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Potential of organic residues in producing oyster mushroom, *Pleurotus ostreatus* Fr. (Polyporaceae)

Wonder NGEZIMANA*, T. A. MTAITA and Itai MTUKWA

Africa University, Faculty of Agriculture and Natural Resources, P. O Box 1320, Mutare, Zimbabwe.
** Corresponding author, E-mail: tamtaita@africau.ac.zw, r054481ms@africau.ac.zw*

ABSTRACT

Low mushroom yields are observed when non-supplemented substrates are used for production. The experiments were carried out to evaluate potential benefits of organic supplements in cotton residues, maize stover and wheat straw substrates used for cultivation of oyster mushroom. Mixed formulations at various doses of 0, 4, 8 and 14 % sunflower seed cake supplement levels were autoclaved before inoculation with *P. ostreatus* spawn. Duration of developmental stages, mycelium vigour and yield of oyster mushroom on substrates formulations were evaluated. The mycelium vigour significantly increased ($P<0.01$) as supplement levels increase in both wheat straw and maize stover substrates. First flushes contributed more to total yield than subsequent flushes. There was significant increase in biological efficiency ($P<0.001$) with increase in supplement levels across all substrates. However, in contrast to supplemented cotton residues and wheat straw substrates, further increase of supplements (14 % level) in maize stover decreased the yields. Non supplemented substrates produced significantly low yields in both experiments. Cotton residues gave significantly higher yields (134.8 biological efficiency) at 14 % supplement level.

Keywords: organic, substrates, mycelium vigour, biological efficiency.

INTRODUCTION

Oyster mushrooms (*Pleurotus* spp.) are amongst the major mushrooms in production in the world, accounting for about 25 % of total world production of cultivated mushrooms (Tan et al., 2005). In Africa; Ethiopia, Malawi, Nigeria, South Africa, Zambia and Zimbabwe are some of the countries producing oyster mushroom. According to Chang (1999), these countries have a total annual production of approximately 200 tonnes (dry weight) of mushrooms. However, the contribution of Africa is negligible, constituting less than one percent of world production (Chang, 1999). In Zimbabwe there are about 60 species of edible mushrooms (Boa, 2004). Most of these mushrooms are found in the wild. According to Masuka (2002), both indigenous woodlands, particularly the miombo and exotic pine plantations are an important source of the mushrooms. The wild mushrooms found in Zimbabwe are produced in the summer (rainy season) months, so the mushroom demand is not met off summer period. In Zimbabwe, the annual mushroom commercially produced is estimated at 250 tonnes (fresh weight) and the demand for the produce is 700 tonnes (Anonymous, 2006).

The economic importance of oyster mushroom lies primarily in its use as food of high nutritional and medicinal value. The ability of the mushroom strains to convert substrate into mushrooms is known as their biological efficiency, which is the yield obtained per dry mass of the initial substrate (Stamets, 1993). Most of the materials used as substrate have low nutrients (Nitrogen) which results in low yields hence low biological efficiency. The nitrogen is usually depleted by fructification therefore becoming inadequate and limiting mushroom yield (Upadhyay et al., 2002). Organic supplements such as soybean meal, chicken manure and cotton

seed meal with N content which varies between 3 to 7 % can be added to substrates to provide organic sources of nitrogen (Upadhyay and Verma, 2000). The major aims of this work were (1) to evaluate the potential ability of various locally available substrates to produce mushrooms and (2) to establish the effects of sunflower seed cake supplements on improving yield of oyster mushrooms grown on cotton residues, maize stover and wheat straw.

MATERIALS AND METHODS

Two experiments were carried out in 2006-2007 at Africa University located in Mutare, Zimbabwe. In the first experiment, seven base substrates (wheat straw, banana leaves, maize stover, maize cobs, bean shells, cotton residues and grass) of 1 kg dry weight were evaluated for their potential to produce mushrooms. The design used was a randomized complete block design with 4 replicates. Substrates were ground to small units of about 2 cm long and weighed into 1 kg units. The weighed substrates were then boiled in water for 30 minutes, allowed to cool, and the water drained out. The substrate was then packed into plastic fruiting bags of 22 cm diameter and 45 cm height. Experimental units constituted of two bags of substrate. Spawning was done with grain spawn using triple spawning where spawn was placed at three levels in the substrate. The first level was placed at one third of the substrate height in the bag, the second at two thirds and the third at the top. The amount of spawn used per bag was 75 grams. Fruiting bags were then loosely tied to allow air to circulate. The spawned substrates were kept in the lab at room temperature, until spawn run was complete and then the fruiting bags were taken to the fruiting house.

In the second experiment, mushroom yield from cotton residues, maize stover and wheat straw substrates supplemented with sunflower seed cake at 0, 4, 8 and 14 % levels was evaluated. Substrates were soaked for 2 hours in water and strained up to when a few drops of water were released with some pressure. The substrates were mixed at 100 %, 96 %, 92 % and 86 % (dry weight of substrate) by mass with the sunflower seed cake supplement. Each substrate was filled in polypropylene-ethythene heat resistant bag. After filling, the bags were closed by putting a PVC plastic ring and a cotton plug in the formed mouth on top of the bag. Aluminium foil was used to cover the cotton wool for moisture control during autoclaving. The mixed formulations were autoclaved before inoculation. Cooled bags were top spawned with oyster mushroom spawn at the inoculation rate of 7 % per bag according to the dry weight of the substrates. Fruiting bags were loosely plugged with cotton wool to allow air to circulate. The spawned substrates were kept in the lab at room temperature, until spawn run was complete and then the fruiting bags were taken to the fruiting house and laid on shelves. The design used was a randomized complete block design with 3 replicates. The bags were opened by removing the cotton plug and the PVC plastic neck. Duration of developmental stages, mycelium vigour, biological efficiency, yield and quality of oyster mushroom was recorded and analyzed using Genstat 4DE.

RESULTS

Experiment 1

Duration of developmental stages

Spawn run and pinhead formation

The life cycle of the mushroom is useful in the understanding of its biology. Mushroom spores germinated, forming mycelia which grew into a network of strands in the culture medium on which it was grown. Fruiting bodies emerged as pinheads and developed to maturity. Duration of such developmental stages varied with substrate type as shown in Table 1.

Days from spawning to 100 % spawn run

Period from spawning to completion of spawn run ranged from 11 to 20 days. There was no significant difference in the time to spawn run completion in wheat straw, grass, maize stover and banana leaves (Table 1). The slowest was in cotton waste (20.75 days).

Time taken from 100 % spawn run to pinhead formation

Days taken from 100 % spawn run to pin head formation varied significantly among the substrates with cotton being the quickest and wheat straw being the slowest which is contrary to the substrate's trends in days to completion of spawn run (Table 1). Days from 100 % spawn run completion to pin head formation ranged from 7 to 16 days. Days

taken from the time fruiting bags were taken to the fruiting house to the time of pinhead formation varied significantly. The earliest pinhead formation was observed from the substrates maize stover, grass, maize cobs and bean waste and the latest on cotton waste (Table 1).

Time taken from spawning to pin head formation

There was no significant difference in the days taken from spawning to pin head formation.

Biological efficiency

There was no significant ($P>0.05$) difference in the biological efficiency obtained on the different seven substrates (Figure 1). The yield averages ranged from 738 to 440 grams and these were obtained from maize stover and maize cobs respectively. Cotton waste gave an average yield of 721grams. Maize Stover however had a yield average of 738 grams. The yields obtained were from 38 cumulative days. The biological efficiency ranged from 22.9 % to 39 %.

Experiment 2

Duration of developmental stages

As shown in Table 2, there were significant differences in mushroom growth stages in different substrates.

Days from spawning to full spawn run.

The length of time from spawning to full spawn run varied significantly ($P<0.001$) among the different substrates types. However, there was no significant interaction between substrate type and supplement level on days from spawning to full spawn run. Supplement levels had no effects on days to full spawn growth. Spawn growth showed no significant difference in both high and no supplement substrates. Days from spawning to full spawn run ranged from 19 to 22 in cotton residues, 23 to 36 in maize stover and 24 to 33 in wheat straw. There were no significant differences in maize stover and wheat straw substrates in days to full spawn run, though both were significantly different from cotton residues. Spawn run in cotton residues substrate took lesser number of days (19.75), with no significant differences at various supplement levels.

Days between full spawn run and first primordial formation

There were significant differences ($P<0.05$) in time from full spawn run to first primordial formation in the different substrate types. Maize stover and wheat straw substrates took the least number of days to reach primordial formation after full spawn run than cotton residues. Supplementation levels did not show any significant difference in time from full spawn run to first primordial formation. There was also no significant interaction effect between substrate and supplement levels on days taken from 100 % spawn run to pin head formation.

Table 1: Means of days from spawning to 100% spawn run, days from 100% spawn run to pin head formation and days from time fruiting bags were taken to fruiting house to pin head formation from seven different substrates used for growing oyster mushroom.

Treatments	DTFSR	DFSRPF	DPFTH	DTPHF
Cotton residues	20.75^{c}	7.00^{a}	6.00^{b}	28.00
Maize Cobs	16.50^{b}	11.25^{ab}	4.50^{a}	26.00
Banana Leaves	13.75^{a}	14.00^{b}	5.75^{ab}	27.75
Grass	13.50^{a}	12.00^{b}	3.75^{a}	25.75
Maize stover	13.75^{a}	11.25^{ab}	3.00^{a}	25.00
Wheat straw	11.00^{a}	16.00^{bc}	5.75^{ab}	27.00
Bean residues	16.25^{ab}	9.75^{a}	4.00^{a}	26.00
Grand mean	15.07	11.61	4.68	26.50
SED	1.485	1.473	0.955	1.053
$LSD_{0.05}$	3.121	3.095	2.007	NS
CV%	6.1	5.4	15.0	3.2

Within the columns, means with at least a common superscript letter are not significantly different using the Duncan's Multiple Range test. NS denotes non significance at P=0.05.

DTFSR- Days from spawning to 100% spawn run; DFSRPF- Days from 100% spawn run to pin head formation; DPFTH- Days from pinhead formation to harvesting; DTPHF- Days from spawning to pinhead formation.

Table 2: Number of days taken for the different developmental stages in cotton residues, maize stover and wheat straw substrates.

Substrate type	Variables				
	DTFSR	DBFSR-PF	DFPFTH1	CDTF1H	TNF
Cotton residues	19.75^{a}	21.58^{b}	6.17^{b}	47.50^{a}	2.33^{b}
Maize stover	28.50^{b}	15.33^{a}	3.75^{a}	47.58^{a}	2.75^{b}
Wheat straw	26.83^{b}	16.87^{ab}	3.96^{a}	46.58^{a}	1.50^{a}
Mean	25.03	17.93	4.62	47.22	2.19
P Values	***	*	**	Ns	**
$LSD_{0.05}$	3.115	4.976	1.465	4.766	0.659
CV%	14.8	32.9	37.5	12.0	35.6

*, **, *** Significant at P<0.05, P<0.01, P<0.001 respectively; ns = not significant at P<0.05. Within the columns, means followed by the same superscript letter are not significantly different, $LSD_{0.05}$.
DTFSR- days from spawning to 100% spawn run ; DBFSR-PF- days between full spawn run and primordial formation ; DFPFTH1-days from primordial formation to first harvesting ; CDTF1H- cumulative days to Flush 1 harvesting ; TNF- total number of flushes harvested.

Days from primordial formation to first harvesting

Days taken from primordial formation to harvesting varied significantly (P<0.01) in the different substrates types. However, days from spawning to primordial formation for the first flush did not show any significant difference (P>0.05) for the supplement levels. The interaction effects between substrate and supplement levels did not show any significance on days from primordial formation to first harvesting. Development of primordial to harvestable basidiome size took significantly more days in cotton residues than in maize stover or wheat straw substrates.

Cumulative days to harvesting of first flush

There was no significant difference on the different substrate types in the total number of days from spawning to harvesting at P<0.05. Supplementing the substrates, did not significantly vary the cumulative days to first flush harvesting. There was no significant difference (P>0.05) in cumulative days to harvesting of the first flush in the interaction effects between substrate type and supplement level.

Total number of flushes

Total number of flushes harvested showed significant differences (P<0.01) due to substrate type. In cotton residues and maize stover, flushes harvested ranged from one to four and in wheat straw, from no flush to three flushes. There was no significant difference in total number of flushes in supplement levels at P<0.05. The interaction between substrate type and supplement level did not show any significant difference in the number of flushes harvested.

Cumulative days to harvesting of last flush

Cumulative number of days to last flush harvest varied significantly with substrate type (P<0.001) and supplement levels (P<0.05). There were no significant interaction effects that occurred between the two main factors on the cumulative days to last harvest (Table 3).

Production cycles per year

The interaction effect between substrate type and supplement level on total number of production cycles was significant (P<0.01). Production cycles differed significantly in different substrates types (P<0.001) and supplement levels (P<0.01). At high supplement levels, there was lesser number of production cycles to be produced (Table 4). However, there were higher yields produced at the high supplement levels across all substrates (more than 90% biological efficient).

Percentage Mycelium vigour

There was significant difference in mycelium vigour between the different substrates (P<0.001) and between the different supplement levels (P<0.01) (Table 5). The interaction effect between substrate types and supplement levels was not significant (P>0.05) on mycelium vigour. This relates with the total days to full spawn run which did not show any significant difference on the interaction of substrates and different supplement levels.

Mushroom Yield

When the three different substrates were supplemented with increasing doses of sunflower seed cake, there was significant

Table 3: Cumulative number of days to last flush harvesting for the period of experiment run

Substrate Type	Supplement Level (%)				Mean	P Value	$LSD_{0.05}$
	0	4	8	14			
Cotton residues	71.7	69.0	76.0	76.0	73.2	***	11.06
Maize stover	77.3	73.3	91.3	74.7	79.2	***	11.06
Wheat straw	36.7	54.7	62.0	69.7	55.7	***	11.06
Mean	61.9	65.7	76.4	73.4	69.4		
P values	*	*	*	*			
$LSD_{0.05}$	9.58	9.58	9.58	9.58			
CV%	16.4	16.4	16.4	16.4			

*, *** Significant at P<0.05, P<0.001 respectively.

Table 4: Production cycles per year in supplemented cotton residues, maize stover and wheat straw substrates

Substrate Type	Supplement Level (%)				Mean	P Value
	0	4	8	14		
Cotton residues	5.12^a	5.44^a	4.94^a	4.84^a	5.08	***
Maize stover	4.79^a	5.19^a	4.04^a	4.91^a	4.73	***
Wheat straw	10.52^b	6.75^a	5.99^a	5.28^a	7.13	***
Mean	6.81	5.79	4.99	5.01	5.65	
P Value	**	**	**	**		
$LSD_{0.05}$	1.896	1.896	1.896	1.896		
CV%	19.9	19.9	19.9	19.9		

** Significant at P<0.01, *** Significant at P<0.001. Means in the same row with at least a common superscript letter are not significantly different, $LSD_{0.05}$

Table 5: Mycelium vigour in supplemented cotton residues, maize stover and wheat straw substrates.

Substrate Type	Supplement Level (%)				Mean	P Value	$LSD_{0.05}$
	0	4	8	14			
Cotton residues	96.7	100	93.3	100	97.5	***	11.23
Maize stover	66.7	66.7	66.7	86.7	71.7	***	11.23
Wheat straw	40.0	63.3	60.0	90.0	63.3	***	11.23
Mean	67.8	76.7	73.3	92.2	77.5		
P Value	**	**	**	**			
$LSD_{0.05}$	12.97	12.97	12.97	12.97			
CV%	17.2	17.2	17.2	17.2			

** Significant at P<0.01, *** Significant at P<0.001.

difference (P<0.01) in the biological efficiency obtained in the different substrate types. Supplement levels showed significant differences (P<0.001) in the biological efficiency of the first flush. Significant variation in the biological efficiency (P<0.05) of the first flush harvested is also shown in the interaction between substrate type and supplement level. The different flushes contributed differently to the total biological efficiency with the first two flushes contributing more than 70% of the yield (Fig. 2).

There were significant differences (P<0.001) in fresh yield, dry matter yield and total biological efficiency on different substrate types and supplement levels. The substrate type and supplement levels showed a significant interaction on either fresh yield, dry matter yield and biological efficiency with increase in ability to convert substrate to sporophores as supplement levels increased. Cotton residues gave the highest fresh yield at 14 % supplementation. The yields obtained at 14 % supplementation in maize stover did not significantly vary with the yields observed in the same substrate at no supplementation.

Linear regression models on total biological efficiency observed due to supplement application showed strong linear

relationships between supplement level and the biological efficiency for both cotton residues and wheat straw as shown in equations on Figure 3. In contrast, the overall supplement effect on biological efficiency for maize stover substrate could best be accommodated polynomial.

The biological efficiency observed in cotton residues at 14 % supplement level was significantly higher than the lower supplement levels in the same substrate. In maize stover, 4 and 8 % supplement levels gave the highest yields than the control and 14 % level. In maize stover supplementation, there was an increase in yield (from 27.2 to 105.6 biological efficiency) up to the 8 % supplement level, and then decrease onwards to the 14 % level (60.7 biological efficiency). As indicated in Figure 4, there was a relationship between the dry matter yields observed for the total flushes and the fresh weight.

Basidiome size

Basidiome size did not vary significantly (P>0.05) in substrate alone, different supplement levels and in the interaction between substrate and supplement levels.

Basidiome number

Substrate type did not show any significant difference (P>0.05) in the total basidiome number harvested per flush. The supplement levels showed significant differences (P<0.01), though the substrate and supplement level interaction did not show any significant differences. Addition of supplements increased the number of basidiome observed (Figure 5). However, the 4 % supplement level produced significantly higher yield than 8 % and 14 % levels.

Marketable basidiome

Total marketable basidiome per flush varied significantly (P<0.01) with different supplement levels but did not vary with substrate types. Increasing the supplement levels increased the number of marketable basidiomes, due to improvement in development of the basidiome in supplemented substrates. The interaction effects between substrate and supplement level did not show any significance (P>0.05) on number of total marketable basidiome per flush.

Ratio of total marketable basidiomes to total basidiomes harvested per flush.

Substrate types did not show any significant difference (P>0.05) in ratio of total marketable basidiomes to total basidiomes harvested per flush. There was significant difference (P<0.01) in total marketable basidiome to total basidiome produced per flush in the supplement levels. Interaction between substrate and supplement levels (P<0.01) on the ratio of total marketable basidiome to total basidiomes harvested per flush were also significant. Wheat straw supplemented at 8 % produced the highest ratio of marketable basidiome number to harvested basidiome (Fig 6) followed by maize stover at 4 % supplement level.

Quality

There was significant difference in the quality of the basidiome produced per flush in the different substrates (P<0.05) and supplement levels (P<0.001). The results also showed significant (P<0.05) interaction effects between substrate type and supplement level on the quality of mushrooms produced (Table 6). Substrates that had shown highest biological efficiency also gave the best mushroom quality.

Table 6: Quality of the basidiomes harvested per flush in sunflower supplemented cotton residues, maize stover and wheat straw substrates.

Substrate Type	Supplement Level (%)				Mean	P Value
	0	4	8	14		
Cotton residues	3.28^{a}	3.00^{a}	3.83^{a}	3.44^{a}	3.39	*
Maize stover	1.22^{a}	3.50^{b}	3.25^{b}	2.33^{ab}	2.58	*
Wheat straw	0.00^{a}	1.67^{b}	3.83^{c}	3.22^{bc}	2.18	*
Mean	1.50	2.72	3.64	3.00	2.72	
P Values	***	***	***	***		
$LSD_{0.05}$	1.647	1.647	1.647	1.647		
CV%	35.8	35.8	35.8	35.8		

* Significant at P<0.05, *** Significant at P<0.001. Means in the same row with at least a common superscript letter are not significantly different, $LSD_{0.05}$

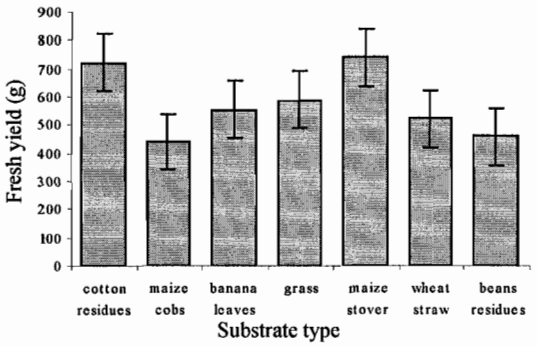

Figure 1: Average yield of oyster mushroom (per fresh weight basis) grown on seven different substrates, obtained over 38 days of harvesting.

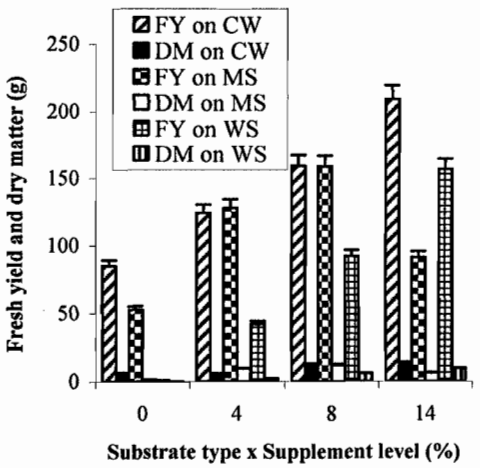

Figure 4: Relationship between fresh yield (FY) and dry matter (DM) yield in cotton residues (CW), maize stover (MS) and wheat straw (WS) substrates at 4 supplement levels.

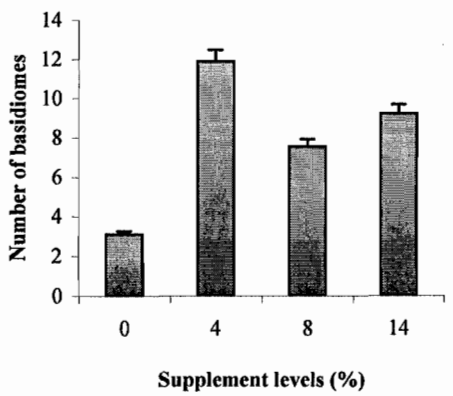

Figure 5: Number of basidiome produced per flush at 0, 4, 8 and 14 % supplement levels.

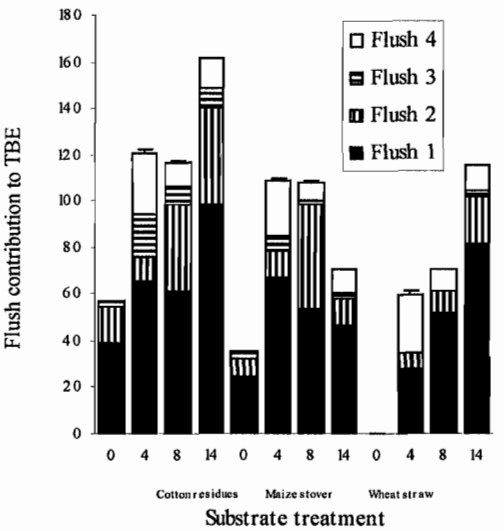

Figure 2: Contribution of flushes to the total biological efficiency (TBE %) in supplemented cotton residues maize stover and wheat straw substrates.

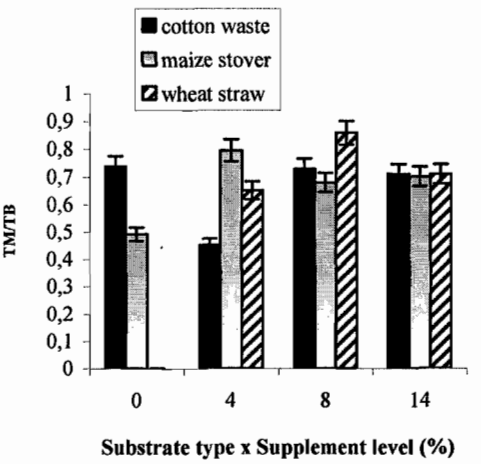

Figure 6: Ratio of total marketable basidiome to total basidiome harvested per flush in supplemented cotton residues, maize stover and wheat straw substrates. TM= total marketable; TB= total basidiome.

Mushroom quality from maize stover at 14 % did not differ significantly from the non-supplement treatment in the same substrate. Quality in cotton residue substrate did not vary at the four supplement levels, yet it increased in wheat straw substrate with increasing supplement level.

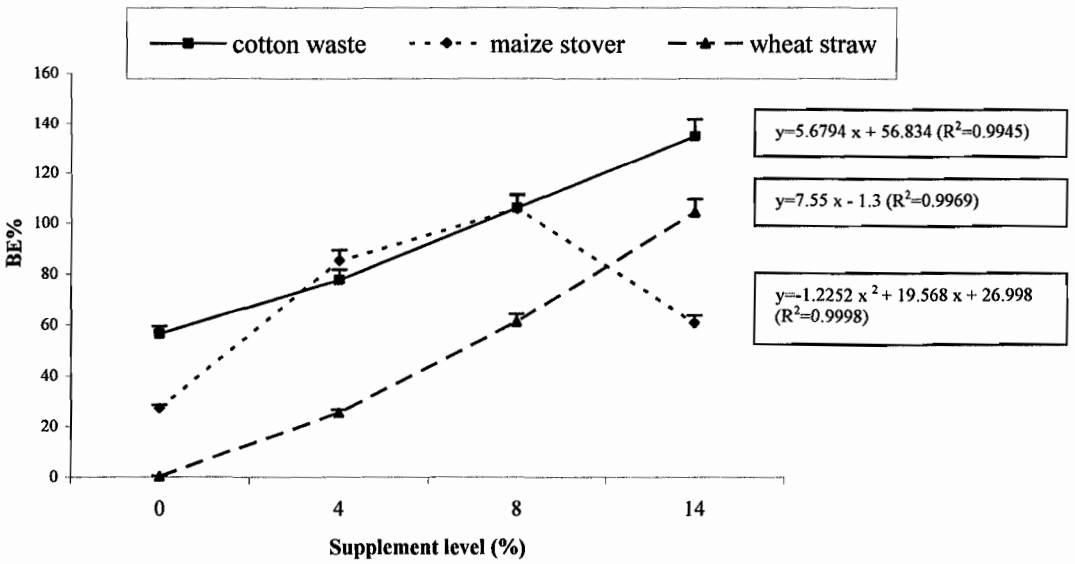

Figure 3: Total biological efficiency of all flushes harvested in cotton residues, maize stover and wheat straw substrates supplemented at 0, 4, 8 and 14 % levels with sunflower seed cake.
Equations: y=biological efficiency, and x=supplement level. BE= biological efficiency.

Crude Protein

There was no significant difference ($P<0.05$) in crude protein found from either the different substrate types, supplement levels or substrates type-supplement level interaction effects. The crude protein means ranged from 12.25 to 21.28 %.

DISCUSSION

Experiment 1

The spawn run period compares well with Stamets (1993), Singh (1996) and Gour (1998) who reported the period of spawn run of 12-21, 15-20 and 20-30 days from spawning to spawn run completion respectively. On the contrary, Oei (1991) reported that in *P. ostreatus,* spawn run takes 4-5 weeks. Stamets (1993), reported 2-14 days for primordial formation and he argued that it takes only 3-5 days for primordial formation at a relative humidity of 95-100 % and temperature of 10-16 °C. Gour (1998) and Singh (1996) also reported that primordial development takes 2-5 and 3-4 days respectively. The longer duration observed in this experiment could have been due to variations in humidity (40-90 %) and temperature (20-28 °C). Wetting the floor of the fruiting house and sprinkling a fine spray of water on the fruiting bags might not have been efficient to maintain humidity thus contributing to a longer period taken for primordial formation. The days from spawning to pinhead formation (25-28 days) compared favorably with Singh (1996), who reported that it takes 20-25 days for pinheads to start appearing after spawning.

Even though Oei (1991) suggests harvesting to be done for as long as the mycelium remains white and firm, at the end of our experiment (38 days of harvesting), some of the substrates like bean residues had stopped fruiting, as their production was concentrated at the beginning of the harvest period. For maize cobs and banana leaves, the yield had tapered down and was very low per harvest. Yield from all substrates was decreasing with subsequent flushes during the harvest period as the nutrients for growth and development decreased in the substrates. Stamets (1993) also observed that the first three flushes are usually the best with a decrease in yield with each successive flush.

Since maize stover and cotton have a longer fruiting life, they have potentials to ultimately give more yields than those obtained in this experiment. For *P. ostreatus*, Stamets (1993) reported three to four flushes

that are seven to fourteen days apart within 45-55 days of harvest. The biological efficiency found in the first experiment compares well with that observed by Oei (1991) who recorded four flushes of *P. sajor caju* with a yield of around 20% of the weight of the substrate. The low biological efficiency found in this experiment contradicts Stamets (1993) who reported yield potential of oyster mushrooms expressed on a biological efficiency basis ranging from 75-200 % and in growing *P. ostreatus*, yield often exceeds 100 %. However, like what Kasuya et al. (2005) and Tan et al. (2005) observed, most of the substrates used for cultivation are of low-value lignocellulosic wastes that are primarily derived from agricultural practices or the agro-industry. Platt et al (1989) also reported a biological efficiency of 60-70 % when growing *P. ostreatus* on cotton residues which is greater than what was obtained with all the substrates used in this experiment within the 38 days of harvesting. The large difference in the biological efficiency obtained in this experiment and that obtained by Stamets (1993) and Platt et al. (1989) is a clear indication that to improve biological efficiency, nutrient supplements must be used.

Experiment 2

In experiment 2, the less number of days (19.75) taken to reach full spawn run taken by the cotton residues substrate can be best attributed to its physical structure. The lint fiber has particles that are finely separated and close together such that there is less energy utilized by the mycelium to move from one particle to the other for cellular digestion. As the mycelium grows away in the surrounding substrates, each individual thread of the mycelium produces a wide range of extra cellular hydrolytic and oxidative enzymes. These enzymes degrade and utilize the substrate in the period from inoculation to cropping end. Wood and Smith (1988) also observed that after inoculation, the process of growth and establishment depends on the physical, chemical and biological environment of the substrate. However, the physical characteristics of the cotton residues enhanced more time for the development of primordial. After primordial initiation, subsequent growth involves the digestion and absorption of nutrients from the media, which was affected by the ability of the substrate to be easily digested. The effect of the different substrates on the duration of developmental stages have been explained by Vladimir et al. (2005), who attributed such difference to chemical and physical characteristics of lignocellulosic substrates.

The volume of the cotton residues regulated the days to full spawn run. When water was added to the units of the three substrates, weighing the same mass and further strained, cotton residue had less volume than maize stover and wheat straw. When top spawned, the mushroom inoculum therefore moves faster to fully colonize the substrate in cotton residue than maize stover and wheat straw substrates though they both have the same dry weight. Oei (1991) emphasizes on the similarities in size and character of the substrates used for production with given water content resulting in more or less the same free water activity if the substrates are similar.

Primordial formation occurred under high (above 80 %) relative humidity in the growing room, with good air circulation and a constant temperature (less than 30 °C) that is within the fruiting range. Stamets and Chilton (1983), argues that once pins are set, they need a constant environment until they develop to harvestable mushrooms. Usually addition of water would raise the relative humidity of the fruiting room facilitating pin set. The extra-cellular enzymes digest the organic sources of food to components that can be easily leached off if there is forceful watering.

The significantly low number of flushes observed in wheat straw was caused by the low nitrogen and carbon to nitrogen ratio (1:98) in the substrate. This concurs with Chang and Hayes (1978) who argued that, lower carbon to nitrogen ratios reduce the amount of energy required for mycelium development, consequently reducing the flushes obtained. The number of flushes harvested in this experiment does not strongly relate to the total yield produced. Saxena and Rai (1994), revealed in a mathematical model of flushing, a hypothetical substance which is absorbed and accumulated in the mycelium to a threshold level sufficient to trigger flush initiation. Flush growth then depletes the intra-mycelial substrates and prevents new

initiation. The substrates accumulate again after harvesting and trigger another flush. This is similar to what was observed in this experiment, as flush development was also preceded by a break before new flush was initiated. Increasing supplement levels increased the number of days to last flush harvesting due to increased organic nutrients in the substrates available for mushroom flush development. However, the results are contrary to Gupta and Vijay (1994) who observed less number of days to last flush when supplements were used.

At no supplement, wheat straw gave only a single flush. This was probably due to the poor mycelium vigour that led to contamination before subsequent flushes developed. However, terminating the crop after the first harvest would increase the production cycles per year, hence increase in the overall yields obtained with time. These results are contrary to observation by Gupta and Vijay (1994) who observed increase in cropping cycles per year (11-12 cycles) in supplemented treatments compared to 4-5 cycles in un-supplemented treatments. Results of our work are also contrary to Stamets and Chilton (1983), who concluded that 60-70 % of the total yield is normally harvested from the first two flushes and that the greater the number of pins set for the first flush the higher the yield, provided sufficient nutrients are available.

Substrates determined the type of spawn growth with high vigour and colonization rates in lint fibre particles of cotton residues than in maize stover and wheat straw substrates. Beyer and Wilkinson (2002) noted that problems with substrate formulation may first develop during the spawn growing period. According to Beyer and Wilkinson (2002), the relationship between mycelium vigour and yield can be used in the evaluation of the potential yield from substrates during the colonization of the substrate by spawn. Our results gave an indication that organic nutrients supplements can be of immediate use during the growth and development processes of the mycelium which take place from inoculation to full spawn run.

Most contamination occurred on treatments with poor mycelium growth because mycelium growth is out competed by the contaminants as observed in the mycelium vigour of wheat straw at no supplementation. Contaminants (weeds and indicators molds) indicates the possibility of limited or excess nutrients (Stamets and Chilton, 1983; Beyer and Wilkinson, 2002).

The low volumes of substrate used (150g dry weight), might have contributed to the low fresh yields obtained. Bisaria et al. (1989) argued that increasing the size of the containers and volume of substrate used would increase the yield obtained, though the overall biological efficiency remains the same. However, increasing supplement levels increased the organic nitrogen availability which increases yield. This contradicts to Upadhyay et al. (2002), who observed that when soybean cake is used at 1 %, 2.5 %, 5 % and 7.5 % supplement levels, there was no significant difference in yield. According to Youri and Kwaku (2005), carbon is readily available from cellulose, hemi-cellulose and lignin from cotton residues, maize stover or wheat straw, but N occurs mainly in a bound form unavailable until it is enzymatically released. The yield increase with addition of supplements in this experiment may be attributed to extra amino acids from the organic supplement and proteins available to the mushroom mycelium. Supplements were most effective in already high yielding substrates, which have optimal level of nutrients for growth of mushroom mycelium.

The inherent characteristics of the base substrates used differ significantly such that the contribution of the supplements would be based on either inherent nutrient content or physical characteristics that support growth and development of the sporophores. Beyer and Wilkinson (2002), observed a direct correlation between substrate ammonia content and subsequent growth and yield of mushrooms. They also noted that ammonia content above 0.2 % kills spawn. Nitrogen could also either reduce the carbon to nitrogen ratio such that the fungi would not have enough carbon for growth or the nitrogen may cause increase in the temperature within the growing substrate through increasing the metabolic processes.

The low average biological efficiency in the wheat straw (0.3) at 0 % supplementation, as well as the biological efficiency variation in different flushes and

within flushes could be partially attributed to the losses from contaminations. This concurs with Perry (1987), who obtained higher dry matter from supplemented substrates compared to the non supplemented controls at all flushes. The high coefficient of variation in the dry matter yield observed in this experiment might be caused by effects of cockroaches during the drying period which fed on some of the samples. Control was done but relatively late. Moda et al. (2005) also pointed out the intrinsic variability of the biological material, its phenotypic plasticity and the type of substrate used as major influences to the coefficient of variation of the mushrooms yields in flushes.

Dry matter of the mushrooms was approximately equal to 10 % and water content around 90 % of the fresh weight. Oei (1991) also obtained the same composition in mushrooms. Organic nitrogen in the supplement facilitated initiation of primordial and growth of mushrooms basidiomes.

Even though Stamets (1993) argued that the substrate will only support the development of a certain number of primordial per flush, results of this experiment has shown that the number of pins do not always relate to the total marketable yield that can be obtained. More pinheads tend to compete for the nutrient base resulting in abortion before they attain the harvestable size. This reduces the number of marketable basidiomes or the mean basidiome size as the numbers of basidiomes are many but low fresh yield. The low yield is caused by insufficient nutrients or late formation of the primordial (Stamets and Chilton, 1983). The ratio of total number of marketable basidiome harvested per flush to the total basidiome harvested indicates the number of sporophores that failed to meet the market standards especially with relation to size.

Addition of supplements improved appearance of mushroom in terms of shape and size. This is in agreement with observations by Rossi et al. (2003) who concluded that increasing amount of rice bran added to the sugarcane bagasse improved the mushroom quality. The observed crude proteins means were similar to those recorded by Stamets (1993), whose range varied from 19-35 % of the dry weight. Oyster mushrooms crude proteins found were comparable to other foodstuffs and have potential to benefit consumers nutritionally when included in the diet.

Conclusion

All the substrates (maize stover, cotton waste, banana leaves, maize cobs, bean shells, grass and wheat) can successfully be used to cultivate oyster mushroom and there is no significant difference in yield per fresh weight basis. However the biological efficiency obtained from these substrates when used without supplementation was low (22-39 %). The performance of the mushrooms can be increased in the different production substrates through addition of cheap organic nitrogenous materials as supplements. Yields increased linearly in cotton residues and wheat straw substrates at 0-14 % sunflower seed cake supplement range used in the experiment. For maize stover substrate, yields increased linearly up to the 8 % level and then decreased when supplements were increased to the 14 % level. The mycelium vigor in maize stover and wheat straw indicates the importance of increasing supplements rates with relation to the yields observed.

Further work needs to be carried out to find out the effect of other organic supplements like chicken manure, cotton seed cake and soybean cake. It will also be useful to evaluate the effects of changes in volume of substrates on final yield of oyster mushrooms.

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