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Effect of additives on mycelial growth and fructification of *Pleurotus squarrosulus* (Polyporales: Polyporaceae)

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Abstract: The effects of dungs (cow, horse, poultry), yam peels, rice bran, chaff (*Sorghum*, maize) and several sugars on mycelial growth and fructification of *Pleurotus squarrosulus* (Mont.). Singer were investigated. The concentrations were 5%, 10% and 15% (for sugars: 1%, 3% and 5%). The substrate raw materials were maize and rice straw and cotton waste (substrates without the additives used as controls). Optimum mycelial growth was obtained with 10% rice bran, or *Sorghum* or maize chaff, 5% dung (any) and 5% sugars. Mycelial growth increased with concentration of the sugars and decreased with dung and yam peel concentration. All additives accelerated primordial formation and increased the number of fruitbodies. Rice bran was the best in promoting fructificaction, followed by glucose and fructose; *Sorghum* chaff was the poorest. Sugars promoted fructification more han dungs or chaffs but less than rice bran.

Key words: Fungi, cultured fungus, growth, tropical food.

Pleurotus squarrosulus (Mont.) Singer is an edible Nigerian mushroom that grows on fallen trees and logs (Zoberi 1972, Nicholson 1989). The fruitbodies are tough and are locally uses to prepare soup (Zoberi 1972).

In mushroom cultivation, supplementation of the lignocellulosic organic wastes has been found to increase yield. Zadrazil (1980) and Gunasegaran and Graham (1987) obtained yield increases of *Pleurotus* by adding ligno-cellulosic eastes. Some organic additives are cotton seed, soybean and peanut cakes, plant hormones, urea, glucose, sucrose, rice and wheat, bran, maize chaff, peptone, spent brewer's grain and animal dungs (Chua and Ho. 1973, Han *et al.* 1981, Quimio 1981, Bahl 1988, El-Kattan *et al.* 1991). Waste materials such as sawdust, rice straw, sugar cane debris and non-controlled environmental conditions have been utilized for *Pleurotus ostreatus* cultivation (Macaya-Lizano 1988).

Literature on the cultivation of *P. squarrosulus* is scarce. This study examines the effect of locally available substrate materials and how organic additives influence growth and fructification.

MATERIAL AND METHODS

Mycelium was established on potato dextrose agar by tissue culture of a young fruitbody obtained from decaying logs of wood. Sun-dried maize straw, rice straw and cotton waste (abundantly available in Nigeria) were shreded in 0.5 cm pieces with an office guillotine.

Fifty grams of maize straw, rice straw and cotton waste were separately soaked overnight in 100 ml of sterile distiled water and excess water squeesed out through a muslin cloth. This procedure was repeated many tines. Some batches of these materials were mixed each with cow, horse or poultry dung, ground yam peels, rice bran, Sorghum chaff and maize chaff at 5%, 10% and 15% concentrations. Controls were similarly soaked but received no supplements. Substrate materials were packed to the 10cm mark in boiling tubes which were covered with aluminium foil and autoclaved at 1210C for 15 min. After cooling, the substrates were inoculayted with 8 mm discs of 6 day old mycelium, incubated at 30 ± 2 0C for 10 days, and the liner growth of the colony measured to the nearest cm.

Three hundred grams of maize straw, rice straw and cotton wastes, treated as mentioned above, recived 5% cow dung, horse dung, glucose, maltose, sucrose and furctose, and 10% rice bran or Sorghum chaff each. Treated and control (no additive) substratres were packed in 2L glass flask which were autoclaved as described above. Three replicates of each case were inoculated with 20 g of spawn obtained as active mycelia from a mixture of rice bran and rice straw inoculated with *P. squarrosulus* agar mycelium.

A completely randomized blick design with the treated and control experiments in separate blocks was used.

RESULTS

The various concentrations of additives enhanced the mycelial growth of *P. squarrosulus* (Tables 1 and 2). Among the organic waste additives, the use of 5% and 10% dungs and yam peels, 5% 10% and 15% of rice bran and 10% of Sorghum and maize chaffs as additives resulted in increased mycelial growth (Table 1). Higher mycelial extension was associated with various concentrarions of glucose, fructose, sucrose and maltose, as well as 3% and 5% lactose, arabinose and galactose (Table 2). With rice bran, and *Sorghum* and maize chaff, the greatest mycelial extensions were observed at 10% additive levels for cottom waste, and maize and rice straw (Table 1). Five percent supolentation of dungs, yam peels and sugars resulted in the highest growth for the three materials (Tables 1 and 2). The greatest mycelial extension in the three substrates was stimulated by 10% rice bran, 5% glucose and 5% fructose (Tables 1 and 2).

All the additives enhanced fruitbody production (Table 3). Apart from 5% dungs and 10% *Sorghum* chaff, the additives accelerated primordial development (Table 3). Rice bran was the best additive to promote fructification (Table 3). Rice bran was followed by glucose and fructose in promoting fructifiactions while *Sorghum* chaff was the poorest (Table 3). In decreasing order, frutification was accelerated by rice bran, sugars, dugns and *Sorghum* chaff (Table 3).

Rice straw was consitently the best in supporting mycelial growth and fructification, followed by maize straw; cotton waste was the porest (Tables 1-3).

TABLE 1

Mean mycelial extension (cm) of P. squarrosulus *in substates of various raw materials mixed with several organic waste additives 10 days after inoculation*

Organic waste additive and concentration	Substrate types		
	Maize straw	Rice straw	Cotton waste
Cow dung			
5%	5.7	6.4	3.9
10%	5.1	5.6	3.3
15%	4.6	4.9	2.8
Horse dung			
5%	5.9	6.6	4.1
10%	5.2	5.8	3.5
15%	4.7	5.0	3.0
Pultry dung			
5%	5.7	6.3	3.8
10%	5.0	5.6	3.2
15%	4.5	4.9	2.6
Yam peels			
5%	5.7	6.5	3.8
10%	5.0	5.7	3.3
15%	4.5	5.0	2.5
Rice bran			
5%	5.6	6.1	3.4
10%	7.1	7.8	4.8
15%	5.0	5.7	3.0
Sorghum chaff			
5%	4.9	5.5	3.4
10%	5.6	6.2	4.2
15%	4.6	5.2	2.9
Maize chaff			
5%	4.7	5.1	3.3
10%	5.5	6.0	4.1
15%	4.5	5.0	2.7
Control (no additive)	4.3	4.7	2.3
Least significant difference (LSD)	0.6	0.8	0.4
Coefficient of variation (CV)	6.1	5.9	6.4

TABLE 2

Mean mycelial extension (cm) of P. squarrosulus *in substrates of various raw materials mixed with several organic sugar additives 10 days after inoculation*

Organic waste additive and concentration	Substrate types: Maize straw	Rice straw	Cotton waste
Glucose			
1%	5.8	6.0	3.0
3%	6.3	6.8	3.5
5%	7.0	7.5	4.3
Lactose			
1%	5.0	5.2	2.8
3%	5.6	5.7	3.2
5%	6.2	6.3	3.7
Maltose			
1%	5.4	5.6	2.9
3%	6.1	6.3	3.4
5%	6.7	7.0	4.0
Arabionose			
1%	4.9	5.1	2.6
3%	5.4	5.6	3.0
5%	6.0	6.1	3.5
Galactose			
1%	5.2	5.3	2.8
3%	5.8	6.0	3.2
5%	6.5	6.6	3.8
Sucrose			
1%	5.3	5.5	3.0
3%	6.0	6.3	3.5
5%	6.8	7.0	4.1
Fructose			
1%	5.6	5.9	3.0
3%	6.2	6.6	3.5
5%	7.0	7.3	4.1
Control (no additive)	4.4	4.7	2.4
Least significant difference (LSD)	0.7	0.8	0.4
Coefficient of variation (CV)	5.7	6.1	6.5

TABLE 3

Mean number of P. squarrosulus *fruitbodies produced and time of primordia formation (days) for substrates of various raw materials mixed with different organic waste and sugar additives*

Additive type and concentration	Substrate types: Maize straw A	Maize straw B	Rice straw A	Rice straw B	Cotton waste A	Cotton waste B
Cow dung 5%	4	27	5	27	2	28
Horse dung 5%	5	27	6	26	2	27
Rice bran 10%	7	20	11	20	4	20
Sorghum chaff 10%	4	29	5	28	2	29
Glucose 5%	6	22	9	22	3	22
Maltose 5%	5	25	7	24	2	25
Surcrose 5%	5	24	7	24	2	25
Fructose 5%	6	22	8	22	3	23
Control (no additive)	3	31	4	31	1	32
Least significant difference (LSD)	0.8	3.0	0.9	3.1	0.4	3.2
Coefficient of variation (CV)	12.3	11.4	12.7	10.4	13.8	10.5

A = No. of fruitbodies
B = Primordia formation (Nº of days after inoculation)

DISCUSSION

Treatments of the 3 substrate types with the different organic additives of dungs, chaffs, yam peels, rice bran and sugars led to enhanced mycelial growth. Other workers achieved enhanced mycelial growth of mushrooms by using additives. Quimio (1981) did so for *Auricularia* sp. cultured on sawdust: a 5% sugar supplement produced the best growth. Han *et al.* (1981) also obtained a similar result using lentinus edodes colonized sawdust with wheat bran, and soybean, sesame and peanut cake, increasing mycelial growth optimally at 5%.

High sugar concentrarions were more favourable than high dung, bran or chaff concentrations, perhaps because sugars are needed in large quantities as carbon sources, while dungs, bran and chaffs could have made the substrate highly alkaline. Alkalinity inhibits mycelial growth and the pHs of the dungs, rice bran and chaff as detected by Kadiri (1990) are as follows: cattle dung 8.2, horse 7.8, poultry 8.6, rice bran 7.1., Sorghum chaff 7.6 and maize chaff 8.6.

All supplements favoured fructifiaction, as expected. El-Kattan *et al.* (1991) reported that supplementing *Pleurotus florida* and *P. sajor-caju* at spawning with soybean flour and cotton seed cake (0.30 to 0.60% of dry rice straw substrate) led to increases in both mushroom yields and net protein production occurred at

0.45% supplementations with either soybean flour or cotton see cake. Han et al. (1981) likewise observed that additive treatments of *L. edodes* mycelia colonized sawdust with 0.5 to 20 ppm of 2,4 - dichlorophenoxyacetic acid and indole -3-acetic acid enhanced the time of primordia formation.

The 10% rice bran treartment produced the highest mycelial growth and fructification, followed by glucose and fructose and finally *Sorghum* chaff. Earlier studies had shown rice bran to be a good nutrient supplement to sawdust for mycelial growth of *Auricularia* sp. (Quimio 1981) and *L. edodes* (han *et al.* 1981). The stimulatory and enhancement substances present in rice bran are likely to be sugars, amino acids, vitamins and mineral elements (Oyenuga 1968, Botton and Blair 1982). This study supports the use of rice bran (10%) and glucose, fructose, sucrose, maltose and horse dung (5%) as additives to improve the yild of *P. squarrosulus*.

RESUMEN

Se investigó el efecto de heces (vaca, caballo, gallinas), cáscaras (yam), afrecho de arroz, cascarilla de sorgo y maíz y varios azúcares, en el crecimiento y fructificación del hongo *Pleurotus squarrosulus* (Mont.) Singer, usando concentraciones de 5, 10 y 15% (excepto para azúcares, que fueron 1, 3 y 5%). Los sustratos fueron "paja" de maíz y arroz, y desechos de algodón (se usó como testigos sustratos sin los aditivos). Se obtuvo un crecimiento micelial óptimo con 10% de afrecho de arroz, o cascarilla de sorgo y maíz; con 5% de heces (todas) y con 5% de azúcares. El crecimineto del micelio aumentó con la concentración de azúcar y disminuyó con la de heces o cáscara. Todos los aditivos aceleraron la formación de primordios y aumentaron el número de cuerpos fructíferos. El afrecho de arroz fue el mejor para la fructificación (seguido de glucosa y fructosa) y la cascarilla de sorgo fue la peor. Los azúcares favorecieron la fructificación más que las heces o cascarillas pero menos que el afrecho de arroz.

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