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Effect of different agricultural by-products on the growth and yield of the mushroom *Volvariella volvacea*

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EFFECT OF DIFFERENT AGRICULTURAL BY-PRODUCTS ON THE GROWTH AND YIELD OF THE MUSHROOM *Volvariella volvacea*

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ABSTRACT

The growth, yield and biological efficiency of *Volvariella volvacea* strain VVL on three agricultural by-products: rice straw, cotton waste and composted sawdust of *Triplochiton scleroxylon* were studied. These were used singly and in a combination of a 1:1 ratio. Primordia were observed after 15 to 20 days on all the substrates with the exception of the composted sawdust, from which no flush was observed. The yields of mushroom on the different substrates were 565.50g, 148.25g, 42.25g and 0g for cotton waste, combination of rice straw and cotton waste, rice straw only and composted sawdust only respectively. The biological efficiency (BE) followed the same pattern and ranged from 128.50% for cotton waste to 0.0% for composted sawdust. Based on the yield and BE values, cotton waste is recommended as a substrate to be used in growing the oil palm mushroom, *V. volvacea.*

INTRODUCTION

Volvariella volvacea (the oil palm or paddy straw mushroom), commonly known in Ghana as 'domo', is an edible and nutritious mushroom with some known health benefits. These health benefits include immunomodulating, antitumor, and hypocholesteroiaemic activity, which are typically ascribed to various components isolated from its fruit bodies and mycelia (Lin and Chou, 1984; Kishida *et al.* 1989, 1992; Benzie and Strain, 1996; Hsu *et al.* 1997; Cheung 1998; Liua *et al.* 2001; Shi *et al.* 2002). It's nutritional properties are in the range of 21.34-30.9% for crude protein, 4% fat, 15.2% ash and 49.3% for carbohydrate among others (Li and Chang, 1982; Obodai and Apetorgbor, 2008). This mushroom grows in the wild on felled oil palm trunk, hence the common name, oil palm mushroom. *V. volvacea* is one of the fastest growing mushrooms with a cropping cycle of 7-12 days, it thrives at warm temperatures (24-35°C), and dies at temperatures below 7°C (Stamets, 2000). It is thus a thermophilic fungus.

V. volvacea is a popular edible fungus of the tropics and subtropics, which grows well on cellulosic agricultural residues and industrial wastes (Yau and Chang, 1971; Chang, 1978). These agricultural wastes include cotton waste (Chang, 1972, 1974, 1978, 1979), rice straw (Chang, 1965, 1978), dry banana leaves (Chang, 1978, Obodai *et. al.* 2003a), sugarcane bagasse (Chang, 1978), water hyacinth and oil palm pericarp waste (Yong and Graham, 1973). Descriptions of methods of cultivation of this mushroom are given by Chang and Miles (2004) and Kaul and Dhar (2007). The cultivation of edible mushrooms like *V. volvacea* on these wastes is a value added process capable of converting these materials, which are otherwise considered as wastes, into foods and feeds (Bisaria *et al.*, 1997).

Due to industrialization of most parts of the world resulting in increased production of both industrial and domestic wastes and the consequent pollution of the air as well as water bodies, adequate food production is now increasingly becoming a major problem for most governments, especially in the under-developed countries. The environmental pollution problems associated with conventional disposal methods of these waste materials necessitate the search for alternative, environmentally friendly methods of handling agrowastes. Mushrooms have been reported to be capable of transforming nutritionally worthless wastes into protein rich food and have been confirmed to be sources of single cell protein (Kurtzman, 1981; Alofe *et al.*, 1998).

This paper reports on the efficiency of rice straw, cotton waste and sawdust used singly and in a combination of a 1:1 ratio as substrates for the cultivation of *V. volvacea* strain VVL under Ghanaian conditions.

MATERIALS AND METHODS

Culture maintenance

The strain of *V. volvacea* used in this study was VVL from Legon, Ghana. The strain was maintained on potato dextrose agar slants and the spawn was prepared on sorghum grains (Oei, 1996). Both the cultures and the spawns were incubated at a temperature of 32°C and a relative humidity of 75%.

Preparation of substrates

Rice straw only (RS) and Cotton waste only (CW)

Rice bran (4% of the dry weight of each of the substrates) was added to rice straw and cotton waste separately, and mixed thoroughly. Fifteen liters of water in a plastic basin was mixed with 5g of powdered lime. The rice straw and cotton waste were separately steeped in the water containing lime for 30 min. The substrates were then removed and kept in baskets for excess water to drain out until a moisture content of 75% was attained.

Sawdust only (SD)

Two month old composted sawdust of *Triplochiton scleroxylon* was weighed and mixed thoroughly with 4% rice bran on a concrete floor. Water containing lime at a concentration as described above was sprinkled on the sawdust and mixed thoroughly until a moisture content of 75% was attained.

Combined substrates

For each of the combinations of substrates in this experiment, the prepared substrates were weighed in a ratio of 1:1 (w/w) and mixed thoroughly. The combinations were: Rice Straw and Sawdust (RS+SD), Rice Straw and Cotton Waste (RS+CW) and Cotton Waste and Sawdust (CW+SD).

The moisture content of all the substrates was determined by performing the squeeze test (Buswell, 1984). All experiments were carried out in replicates.

Construction of beds and spawning

The beds were constructed and spawned as described by Obodai *et al.* (2003a). The beds were then covered first with translucent plastic sheets and then with straw mats to retain the moisture in the substrates, to maintain a high internal temperature and to create the low light intensity required by the mushroom for the spawn run period. On the tenth day of spawn run, the plastic sheets and the mats were raised about 10 cm above the surface of the bed to allow ventilation and light exposure to induce fruit body formation.

Harvesting

The fruit bodies were mostly harvested at the egg stage, when the gills had not yet been exposed. The number of days until the first appearance of the primordia was recorded. Occurrences of different mushrooms and lower fungi were also noted. The yield per flush and the biological efficiency (BE), which is expressed as the weight of the fresh mushroom as a percentage of the dry weight of the substrate (Mueller *et al.* 1985), was calculated.

Statistical analysis

The means and standard errors (SE) were determined using Microsoft Office Excel, 2007 and reported as mean ± SE. Data analysis was conducted by the separation of means by Fisher's Least Significant Difference (LSD) at a 95% level of probability.

RESULTS AND DISCUSSION

Days till primordial formation on substrates

The number of days until the first appearance of fruit bodies recorded in this study (between 15-20 days) (Table 1) was higher than that recorded by Stamets (2000) (7-12 days). This can be attributed to the lower temperature of 26±2°C recorded during this study. Chang and Chu (1969) and Stamets (2000) have recorded 30-35°C and 24-35°C, respectively, as the optimum temperature range, with 32°C as the most suitable temperature in both cases, for *V. volvacea* cultivation.

Cotton waste only (CW) recorded 15 days for the primordial formation. This was followed by the combination of cotton waste and rice straw (CW+RS) and that of cotton waste and sawdust (CW+SD), which both took 18 days for primordial formation. Rice straw only (RS) took the longest period of 20 days (Table 1) for primordial formation. This indicates that spawn run for *V. volvacea* strain VVL cultivation is best on cotton waste only. *V. volvacea* mushroom has been found to prefer high cellulose-low lignin containing substrates such as cotton waste.

This mushroom produces a family of cellulolytic enzymes including at least five endoglucanases, five cellobiohydrolases and two ß-glucosidases, but none of the recognised lignin-degrading enzymes (Chang, 2008). Cott on waste was also the best substrate for the cultivation of *V. volvacea* strain VVL due to its relatively compact nature when wet (Chang, 1983). Rice straw on the other hand contains cellulose (35.5%), hemicellulose (24.2%) and lignin (24%) and is therefore not a good substrate for growth and colonization by *V. volvacea* in comparison with cotton waste (Rajarathanam *et al.*, 1997; Datta and Chakravarty, 2001).

Table 1: Mean fresh weight of fruit bodies per flush and yield of *V. volvacea* strain VVL on different substrates.

Substrate	Day of appearance of fruit body	Mean fresh weight of fresh fruit bodies/flush (g)							Total mean weight of fresh fruit bodies (g)
		1st flush	2nd flush	3rd flush	4th flush	5th flush	6th flush	7th flush	
RS	20	13.0±4.5^{c}	16.5±16.5^{c}	12.8±12.8^{bc}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	42.25
CW	15	233.0±35.0^{f}	145.0±75.0^{e}	47.0±28.0^{d}	35.0±7.0^{cd}	53.8±0.3^{d}	49.8±24.3^{d}	2.0±2.0^{ab}	565.50
SD	nd	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.00
RS+CW	18	107.0±51.0^{e}	11.3±2.3^{bc}	26.5±8.5^{c}	3.5±3.5^{bc}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	148.25
RS+SD	nd	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.00
CW+SD	18	5.5±5.5^{b}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	5.50

Values within a column followed by a different letter are significantly different at $p<0.05$; $n=2$.

RS : Rice straw only
CW : Cotton waste only
SD : Sawdust only
RS+CW : Rice straw and cotton waste
RS+SD : Rice straw and sawdust
CW+SD : Cotton waste and sawdust
nd : Not determined

Yield per flush across the substrates

The mean yield of the fresh fruit bodies decreased with time, with the first flush being significantly different from all the other flushes ($P<0.05$) (Fig. 1, Plate 1). This indicates that there is a loss of nutrients and a form of substrate modification with the harvest of the fresh mushrooms from the substrates from flush to flush (Fig 1). For each flush, the cotton waste only treatment (CW) had a significantly higher yield ($P<0.05$) than all the other treatments, followed by the combination of rice straw and cotton waste (RS+CW) and then by the rice straw only treatment (RS) (Table 1).

Figure 1: Mean yield of fresh fruit bodies per flush across the substrates.

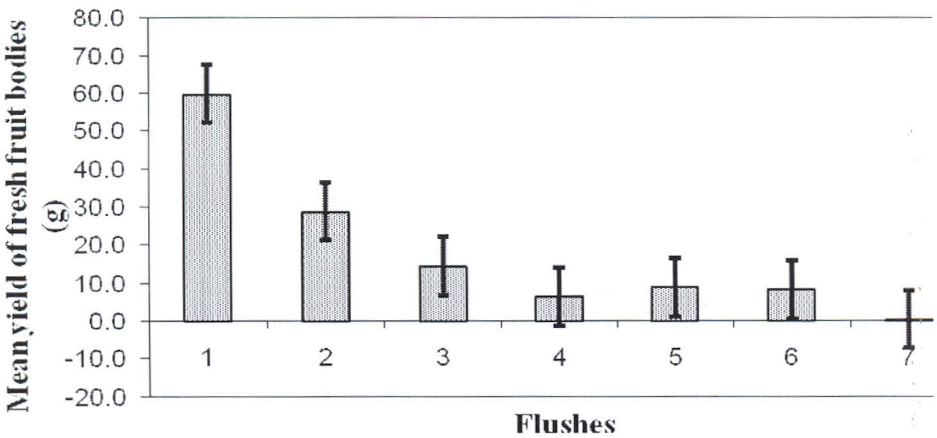
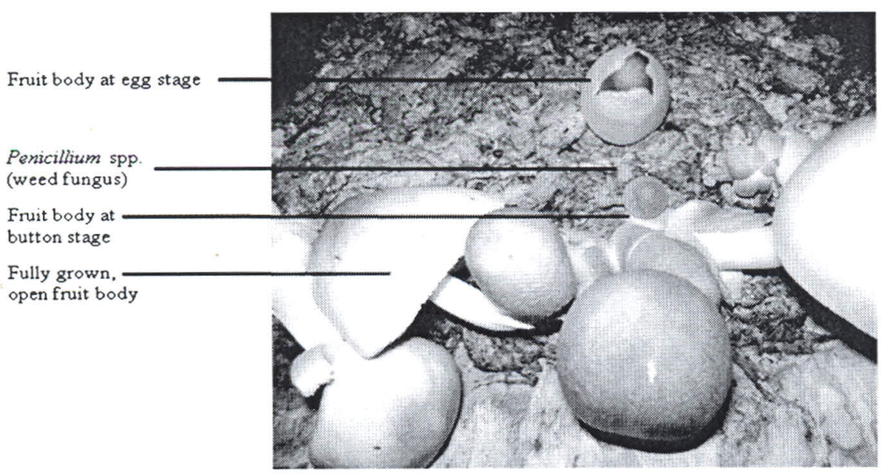


Plate 1: *V. volvacea* strain VVL flushing at various stages on cotton waste only (CW).



Mycelial densities and biological efficiencies of *V. volvacea* on the various substrates

The mycelial densities and the biological efficiencies (BEs) of the various substrates showed the same trend (Table 2). In descending order, the BE values were cotton waste, combination of rice straw and cotton waste, rice straw only and the combination of cotton waste and sawdust, which were 128.50%, 39.00%, 12.10% and 1.40% respectively (Table 2). This is in agreement with Thomas *et al* (1998) who stated that the yield of the mushroom is directly related to the spread of mycelium into the substrate. The trend in the mycelial density on cotton waste only, rice straw only and composted sawdust only followed the same pattern seen in the results obtained by Fasidi (1996), who worked with *V. esculenta*. In both cases, cotton waste showed very dense mycelial growth, rice straw showed moderate mycelial growth, whereas composted sawdust showed no mycelial growth. This is an indication that the two species of *Volvariella* demonstrated similar requirements for substrate colonization.

Table 2: Mycelial density during spawn run and biological efficiency on the substrates used for the cultivation of *V. volvacea* strain VVL.

Substrate	**Mycelial density**	**Biological Efficiency (%)**
Rice straw only (RS)	4+	12.10^{b}
Cotton waste only (CW)	7+	128.50^{d}
Sawdust only (SD)	-	0.00^{a}
Rice straw and cotton waste (RS+CW)	5+	39.00^{c}
Rice straw and sawdust (RS+SD)	1+	0.00^{a}
Cotton waste and sawdust (CW+SD)	2+	1.40^{b}

Values within a column followed by a different letter are significantly different at $p<0.05$; $n=2$.

- : no mycelial growth recorded.

<3+: scanty mycelia growth

4+: Moderate mycelia growth

>4+: Very abundant mycelia growth

Though there was scanty mycelial growth on the combination of rice straw and sawdust (RS+SD) treatment, no flush (BE=0.00%) was recorded on this treatment (Table 2). It was however, observed that the rice straw in the combination of rice straw and sawdust (RS+SD) treatment started germinating, indicating that the sawdust served as a substrate for the growth of the rice straw. This resulted in the unavailability of the nutrients in the rice straw for mycelial growth in this treatment. Thus, it would be necessary to give the rice straw some heat treatment to prevent its germination in further studies.

Neither a sign of mycelial growth nor flushes (BE=0.00%) (Table 2) were observed on the composted sawdust treatment; an indication that composted sawdust is a very poor substrate for the cultivation of *V. volvacea* strain VVL. The ratio of cellulose to hemicellulose to lignin is approximately 6:1:4 for composted sawdust of *Triplochiton scleroxylon*, and 7:5:1 for rice straw (Table 3). *Volvariella volvacea* utilizes cellulose and hemicellulose throughout the spawn run and cropping phases, but is unable to utilize lignin at any stage due to lack of a lignolytic system (Datta and Chakravarty, 2001). Datta and Chakravarty (2001) also established that the utilization of hemicellulose by *V. volvacea* was higher (5.72 in 100g of dry substrate) than the utilization of cellulose (4.82 in 100g of dry substrate) by the mushroom. An interesting observation made however, was that on very old decomposed sawdust of *T. scleroxylon* on a composting platform, some strains of *V. volvacea* and *Coprinus* species were found actively growing.

Table 3: Proximate and chemical composition of some of the lignocellulosic materials used.

Component	Composted sawdust	Rice straw
Nitrogen	0.16±0.09	0.91±0.11
Cellulose	46.47±0.14	38.42±0.32
Hemicellulose	8.82±0.25	28.57±0.01
Lignin	31.68±0.51	6.73±0.21
Crude fibre	63.28±0.19	28.78±0.41
Ash	15. 22±0.03	8.37±0.53

Source: Obodai *et al.* (2003b), *Microbiol. Biotechnol.,* 30, 146-149

Other fungi (weed fungi) observed

Coprinus spp. were observed as the weed macrofungus in this study, being observed on the cotton waste only (CW) and the rice straw only (RS) treatments after the second flush of the *V. volvacea* fruit bodies were harvested. The yield of the fresh fruit bodies reduced after the second harvest when the *Coprinus* spp. were observed on the beds. This observation is in line with Chang-Ho and Yee (1977) as well as Yee and Yung (1980), who have stated that the growth of *V. volvacea* is inhibited by the growth of *Coprinus cinereus*. According to Yee and Yung (1980), this inhibition is better explained by the competition for nutrients between the two mushroom species rather than by the production of metabolites by the weed macrofungus.

Coprinus spp. were not observed on the beds with the RS+CW and CW+SD treatments even though there were *V. volvacea* fruit bodies present on these beds, an indication that the *Coprinus* spp. has similar nutrient and/or characteristic requirement as *V. volvacea*. The high lignin content of both rice straw and composted sawdust, according to Datta and Chakravarty (2001), restricts *V. volvacea*'s access to cellulose and hemicellulose when being cultivated on these substrates. After *V. volvacea* had utilized the substrate and flushed twice, there was either an unavailability or significantly low amounts of these components (cellulose and hemicellulose) in the combined substrates (RS+CW and CW+SD). For this reason, the *Coprinus* spp. was unable to colonize the substrate to compete with the *V. volvacea* on the RS+CW and CW+SD treatments.

Another fungus observed in this experiment was *Penicillium* spp. However, there was no antagonistic effect observed between the two fungi, since the *V. volvacea* fruit bodies were seen to flush very close to the *Penicillium* colonies on the substrate. This shows that there is a likely symbiotic association between the two fungi. This is the subject of another study for which a manuscript is in preparation.

CONCLUSION

The suitability of cotton waste for the cultivation of *V. volvacea* strain VVL under Ghanaian conditions was established in this study.

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