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The Improvement of *Pleuorotus* Species Cultivated On Soybean Straw Bed Supplemented with Flax Seed Meal

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Abstract: ***Mushrooms are rich sources of nutraceuticals and are known for antioxidant, antitumor, antimicrobial properties. Flax, Linumusitatissimum, is a unique plant having a rich combination of fat, protein and dietary fibre, which constitute approximately 40%, 20% and 28% respectively. Flax oil is the richest source of ALA (50-60%), the parent compound of ώ-3 fatty acids. Soybean straw bed supplemented with flaxseed meal (10% to50% dry weight) was used as potential raw material to improve nutritional quality of mushrooms. The maximum protein content of 44.268g % was obtained in fruiting bodies produced when soybean straw was supplanted with 20% flax seed meal (on dry weight basis). Fatty acid profile analysis suggests that mushroom species could not bioconvert flax ALA into Poly Unsaturated Fatty Acids (PUFA).***

Keywords: Flax, *Pleurotus*, PUFA, Fatty acid profile.

1. Introduction

Protein malnutrition is one of the key problems in developing countries including India (Paruchuri 2012). Nutritional imbalance is often correlated with an increase in number of patients suffering of cardiac problems, arthritis, allergies, that could be avoided by correcting dietary imbalance (Guillamón 2010,Mark Houston, 2010). In this context, unconventional alternative sources of essential nutrients such as mushrooms are gaining importance to salvage these situations (Dundar 2008). Mushrooms are rich sources of nutraceuticals and are known for antioxidant, antitumor, antimicrobial properties. More than 2,000 species of mushrooms exist in nature (Makarov 2002). However, less than 25 species are accepted for human consumption of which only a few have attained commercial status. The edible cellulose-degrading higher fungi, oyster capmushroom (*Pleurotusostreatus*) and honey mushroom (*Flammulinavelutipes*) are cultivated commonly as dietetic food in India, Japan and China (Aida 2009).

The oyster mushroom *Pleurotus*spp is a saprophytic fungus, commercially cultivated throughout the world because of its tasty basidiocarp and simple cultivation technology. Mushrooms normally ranges between 20 and 40% of protein, which is considered better than many legume sources like soybeans and peanuts, and protein-yielding vegetable foods (Chang and Buswell, 1996; Chang and Mshigeni, 2001). Moreover, mushroom proteins are excellent source of essential amino acids required for humans and are especially rich in lysine and leucine, which are lacking in most staple cereal foods (Chang and Buswell, 1996; Sadler, 2003). Mushrooms are low in total fat content but have an appreciable proportion of polyunsaturated fatty acids (72 to 85%), primarily due to linoleic acid (Chang and Mshigeni, 2001; Sadler, 2003). Oyster mushrooms are mainly cultivated on cheaper residues from agricultural crops such as wheat, paddy, cotton, sugar cane or soybean (Sohi and Upadhyay 1989, Savalgi and Savalgi 1994). Carbon is readily available from degradation of cellulose, hemicellulose and lignin from the straw. The nitrogen in these residues is typically low (0.5 to 0.8%) occurs mainly in bound form and is not available until it is enzymatically released. Use of protein rich medium for mushroom is thought to be an alternative strategy to increase its protein content.

Flax, *Linumusitatissimum* is a unique plant having a rich combination of fat, protein and dietary fibre, which constitute approximately 40%, 20% and 28%, respectively. Flax is mainly grown for its oil and stem fibre. Flax oil is the richest source of ALA (50-60%), the parent compound of ώ-3 fatty acids. (One tablespoon of milled flax contains 1.8 g of ALA, while one tablespoon of flax oil contains 8 g of ALA), which can be used directly for human consumption through fortification. Flax seed protein, although relatively neglected is an important dietary component. Flax seed protein content ranges between 25 to 45%. Flaxseed protein is reported to release physiologically important amino acids and bioactive peptides. Most interesting are the favourable functional properties of flaxseed proteins and the wide range of potential products in which they can be incorporated.

Various studies report supplementation of wheat straw with various oil rich seeds but there are no reports on use of flax seed oil or seedcake for mushroom cultivation. In the present study, flaxseed meal was used as nutritional supplement for oyster mushrooms cultivated on soybean straw.

2. Materials and Methods

In the present study three species of *Pleurotusviz, P. sajor-caju* , *P. ostreaous, P. florida*were used. Mushroom culture spawns were obtained from Mushroom Department of Mahatma KrishiVidyapeeth, Pune. The spawns were cultivated on soybean straw, with or without flax seed meal in different concentrations. Chopped Soybean straw (2-3cm size) was soaked in tap water for 10-12h. Wet straw was filled in autoclavable plastic bags 35x55cm size and pasteurised at 120lbs for 1h. Sterilised straw bags in six replicates of each treatment were spawned (at 2% on wet weight basis) along with 0%, 10%,20%, 50% supplementation of sterilised flax meal.

2.1 Mushroom growth and yield

The mushroom beds were kept in dark for 7 days. The bags were cut open as pinhead primordiacould be seen. The exposed straw blocks were watered and maintained at 25^{o}C +/_2^{o}C and relative humidity of about 85-90%. Mushroom heads were harvested after a week from the day of formation of fruiting primordia.

2.2 Estimation of protein content of mushroom fruiting bodies

Harvested mushroomswere dried at 60^{o}C till constant dry weight and total nitrogen in fruiting bodies was estimated using micro-Kjeldhal method. Total protein value was calculated using protein factor of 6.38.

2.3 Fatty acid analysis of mushroom fruiting bodies/mycelial filaments using Gas chromatography

Lipids in dried fruiting bodies were extracted in the form of FAMEs using 0.6N methanolic HCL and were analysed by gas- liquid chromatography.

3. Results and Discussion

Several workers have reported variable yields of fresh oyster mushroom using different crop residues (Sohi and Upadhyay 1987, Madan*et. al.* 1987). Cultivation of *Pleurotus*spp on non-conventional, cheaper raw materials containing lignin, cellulose or hemicellulose such as dried *Populus* leaves (Upadhyay and Verma (2000) and industrial waste materials like tea (Upadhyay*et. al.* 1996), apple pomace (Upadhyay and Sohi 1988) chicken manure is also reported. Various workers have used supplements from animal and plant origins, including protein-rich, carbohydrate-rich or oil-rich substances, for *Agaricus and Pleurotus*cultivation (Sinden and Schisler 1962, Gerits 1983, Gupta and Vijay 1992). Supplementation of wheat straw with different organic nitrogen sources like wheat bran, rice bran, soybean floor, de-oiled soybean meal, mustard cake, cotton seed cake and cotton seed meal were evaluated for their effect on mushroom yield (Upadhyay*et. al.* 2002). Rinker (1989) found 37 and 42.6% more total yield in *P. ostreatus* from supplementation with barley straw with brewer's grain and 17, 27, 65 and 118% more yield by addition of alfalfa hay at 5, 10, 20 and 40% (dry wt. basis).Cotton and wheat straw supplemented with groundnut haulms and soybean straw, produced relatively higher yields. This is probably due to the high nitrogen content of groundnut and soybean.Some fatty acid esters like methyl oleate, ethyl oleate, methyl palmitate, methyl palmitoleate , methyl stearate were reported as suitable carbon and energy source. The effects of various lipids on growth andyields were observed in different mushroom species among these ethyl oleate, giving highest yield for *Agaricusbisporus*and for*Coprinuscomatus*(Wardle, Schisler1969). The variations may be due to the nutrient status of the substrate used for cultivation and also depends on the genotype of the cultured mushroom. (Laborde*et al.*1993, Sangwan and Saini, 1995).

In the view of nutritional merits of flax seed, it was thought interesting to check if it could be translated in an improved nutritional quality of mushrooms. It was important to check the increase in total protein as well as fatty acids and the efficiency of the studied strains to convert flax ALA into PUFA. The *Pleuorotusflorida* and other species grown on soybean straw supplemented with flax seed meal showed increased yield as compared to the control (soybean straw bed without flaxseed meal). As reported earlier, (Laborde*etal.*,1993, Sangwan and Saini, 1995), these yield differences depend on the genotype of the cultured mushroom.

The protein contents of mushrooms range from 19 to 39 g per 100 g dry weight (Cuptapun2010). In our study we found that the protein values (g/100 g dried matter) were 13.95 gm in *P. sajor-caju*and 17.12 g in *P.eous*and 22.12g in *P.florida*, when grown on soybean straw without any supplement (Table1a). Protein content in fruiting bodies was found to increase with increasing levels of flax from 0% to 20%. The maximum protein content of 44.268g % was obtained in fruiting bodies grown on soybean straw supplanted with 20% flax seed meal (on dry weight basis). Substrates rich in usable nitrogen may be a factor in enhancing the mushroom yield along withnutritional quality (Patil*et al.,*2008), wherein bioconversion and bioaccumulation efficiency of the mushroom species plays a major role. Increase in protein could be attributed to rapid metabolic activity triggered by extra nitrogen available through flax meal, (Upadhyay*et.al* 2002). The higher yield and biological efficiency could be achieved by combining high fiber substrates and high protein complements.

According to studies by Wardle and Schisler (1969) the lipids when added in small amounts to a medium, containing sugar as the principal carbon source, stimulated the growth of *Agaricusbisporus*and*Coprinuscomatus*. In the present study, all three *Pleurotus* species showed increase in total LA content with increasing flax meal content of the bed, however showed no increase in omega -3 fatty acids was observed. The fatty acid profile analysis suggests that mushroom species could not bioconvert flax ALA into PUFA into long chain fatty acids (Table1 b). Perhaps the available fat was primarily hydrolyzed and used as carbon source for growth. The effects of various lipids on growth of mycelium and lipogenesis are species dependent. The chemical nature of carbon and nitrogen sources, as well as their concentrations and ratio, significantly affect the rate of lipogenesis. The C/N ratio and general proportions of medium components determine not only the rate of biomass accumulation but also the contents of proteins, lipids, and carbohydrates. The unsaturation degree of total extractable lipids (membrane/ storage) also depends on the rate of lipogenesis. The features of lipogenesis on physiologically different media were described only for lower fungi but not for higher ones, basidiomycetes (Bespalova 2002). Higher mycelial fungi are foods and sources of essential lipids for humans, therefore, investigation of lipids in basidiomycetes is of commercial significance.

There is an increasing emphasis on cost reduction of processes as well as on value addition for agro-industrial residues. Crop residues such as bran, husk, bagasse, straw and fruits serveas an excellent substratum for the growth of microorganism and serve as cheaper source of nutrients for their growth.It is reported that the substrates enriched by plant origin led to slow release of organic materials which could be optimally absorbed by mycelium structures (Royse et al., 1991).The bioconversion and biotransformation efficiencies can be improved by supplementation of straw substrate with oil seed cakes (Zakia Bano1993).However it depends on the ability of the fungi to use the available fatty acid skeletons for synthesis of LCPUFA or mear hydrolyzing it for use as carbon source.The increased biomass and protein content observed in these mushroom species suggests its assimilation without bioconversion or biotransformation.

4. Conclusion

As revealed by the present data, flax seed meal could be ideal source of proteinaceous nutrients and as support matrix for mushrooms. The cultivation of edible mushrooms on leguminous straw offers one of the most feasible and economic method for the bioconversion of agro-lignocellulosic wastes. The use of leguminous straw supplemented with flax seed meal offers a valuable system for improving nutritional quality of mushrooms.

Figure 1: Effect of flax seed meal(10% to 50%) on mycelial growth of *P. sajorcaju* cultivated on soybean straw

Figure 2: Effect of flax seed meal (10%,20%) on yield of *P. oeous* cultivated on soybean straw

Figure 3: Effect of flax seed meal(0%,10%,20%) on yield of *P. sajorcaju* cultivated on soybean straw

Figure 4: Effect of flax seed meal (10%,20%) on yield of P.florida cultivated on soybean straw

Table 1: Effect on protein content of *P. sajorcaju* cultivated on flax seed powder

Percentage of flax seed meal	Total protein content(micro-Kjeldhal method)	ALA fatty acid content
Control	13.995g%	52.48
10%	23.067g%	55.31
20 %	44.268g%	59.16
50%	No fruiting bodies	--

Table 2: Fatty acid profile of *Pleuorotus* spp. Grown on soybean bed supplemented with flax seed (ND-not detectable)

Mushroom bed composition	**MTR**	**OLE**	**PAL**	**PAL**	**STE**	**OLE**	**LA**
Pleuorotuseous Control	4.68	1.93	16.91	ND	4.97	12.38	59.13
P. eous 10% flax meal	2.54	0.92	5.11	25.99	ND	4.96	60.48
P.eous 20% flax meal	5.15	2.58	14.54	ND	4.24	7.82	65.66
Pleuorotus florida Control	3.46	15.58	15.01	ND	3.46	14.12	62.40
P.florida 10% flax meal	5.57	2.35	13.73	0.82	3.49	7.75	66.29
P.florida 20% flax meal	4.22	1.60	11.04	ND	3.35	12.43	65.63
P.sajorcaju Control	10.12	1.22	16.01	ND	5.92	12.10	52.48
P.sajorcaju 10 % flax meal	14.46	5.58	12.06	1.73	8.13	4.47	55.31
P.sajorcaju 20% flax meal	17.21	7.29	9.34	ND	9.02	11.23	59.16

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