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Physical, chemical and fungal phenology associated with the composting of 'wawa' sawdust (*Triplochiton scleroxylon*) used in the cultivation of oyster mushrooms in Ghana

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Abstract: The physical, chemical and fungal phenology during the composting of *Triplochiton scleroxylon* K. (Schum) sawdust was studied. Composting was carried out for 28 days. During the 28 days of composting the moisture content of the sawdust from the core portion of heap varied between 58.44 and 71.02%, whilst the pH ranged between 6.42 and 7.03. The dry weight of cellulose, hemicellulose, crude fibre and organic matter (% per unit) decreased as lignin increased. The microbial population also decreased during composting with a fungal population difference of 1.23 log cycles and bacterial population difference of 0.96 log cycles. The phenology of fungi encountered during composting and after fruiting were found to be *Aspergillus* species, *Cladosporum herbarum*, *Penicillium digitatum* and *Trichoderma viride*.

Keywords: Composting, *Triplochiton scleroxylon*, phenology, fungi, bacteria

Introduction

Mushroom cultivation exploits the natural ability of fungi to bio-convert solid waste generated by industry and agriculture into food (Tripothi and Yadar, 1992; Chiu *et al*., 2001). In nature, several mushroom species including *Pleurotus, Auricularia*, and *Lentinula* species have been isolated from decomposing wood of different tree species. This observation has led to the development of the bag system in the cultivation of these mushrooms using sawdust as the main medium. Invariably, the sawdust undergoes a period of composting which is required to break-down the cellulose and lignin components of the wood to release materials for the establishment of the mushroom mycelium (Sawyerr, 1994: Obodai *et al*., 2002). Composting is a solid-waste fermentation process, which exploits the phenomenon of microbial degradation and mineralization (Mckinley and Vestal, 1984). The main purpose of composting to a mushroom grower is to prepare a substrate in which the growth of mushroom is promoted to the practical exclusion of other microorganisms. 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 improve both the physical and chemical conditions for mushroom growth (Oei, 1991). Microorganisms colonizing mushroom compost during the composting process are regarded as active agents, which determine the chemical composition of the substrate thus making it possible for mushroom growth (Fermor *et al*., 1985). Very few studies describe the diversity of fungi in an entire composting process therefore this study was conducted to study the physical, chemical and fungal changes that occur during the composting of *Triplochiton scleroxylon* thus making it suitable for the cultivation of mushrooms.

Materials and Methods

Mushroom cultures

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Cultures of *Pleurotus sajor - caju* (Fr.) Sing strain PSCH and strain PSCM, *Pleurotus eous* strain OT-3 were used in this study. The cultures were maintained on Potato Dextrose Agar slants incubated at 28 ±2ºC and subcultured every 2 weeks. This was used for the preparation of sorghum spawn as described by Zadrazil (1978).

Substrate preparation

The compost was prepared by the outdoor single-phase solid waste fermentation. Fresh sawdust of *Triplochiton scleroxylon* K. Schum locally known as 'wawa' obtained from Timber Market, Accra was mixed and composted as described by Obodai *et al*. (2000). The compost was then stacked into a heap of about 1.5m high, 1.5m long and 1.5m wide. This heap was left for composting for 28 days with regular turning every 4 days. At weekly intervals samples of the compost were adjusted to approximately 68 - 70% (Buswell, 1984) and then supplemented with ricebran (12%) and lime (0.5%). The mixtures were bagged, sterilized, incubated and mushrooms harvested as described by Obodai *et al*. (2002).

Changes in the fermenting compost such as temperature, pH, moisture, chemical composition, and microbiota were also evaluated at weekly intervals. Samples used for these analyses were taken from the core region of the compost with a pair of sterile forceps. All samples were taken in triplicates.

Moisture content and pH of substrate

The moisture content was determined by drying the samples at 107ºC overnight in an electrically heated oven (Gallenkamp oven 300 plus series). Acidity (pH) was measured by soaking 1 g of compost in 10 ml distilled water for 6 h and using the supernatant to determine pH using an Alpha 500 model laboratory pH/mv meter (Obodai, 1992).

Chemical analysis

Samples of composting sawdust taken at weekly intervals (0, 7, 14, 21 and 28 days) from the central portion of the heap were put into sterile bags and quantitative estimation of crude protein, crude fibre, cellulose, hemicellulose, lignin, organic matter and ash 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:

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

To calculate total nitrogen in the samples, the specimens were dried at 60ºC 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 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 30-32ºC for 5 to 7 days. Aerobic bacterial population was enumerated on Plate Count Agar (PCA, Oxoid, Basingstoke Hampshire, England) incubated at 37ºC for 24h. The fungi were identified using their morphological and cultural characteristics as outlined by Samson *et al*. (1995).

Results and Discussion

Effect of composting on the physical and chemical characteristics of 'wawa' sawdust.

The selection of 'wawa' sawdust as substrate was based on its relative abundance and availability. The pH values of the fermenting substrate ranged initially at a neutral pH of 7.03 and reduced finally to a slightly acidic value of 6.47 at day 28 of composting (Table 1). These values are within the optimal pH range (5.5 to 7.0) required for best growth of *Pleurotus* species (Stamets, 1983). This drop in pH could probably be due to rapid breakdown of soluble and easily degradable carbon sources, resulting in a pH drop due to organic acids formation (Gray *et al*., 1971; Finstein and Morris, 1975; Beffa *et al*., 1996b). The moisture content ranged between 58.44 and 71.02 percent for day 28 and 0 respectively (Table 1). These are within the optimal moisture content required for best growth

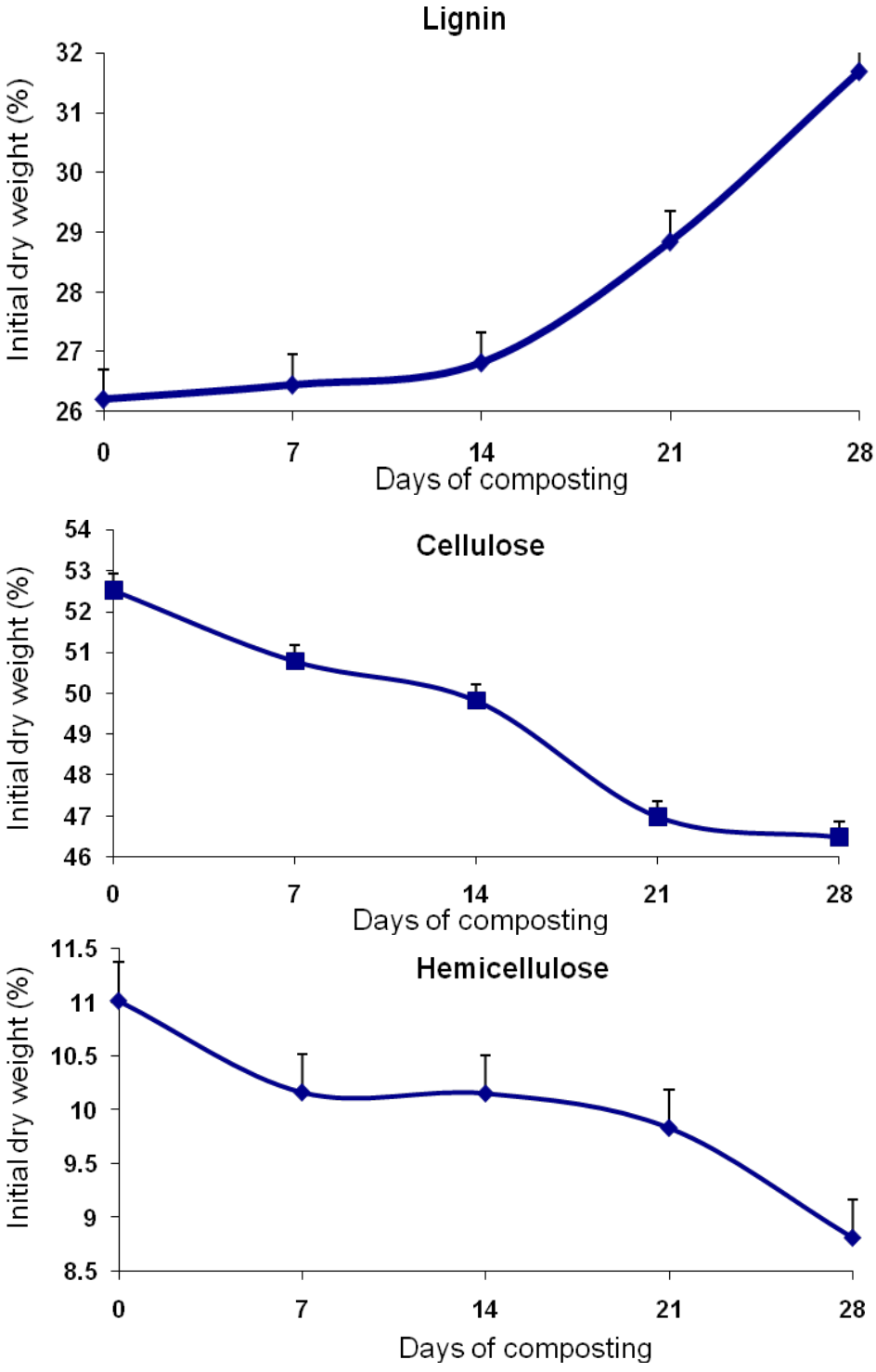

Figure 1-3. Changes in lignin, cellulose and hemicelluloses during composting of 'wawa' sawdust for 28 days

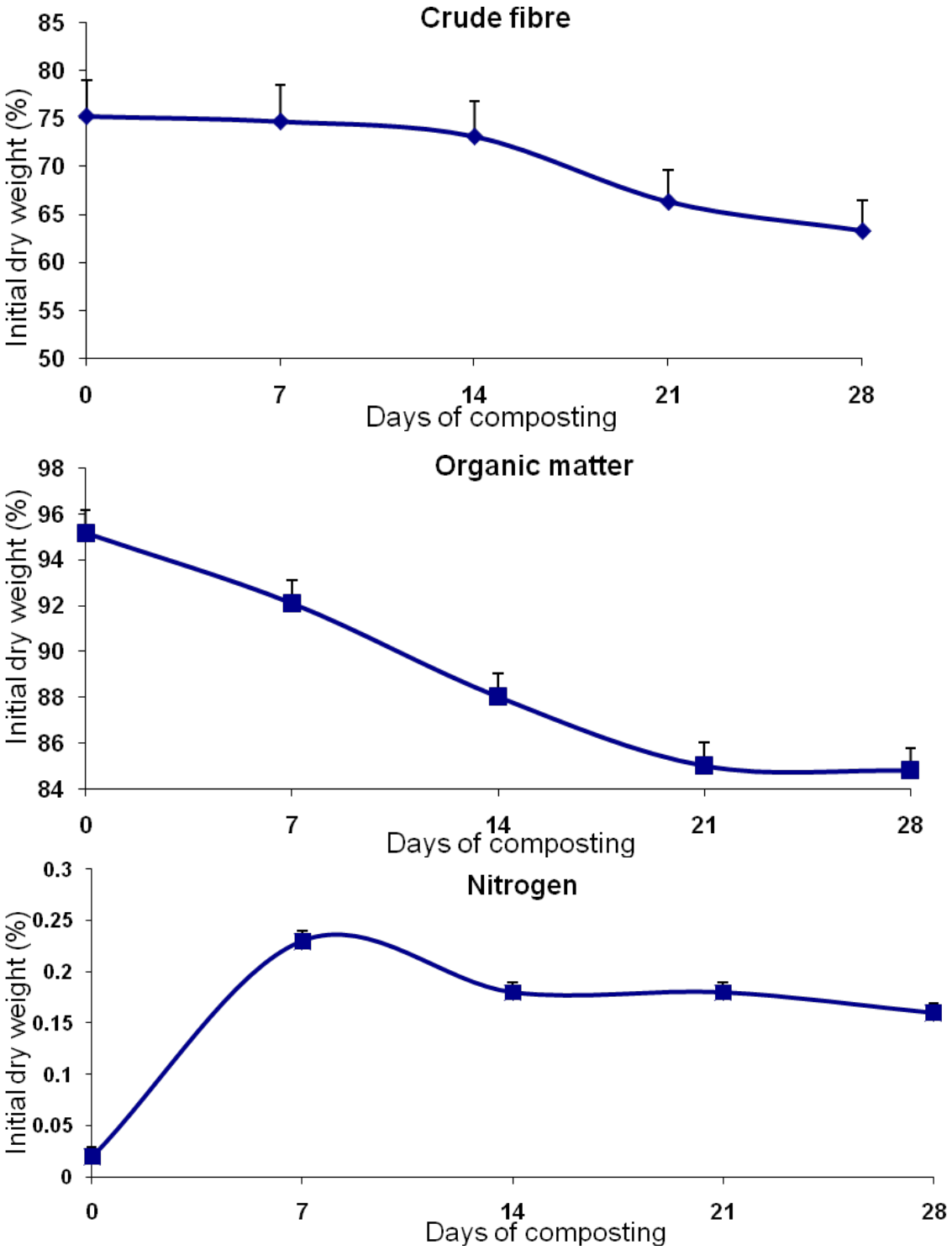

Figure 4-6. Changes in crude fibre, organic matter and nitrogen during composting of 'wawa' sawdust for 28 days

Table 1. Changes in the physical characteristics of 'wawa' sawdust during compositing

Period of composting	pH	Moisture	Temperature
0	7.03 (±0.03)	71.02(±0.01)	31.5 (±0.02)
7	6.74 (±0.01)	70.03(±0.09)	56.0 (±0.01)
14	6.42 (±0.12)	69.62(±0.10)	34.0 (±0.04)
21	7.34 (±0.09)	63.54(±0.03)	31.0 (±0.02)
28	6.47 (±0.01)	58.44(±0.02)	29.5 (±0.01)

Each mean represents analyses of three samples

Table 2. Changes in microbial profile before and after fruiting for 3 months for three *Pleurotus* species

Mushroom species	Microbial population of sawdust						
	Fungi		Bacteria		Period of composting	pH	
	Initial	After	Initial	After		Initial	After
Pleurotus sajor-caju Strain PSCH	6.23	3.78	7.24	6.14	0	7.03	5.73
	5.46	3.49	6.69	5.95	7	6.74	5.21
	5.36	3.41	6.48	6.11	14	6.42	5.46
	5.11	3.11	6.45	6.04	21	7.34	5.31
	5.00	3.04	6.28	6.00	28	6.47	5.49
Pleurotus sajor-caju Strain PSCM	6.23	3.30	7.24	6.46	0	7.03	6.98
	5.46	3.69	6.69	6.32	7	6.74	5.49
	5.36	3.25	6.48	6.27	14	6.42	6.16
	5.11	3.30	6.45	6.00	21	7.34	5.44
	5.00	3.39	6.28	5.60	28	6.47	5.45
Pleurotus ostreatus Strain OT3	6.23	3.38	7.24	6.04	0	7.03	5.61
	5.46	3.20	6.69	5.94	7	6.74	6.29
	5.36	3.23	6.48	5.77	14	6.42	5.74
	5.11	3.08	6.45	5.69	21	7.34	5.41
	5.00	2.84	6.28	5.60	28	6.47	4.59

of *Pleurotus* species (Kurtzman and Zadrazil, 1982; Stamets, 1983). Moisture content below a critical level (<30%), will decrease microbial activity and the microorganisms will become dormant. On the other hand, a moisture content that is too high (>65%) can cause oxygen depletion and losses of nutrients through leaching. In subsequent anaerobic conditions the decomposition rate decreases and odor problems arise (de Bertoldi *et al*., 1985; Golueke, 1992; Fogarty and Tuovinen, 1991; Tiquia *et al*., 1996).

Temperature values ranged from 56°C at day 7 to 29.5°C on day 28 (Table 1) indicating very high microbial activity on day 7 (Table 1). Bacteria are responsible for most of the initial decomposition and heat generation in compost provided that the major growth requirements are met. For bacteria, the optimal moisture content ranges from 50 to 60% (Fogarty and Tuovinen, 1991; Golueke, 1992) and they favor a near-neutral pH.

Analyses of the various organic constituents of 'wawa' during the 28 days of composting showed that there was a gradual decline in nitrogen. This indicates its utilization by the microorganisms during decomposition of the fermenting substrate thus it is essential that the nitrogen content of the substrate be amended for utilization of the mushroom mycelium at the bagging stage. Besides a C source, microorganisms require macronutrients such as N, P and K, and trace elements for their growth. Nitrogen is a critical element for microbial growth. If N is limiting during composting the degradation process will be slow (Ryckeboer *et al*., 2003).

Cellulose, hemicellulose, crude fibre, and organic matter decreased with increasing days of composting as compared to lignin (Figures 1-6). This was possibly because these were easily degradable by the fungi and bacteria present as compared to lignin a polymer of aromatic compounds which is very resistant and relatively difficult for cellulolytic organisms to decompose (Chang – Ho, 1982; Insam and de Bertoldi, 2003). These decreases occurred mainly at the end of composting (21 to 28 days) when

Table 3. Changes in fungal phenology during composting of 'wawa' sawdust for 28 days

Fungi recorded	Occurrence (%) of fungi in sawdust during composting (Days)				
	0	7	14	21	28
Aspergillus flavus	-	-	-	40.0	26.09
A. fumigatus	-	23.03	31.03	-	56.52
A. niger	-	-	-	30.0	-
Mucor pusillus	30.0	-	-	-	-
Paeciliomyces varioti	70.0	-	-	-	-
Rhizopus oryzae	-	76.92	68.97	30.0	-

Table 4. Changes in fungal phenology after fruiting for 3 months for *Pleurotus sajor-caju* strain PSCH and PSCM on 'wawa' sawdust

Fungi recorded	Occurrence (%) of fungi in sawdust inoculated with									
	P. sajor-caju strain PSCH					*P. sajor-caju* strain PSCM				
	0	7	14	21	28	0	7	14	21	28
Aspergillus flavus	-	-	-	40.0	26.09	-	17.91	-	-	-
A. fumigatus	-	23.03	31.03	-	56.52	10.0	-	47.5	20.0	8.0
A. niger	-	-	-	30.0	-	-	18.2	5.5	10.0	-
A. ochraceus	-	-	-	-	-	5.0	-	-	-	-
Cladosporum herbarum	-	-	-	-	-	5.5	13.56	47.5	60.0	60.0
Mucor pusillus	30.0	-	-	-	-	-	-	-	-	-
Penicillum cyclopium	-	-	-	-	-	15.0	-	-	-	4.0
P. digitatum	-	-	-	-	-	-	30.21	5.5	20.0	8.0
Paeciliomyces varioti	70.0	-	-	-	-	-	-	-	-	8.0
Rhizopus oryzae	-	76.92	68.97	30.0	-	-	-	-	-	-
Trichoderma viride	-	-	-	-	-	12.0	20.11	-	-	22.5

Table 5. Changes in fungal phenology after fruiting for 3 months for *P. ostreatus* strain OT-3 on 'wawa' sawdust

Fungi recorded	Occurrence (%) of fungi in sawdust inoculated with *P. ostreatus* strain OT-3				
	0	7	14	21	28
Aspergillus flavus	20.21	nd	nd	nd	nd
A. fumigatus	7.31	nd	11.76	25.41	-
A. niger	-	6.25	23.53	21.31	14.28
A. ochraceus	21.0	nd	nd	nd	nd
Cladosporum herbarum	nd	nd	41.17	nd	28.75
Penicillium digitatum	30.38	48.25	nd	27.97	14.28
Mycelia sterilia	nd	nd	nd	nd	14.28
Trichoderma viride	12.0	45.5	nd	26.31	14.28

temperatures had dropped and the environment was conducive for their growth (Figures 1-6) (Internet).

The microflora population during composting

Ammonification causes an increase of pH favourable for bacteria that subsequently out-compete fungi within a few hours or days of fermentation. Though there were decreases in the microbial counts these differences were marginal with a fungal population difference of 1.23 log cycles and bacterial population difference of 0.96 log cycles by day 28 of composting (Table 2). This indicated that relatively high numbers of microorganisms were involved in the decomposition of the compost making it suitable for growth of mushrooms. Table 2 also shows that the microbial populations of the sawdust decreased with increasing days of composting, and different fungi appeared at different periods of composting, this agrees with Stanek, (1972) who reported that the number of micro-organisms decreased during fermentation process in wheat straw composted for 30 days. The environmental and nutritional conditions created during composting selectively favoured certain fungi to the detriment of others.

At the different periods of composting the microbial populations in all instances were lower within three months of fruiting. The shorter the period of composting, the greater the difference between the initial and final microbial population (Table 2). A resident fungal and bacterial population difference of 2.45 log cycles and 0.83 log cycles respectively were recorded for day 0 as compared to 1.96 log cycles and 0.28 log cycles for 28 days for *P. sajor-caju* strain PSCH (Table 2).

Although there were higher counts of bacteria at all stages of fermentation and after fruiting, this paper is on the changes of fungi that occurred during the decomposition of 'wawa' sawdust before and after fruiting, this is because the characteristics of substrate in play here that is cellulose, hemicellulose and lignin are degraded mainly by fungi although bacteria have a role to play (Insam and de Bertoldi, 2003).

The fungal phenology during composting

Paeciliomyces varioti and *Mucor pusillus* initially dominated in the 'wawa' sawdust at 70% and 30% occurrences respectively (Table 3). These two fungi have also been found to be dominant in initial stages of straw undergoing fermentation (Anastasi *et al*., 2002). These, however disappeared by the 7th day of composting and was replaced by *Aspergillus fumigatus* found to be dominant in substrates at temperatures of 30-50^{o}C (Anastasi *et al*., 2002). This fungus prevailed and was observed at day 28. It was interesting to note that *A. flavus* was found at day 21 and persisted to day 28 (Table 3). This interplay of fungi could be attributed to antibiosis displayed by the different fungi which was very dependent on the prevailing conditions of the substrate (sawdust). Carlile and Watkinson (1996) have suggested that temperature has significant effect on the succession of microorganisms involved in the fermentation process of composting. The fungi identified have been found to degrade cellulose, hemicellulose, starch and to some extent lignin (Ryckeboer *et al*., 2003). The composting process involves microbial activity, chemical reactions, aeration, temperature and nutritional factors. During composting, the fungal succession in the compost were influenced by the listed factors (Chang - Ho, 1982). At different stages of composting different groups of micro-organisms dominated (Hayes, 1977).

Phenology of the fungal species in the sawdust varied with the period of composting and the species of *Pleurotus* that utilized the substrate. Generally, thirteen fungal species belonging to eight genera namely: *Aspergillus*, *Cladosporium*, *Mucor*, *Neurospora*, *Paeciliomyces*, *Penicillium*, *Rhizopus*, and *Trichoderma* were encountered. *Aspergillus* species predominated followed by *Penicillum* species. At any composting period (0, 7, 14, 21 or 28 days) the species of fungi which appeared varied depending on the species of the mushroom utilizing the substrate. For example, although percentage occurrence of *A. flavus* in the substrate was moderately high (17.91-47.92 percent) at the onset of the experiment, it could not be isolated after 28 days in all the mushroom species utilizing it.

Similarly *A. fumigatus* although present in the substrate at the beginning (7.31-56.52 percent) of the experiment, it could not be isolated after 28 days (Table 3-5). Conversely, occurrence of *A. niger* in the compost was initially low in substrates supporting growth of *P. sajor-caju* strain PSCH and *P. ostreatus* strain OT-3 but this fungus persisted in all substrates (10.00 – 14.28 percent) till the end of the experiment: *Cladosporium herbarum* and *Trichoderma viride* behaved similarly (Table 3 - 5). Presumably, antibiosis was at play in the composted 'wawa' substrate.

Conclusion

Different fungal genera namely *Aspergillus*, *Mucor*, *Paeciliomyces*, and *Rhizopus* were involved in the decomposition of the 'wawa' sawdust. These fungi in addition to the conducive environmental

and chemical characteristics created in the sawdust made the compost suitable for the growth and yield of *Pleurotus sajor-caju* strain PSCH, PSCM and *P. ostreatus* strain OT-3.

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