EFFECT OF ORGANIC NITROGEN SUPPLEMENTATION IN *PLEUROTUS* SPECIES.

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ABSTRACT

Oyster mushrooms *Pleurotus* spp draw their nutritional requirement from a host substrate or from the agricultural wastes rich in lignin, cellulose and hemicellulose used for their cultivation. Due to varying nutrients in the substrates, different mushroom yields have been recorded by various workers. Nitrogen is an essential element for cellular functions for growth and various metabolic activities particularly protein and enzymes synthesis. The nitrogen content of mycelium ranges between 3 to 6%. Cereal straw used for cultivation of oyster mushroom is a poor source of nitrogen (0.5 to 0.8%) and at the time of fructification when most of the nitrogen is utilized for mycelial growth, the depleted nitrogen in the substrate becomes inadequate and limits mushroom yield. In the present studies seven different organic nitrogen sources: 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. Cotton seed cake and de-oiled soybean meal gave significantly higher yield than unsupplemented bags. Mustard cake supplemented bags gave the lowest yield, which obviously was due to its anti-fungal properties. Cotton seed cake and de-oiled soybean meal were further evaluated – on a 1, 2.5, 5, 7.5 and 10 % per dry wt. basis, to find out the minimum dose for optimum yield. Cotton seed cake - 2.5%, 5%, 7.5% and 10% - gave similar yields although significantly higher than 1%. However, 1% soybean meal was found the best, and all higher rates of supplementation gave lower yields. It could thus be concluded that addition of 1% de-oiled soybean meal and 2.5% cotton seed cake are the optimum doses for these supplements to enhance the yields of *P. ostreatus* var *florida*. Their supplementation also gave higher dry matter than unsupplemented bags.

INTRODUCTION

The oyster mushroom *Pleurotus* spp is a saprophytic fungus commercially cultivated throughout the world because of its tasty basidiocarp and simple cultivation technology. It is also one of the choicest white rot fungi for research scientists to investigate. *Pleurotus* spp's lignocellulolytic enzymes for bioremediation (Arisoy and Kalan Kayan 1997, Walter *et. al.* 1997), its flavour compounds, (Mau *et. al.* 1998), its synthesis of diterpene and polysaccharide (Gutirrez *et. al*. 1996) and its natural pigment extraction (Shirata and Kato 1998) make it a promising subject for study. Oyster mushrooms are mainly cultivated on residues from agricultural crops such as wheat, paddy, cotton, sugar cane or soybean (Sohi and Upadhyay 1989, Savalgi and Savalgi 1994). *Pleurotus* spp also have the potential to mineralize and grow on industrial wastes such as tea (Upadhyay *et. al*. 1996), apple pomace (Upadhyay and Sohi 1988) or non-conventional substrates containing lignin, cellulose or hemicellulose such as dried *Populus* leaves. (Upadhyay and Verma 2000).

These residues are low (0.5 to 0.8%) in nitrogen content. Several workers have reported varying fresh oyster mushroom yields using crop residues (Sohi and Upadhyay 1987, Madan *et. al.* 1987). The variations may be due to the nutrient status of the substrate used for cultivation. The production of oyster mushrooms after the first flush is drastically reduced and there is a flush break of 10 to 20 days depending upon the species of oyster mushroom. The yield decline could be due to either depletion of nutrients or accumulation of toxic substances unfavourable to fruiting. In *Agaricus bisporus*, increased yields have been reported by supplementing with various proteins, carbohydrate or oil rich supplements like soybean meal, cotton seed meal, alfalfa meal or corn gluten meal. (Sinder and Schisler 1962, Gerrits 1983). In the present studies seven different organic nitrogenous materials were evaluated to find out their effect on yield. The best substrates were further evaluated for their optimal dose with maximum yield.

MATERIALS AND METHODS

Prewetted chopped wheat straw (2-3cm) was mixed with calcium sulphate (4% w/w) and carbendazim 50% w.p. (15g/quintal) and a rectangular pile prepared. It was given two turnings on alternate days for four days so that the temperature did not exceed more than 60°C during fermentation. After four days, the partially fermented straw was pasteurized in a tunnel at 70°C for 6h and subsequently conditioned at 45°C for 36h. Supplements (wheat bran, rice bran, cotton seed meal, cotton seed cake, soybean meal, de-oiled soybean cake and mustard cake) were separately treated in a solution of carbendazim (100ppm) for 16h. The rate of addition of all the supplements was 5% (dry wt.) except wheat and rice bran (10%). Treated supplements were thoroughly mixed at the time of spawning with pasteurized straw. Twenty-day-old spawn of *P. ostratuas* var *florida* was added at a 3% wet wt. Five kg spawned substrate was filled into 45x30cm polyethelene bags with 10 holes (5mm dia.). Each supplement had six replications. Spawned bags were incubated in a dark cropping room (temp. 13-18°C).

Colonized bags were opened after 25 days. A relative humidity of 70-75% was maintained by spraying water twice a day; 6-8h light was provided with fluorescent tubes; and carbon dioxide concentration was maintained at 700-780 ppm in the cropping room. Mushrooms were harvested daily before spraying and data were recorded. Biological efficiency (BE) was calculated on the basis of fresh mushrooms from 100 kg dry substrate weight. In the second experiment, cotton seed cake and de-oiled soybean cake were further evaluated for their effective optimum dose. The supplements were treated as before, at a rate of 1, 2.5, 5, 7.5 and 10% substrate dry weight.

Temperature record

The temperature of the bags was recorded daily from days 3 - 16. Three bags from each treatment were selected and temperatures at three places - in the center, 5cm deep from top and 5cm deep from the periphery - were recorded. Average temperatures were then calculated.

Dry weight determination

Three samples of one hundred grams of fresh mushrooms from all the supplements were oven dried at (45°C) to a constant weight.

RESULTS

Best organic nitrogen source

The effect of organic nitrogen supplementation in wheat straw on yield of *P. ostreatus* var *florida* is presented in Table1. Among the various supplements tried, cotton seed cake and de-oiled soybean cake gave significantly higher yield than unsupplemented bags. Lowest yield was recorded with mustard cake (53.2% BE) followed by cotton seed meal (64.6% BE) which were even lower than the control. Wheat and rice bran were equally effective and gave 20.6% and 17.6% higher yield than unsupplemented bags.

Optimum dose of best nitrogen source

No significant differences were observed in the doses of soybean cake (1% de-oiled soybean cake and 7.5, 5.0 and 2.5% rates of supplementation). However, addition of cotton seed cake gave the highest mushroom yield at 10%, followed by 5.0 and 7.5% rates. In general, with cotton seed cake addition, higher mushroom yield was obtained (Table 2 and Table 3).

Table 1. Effect of organic supplements to wheat straw on fresh mushroom yield of *Pleurotus ostreatus* var *florida* in 60 days.

S. No.	Substrate + Supplements	Average Yield (kg) per kg substrate	Biological efficiency (BE) (%)	Percent increase (+) or decrease (-) from control
1.	Wheat straw + wheat bran (10%)	0.860	86.0	+20.6
2.	Wheat straw + rice bran (10%)	0.838	83.8	+17.5
3.	Wheat straw + cotton seed cake (5%)	0.946	94.6	-12.2
4.	Wheat straw + cotton seed meal (5%)	0.646	64.6	-12.2
5.	Wheat straw + soybean meal (5%)	0.732	73.2	2.6
6.	Wheat straw + de-oiled soybean cake (5%)	0.928	92.8	+30.1
7.	Wheat straw + mustard cake (5%)	0.532	53.2	-25.3
8.	Wheat straw (control)	0.713	71.3	

CD at 5% = 0.211

Table 2. Effect of different doses of de-oiled soybean cake on fresh mushroom yield and dry matter of *Pleurotus ostreatus* var *florida*.

S. No.	Rate of supplementation (Dry wt.)	B.E.%	% increase over control	% dry matter content in 1st and 2nd flushes	
1.	Wheat straw 1% soybean	92.2	+21	9.85	10.04
2.	2.5% soybean	84.8	+11.57	9.45	10.0
3.	5% soybean	84.5	+11.18	10.29	10.25
4.	7.5% soybean	87.8	+15.52	8.8	9.75
5.	10.0% soybean	83.2	+8.15	9.3	10.55
6.	Wheat straw (control)	76.0	--	6.9	9.3

CD at 5% = 23

The dry matter content of mushrooms harvested from both the supplements at different doses are shown in Table 2 and Table 3. Soybean supplementation generally yielded heavier mushrooms in the first flush than cotton seed cake supplementation. Maximum dry matter content was recorded from bags supplemented with 5% de-oiled soybean cake and further increase in supplementation did not yield heavier mushrooms. In cotton seed cake, the heaviest mushroom fruit bodies were observed with 10% dose in the first flush, while in the second flush, the lowest rate of application gave the heaviest mushrooms. The dry matter content in the second flush was generally more than the first flush for both the supplements. Interestingly the spore print color of the mushrooms from 10% soybean was a creamy yellow. The nutritional analysis of mushrooms obtained from different supplements is under investigation. The addition of cotton seed cake gave the maximum yield increase (+47.7%) while soybean cake gave heavier mushroom fruit bodies than cotton seed cake.

The use of supplementation increased the substrate temperature (Figure1 and Figure 2) from the fourth day onwards to the sixteenth day. Bags with de-oiled soybean cakes showed a rise in temperature from 3 to 9°C over room temperature and 3 to 5°C over unsupplemented bag temperature. Cotton seed cake addition showed less temperature rise compared with similar doses of soybean cake. The maximum rise in temperature was between the fourth day and the ninth day.

Table-3: Effect of different doses of cotton seed cake on fresh mushroom yield and dry matter of *Pleurotus ostreatus* var *florida.*

S. No.	Substrate used	Biological Efficiency (%)	% increase over control	% Dry matter content in 1st and 2nd flush	
1.	Wheat straw + cotton seed cake 1%	90.4	+18.94	7.3	10.5
2.	Wheat straw + cotton seed cake 2.5%	100.3	+31.97	8.1	9.82
3.	Wheat straw + cotton seed cake 5.0%	112.0	+47.36	7.8	9.49
4.	Wheat straw + cotton seed cake 7.5%	105.3	+38.5	8.0	9.45
5.	Wheat straw + cotton seed cake 10%	112.3	+47.7	9.01	9.0
6.	Wheat straw (control)	76.0	--	6.9	9.28

CD at 5% =23

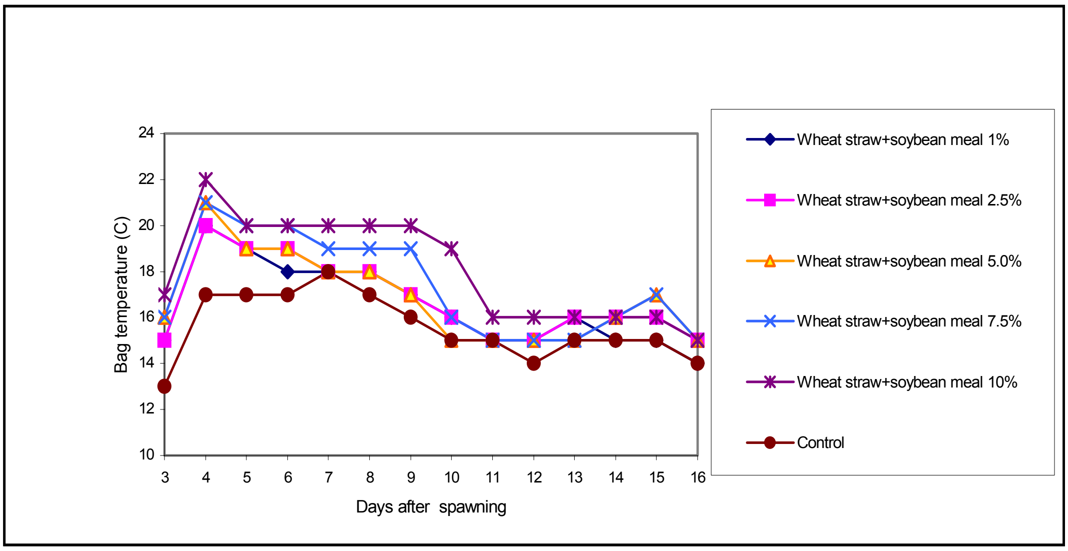

Figure 1. Rise in substrate temperature due to supplementation from 3rd day of spawning to 16th day

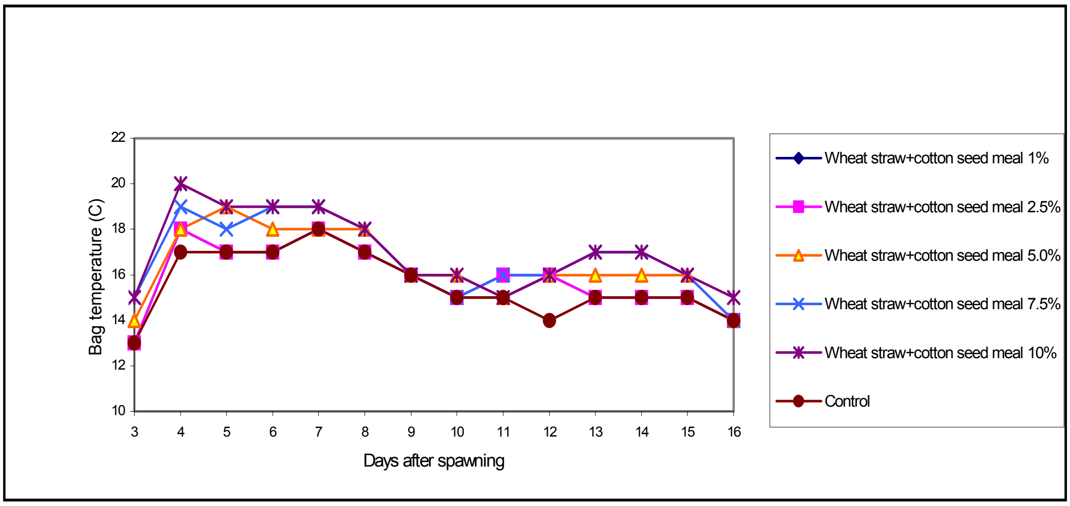

Figure 2. Rise in substrate temperature due to supplementation from 3rd day of spawning to 16th day

DISCUSSION

Although commercial cultivation of oyster mushroom *Pleurotus* spp started very late compared to *Agericus bisporus* (1650 A. D.), *Lentinula edodes* (1100 A. D.) and *Auricularia* spp (600 A. D.), it occupies the third position in the world among the cultivated mushrooms. Successful cultivation of oyster mushroom using cereal straw was reported in 1962 by Bano and Srivastava from India. Still, it is not widely cultivated due to inconsistent yields. The fresh mushroom yield or biological efficiency of a species is directly related to strain, substrate nutrition and growth conditions. Sustainable oyster mushroom production can be achieved by employing cultural practices which optimize and integrate nutrient management. Agricultural residues used for oyster mushroom cultivation provide most of the nutrients and vitamins for growth. Carbon is readily available from cellulose, hemicellulose and lignin from straw, but nitrogen occurs mainly in a bound form and is not available until it is enzymatically released. Various workers have also reported that *Pleurotus* spp have the capability to fix atmospheric nitrogen (Rangaswamy *et. al.* 1975, Jandaik and Rangad 1977) but this has not been proved conclusively. In the cultivation of *A. bisporus* the addition of protein rich supplements is a common practice, which indicates that either the compost is deficient in nitrogen or the bacterial proteins present in the compost are inadequate. Various workers have used supplements from animal and plant origins, including protein-rich, carbohydrate-rich or oil-rich substances, for *A. bisporus* cultivation (Sinden and Schisler 1962, Gerits 1983, Gupta and Vijay 1992). Among the seven supplements used at the time of spawning in *P. ostreatus* var *florida*, cotton seed cake (5%) and de-oiled soybean (5%) gave significantly higher yield (94.6 and 92.8% BE respectively) than the unsupplemented bags. Yield increase may be due to extra amino acids or proteins available to the mushroom mycelium. Lowest yield was recorded in bags where mustard cake was added, possibly due to anti-fungal properties in mustard. Further, cotton seed meal and soybean meal gave lower yield than unsupplemented bag, indicating that oils and fatty acids have negative effect on yield. Dhandha *et. al.* (1995) also observed no change in total mushroom yield in paddy straw mushrooms from the addition of oils of mustard, sunflower, ground nuts and cotton seed at 0.1 to 0.5. He concluded that *Pleurotus* spp prefer unsupplemented and unfermented straw. Mo *et. al.* (1999) found soybean flour superior to other nitrogen sources for eight oyster mushroom species. Zadrazil and Kamara (1997), reported a 300% increase in the yield of *P. sajor-caju* from the addition of either 30% soybean or 40% alfalfa meal. Industrial wastes such as apple pomace and chicken manure have also been reported as cheap nitrogen sources for higher mushroom yield with high dry matter content for several *Pleurotus* and *Auricularia* spp (Vijay and Upadhyay 1989, Upadhyay and Sohi 1987). 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). He also found that supplementation prior to pasteurization increased the total yield, but mushroom size was negatively affected with increased supplementation. In our studies we have not found any significant effect on mushroom size. Influence of supplementation is also species and strain-specific. Somycel 3200 reacted poorly to alfalfa meal and negatively to chicken manure, and Somycel 3001 reacted positively to rice bran and alfalfa meal at the time of filling (Visscher 1989). Upadhyay and Vijay (1989) also observed cotton seed meal as better supplement for *P. fossulatus* and rice bran for *P. ostreatus*. Supplementation is absolutely necessary for getting fructification is some strains of *P. eryngii* (Royse 1999, Upadhyay and Vijay 1991).

With supplementation came a rise in substrate temperature, possibly due to faster metabolic activities triggered by extra nitrogen. Royse and Schisler (1986) also observed overheating (from 30°C to 47°C) in bags where Spawnmate was applied without benomyl treatment, and proposed that it could be due to the growth of competitor moulds. Gurjar and Doshi (1995) did not find any effect on yield of *P. cornucopiae* with 5 and 7.5% addition of soybean meal in wheat straw and assumed this could be due to a rise in temperature. We identified increases in the temperature of beds from 5 to 9°C over room temperature. Therefore, supplements should be cautiously used, because excessive bed temperature (more than 35°C) may kill the mycelium. Overstijns (1995) observed an increase of 19% in mushrooms with the addition of only 0.5% corn steep liquor and recorded a rise in temperature from 0.3, 1.4 and 2.3°C with the addition of only 0.5, 1 and 2% corn steep liquor. Higher supplement doses gave even higher temperatures, which were harmful and attracted growth of *Coprinus* sp (Guna segaran and Graham 1987). In *A. bisporus*, the addition of formaldehyde-pretreated 1 and 2% cotton seed meal and soybean meal at the time of casing produced 20 and 30% higher yields respectively, but higher doses of supplement attracted a lot of contamination (Gupta and Vijay 1992). Supplementation has also been found to facilitate higher mushroom yield in other mushrooms such as *Agrocybe aegerita* and *L. edodes* (Zadrazil 1994, Jong 1989). Higher supplementation (3 to 4% w/w) of NPK in rice husk, melon husk and coconut fruit fibers did not give either mycelium growth or basidiocarp from the tubers of *P. tuberregium* (Isikhuemhen and Okhuoya 1998). Supplementation with de-oiled soybean and cotton seed cake not only gave higher biological efficiency but the fruit bodies were significantly heavier than in unsupplemented bags. The percent conversion of waste into utilizable mushroom is much higher with additional organic nitrogen. Mushrooms in the second flush were heavier than in the first flush, possibly because there were less mushrooms in the second flush than in the first. De-oiled soybean supplementation generally gave heavier fruit bodies than cotton seed cake, possibly due to rapid metabolic activity also evident from higher bed temperatures with the addition of de-oiled soybean. Kalberer (2000) also observed heavier mushroom fruit bodies of *L. edodes* with the addition of inorganic nitrogen sources such as urea and ammonium chloride.

CONCLUSION

Supplementation of wheat straw with organic sources like de-oiled soybean and cotton seed cake are suitable for *P. ostreatus* var *florida* during winter (15 to 20°C) or in temperature-controlled cropping rooms. An additional 21 kg of mushrooms could be harvested using 1kg de-oiled soybean and 47 additional kg of mushrooms could be produced using 5kg of cotton seed cake. Supplementation with these organic N-sources also yielded heavier mushroom fruit bodies. Extra income and better utilization of wastes for protein-rich mushroom are desirable. Supplementation of oyster mushrooms does not require extra labour and is recommendable (especially in winter) to raise bed temperature, to encourage mycelial growth and to increase mushroom yield.

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