problem for gardening management. This research was conducted to study the growth rate of *Pleurotus* fungi on the wheat stubble and date palm leaf and to determine the effect of fungal treatment on the dry mater and organic matter losses and increasing of crude protein of these substrates.

MATERIAL AND METHODS

Cultures

Four fungi including; *Pleurotus*(*P. florida, P. ostreatus A., P. ostreatus M.* and *P. ostreatus T.*) were obtained from Plant Pests and Diseases Research Institute of Iran.

Preparation and inoculation of substrates

Wheat straw (WS) and date palm leaf (DPL) were milled 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) for obtain 75% moisture and left for 24h at room temperature to let the substrate absorb water enough. In next day, all plates were autoclaved for 20min at 121$^{\circ C}$ in 1.5 bar pressure. The plates were then inoculated with the prepared cultures, using soap beside the flame in sterile conditions. In addition to the WS and DPL, separate plates contained potato dextrose agar (PDA), were used as control (Kiani, 1999; Fazaeli *et al.,* 1999).

Measurement of mycelium growth

After the first and second weeks of incubation (at 28 ˚C), all plates were controlled for growth rate and probability contamination, using direct visual method (Calzada and rolz, 1990; Moorty and Mohanan, 1991). The mycellial running measured through an attachment a plastic sheet on the top of packed plates and the borderline of surface area covered by mycelium was copied and then measured by a handle plan meter (Fazaeli, 2001).

Proximate analysis

After the end of second week, samples were removed from incubator, dried and analyzed for DM, OM and CP values. DM was determined in 65˚C for 48h, OM estimated by ashing the samples at 500˚C for 4h and kejltek auto analyzer according the standard procedures (AOAC, 1990) measured CP.

Experimental design

A completely randomized design with factorial arrangement of 12 treatments with four replicates (4×3, n=4) was used. The factors were four strains of fungi and three substrates, which were PDA, WS and PDL. The data analyzed, using general linear model procedure of SAS (1992). The major sources of variation were due to the effects of strains, substrates and their interactions.

RESULTS AND DISCUSSION

Influences of strains on mycelium growth rate

The mycelium growth rate of various fungi is shown in Table 1. The periodic growth of the mycelium were significantly different (P<0.05) among the strains. At the first week of incubation, the highest and the lowest growth rate showed by *P. ostreatus A.* and *P.ostreatus M.*, respectively, but at the second week (PW2), *P.ostreatus M.* showed the highest growth rate followed by *P. Florida* (P<0.05). However, cumulative growth rate was not significantly (P>0.05) different among the strains. The differences of mycellial running speed may be due to the specific characteristics of the fungi that are in accordance with the findings of Fazaeli *et al*. (1999). Chahal *et al.* (1991) studied the growing ability of some*Pleurotus* fungi on the rice straw and reported that *P. sajor-caju* was found to have more ability for growing and production of biomass. Moyson and Verachtert (1991) reported that *P. sajor-caju* and *P. pulmunarius* grew rapidly on wheat straw so that 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 species.

Influences of substrate on mycelium growth rate of fungi

In spite of the fungal strains, the growth rate of mycelium was significantly (P<0.05) different on the various substrates (Table 2). At the first week, PDA and WS showed the highest and the lowest mycellial running rate (P<0.05), respectively. It could be due to the soluble nutrients and more available energy source in PDA that is required for initial growth and primary metabolism of the fungi (Quimino *et al.,* 1990; Kiani, 1999). According to Kiani (1999) who studied the growth rate of five *Pleurotus* fungi on WS and PDA, all fungi showed higher mycellial running on PDA than WS. At the second week of incubation, WS showed the highest amount of mycellial running in comparison to the other substrates, whereas PDA resulted the lowest growth rate (P<0.05). Meanwhile, 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 and reported that responses of different species were different in various substrates.

Interaction of fungal strains with substrates on mycelium running

The interaction of fungal stains with substrates on the growth rate of mycelium showed that (Table 3), there was a significant (P<0.05) variation among the treatments. During the first week of incubation (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).

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

Cultures	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 different superscripts within column are significantly different ($p<0.05$)
se= standard error, PW1= first weeks of incubation, PW2= second weeks of incubation, CW2= cumulative growth of mycelium after two weeks of incubation.

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

Substrates	Periodic growth		Cumulative growth
	PW1	PW2	CW2
WS	$40.9^c \pm 2.5$	$51.8^a \pm 3.3$	$92.8^a \pm 0.7$
DPL	$53.4^b \pm 3.6$	$37.3^b \pm 2.7$	$90.7^a \pm 1.7$
PDA	$67.9^a \pm 3.2$	$27.0^c \pm 4.3$	$95.0^a \pm 0.0$

Means with different superscripts within column are significantly different ($p<0.05$)
se= standard error, PW1= first week of incubation, PW2= second weeks of incubation, CW2= cumulative growth at the end of second week of incubation
WS= wheat stopple, DPL= date palm leaf, PDA= potato dextrose agar

In general, *P. ostreatus M.* had the lowest ($p<0.05$) mycellial running on all substrates at the end of first week. The interaction effect of strains with substrates was statistically significant ($p<0.05$) on the growth rate, during the second week (PW2) where *P. floriada* showed the highest but *P. ostreatus A.* had the lowest growth rate on wheat straw. With exception to the *P. ostreatus M.*, all cultures had the lowest ($P<0.05$) amount of mycelium running on PDA during the second week that is due to high-speed growth, during the first week. This variation may be due to the culturing behaviors and growth ability of the fungi as well as the chemical composition and physical structure of the substrates. According to Ragunathan *et al.* (1996) the growth ability of three species of *Pleurotus* on rice straw, maize straw, and sugar cane bagasse were different. The enzymes that produce by fungi could affect their growing ability. Pelaez *et al.* (1995) reported that the ability of enzyme production was varied among the white-rot fungi. They concluded that *Pleurotuseryngi* could show simultaneous production of lacase, aryl-alcohol oxidase and Mn-peroxidase.

However, those treatments that were retard in mycelium running during the first week, compensate the growth rate and reach to the other treatments at the end of second week when the surface area of all plates were covered by the mycelium.

As it is presented in Table 4, the amount of mycellial running rate (cm^2 per day) was significantly ($P<0.05$) affected by the fungal strains on the different substrates. It may be due to the higher initial speed of mycelium running of the fungi on PDA (Fazaeli *et al.*, 1999).

Table 3. Average of (±se) cumulative growth (cm^2) of various treatments after two weeks of incubation

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

Means with different superscripts (for either PW1, PW2 or CW2) are significantly different ($p<0.05$), se = Standard error,
PW1= during first week of incubation, PW2 = during second week of incubation,
CW2= cumulative growth at the end of second week.
WS = wheat stopple, DPL= date palm leaf, PDA= potato dextrose agar.

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

Substrate	Cultures			
	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
PDF	9.1^{ab} ±0.2	9.5^{a} ± 0.01	9.5^{a} ±0.01	9.5^{a} ±0.01

Means with different superscripts are significantly different ($p<0.05$), se= standard error, WS= wheat stopple, DPL= date palm leaf, PDA= potato dextrose agar,

DM and OM loss

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

There was no interaction effect on the treatments for DM and OM loss on 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 for *P. ostreatus T.* and *P. Florida* on DPL. The lowest DM loss (45 g/kg) was found when WS cultured with *P. ostreatus A.* but the lowest OM loss (50 g/kg) was found in PDL when cultured by *P. ostreatus A.* It was found that growth rate and saprophytic ability of fungi were higher in WS than DPL that causes the greater DM and OM losses. The differences in DM and OM losses among the different substrates and cultures could be due to the specific characteristics of fungi that could have different responses to the substrates. According to Fazaeli *et al.* (1999) the ability of *Pleurotus* species was different to deplete the OM when wheat straw and PDA were inoculated with four species of *Pleurotus* fungi. Additionally, DM and OM loss may be affected by the duration of incubation. Zadrazil (1997) reported that the OM loss of 12 strains of *Pleurotusostreatus*and*Pleurotuseryngii* variedfrom 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 *Pleurotusostreatus*, was 22.1%. Fazeli *et al.*, (2004) reported a 15% DM loss, when they cultured *Pleurotusflorida* on wheat straw.

Table 5. Average (±se) DM and OM losses (g/kg) in the treatments at the end of incubation period

Substare	DM losses	OM losses
WS	100.0 ^{a} ± 12.0	93.0 ^{a} ± 11.0
DPL	56.0 ^{b} ± 11.0	49.0 ^{b} ± 9.0
Fungi		
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 strains are significantly different (p<0.05), se= standard error.
WS = wheat stopple, DPL = date palm leaf, PDA= potato dextrose agar.

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

Variables	Substrates	Fungi			
		P. florida	*P. ostreatus A.*	*P. ostreatus M.*	*P. ostreatus T*
DM losses	WS	$102.0^{a} \pm 4.0$	$86.0^{a} \pm 7.0$	$100.0^{a} \pm 10.0$	$84.0^{a} \pm 11.0$
	DPL	$50.0^{b} \pm 8.0$	$45.0^{b} \pm 9.0$	$53.0^{b} \pm 12.0$	$50.0^{b} \pm 12.0$
OM losses	WS	$108.0^{a} \pm 5.0$	$95.0^{a} \pm 8.0$	$107.0^{a} \pm 10.0$	$90.0^{a} \pm 14.0$
	DPL	$55.0^{b} \pm 8.0$	$50.0^{b} \pm 12.0$	$58.0^{b} \pm 13.0$	$59.0^{b} \pm 14.0$

Means with the different superscripts are significantly different ($p<0.05$), se= standard error,
WS= wheat stopple, DPL= date palm leaf, PDA= potato dextrose agar

Crude protein content

The CP value was significantly ($P<0.05$) increased 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 differences were observed between the treated 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 strains with 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 it was 4.5% for the untreated straw. Gupta and langar (1988) reported that the CP value of wheat straw increased ($P<0.05$) when cultured by *P. florida*. 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 fungal mycelium to have a higher concentration of CP. Increasing of CP content in wheat straw incubated with *Pleurotus*species had also been reported (Ardon *et al*., 1996; Zadrazil *et al*., 1996). Kundu (1994) reported increasing of CP in wheat straw up to 2-folds, when it was treated with *Poecilomycesvoriotii*and*Aspergilusniger*.

CONCLUSION

It can be concluded that, there is a potential application of *Pleurotus* fungi growing on wheat stubble and date palm leaf, but it was found that the fungi could growth faster on date palm leaf than wheat stubble, however the speed of mycelilal running of fungi was the highest on PDA as a basal media. Among the four strains, *P. ostreatus A.* and *P. ostreatus T.* showed a greater speed of mycelium running than the others. Therefore, they seem to be more suitable due to quick growth to prevent contamination by other microorganisms, lower OM loss and increasing higher crude protein value.

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