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Compost Physico-chemical Factors that Impact on Yield in Button Mushrooms, *Agaricus bisporus* (Lge) and *Agaricus bitorquis* (Quel) *Saccardo*

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ABSTRACT Button mushrooms, *Agaricus* sp. are secondary decomposers that require nutritious and very selective composts for their growth. Selectivity of these composts is influenced by both biological and physico-chemical characteristics of composting process. The conventional method of preparing button mushroom composts is to use a combination of horse manure, hay and a high nitrogenous source such as chicken manure. These materials are composted over time until the compost is 'mature'. In the absence of the materials above, several alternatives can be used. An experiment was designed to use locally available materials in making two synthetic composts of grass and maize stalk. The objective was to find out factors that contribute to making compost that give high yields of button mushrooms. Three strains of mushrooms *A. bisporus*, *A. brunescence* and *A. bitorquis* were planted in three different composts; horse manure was used as control, maize and grass as synthetic in a complete randomized design. Nitrogen content of each compost was calculated, moisture content and temperature of the compost were recorded throughout composting and during conditioning period. Yields of mushrooms were taken in three different flushes. Results of the mean yields of three mushroom strains grown indicated that the two composts were significantly different; the grass compost gave significantly superior yields of mushrooms of 549 gm/kg of compost compared to the conventional horse manure which gave 435.66 gm/ kg of compost. The maize straw synthetic type gave 327.33 gm/ kg of compost (pd"0.05). Significantly very low yields were realized in the maize straw synthetic type. Though many factors contribute to the yield of mushrooms, the superiority of the grass synthetic compost may be attributed to three main factors: sufficient nitrogen levels at spawning, a more open structure that facilitated aeration during composting as well as conditioning, and the high temperature regime maintained during composting. Maize straw synthetic compost on the other hand remained soggy due to the spongy stalks which imbibed water readily, especially during steam pasteurization. This resulted in the failure of a temperature build up. Temperature build -up in phase I as well as at peak heating. Moisture content during composting and aeration of the compost heap are significant factors in mushroom compost preparation.

INTRODUCTION

White button mushroom (*Agaricus spp)* cultivation began in France two hundred years ago and has developed into a thriving industry not only in Europe, but world over (Piet et al.1998). A large body of knowledge has been developed with the growth of this industry particularly on the composting of substrates, sporophore development and improvement of yields. There are four main steps involved in mushroom cultivation: composting, conditioning, spawning and casing.

Composting prepares a selective medium that will favour growth of the mushrooms relative to other microorganisms (Femor et al.1985). Baar, a researcher with the University of Wageningen, Netherlands in a composting workshop emphasized the importance of research in composting for mushroom growing. He lauded the importance of using novel molecular techniques which enable us to study microbes in the compost to reconsider the temperature requirements. He reported that the assessment of the starting materials may result in optimal process, we must therefore define what parameters we need to measure (Barr and Istvan Parad 2004).

The compost procedure involves two to three weeks of uncontrolled self- heating, followed by pasteurization. Conditioning involves placing the compost at 45-60^{0}C for a period of six to nine days (Gerrits 1988; Gerben et al.1995). Conditioning is a controlled process during which the thermophilic fungus *Scytalidium thermophilium* becomes abundant. The density of this fungus in finished compost is a positive correlate of the yield of mushrooms (Gerben et al. 1995). *Scytalidium thermophilium* has been confirmed to enhance and promote the growth of *Agaricus bisporus* (Gerben 1993; Samson 1993). It has been suggested that this fungal species provides a trigger for enhanced growth of button mushrooms (Gerben et al.1995). The basic function of the thermophilic fungi is to

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utilize and exhaust the readily available carbohydrates and the free ammonia. A more recent development calls for further research on this, Gerben et al. (1995) depicted an up to date overview on the latest results of compost microbiology and processing. He states that Thermophilic *Scytalidium* varieties possess the largest biomass and create selectivity in the compost. However, the numbers of micro-organisms decline with at least a factor of 100 after spawn run due to the dominating position of Agaricus; which produces inhibitory substances. Agaricus degrades much of the compost when the growing fruit bodies need the nutrients. (Barr and Istvan Parad 2004). Significance of thermophilic fungi in mushroom compost preparation and measurement of microbial biomass is still an issue (Sparling et al. 1982; Salar and Anejar 2007)

The preparation of proper mushroom compost is still therefore a prerequisite for high yields of *Agaricus bisporus*. In Europe horse manure is commonly used because it is readily available. The alternative to horse manure is referred to here as synthetic compost. This research investigated conditions that are necessary in making successful synthetic composts as an alternative to the conventional horse manure.

METHODS

Compost Preparation

Cellulose materials and the supplements, chicken manure, soya meal and cotton meal were analyzed for their carbon and nitrogen content using the methods recommended by Anderson and Ingram (1993) to determine nitrogen ratios to be applied. The conventional horse manure compost was prepared from stable horse manure, supplemented with chicken manure, while the first synthetic compost was prepared using a mixture of maize straws and soya meal. The second synthetic compost was prepared from grass and cotton meal. The nitrogen ratios at the start of composting were calculated as shown on the Tables 1a, b and c.

Table 1a: Maize synthetic compost composition

Item	*Wet weight g*	*Moisture content %*	*Dry weight g*	*Percentage Nitrogen%*	*Total Nitrogen g*
Maize	250	10	225	0.9	2.025
Straw	75.4	40	45.22	1.0	0.452
Soya meal	40.0	10	36	6.65	92.65
Gypsum	3.0	-	3.0	0	0
Total	368.4	60.0	306.22	8.55	4.843

Table 1b: Grass compost composition

Item	*Wet weight g*	*Moisture content %*	*Dry weight g*	*Percentage Nitrogen%*	*Total Nitrogen g*
Grass straw	250	10	225	0.75	1.68
Chicken manure	400	40	240	3.6	8.60
Gypsum	-	-	6.9	-	6.9
Total	650.0	50.0	471.9	696.9	11.98

Table 1c: Horse synthetic compost composition

Item	*Wet weight g*	*Moisture content %*	*Dry weight g*	*Percentage Nitrogen%*	*Total Nitrogen g*
Maize	250.0	10.0	225.0	0.9	2.025
Straw	75.37	40.0	45.22	1.0	0.452
Cotton meal	40.0	10.0	36.0	5.4	2.666
Gypsum	3.0	-	3.0	-	6.0
Total	368.37	50.0	309.22	5.143	11.143

The nitrogen compost levels were within the required ratio of 1.5 to 2% as has been suggested by Lebanon (1993).

Pre Wetting, Piling and Turning of Composts

The compost was wetted every time the different components were added to the compost pile, starting with the cellulose material. The compost measured 1.5mx2m in dimensions. Wooden pellets were placed at the base of each compost pile to facilitate air circulation below. Each heap was incubated for four days during which temperatures were taken daily. These included the ambient minimum and maximum, compost temperature at the periphery, at 15cm into the heap, and at 30cm into the compost heap. For each temperature measurement, three temperature readings were taken and an average calculated. On the fourth day the compost was aerated by turning, mixing and reconstituting the pile. Dry parches were re-watered and gypsum was added at a rate of 3%. Three samples from each compost heap were then taken for analysis of moisture content, pH, carbon and nitrogen contents. The heap was incubated for a further two days and temperature measurements repeated as before. This process was thereafter repeated every two days until the compost was ten days old. A quantity of 2 kg of compost was then placed in polythene bags for analysis.

Pasteurization and Conditioning (Peak Heating) of Compost

The bagged compost was placed in a steaming chamber at 70^{0}C for two hours. The bags were then moved to the growing room where the temperature was stabilized to between 50^{0}C-60^{0}C for four days. The temperature in the room was then lowered to 25^{0}C and the bags spawned with 1% spawn to the weight of the compost.

Spawn Preparation

The spawn was prepared from a strain of *Agaricus bisporus* 130s, *Agaricus bitorquis* 802, and *Agaricus brunnescens*. Pure cultures were aseptically prepared and stored as slants for future use. The plate cultures were prepared and used for making spawn.

Spawning and Spawn Run

One hundred (100) gms of the ready spawn was used to 'seed' the compost in bags. The ratio of spawn to compost was 1. The grains were distributed evenly within the compost and the compost bags kept in the growing room. Temperature in the growing room was kept at 23^{0}C for *A.bisporus* and *A.brunnescens* and at 25^{0}C for *A.bitorquis* for four weeks. This is the optimum temperature at which these species are normally incubated (Gerben et al. 1995). Two weeks after seeding, the casing material was added. Casing material was a mixture of peat with 70 percent moisture content. It was pasteurized and its pH adjusted by liming to pH 7. Temperature in the growing room was further lowered one month after spawning to 16^{0}C and 20^{0}C respectively for the two different mushrooms species for two days. This is the way *Agaricus* mushrooms are 'shocked' into fruiting (Femor et al. 1985). Thereafter, the temperature was raised to 18^{0}C and 22^{0}C for the two mushroom species for the whole of the cropping period. Humidity was maintained at 95 percent for first two weeks then reduced to 92 percent. At cropping time (one month after seeding), the vents were opened to let in fresh air and release carbon dioxide. Harvesting of first flush was initiated 5 weeks after seeding while the 2^{nd} and 3^{rd} flushes were harvested at an interval of 7-10 days and their total weights taken at each harvesting. Individual flush weights as well as dates of harvest were recorded.

Data Analysis

Weights of harvested mushroom from different substrates were subjected to simple analysis of variance to determine significant differences at 95 percent level of significance. Separation of means to test for their independence was carried out by LSD at 95% confidence level

RESULTS AND DISCUSSION

The three experimental composts produced significantly different weights of mushroom harvests (Table 2) ($p \leq 0.05$). The grass compost produced significantly superior yields than the conventional horse manure and the maize straw synthetic type ($p \leq 0.05$). The lowest yields were realized in the maize straw synthetic compost type. While many factors contribute to the yield of mushrooms, our results clearly show that grass synthetic compost was superior to conventional horse manure and maize straw synthetic compost. Three factors may explain the superiority of the grass synthetic compost (i) sufficient nitrogen levels at spawning of 1.66%. These are very close to the optimal composition of synthetic composts (Levanon 1993). Baar and Paradi (2004) presented an extensive report on the fate of nitrogen during composting. He said that nitrogen is a versatile component, which can undergo a wide series of microbial mediated redox reactions and can have several species with highly different physico-chemical characteristics. The amount of nitrogen should be sufficient in the compost, because it is an energy supply for the micro-organisms and a basic nutrient for mushroom growth (Baar and Paradi 2004). (ii) A more open structure that facilitates aeration during composting as well as conditioning is most important. Francisco (2010), has emphasized the importance of conditioning in the growing of mushrooms. He showed clearly that the results of a growing cycle are largely determined by using correct procedures during peak-heating. He further advised that controlled temperatures and quantities of air used during ventilation should be monitored and controlled. This clearly points to the importance of proper ventilation and aeration during composting. Anae-robic conditions promote decarboxylation which produces a volatile basic amine. The amine causes high dispersion of the constituents of the compost which affects fungal mycelia growth in the compost.

Means in the same column with different superscript letters were significantly different by LSD at 95%confidenc level.

(iii) The high temperature regime during the first phase of composting (Fig.1), sufficient and readily available sugars are necessary for the

Table 2: Mean yields of the different species of mushrooms grown on three experimental composts

Compost	*Variety and mean yields in grams*			*Mean yields of mush-rooms gm/kg*
	A .bis-porus	*A.brune-scence*	*A. bitor-quis*	
Horse	$543^{b}\pm$ 176.00	$360^{b}\pm$ 16.73	$404^{b}\pm$ 42.45	435.66
Maize	$291^{b}\pm$ 37.61	$351^{b}\pm$ 4.70	$340^{b}\pm$ 30.55	327.33
Grass	$839^{a}\pm$ 180.00	$384^{b}\pm$ 51.20	$426^{b}\pm$ 13.01	549.0

initial rapid growth of mesophilic organisms that initiate the succession of microorganism which raise the temperature due to their metabolic activities. During the second phase of composting, high temperatures have traditionally been thought necessary to induce the browning reaction and fixation of ammonia responsible for compost selectivity (Laborde et al. 1986). Yield and quality of *Agaricus* mushrooms are dependent on the presence of thermophilic microorganisms during composting (Ross and Harris 1983). Aerobic conditioning at 45^{0}C-60^{0}C restores the population of the microbes earlier destroyed by high temperatures. These microbes are an important concentrated source of available nitrogen and possibly other nutrients (Sparling et al.1982). Composting for *Agaricus* mushrooms is therefore not just a science but also an art.

Temperature build up in compost (Fig. 1) are well correlated for the higher yielding grass compost (Table 1). The temperature in the grass compost rises from 60^{0}C to 79^{0}C in the third turn and finally 62^{0}C in the final turn compared to maize compost which rises from 40^{0}C to a

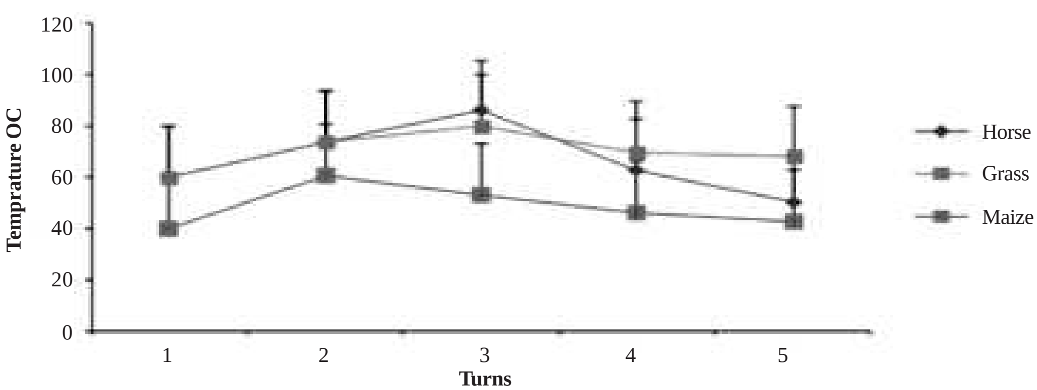

Fig. 1. Temprature range over period of composting on three different compost horse manure, grass and maize

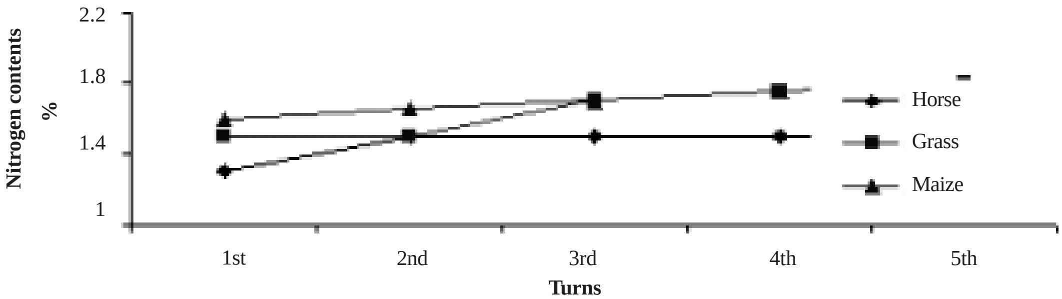

Fig. 2. Compost nitrogen content from Ist tern to 5th turn on horse manure maize and grass synthetic composts

maximum of 60^0C in the second turn then returns to 42^0C. The horse manure compost on the other hand reaches a peak of 80^0C on the third turn but looses heat fairly fast (Fig. 1).

Phase one composting consists of biomass, made up of actinomycetes, bacteria, fungi and yeast. The buildup of these organisms is correlated with the rise in temperature during composting. Through their metabolism, they convert dry matter to sugars, proteins, carbon dioxide ammonia and water (Laborde et al.1986). The initial rise in temperature of about 50^0C–60^0C is a self-heating process, which results from heat generated by the aerobic microbial decomposition. Amino acids and sugars are produced. These nutrients are essential for the phase two composting. The high temperature phase of about 60^0C-80^0C that is realized towards the end of phase I determine ultimate selectivity of the compost towards the cultivated mushroom. This high temperature phase is as a result of physico-chemical reactions resulting in the formation of amino sugars and the lingo-protein complexes (Gerrits et al.1967). The ultimate selectivity of the *Agaricus* sp. mushroom to the compost is mainly brought about by the enzymatic capacity of the mushroom to utilize products from the composted material. At the end of the composting process, the compost consists mainly of the plant derived components such as lignin, cellulose, hemicelluloses, protein and also microbial biomass (Femor and Wood 1979). The lignin fraction encrusts cellulose and is chemically bound to hemicellulose (Cullen and Kersten 2004). This makes the compost selective to *Agaricus* sp.

The horse manure compost had relatively low nitrogen content (Fig. 2). The high moisture content towards the fifth turn (Fig.3) may also have disadvantaged it.

The maize compost was disadvantaged by two factors, it did not realize optimal temperatures (Fig. 1), and the moisture content was high (Fig. 3).

High moisture content towards the fourth turn (Fig. 3) may have disadvantaged maize compost. As a result it did not realize optimal temperatures (Fig. 1), rather high despite high nitrogen content (Fig. 2).

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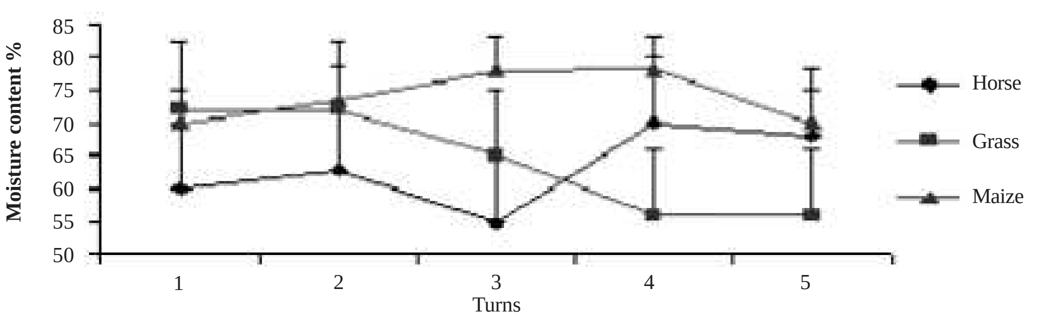

Fig. 3. Compost moisture content in the three different substrates horse manure maize and grass over different periods

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