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Phenology of mycoflora and some physical and organic composition of agricultural waste 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 waste used to cultivate a local isolate of *Volvariella volvacea* (Bull ex Fr) Sing. were studied. The major components studied were cellulose, hemicelluloses, lignin, crude fibre, ash, organic matter and protein. There were considerably variations in the organic and physical compositions of these wastes namely: banana leaves, cocoa shells, maize stover, oil palm pericarp and rice straw. Maize stover and rice straw recorded the highest value for cellulose (39.04-38.42%) and hemicelluloses (28.57-25.27%) and the lowest for lignin (6.73-6.15%). The pH of the composts varied between pH 5.37-7.34 which was within the optimum pH for best growth of V. *volvacea.* Moisture content ranged from 69.0-79.1% with the attendant Equilibrum Relative Humidity, ERH, between 75-85% conducive for fungal growth. 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 unamended dry banana leaves waste which permitted fructification was 43.0%. The practical implications of these findings are discussed and further work suggested.

Key words: Phenology, mycoflora, 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 fungi 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 among others (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 (Chang, 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). 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 does soil in higher plant growth. A good compost should have a suitable physical condition that will provide good anchorage for the mushroom as well as maintain 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 fungi or microorganisms (Mason, 1979). Phenology therefore has to do with the science of appearance and disappearance of species in the substrate.

During compost utilization, the phenology of microorganisms: fungi, bacteria, actinomycetes and protozoa is different at different stages, different groups may dominate at different times (Hayes, 1977). The initial microflora may be mesophillic and utilizes the soluble organic carbohydrates and nitrogen. This is followed by increase 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. *Aspergillus* and *Mucor* multiplied quickly in 3-4 days but soon disappeared from the compost. Thermophilic and thermotolerant *Aspergillus fumigatus*,*Chaetomium thermophiles, Humicola* 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 a substrate or compost used in the cultivation of mushrooms. This paper reports of a study conducted to screen five locally available agricultural lignocelluloses waste (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 examined.

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 percent dry weight of *Leuceana leucocephala* leaves. Both the cultures and the spawns were incubated at a temperature of 32°C and an Equilibrium Relative Humidity of 75%.

Substrate preparation

The slow decomposing agricultural lignocelluloses eg. maize stover, rice straw, banana leaves were chopped into pieces (3-4 cm long) and steeped in a 200l oil drum for 24h 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. (2003a). 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 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. 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 107°C overnight in an electrically heated oven (Gallenkamp oven 300 plus series). Acidity (pH) was measured by soaking 1 g of substrate in 10 ml distilled water for 6 h and using the supernatant to determine pH 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 (AOAC, 1990). Hemicellulose content was estimated by neutral detergent solution using 1g of dried sample (AOAC, 1990). The difference between the acid detergent fibre and neutral detergent fibre gave the value for hemicellulose content. Ash, organic matter and crude fibre were determined by AOAC, (1990) method and percentage crude fibre was calculated as:

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

Where A=Weight of residue dried in an oven at 107°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°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 bottles (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°C for 5 to 7 days. Population of fungal appearing was calculated as log10CFU/g sample. Mycoflora 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°C for 24h.

Measurement of yield:

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), were determined.

Results

Organic composition of substrates

Analyses of the various constituents of the substrate used in the cultivation of *V. volvacea* are shown in Tables 1 and 2. Moisture content varied between 69.02% (rice straw) and 79.19% (cocoa shells). Cellulose content ranged from 19.22 to 39.04%, hemicelluloses 6.54-28.57, 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 2) with its corresponding crude protein ranging from 18.41-48.65% (Table 2). Organic matter was high (80.75-91.63%).

Phenology of micro organisms:

The fungal population resident in 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 were 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 were 0.3-1.2 log cycles higher than the initial population recorded (Table 3)

Bacteria population in the dry banana leaves, maize stover, oil palm pericap and rice straw were 0.4-0.6 log cycles lower after 30 days than what was obtained initially (Table 3). The only exception was in the cocoa shell substrate which contained slightly higher final population (0.25 log cycles higher) than what existed at the commencement of the experiment. Acidity (pH) of the

substrates was between pH 5.37 to 7.02 but shifted to basic pH 7.8 to 8.28 after 30 days, except in oil palm pericap compost which recorded a final pH of 5.85 (Table 3).

Phenology of selected resident individual fungal species in the substrate seeded with *V. volvacea* is presented in Fig.1. In almost all the substrates *Aspergillus niger* was initially present and persisted after 30 days with the exception in rice straw (Fig.1). Only *A. flavus* was initially resident in rice straw substrate but was replaced by *Coprinus cinereus* (20.0%) and *Trichoderma viride* (7.1%) at the end of 30 days (Fig.1). Maize stover substrate recorded *A. niger* (5.1%) initially; after 30 days, *A. niger* (9.4%), *A. flavus* (3.1%), *A. fumigatus* (24.4%), *C. cinereus* (10.0%), and *T. viride* (21.9%) could be isolated as well. Cocoa shell substrate initially recorded *A. niger, A. flavus* and *C. cinereus*. *A. fumigatus* predominated (59.2%) after the experiment (Fig.1). The occurrence of fungi of other genera namely; *Cephalosporium*, *Cladosporium*, *Fusarium*, *Gliocladium*, *Mucor*, *Paecilomyces*, *Penicillium*, *Pestalotia* and *Rhizopus* were recorded during the period of observation (Table 4).

Banana leaves initially harboured *A. niger, A. flavus*, *A. fumigatus* constituting 19.7, 20.4, and 19.7% of the total population respectively (Fig.1). After 30 days of growth of *V. volvocea* mycelium, *Penicillium digitatum, C. cinereus* and *T. viride* appeared as well (Fig. 1). 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 stricking 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 491.5g after

13 days and thereafter declined. Biological Efficiency (BE), of this substrate was 43.0 percent. The mycelium of *V. volvacea* ramified the remaining compost (cocoa shells, maize stover, oil palm pericarp and rice straw) but succumbed to infection by *Mycogone* 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 composition of the five substrates tested for cultivation of *V. volvacea* varied (Tables 1 and 2). 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 has been found to prefer high cellulose-low lignin containing substrates such as cotton waste and from results of this study 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).

The pH values range between 5.37-7.37 which is within the optimum for best growth of *V. volvacea* (Ofosu-Asiedu et al. 1986; Oei, 1996) Moisture content of the substrates ranged from 69.0-79.1% with the attendant ERH between 75-85% conducive for 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 (Table 3). 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 were 0.3-1.2 log cycles higher than the initial mycoflora (Table 3).

In rice straw, mesophyllic, Zygomycota and Deuteromycota predominate in fungal succession. *Aspergillus* and *Mucor* multiplied quickly but soon disappear from the center of the substrate (Chang-Ho 1982). In the composting process *Mucor pusillus* initially predominated in rice straw followed by *Paecilomyces varioti, Penicillium oxalium, A. flavus and A. terreus*. But these were replaced by *Coprinus cinereus* (20.0%) and *T.viride* (7.7%) after 30 days (Fig.1). Coprinus and Trichoderma have been amply reported as a contaminat of the edible mushroom production process (Lopez-Arevalo et al. 1996).

Other fungi which were isolated after 30 days in the substrates inoculated with *V. volvacea* were; maize stover: *A. niger* (9.4%), *A. flavus* (3.1%), *A. fumigatus* (24.4%), *C. cinereus* (10.0%) and *T. viride* (21.9%); Cocoa shell waste: *A. niger* (25.9%) and *A. fumigatus* (52.9%); Banana leaves: A. flavus (5.2%), *P. digitatum* (5.2%), *A. niger* (8.9%), *T. viride* (9.2%), *C. cinereus* (10.2%).and

A. fumigatus (18.5%). Members of the genera Aspergillus, Penicillium, and Paecilomyces often 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 (Fig.1). According to Chang-Ho (1982), *A. fumigatus, A.niger and C. cinereus* inhibit 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 infected by 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, this antibiosis effects were reflected in the lack of fruiting on all the substrates with the exception of the banana leaves compost used for the cultivation of *V. volvacea* which yielded a total number of 55 fruitbodies. Future studies will ascertain 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*. There has however been a higher value recorded by another strain V99 (Chinese strain) grown under Ghanaian conditions on banana leaves (Obodai et al. 2003a).

Data from this paper 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, in Japan it is considered edible while the fruit bodies are young (Imezaki et al. 1988). According to Chamdra (1989), *C. cinereus* is edible while the fruit bodies are young and unripe 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. The use of legume plant leaves as supplement to improve the yield of *V. volvacea* on banana leaves compost forms the basis of a subsequent paper.

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

Substrate used	**Moisture content**	**Percentage composition of**			
		Cellulose	Hemicelluloses	Lignin	Ash
Banana leaves	67.72	29.47±0.03	21.83±0.06	15.74±0.11	14.17±0.04
Cocoa shells	79.19	22.24±0.04	6.54±0.34	15.17±0.00	11.25±0.53
Maize stover	69.30	39.04±0.05	25.27±1.21	6.15±0.31	8.65±0.62
Rice straw	69.02	38.42±0.32	28.57±0.01	6.73±0.21	8.37±0.53
Oil palm pericarp	69.54	19.23±0.47	19.23±0.32	13.48±0.32	19.25±0.41

Table 2: Nitrogen, crude protein, fibre and organic matter contents of the indicated substrates used for the cultivation of *Volvariella volvacea*

Substrate used	Percentage composition of (%)			
	Nitrogen	**Crude protein**	**Crude fibre**	**Organic matter**
Banana leaves	0.94±0.51	5.85±0.43	32.72±0.51	85.83±0.04
Cocoa shells	2.07±0.77	14.92±0.53	18.41±0.00	88.75±0.53
Maize stover	0.76±0.32	5.35±0.41	32.35±0.32	91.35±0.62
Rice straw	0.91±0.11	5.65±0.21	28.78±0.41	91.63±0.99
Oil palm pericarp	0.79±0.21	4.96±0.41	48.65±0.64	80.75±0.41

Table 3: Microbial and pH profile of uncomposted indicated substrate used for the cultivated of *Volvariella volvacea* for 30 days at 28°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 4: Percentage occurrence of saprophytic fungi in the indicated substrates used for the cultivation of *Volvariella volvacea*

Saprophytic fungi	Occurrences in Substrate Used For Cultivation (%)									
	Dry banana leaves		Maize stover		Cocoa shells		Oil palm pericarp		Rice straw	
	Initial	Fungi	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Aspergillus flavus	24.4	2.9	-	3.1	11.1	-	-	16.7	10.9	-
A. fumigatus	19.7	25.7	-	24.4	-	59.2	-	52.7	-	-
A. niger	19.7	5.7	5.1	19.4	11.1	40.8	38.9	16.7	-	-
A. ochraceus	-	-	-	-	-	-	-	-	13.0	-
A. terreus	-	-	-	-	-	-	-	-	-	-
Cephalosporium acoemonium	-	-	-	-	16.7	-	-	-	-	-
Coprinus cinereus	-	14.0	-	10.0	-	-	-	14.0	-	20.0
Cladosoporium herbarium	-	-	-	-	33.3	-	-	-	-	-
Fusarium oxysporium	-	-	16.8	-	16.7	-	-	-	-	-
Gliocladium fimbriatum	21.1	38.6	-	31.3	11.1	-	-	-	-	-
Paeciliomyces sp.	-	-	21.0	-	-	-	-	-	15.2	7.1
Pestalotia macrotricha	18.9	-	-	-	-	-	-	-	-	-
Penicillium digitatum	-	2.9	-	-	-	-	-	-	-	-
Rhizopus stolonifer	-	-	-	-	-	-	-	-	-	-
Trichoderma viride	-	10.3	-	21.9	-	-	-	-	-	7.1

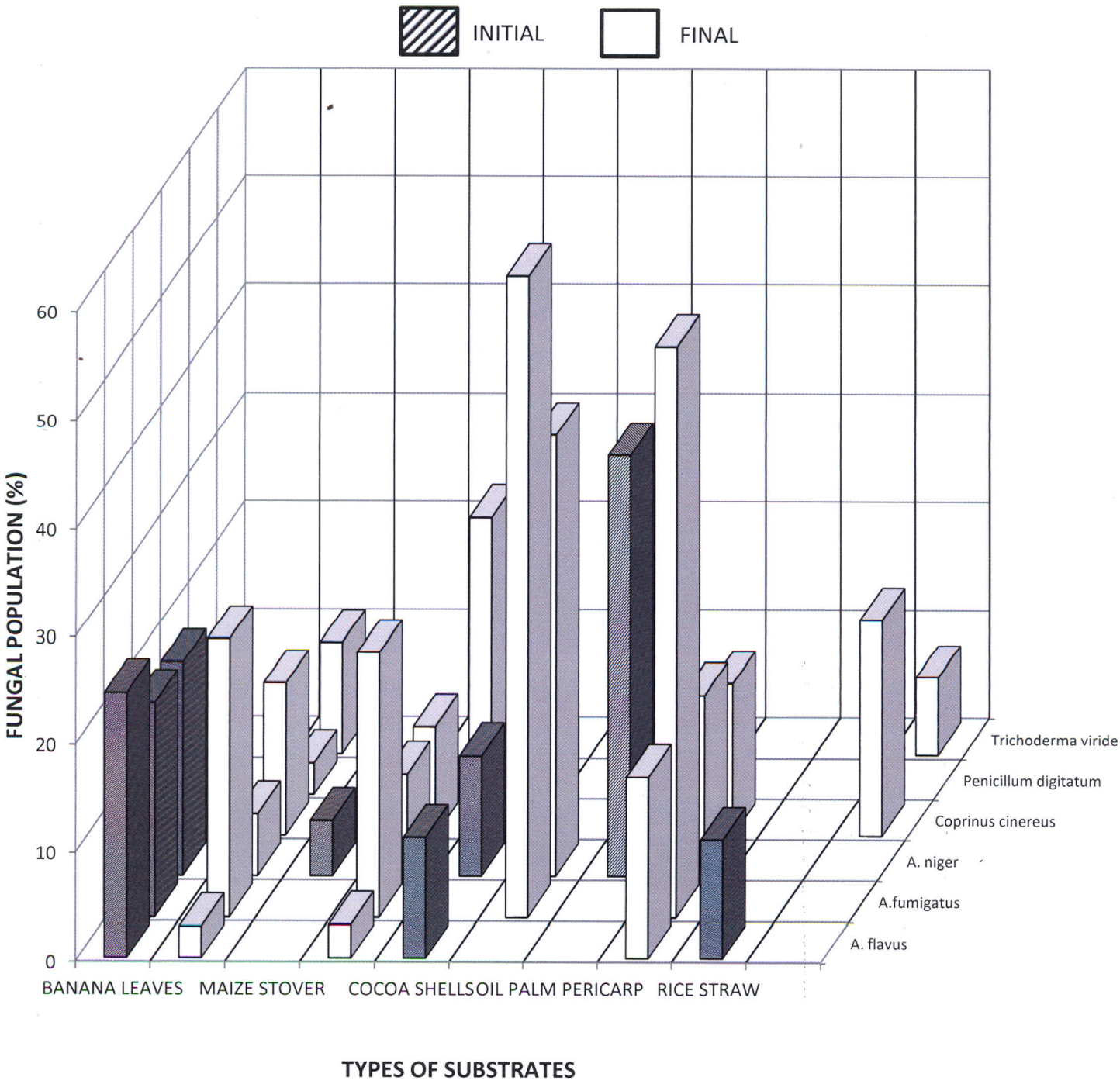

Fig !: Occurrence of some harmful fungi found in the substrate before and after cultivation of *Volvariella volvacea* at 28°C for 30 days.