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Mycobiota and some physical and organic composition of agricultural wastes used in the cultivation of the mushroom *Volvariella volvacea*

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Abstract. The physical, organic and fungal phenology of five unamended agricultural lignocellulose wastes namely: banana leaves, cocoa shells, maize stover, oil palm pericarp and rice straw, used to cultivate a local isolate of *Volvariella volvacea* (Bull. ex Fr.) Sing. were studied. The major components studied were cellulose, hemicellulose, lignin, crude fibre, ash, organic matter and protein. There were considerable variations in the organic and physical compositions of these wastes. Maize stover and rice straw recorded the highest value for cellulose (38.42-39.04%), hemicellulose (25.27-28.57%) and the lowest for lignin (6.15-6.73%). The pH of the composts varied between pH 5.37-8.28 which was within the optimum pH for best growth of *V. volvacea*. Phenology of the resident fungi of the five wastes also varied considerably. However, after 30 days of inoculation of the substrates, fungal species such as *Aspergillus fumigatus, A. niger, Coprinus cinereus*, *Mycogyne* sp. and *Trichoderma viride* persisted in the composts and presumably collectively contributed to the non-fruiting of *V. volvacea* on cocoa shells, maize stover, rice straw and oil palm pericarp wastes. The biological efficiency (BE) of *V. volvacea* on dry banana leaves waste which permitted fructification was 43.0%. The practical implications of these findings are discussed and further work is suggested.

Key words: Phenology, mycobiota, agricultural waste, cultivation, *Volvariella volvacea*

Introduction

Volvariella volvacea (the oil palm or paddy straw mushroom), commonly known in Ghana as 'domo' is the third most popular edible fungus in the world, after button and shiitake mushroom, respectively (Graham *et al*. 2004) and the sixth most cultivated (Aida *et al*. 2009). The market for this mushroom continues to grow due to interest in its culinary, nutritional, and health benefits (Graham *et al*. 2004, Tansakul and Lumyong 2008). These health benefits include immunomodulating, antitumor, and hypocholesteroiaemic activity, which are typically ascribed to various components isolated from its fruit bodies and mycelia (Liua *et al*. 2001, Shi *et al*. 2002). Nutritional properties of *V. volvacea* are in the range of 21.34-30.9% for crude protein, 4% fat, 15.2% ash and 49.3% for carbohydrate (Li and Chang 1982, Obodai and Apetorgbor 2009).

V. volvacea, a high temperature mushroom, is grown largely in tropical and subtropical regions and grows well on cellulosic agricultural residues and industrial wastes (Chang 1978). These agricultural wastes include cotton waste (Chang 1979), rice straw, sugarcane bagasse (Chang 1978), dry banana leaves (Chang 1978, Obodai *et al*. 2003), water hyacinth and oil palm pericarp waste (Yong and Graham 1973). The cultivation of edible mushrooms like *V. volvacea* on these substrates or compost is a value added process capable of converting these materials, which are otherwise considered as wastes, into foods and feeds (Bisaria *et al*. 1997).

V. volvacea as most edible mushrooms, are heterotrophic, they therefore have to get all the nutritive elements from the substrate. The compost therefore, plays a comprehensive role in mushroom production than soil does in higher plant growth. A good compost should have a suitable physical condition that will provide good anchorage for the mushroom, good aeration and water holding capacity, a good chemical condition that will release some nutrients from the raw materials of the compost during fermentation and pasteurization and a proper condition for microbial activity that will help improve both the physical and chemical conditions for mushroom growth (Oei 1991). During the process of decomposition, the substrate changes continually, both physically and chemically, such that its suitability for colonization by different organisms also changes. An apparent succession of fungi is therefore seen which is referred to as phenology of the resident microorganisms (Mason 1979).

During compost utilization, the phenology of microorganisms: fungi, bacteria, actinomycetes and protozoa is different at different stages where different groups may dominate at different times (Hayes 1977). The initial microflora may be mesophilic and utilizes the soluble organic carbohydrates and nitrogen. This is followed by increase in growth of more tolerant organisms and the release of carbon dioxide, ammonia, and a considerable amount of heat. At the later stage of

composting, the temperature is higher (35-45^{o}C) and thermophilic microorganisms become dominant. Chang-Ho (1982) showed that the fungal succession in the compost used in the cultivation of *V. volvacea* is controlled by factors such as pH, moisture, temperature and nutrition. Species of *Aspergillus* and *Mucor* multiplied quickly in 3-4 days, but soon disappeared from the compost. Thermophilic and thermotolerant *Aspergillus fumigatus*, *Chaetomium thermophile* and *Humicola* sp. took over followed by *Coprinus cinereus* in 10-14 days (Chang-Ho 1982). Some of these fungi may interfere with the growth and yield of *V. volvacea*.

There are limited studies which describe the diversity of fungi in compost used in the cultivation of mushrooms. This study is conducted to screen five locally agricultural lignocellulosic wastes (maize stover, rice straw, banana leaves, oil palm pericarp and cocoa shells) for suitability as compost in the cultivation of *V. volvacea* on a commercial scale. The chemical composition of the compost and the phenology of the attendant fungi and their effect on the yield of the mushroom were also studied.

Materials and Methods

Mushroom Cultures

Volvariella volvacea strain VVL from Legon, Ghana was used in this study. The strain was maintained on potato dextrose agar slants and spawn was prepared on sorghum grains (Oei 1996) and amended with 10% dry weight of *Leuceana leucocephala* leaves. Both the cultures and the spawns were incubated at 32ºC and at an equilibrium relative humidity (ERH) of 75%.

Substrate preparation

The slow decomposing agricultural lignocelluloses eg. maize stover, rice straw, and banana leaves were chopped into pieces (3-4 cm long) and steeped in a 200 l oil drum for 24 h to allow the material to absorb water. The rapidly decomposing substrates eg. cocoa shells and oil palm pericarp were steeped for 25-30 min before being used to make mushroom beds.

Construction of beds and spawning

The beds were constructed and spawned as described by Obodai *et al.* (2003). The beds were covered first with translucent plastic sheets and then with straw mats to retain the moisture in the substrates, maintain a high internal temperature and create the low light intensity required by the mushroom for the spawn run period. On the 10th 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. Visible fruiting of the mushrooms normally could be seen 6 days after raising the plastic sheets off the beds. The environmental relative humidity was between 75-85%. Each bed of substrate was made in four replicates.

Moisture content and pH of substrates

The moisture content was determined by drying the samples at 107ºC overnight in an oven (Gallenkamp oven 300 plus series). PH was measured using the supernatant resulted from soaking 1 g of substrate in 10 ml distilled water for 6 h using an Alpha 500 model laboratory pH/mv meter.

Organic composition of substrates

Quantitative estimation of cellulose, hemicellulose, lignin, ash, crude protein, crude fibre, and organic matter were carried out, using the standard methods as described by AOAC (1990). Lignin and cellulose were determined by acid detergent fibre (ADF) method. Hemicellulose content was estimated by neutral detergent solution using 1 g of dried sample. The difference between the acid detergent fibre and neutral detergent fibre gave the value for hemicellulose content. Ash, organic matter and crude fibre were also determined and percentage crude fibre was calculated from the following equation:

$$\frac{\text{Loss in weight on ignition (A-B)}}{\text{Initial sample weight}} \times 100$$

Where A=Weight of residue dried in an oven at 107^{o}C overnight and cooled in a dessicator

B =Weight of contents in a crucible after ignition

To calculate total nitrogen in the samples, the specimens were dried at 60^{o}C and analysed by the Microkjeldahl method (AOAC 1990). To obtain crude protein value, nitrogen content values were multiplied by 4.38 (Crisan and Sands 1978).

Phenology of microorganisms

The decimal dilution plate technique was used in estimating fungal and bacterial populations. About 10 g fresh weight of sample was placed in 250 ml Erlenmeyer flask containing 100 ml sterile distilled water. The mixture was shaken at 140 rev. min^{-1} in a Gallenkamp Orbital Shaker for 30 min. Aliquot (1ml) of the suspension was placed in sterile universal bottle (MaCartney tubes) containing 9 ml of 0.1% peptone, and was serially diluted up to 1:10^{5}. The fungal population was enumerated on modified Cooke's medium (Cooke 1954) incubated at 30-32^{o}C for 5 to 7 days. Population of fungal species appearing was calculated as $\log_{10}$ CFU/g sample. Mycobiota were identified using their morphological and cultural characteristics as outlined by Samson *et al.* (1995). Aerobic bacterial population was enumerated on plate count agar (PCA, Oxoid, Basingstoke Hampshire, England) incubated at 37^{o}C for 24 h.

Measurement of yield

The yield per flush and the biological efficiency (BE), which is expressed as the weight of the fresh fruiting bodies as a percentage of the dry weight of the substrate (Mueller *et al.* 1985) was determined.

Results

Organic composition of substrates

Analyses of the various constituents of the substrates used in the cultivation of *V. volvacea* are shown in Table (1). Moisture content varied between 67.72% (banana leaves) and 79.19% (cocoa shells). Cellulose content ranged from 19.23 to 39.04%, hemicellulose 6.54 to 28.57%, lignin 6.15 to 15.74%, ash 8.37 to 19.25% (Table 1). Nitrogen content was low (0.76-0.94%) for all substrates except cocoa shells which yielded 2.07% nitrogen (Table 1). Crude protein ranged from 4.96-14.92% and crude fibre from 18.41 to 48.65% (Table 1). Organic matter was high (80.75-91.63%).

Phenology of microorganisms

The fungal population resident in the five substrates varied within 30 days of spawning with *V. volvacea.* Whilst the final fungal population in dry banana leaves, maize stover and rice straw was 0.15-0.99 log cycles lower than what existed at the beginning of the experiment. The final population of fungal residents in oil palm pericarp waste and cocoa shells was 0.3-1.2 log cycles higher than the initial population recorded (Table 2).

Bacterial population in the dry banana leaves, maize stover, oil palm pericarp and rice straw was 0.4-0.6 log cycles lower after 30 days than what was obtained initially (Table 2). The only exception was in the cocoa shells which contained slightly higher final population (0.25 log cycles higher) than what existed at the commencement of the experiment. Initial pH of the substrates was between 5.37 to 7.02 but shifted to basic pH 7.63 to 8.28 after 30 days, except in oil palm pericarp compost which recorded a final pH of 5.85 (Table 2).

Phenology of selected resident individual fungal species in the substrates seeded with *V. volvacea* is presented in Table (3). In almost all substrates *Aspergillus niger* was initially present and persisted after 30 days with the exception in rice straw. Only *A. flavus, A. ochraceus* and *Paecilomyces* sp. were initially resident in rice straw substrate but were replaced by *Coprinus cinereus* (20.0% of the total count of fungi) and *Trichoderma viride* (7.1%) at the end of 30 days. Maize stover substrate recorded *A. niger* (5.1%) initially; after 30 days, *A. niger* (19.4%), *A. flavus* (3.1%), *A. fumigatus* (24.4%), *C. cinereus* (10.0%), *Gliocladium fimbriatum* (31.3%) and *T. viride* (21.9%) were isolated as well. Cocoa shells initially recorded *A. niger, A. flavus* and *Cladosporium herbarum* while *A. fumigatus* and *A. niger* predominated (59.2%) at the end of the experiment (Table 3). The occurrence of species of *Acremonium, Cladosporium, Fusarium, Gliocladium, Paecilomyces, Penicillium,* and *Pestalotia* were recorded during the period of study (Table 3).

Banana leaves initially harboured *A. niger, A. flavus* and *A. fumigatus* constituting 19.7, 24.4, and 19.7% of the total population respectively (Table 3). After 30 days of growth of *V. volvocea* mycelia, *Penicillium digitatum, C. cinereus* and *T. viride* appeared as well. Finally, while *A. niger* persisted in the oil palm pericarp substrate throughout the experiment, *A. flavus, A. fumigatus* and *C. cinereus* were isolated in the compost at the end of the experiment. It is striking that the species composition of the substrates differed at the beginning of the experiment and also varied considerably after utilization of the substrate by *V. volvacea* mycelium. *V. volvacea* produced 55 fruit bodies on dry banana leaves only yielding 491.5 g after 13 days and thereafter declined. Biological efficiency (BE) of this substrate was 43.0 %. The mycelium of *V. volvacea* ramified the remaining compost (cocoa shells, maize stover, oil palm pericarp and rice straw) but succumbed to infection by *Mycogyne* sp.

Discussion

Geographical and climatic differences between tropical areas do not always allow transfer of knowledge into new regions. It is therefore necessary to investigate, under local environmental conditions, the growth and fruiting of mushrooms in order to arrive at data which provide defined techniques for particular species and allow for cultivation without extra cost.

The organic and physical compositions of the five substrates tested for cultivation of *V. volvacea* were variable. The major components included cellulose, hemicellulose, lignin, crude fibre, ash and organic matter. Nitrogen content was low in all the substrates used. *V. volvacea* mushroom was found to prefer high cellulose-low lignin containing substrates, namely maize stover and rice straw. This is because it 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).

Table 1: Components of composts (g/100 g dry sample) used in the cultivation of *Volvariella volvacea*

Substrate	Moisture content	Cellulose	Hemicelluloses	Lignin	Ash	Nitrogen	Crude protein	Crude fibre	Organic matter
Banana leaves	67.72	29.47±0.03	21.83±0.06	15.74±0.11	14.17±0.04	0.94±0.51	5.85±0.43	32.72±0.51	85.83±0.04
Cocoa shells	79.19	22.24±0.04	6.54±0.34	15.17±0.00	11.25±0.53	2.07±0.77	14.92±0.53	18.41±0.00	88.75±0.53
Maize stover	69.30	39.04±0.05	25.27±1.21	6.15±0.31	8.65±0.62	0.76±0.32	5.35±0.41	32.35±0.32	91.35±0.62
Rice straw	69.02	38.42±0.32	28.57±0.01	6.73±0.21	8.37±0.53	0.91±0.11	5.65±0.21	28.78±0.41	91.63±0.99
Oil palm pericarp	69.54	19.23±0.47	19.23±0.32	13.48±0.32	19.25±0.41	0.79±0.21	4.96±0.41	48.65±0.64	80.75±0.41

Table 2: Microbial and pH profile of substrates used for the cultivation of *Volvariella volvacea* for 30 days at 28^{o}C

Substrate used	pH of Medium		Fungal population (log_{10} CFU/g)		Bacterial population (log_{10} CFU/g)	
	Initial	Final	Initial	Final	Initial	Final
Banana leaves	6.96	8.20	6.14	5.54	7.34	6.78
Cocoa shells	5.37	8.28	5.56	6.71	6.50	6.75
Maize stover	7.02	7.63	6.50	5.51	7.02	6.49
Rice straw	6.35	7.86	5.65	5.50	6.90	6.53
Oil palm pericarp	6.93	5.58	6.29	6.61	7.24	6.89

Table 3: Percentage occurrence of saprophytic fungi in the substrates used for the cultivation of *V. volvacea*

Saprophytic fungi	Dry banana leaves		Maize stover		Cocoa shells		Oil palm pericarp		Rice straw	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Aspergillus flavus Link	24.4	2.9	-	3.1	11.1	-	-	16.7	10.9	-
A. fumigatus Fresenius	19.7	25.7	-	24.4	-	59.2	-	52.7	-	-
A. niger van Tieghem	19.7	5.7	5.1	19.4	11.1	40.8	38.9	16.7	-	-
A. ochraceus Wilhelm	-	-	-	-	-	-	-	-	13.0	-
Acremonium strictum W. Gams	-	-	-	-	16.7	-	-	-	-	-
Coprinus cinereus (Schaeff. ex Fr.) S. F. Gray	-	14.0	-	10.0	-	-	-	14.0	-	20.0
Cladosoporium herbarum (Persoon) Link	-	-	-	-	33.3	-	-	-	-	-
Fusarium oxysporum Schlechtendal	-	-	16.8	-	16.7	-	-	-	-	-
Myrothecium verrucaria (Alb. & Schwein.) Ditmar	21.1	38.6	-	31.3	11.1	-	-	-	-	-
Paecilomyces sp.	-	-	21.0	-	-	-	-	-	15.2	7.1
Pestalotia macrotricha Klebahn	18.9	-	-	-	-	-	-	-	-	-
Penicillium digitatum Sacc.	-	2.9	-	-	-	-	-	-	-	-
Trichoderma viride Pers.	-	10.3	-	21.9	-	-	-	-	-	7.1

The pH values were ranging between 5.37 and 8.28 which were considered to be within the optimum for best growth of *V. volvacea* (Ofosu-Asiedu *et al.* 1986, Oei 1996). Moisture content of the substrates ranged from 67.72 to 79.19% with the attendant ERH between 75 and 85% conducive for good growth of fungi.

Microbial activities and succession within the compost during utilization by the mushroom is influenced by physical and chemical reactions, aeration, temperature and nutritional factors (Chang-Ho 1982, Carlile and Watkinson 1996). Stanek (1972) showed that the number of microorganisms decreased during fermentation process in wheat compost for 30 days. In this paper, microbial composition of the compost declined or increased slightly in some instances depending on the substrates. For example, the final fungal population in banana leaves, maize stover and rice straw were 0.15-0.99 log cycles lower than what existed at the commencement of the experiment. On the other hand, the final fungal population of oil palm pericarp waste and cocoa shell compost was 0.3-1.2 log cycles higher than the initial mycobiota.

In rice straw, mesophilic Zygomycota and Deuteromycota predominated in fungal succession. *Aspergillus* and *Mucor* multiplied quickly but soon disappeared from the center of the substrate (Chang-Ho 1982). In the composting process, rice straw was initially predominated with *A. flavus, A. ochraceus* and *Paecilomyces* sp.. but these were replaced by *C. cinereus* (20.0%), *T. viride* (7.1%) whilst *Paecilomyces* sp. persisted after 30 days of cultivation. *Coprinus* and *Trichoderma* have been amply reported as a contaminant of the edible mushroom production process (Lopez-Arevalo *et al.* 1996).

Other fungi which were isolated after 30 days from the substrates inoculated with *V. volvacea* comprised *A. niger* (19.4%), *A. flavus* (3.1%), *A. fumigatus* (24.4%), *C. cinereus* (10.0%), *Myrothecium verrucaria* (31.3%) and *T. viride* (21.9%) from maize stover; *A. niger* (40.8%) and *A. fumigatus* (59.2%) from cocoa shells; *A. flavus* (2.9%), *P. digitatum* (2.9%), *A. niger* (5.7%), *T. viride* (10.3%), *C. cinereus* (14.0%), *A. fumigatus* (25.7%) and *M. verrucaria* (38.6%) from banana leaves. Members of the genera *Aspergillus*, *Penicillium*, and *Paecilomyces* have previously been reported to grow in organic material (Stamets and Chilton 1983).

Thermophilic and thermotolerant fungi such as *A. fumigatus, A. niger* and *C. cinereus* took over in 10-14 days and persisted to the 30th day of cultivation of *V. volvacea* in the respective substrates. According to Chang-Ho (1982), *A. fumigatus, A. niger* and *C. cinereus* inhibited growth of *V. volvacea* and that mutual inhibition seemed to exist between *V. volvacea* and *C. cinereus*. Furthermore, the rice straw compost used in the cultivation of *V. volvacea* was infested with a *Mycogone* sp. which adversely affected fruiting body formation. *Mycogone* spp. are widespread mycoparasites of *Agaricus bisporus* (Gandy 1985). The inhibitory effects of the aforementioned fungi on vegetative growth and fruiting of *V. volvacea* are being recorded for the first time in Ghana. Undoubtedly, these antibiosis effects were revealed by the lack of fruiting of *V. volvacea* on all the substrates with the exception of the banana leaves compost. Future studies are needed to test the "in *vitro*" inhibition of *V. volvacea* by metabolites of *A. fumigatus*, *A. niger* and *A. flavus.*

A biological efficiency of 43.0% was obtained on banana leaves compost used for the cultivation of *V. volvacea* strain VVL. There has however been a higher value recorded by another strain V99 (Chinese strain) grown under Ghanaian conditions on banana leaves (Obodai *et al.* 2003). The use of legume plant leaves as supplement to improve the yield of *V. volvacea* on banana leaves compost is in progress.

Data from this study show that *C. cinereus* and other contaminants may be a nuisance in any compost used for the cultivation of *V. volvacea*. *C. cinereus* is usually considered inedible (Michael *et al.* 1981, Buczacki 1989). However, *C. cinereus* is edible while the fruit bodies are young and unripe (Imezaki *et al.* 1988, Chandra 1989) and indeed is eaten in Tanzania when it appears in sisal waste compost (Harkonen *et al*. 1993). In Ghana, *C. cinereus* is not eaten but is removed and discarded as a contaminant. Results from this paper and elsewhere may encourage the use of *C. cinereus* as food at the young stages.

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