



# Evaluating the Effect of Gamma Irradiation and Steam Sterilization on the Survival and Growth of Composted Sawdust Fungi in Ghana

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## Authors' contributions

This work was carried out in collaboration between all authors. Authors NKK and GTO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors VA, MO and MWK managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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## ABSTRACT

The growth and survival of some fungi associated with composted and pretreated sawdust particularly for mushroom cultivation were investigated on two growth media; Cooke's and Oxytetracycline Glucose Yeast Extract (OGYE). Some fungi were isolated during the composting of sawdust over a period of 28 days as well as after pretreatment with gamma irradiation doses of 5, 10, 15, 20, 25 and 32 kGy and moist heat of 100±2°C for 2.5 hours. Fungal counts ranged 4.72-5.77 log<sub>10</sub> CFU/g and 3.4- 4.1 log<sub>10</sub> CFU/g respectively for both media. Both pretreatment methods effectively reduced (p<0.05) fungal counts by an average of 1.48 (irradiation) and 2.22 (steam) log-cycle reductions on OGYE while there was an average of 3.13 (irradiation) and 1.10 (steam)

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log-cycle reduction on Cooke's. Corresponding radiation sensitivities ( $D_{10}$  values) of  $5.94 \pm 2.06$  kGy and  $5.64 \pm 1.12$  kGy were recorded for fungi on both media respectively. Five species belonging to three genera were isolated on OGYE and among the fungi were *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus ustus*, *Mucor racemosus* and *Rhizopus stolonifer*. Ten species belonging to four genera were also isolated on Cooke's medium included *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus ustus*, *Aspergillus terreus*, *Aspergillus parasiticus*, *Aspergillus alutaceus*, *Rhizopus stolonifer*, *Fusarium oxysporum* and *Mucor racemosus*. There was an observed reduction ( $p < 0.05$ ) in species diversity after pretreatment. The presence of these microorganisms on sawdust also has serious repercussions on animal and human health.

**Keywords:** Composting; gamma radiation; sawdust; steam;  $D_{10}$  value; fungi; mushroom.

## 1. INTRODUCTION

Sawdust which is a source of organic waste material is used extensively in horticulture, in soil amelioration, as a substrate for cultivation of mushrooms and in diverse applications. Nonetheless, sawdust attains its optimum utilization if it is well decomposed and the lignin content turned to humus. Improperly decomposed sawdust could result in nitrogen immobilization and produce some toxic substances which will affect plants, fungi, animals and humans [1].

Composting is a process of exothermic biological oxidation of various organic wastes in the presence of air involving microorganisms. Through microbial decomposition, the organic matter is stabilized, matured and deodorized into a product rich in humic substances that can be used as organic soil conditioner, easy to store and distribute [2,3]. Modern concept of environmental management relies on the recycling of wastes. In this perspective, composting appears to be a safe form of treatment of some wastes and the reclamation of the nutrients contained in them [4]. It is environmentally friendly because the process produces a marketable end-product that can be used as a soil conditioner and organic fertilizer. Composting is therefore regarded as a successful strategy for the sustainable recycling of organic wastes [5,6].

The active component mediating the biodegradation and conversion processes during composting is the resident microbial community, among which fungi play a vital role [7]. Therefore, optimization of compost quality is directly linked to the composition and succession of microbial communities in the composting process [8,9]. There is practically no substance existing in nature that is not used by one microorganism or

another [10]. It is therefore necessary to identify the microorganisms present in the different processes, as several different species of microbes are usually involved [11,12]. Microbes play key role in the process monitoring of microbial succession is important for the effective management of the composting process.

Since composting methods and different substrates are associated with different composition of a microbial community, monitoring of the resident microbial population in compost is essential to determine its quality and field of application [8,13].

Fungi use many carbon sources; mainly lignocellulosic polymers and can survive in extreme conditions. They mainly are responsible for compost maturation [14]. They also degrade complex polymers such as polyaromatic compounds or plastics and are being increasingly applied to bioremediate soils contaminated with a wide range of pollutants [15,16].

Steam pretreatment of sawdust for mushroom cultivation, is the most widely used method in Ghana [17,18]. It has become essential to explore other methods of pasteurization and improve efficiency of production. Gamma irradiations which have short wave length, high energy photons, and have deep penetrating power so could serve both as a decontaminating agent and a hydrolytic agent [19,20,21] for the bioconversion of lignocellulosic materials to produce mushrooms [21].

The objective of this paper is to investigate the fungal populations and species diversity associated with the composting process, gamma irradiated and steam / moist heat pretreated sawdust substrate principally for mushroom cultivation.

## 2. MATERIALS AND METHODS

### 2.1 Composting

The compost was prepared by the outdoor single phase solid waste fermentation. Fresh sawdust obtained from Timber market Accra was mixed and composted as described by [21]. The compost was then stacked into a heap of about 1.5 m high, 1.5 m long and 1.5 m wide. This heap was left for composting for 28 days with regular turning every 4 days. At weekly intervals samples, moisture content of the compost were adjusted to approximately 68 - 70% [22] and then supplemented with rice bran (12%) and lime (0.5%).

### 2.2 Irradiation and Pasteurization of Sawdust Compost Bags

A batch of bagged composted sawdust substrates stored in polypropylene packs of 25 x 18 cm dimension were sterilized with moist heat at a temperature of 98-100°C for 2.5 hours. Another batch stored in same packs were subjected to irradiation doses of 0 kGy, 5 kGy, 10 kGy, 15 kGy, 20 kGy, 25 kGy and 32 kGy at the Gamma Irradiation Facility of the Ghana Atomic Energy Commission using a Cobalt- 60 source (SLL-02, Hungary) at a dose rate of 1.7 kGy per hour in air . The absorbed dose was confirmed by ethanol-chlorobenzene (ECB) dosimetry. Each treatment was replicated 6 times.

### 2.3 Enumeration and Characterization of Mycoflora

The dilution plate technique was used in estimating fungal 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 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<sup>3</sup>. The fungal population was enumerated on modified Cooke's medium [23] and OGYE incubated at 30-32°C for 3-5 days for species diversity. The fungi were identified using their microscopic, morphological and cultural characteristics as outlined by [24].

### 2.4 Determination of Occurrence Percentage of Fungi

The incidence of occurrence of the different fungal isolates was done by a modified formular

of [25,26]. The frequency of occurrence of the fungal pathogens from the sawdust was determined. The total number of each isolate in the sawdust sample was obtained against the total number of all the isolates in the sample screened.

The mean value of this gives the occurrence percentage as the following equation shows:

$$\text{occurrence percentage} = X/N \times 100 \quad (1)$$

where X = total number of each isolate in the sample and N = total number of all the isolates in the sample.

### 2.5 D<sub>10</sub> Values Determination

The D<sub>10</sub> value is the reciprocal of the slope of the exponential part of a survival curve. This value may also be obtained from equation (2). The data was subjected to regression analysis. The surviving fractions,  $\log_{10} (N/N_0)$  of microorganisms, was calculated and used as relative changes of their actual viable cell counts. The D<sub>10</sub> values were calculated by plotting  $\log_{10} (N/N_0)$  against dose (D) according to the equation

$$D_{10} = \frac{\text{Radiation Dose (D)}}{\log_{10} (N_0 - N)} \quad (2)$$

where N<sub>0</sub> is the initial viable count; N is the viable count after irradiation with dose D; D is the radiation dose [27,28,29]. The linear correlation coefficient (r<sup>2</sup>) and the regression equations were also calculated.

### 2.6 Statistical Analysis

The values obtained for the fungal counts were done in the standard forms and then transformed to logarithmic values and subjected to analysis of variance (one way ANOVA) using SPSS (Illinois, USA) version 9 for Microsoft windows.

## 3. RESULTS AND DISCUSSION

### 3.1 Fungal Population during Composting

The dynamics of fungal community may be attributed generally to abiotic variables and nature of substrate [30]. There was variation of the fungal species in the sawdust with respect to the period of composting. The total mycoflora population enumerated on Cooke's medium was comparatively higher than on OGYE. The highest

mycofloral population of 5.77 log<sub>10</sub> CFU/g was recorded for 28<sup>th</sup> day of composting while the lowest population of 4.72 log<sub>10</sub> CFU/g for the 4<sup>th</sup> day of composting. Statistically, mycoflora population of 0 (first), 4<sup>th</sup>, 8<sup>th</sup> and 24<sup>th</sup> days of composting which corresponded to 5.00, 4.72, 4.98 and 5.00 log<sub>10</sub> CFU/g respectively differed significantly (p<0.05) from 12<sup>th</sup>, 16<sup>th</sup>, 20<sup>th</sup> and 28<sup>th</sup> days of composting which corresponded to 5.74, 5.69, 5.51 and 5.77 log<sub>10</sub> CFU/g (Fig. 1). However, the highest mycoflora population enumerated on OGYE was 3.8 log<sub>10</sub> CFU/g for 0, 12<sup>th</sup>, 28<sup>th</sup> days while the lowest recorded was 3.4 log<sub>10</sub> CFU/g for 24<sup>th</sup> day of composting. Composting days 0, 4, 12, 28 and 8 corresponding to 3.8, 3.7, 3.8, 3.8 and 4.1 log<sub>10</sub> CFU/g respectively differed significantly (p<0.05) from days 16<sup>th</sup> and 20<sup>th</sup> corresponding to 3.6 and 3.5 log<sub>10</sub> CFU/g respectively which differed significantly (p<0.05) from 24<sup>th</sup> day of 4.1 log<sub>10</sub> CFU/g (Fig. 1).

Generally, at the beginning of composting, there was a comparatively low mycofloral population which suggested a minimal occurrence of factors such as moisture content, relative humidity and a wide fluctuation in air temperature which did not favour the sporulation of fungi on the sawdust substrate [31,32]. Microbial activities however increased as the composting period progressed. There was an increase in temperature, moisture content, pH, relative humidity and electrical

conductivity of the activities according to [7] resulted in an increase in metabolic activities to produce enzymes by resident microorganisms to decompose the lignocellulosic materials. The enzymatic activities in compost piles are effective indicators for stress or adaptive practices of the microorganism to different environmental conditions, particularly to feed stock sources. Various hydrolytic enzymes can control the rate of decomposition of complex polymers during composting [33]. Obodai et al. [34] also suggested that the environmental and nutritional conditions created during composting might have selectively favored certain fungi to the detriment of others. This trend was in agreement with works of some researchers [34,35].

### 3.2 Effect of Gamma Irradiation and Steam Sterilization on Mycofloral Population of Sawdust Substrate

Both irradiation and steam techniques of pretreatment were effective in reducing the mycofloral population of sawdust. Although Comparatively high fungal counts were recorded on Cooke's medium than on OGYE medium (Fig. 2), gamma radiation dose of 5 kGy reduced the initial mycoflora of 5.77 log<sub>10</sub> CFU/g (control) by 0.68 log cycles to 4.89 log<sub>10</sub> CFU/g which did not differ (p>0.05) from the control. Doses 10 and 15 kGy recorded log cycle reductions of 1.97 and

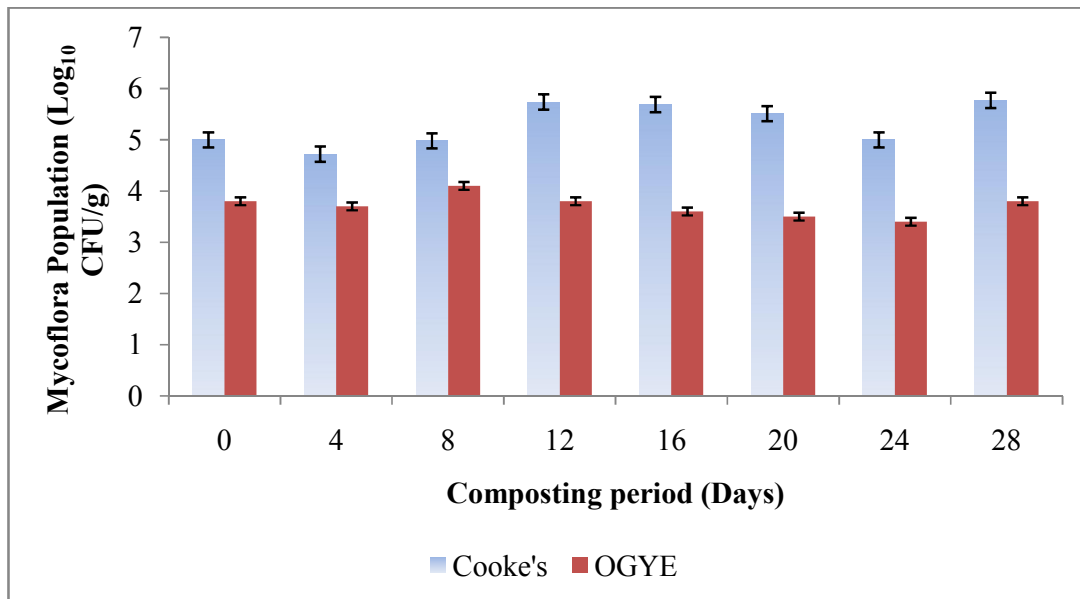


Fig. 1. Mycoflora population of sawdust during composting period enumerated on two media

2.50 respectively. Although their effectiveness did not differ ( $p>0.05$ ), 5 and 10 kGy were similar. Doses 20, 25 and 32 kGy corresponded to log cycle reductions of 3.96, 4.68 and 4.76 respectively which showed no significant difference ( $p>0.05$ ) in effectiveness but differed significantly ( $p<0.05$ ) from the control, 5, 10 and 15 kGy.

Mycoflora population enumerated on OGYE followed a similar trend. Log cycle reductions of 0.5, 0.7, 1.0, 1.8, 2.2 and 2.7 were recorded for doses 5, 10, 15, 20, 25 and 32 kGy respectively (Fig. 2). Gamma radiation causes destruction and ultimately death to microorganisms by two mechanisms of action; first by direct action of producing free radicals by the ionizing energy and secondly by causing direct disruption of the DNA strand sequence which result in injury to the cell [36].

Steam/moist heat treated composted sawdust at temperature of  $100\pm 2^\circ\text{C}$  was able to reduce mycoflora populations by 2.20 and 1.10 log cycles for Cooke's and OGYE respectively. The effectiveness of moist heat in reducing mycoflora populations were comparable ( $p>0.05$ ) to gamma radiation doses of 5, 10 and 15 kGy on both media. However, doses 20, 25, and 32 kGy were more effective in mycoflora reduction by an average reduction range of 2.88, 3.44 and 3.73 log cycles. Reduction by these doses differed significantly ( $p>0.05$ ) from moist heat effectiveness.

### 3.3 Occurrence Percentage of Microorganisms during Composting of Sawdust Substrate

Various mycoflora and microflora are involved in the composting of agricultural wastes and these range from fungi and bacteria that are thermophiles due to the temperature rise of  $50-60^\circ\text{C}$ , mesophiles and tolerant mesophiles.

Ten fungal species belonging to four genera namely: *Aspergillus*, *Mucor*, *Rhizopus* and *Fusarium* were encountered during the composting process. Generally, fungi increased in species diversity as composting proceeded in time (days). On Cooke's medium, 0 day of composting recorded *Aspergillus niger* (38%), *Aspergillus fumigatus* (12%), *Aspergillus ustus* (15%), *Aspergillus terreus* (5%) and *Rhizopus stolonifer* (30%) (Fig. 3). There were a comparatively higher number of microbial counts and fungal species of observed on Cooke's than on OGYE.

On OGYE medium, five species belonging to three genera were enumerated. Fungi recorded were *A. niger* (30%), *A. ustus* (40%), *R. stolonifer* (30%) and *M. racemosus* (30%), *A. niger* (22.7%), *R. stolonifer* (47.3%) which corresponded to composting periods of 0 and 4 days respectively (Fig. 5).

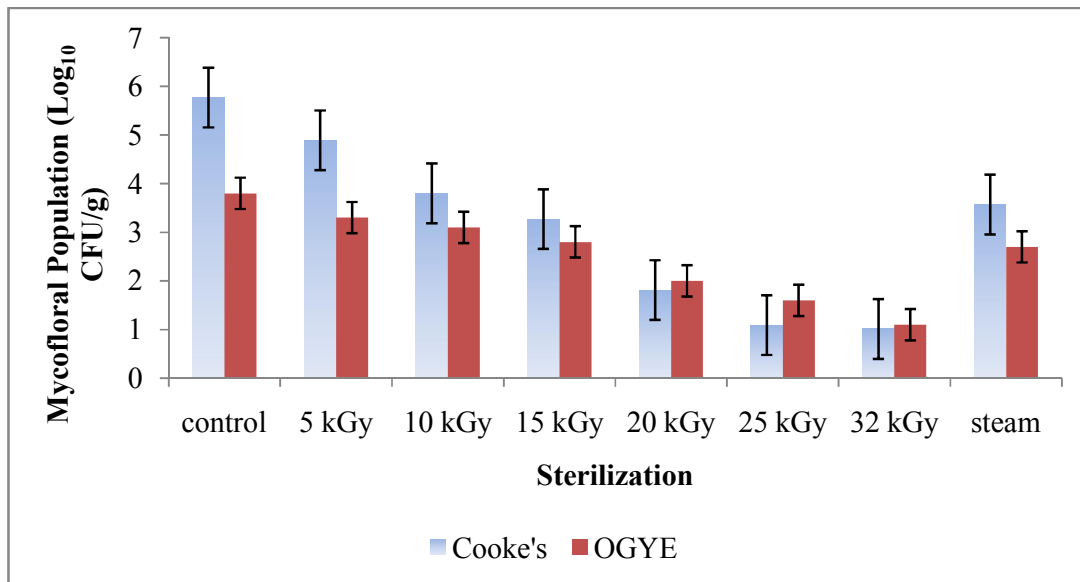


Fig. 2. Mycoflora population of sawdust after pretreatment cultured on two media

Results obtained imply a relatively high numbers of microorganisms were involved in the decomposition of the *T. scleroxylon* sawdust compost making it suitable for the growth of mushrooms as the diversity encourages different contributions biologically, chemically or physically [37,38].

The genus *Aspergillus* was predominant in all composting days of sawdust substrate. Several members of this genus have been reported by several researchers [39,40,41,42,43,44,45] to be capable of hydrolyzing the  $\beta$ -(1-4)-glucosidic linkage in the cellulose chain. According to [7], there is the production of metabolites in the compost during fungal growth which penetrates a cell and inhibits activity by chemical toxicity. Antagonism among fungi may be in the form of competition for nutrients, chemical antibiosis and lysis of mycelium. Antibiosis is the inhibition of one generation by the metabolic product of another. Although it is usually an inhibition of growth and sporulation, it may be toxic. Lysis is destruction and decomposition of biological materials by enzymes of the parasite. Fungal phenology observed in the compost may be partly attributed to antibiosis and lysis of mycelium.

Fungal species occurrence was irregular probably because the environmental and

nutritional conditions created during composting selectively favored certain fungi to the detriment of others.

Results obtained agreed with similar works reported by some researchers [34,46] as they investigated the various fungi associated with the composting of sawdust for mushroom cultivation.

### 3.4 Occurrence Percentage after Irradiation and Steam Sterilization

On Cooke's medium, the non-sterilized (control) sawdust substrate harbored *A. niger* (13.5%), *A. fumigatus* (26.5%), *A. flavus* (15%), *A. ustus* (10%), *A. parasiticus* (5%), *R. stolonifer* (25%), *M. racemosus* (5%). Conversely, radiation dose 5 kGy resulted in the retention of *A. niger* (18.5%), *A. fumigatus* (15.8%), *A. terreus* (10.7%), *A. ochraceus* (20%), *R. stolonifer* (10%) and *M. racemosus* (10.7%). While 10 kGy retained *A. niger* (20%), *A. fumigatus* (12%), *A. flavus* (15.7%), *A. ustus* (23.8%) and *A. parasiticus* (1.5%) (Fig. 4).

Dose 15 kGy, retained only species *A. niger* (24.5%), *A. fumigatus* (60%) and *R. stolonifer* (15.5%). Essentially, doses 20, 25 and 32 kGy retained no mycofloral species.

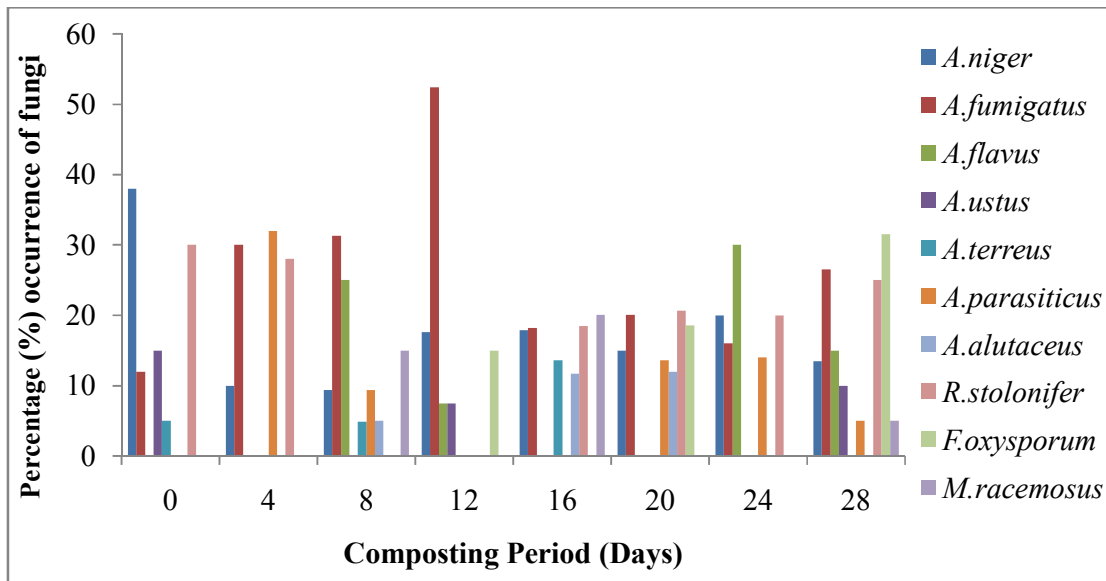


Fig. 3. Occurrence percentage of fungi of sawdust during composting period (Cooke's medium)

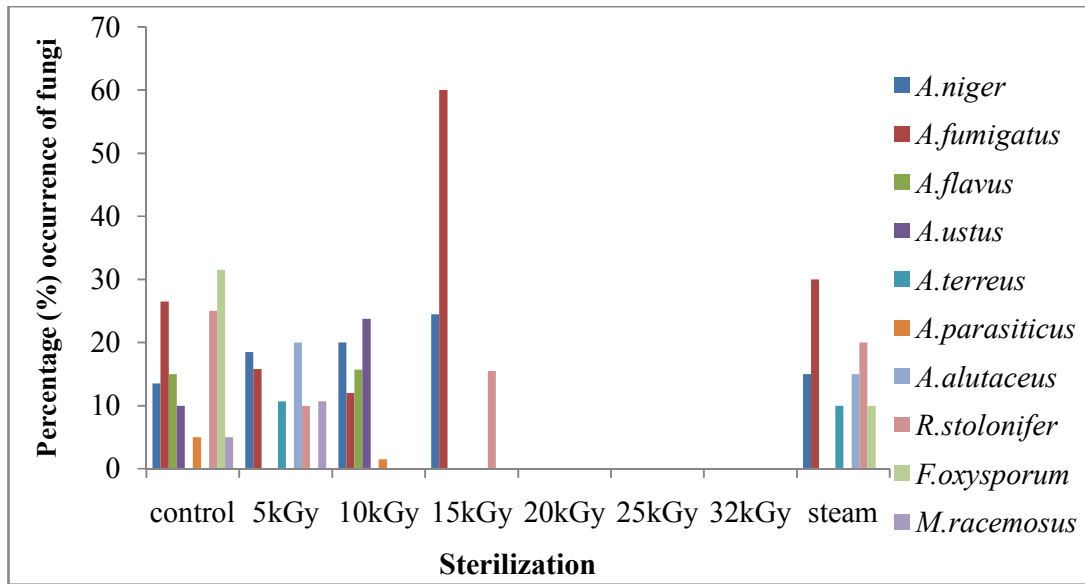


Fig. 4. Occurrence percentage of fungi after sterilization of sawdust (Cooke's medium)

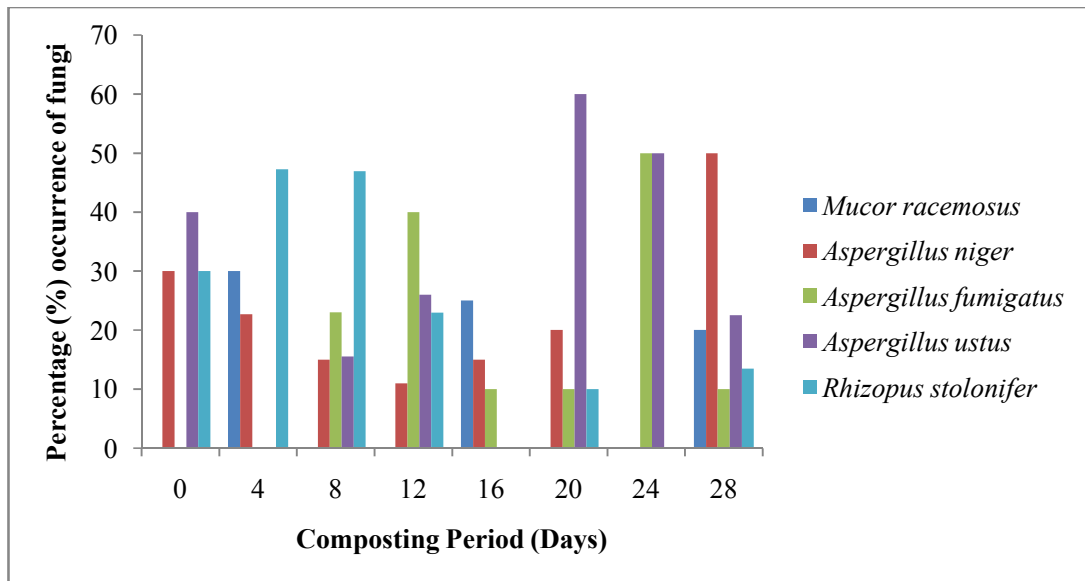


Fig. 5. Occurrence percentage of fungi during composting of sawdust (OGYE)

Moist heat/steam sterilized sawdust retained *A. niger* (15%), *A. fumigatus* (30%), *A. terreus* (10%), *A. ochraceus* (15%), *R. stolonifer* (20%) and *F. oxysporum* (10%) (Fig. 4).

On OGYE medium, enumerated fungal species were comparatively minimal. The non-sterilized (control) retained *M. racemosus* (20%), *A. niger* (34.5%), *A. fumigatus* (27%), *A. ustus* (5%) and *R. stolonifer* (13.5%). Radiation doses 5 retained *M. racemosus* (18.2%), *A. niger* (30%), *A.*

*fumigatus* (50%) and *A. ustus* (1.8%). However 10 kGy retained only *A. niger* (100%) (Fig. 6).

Doses 15, 20, 25 and 32 kGy retained no fungi. However, steam retained *A. niger* (20%) and *A. fumigatus* (80%). The critical radiation target is the chromosomal DNA, damage to which inactivates the microorganism. About 90% of the damage is caused by OH· radicals released from the hydration layer around the DNA molecule. Usually the purine and pyrimidine bases are

chemically changed and the phosphodiester backbone is broken (single-strand breaks) but some 5-10% double-strand breaks also occur [47]. Aquino [48] reported that radiation-resistant organisms are capable of withstanding effects on the plasma membrane of the cells. The rate of dose absorption has no effect on survival except when oxygen replenishment is involved. Elevated temperatures above 45°C are synergistically bactericidal because the higher temperatures damage the repair systems. To achieve this effect in spores, temperatures of 80 - 98°C are needed. Subfreezing temperatures raise the radiation resistance of vegetative cells as the water activity decreases and the diffusion of radicals is restricted.

### 3.5 Radiation Sensitivity ( $D_{10}$ Values) of Fungi on Sawdust

Radiation sensitivity (the killing effect of radiation) in microorganisms is generally expressed by the decimal reduction dose or  $D_{10}$  value [27] which was calculated from equation (1). The relative sensitivity of different microorganisms to ionizing radiation is based on their respective  $D_{10}$  values (which is the dose required to reduce the population by 90%). Lower  $D_{10}$  values indicate greater sensitivity of the organism in question. The data in Table 1. shows that gamma radiation doses achieved significant reduction in mycoflora during pasteurization of sawdust for mushroom production. On OGYE there was a good correlation coefficient of 0.971 with a corresponding mean  $D_{10}$  value of  $5.94 \pm 1.18$  kGy (Fig. 7). Also on Cooke's medium, a good

correlation coefficient of 0.924 was established with a corresponding mean  $D_{10}$  value of  $5.64 \pm 1.12$  kGy was obtained (Fig. 7). This implies that a slightly higher mean dose of 5.94 kGy was required to inactivate fungi of composted sawdust. Hossain et al. [49] obtained a less  $D_{10}$  value of 0.43-1 kGy for yeasts and moulds. However, results were within range of values reported by Addo [50] for  $D_{10}$  values of fungi on irradiated flour (Hausa koko). Results obtained fell within the range of lethal ionization doses of 1.3-11 kGy and 4-11 kGy for moulds and yeasts respectively as reported by [51].

### 3.6 pH

The pH of fermenting substrate, initially recorded 7.39 which was neutral (Table 2). However, pH fluctuated to basic / alkaline (7- 9) and then to acidic (6.81- 8.04) as composting time increased. The 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 [52]. According to Tchobanoglous et al. [53], initial pH values of 7-7.5 is recommended as it aids production of lactic acid and acetic acids during initial degradation of biomass. Interestingly, at the 20<sup>th</sup> day of composting the pH value 9.17 was recorded which is in agreement with some researchers [54,55] who stated that in the thermophilic stage of composting, the pH could rise to 9 resulting in the release of ammonia, and there after the pH returns to near neutral conditions as the compost become mature. After pretreatment, pH ranged 7.45-8.39 (Table 3).

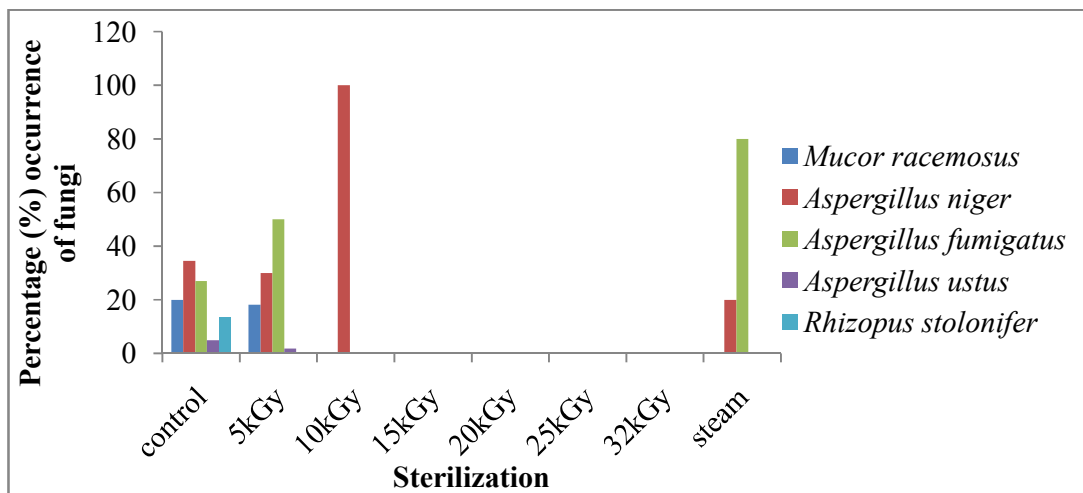


Fig. 6. Occurrence percentage of fungi after sterilization of sawdust (OGYE)



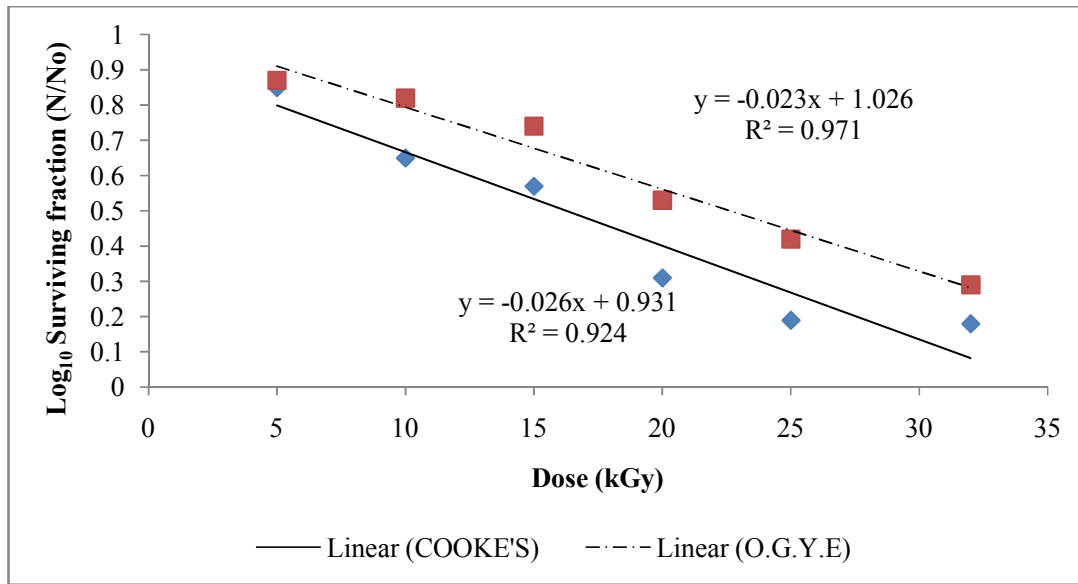


Fig. 7. Radiation sensitivity curves for fungi enumerated on OGYE and Cooke's media

Table 1. Mean D<sub>10</sub> values of fungi associated with sawdust isolated from the two (2) growth media

Substrate	Regression equation	r <sup>2</sup>	D <sub>10</sub> value (kGy)
(a) Cooke's	-0.026x + 0.931	0.924	5.64 ± 1.12
(b) OGYE	-0.023x + 1.026	0.971	5.94 ± 1.18

D<sub>10</sub> values are means of 6 replicates ± S.E

Table 2. Moisture content and pH readings of sawdust recorded during the composting period

Composting time (days)	Moisture content (%)	pH
0	65.1 <sup>b</sup>	7.39 <sup>ab</sup>
4	60.3 <sup>a</sup>	8.04 <sup>b</sup>
8	58.2 <sup>a</sup>	6.81 <sup>a</sup>
12	61.1 <sup>a</sup>	8.20 <sup>b</sup>
16	65.8 <sup>b</sup>	8.75 <sup>b</sup>
20	64.9 <sup>b</sup>	9.17 <sup>c</sup>
24	59.7 <sup>a</sup>	8.93 <sup>b</sup>
28	62.8 <sup>a</sup>	8.85 <sup>b</sup>

Means with same superscripts in a column are not significantly different (P>0.05)

### 3.7 Moisture Content

Moisture content during the composting period ranged between 58.2 - 65.8%. Moisture is important in composting processes for two reasons: it facilitates substrate decomposition through mobilizing microorganism activities and also provides better conditions for nitrogen fixation in the compost. A low moisture content

below a critical level (<30%), would decrease activities of microorganisms by restricting the motility and make them dormant [56]. Under drier conditions, the ammonium and ammonia present generate a higher vapor pressure; thus conditions are more favorable for nitrogen loss. On the other hand, a moisture content which is too high (>65%) could cause oxygen depletion and losses of nutrients through leaching [57].

Table 3. Effect of pretreatments on the pH and moisture contents of composted sawdust (*T. sceroxylon*)

Pretreatment	Moisture content (%)	pH
Steam	62.3 <sup>a</sup>	7.45 <sup>a</sup>
0 kGy	62.7 <sup>a</sup>	8.33 <sup>b</sup>
5 kGy	63.6 <sup>a</sup>	8.13 <sup>b</sup>
10 kGy	64.1 <sup>a</sup>	8.29 <sup>b</sup>
15 kGy	63.7 <sup>a</sup>	8.39 <sup>b</sup>
20 kGy	64.0 <sup>a</sup>	8.08 <sup>b</sup>
24 kGy	63.0 <sup>a</sup>	8.36 <sup>b</sup>
32 kGy	61.8 <sup>a</sup>	8.33 <sup>b</sup>

Means with same superscripts in a column are not significantly different (P>0.05)

#### 4. CONCLUSION

From the results obtained, it was observed that fungal genera *Aspergillus*, *Mucor*, *Fusarium* and *Rhizopus* were involved in the decomposition of sawdust. The present study supports the idea that knowledge regarding species composition of the microorganisms of different composts can help to optimize compost quality.

Irradiation pretreatment with doses 20, 25 and 32 kGy were more effective in pasteurizing than steam. Doses 10 and 15 kGy were comparable to steam. Steam was however more effective than 5 kGy.

Researchers stand to benefit more from this research by acquiring more knowledge on the consortium of fungi that would facilitate the composting process to achieve the desired quality. Knowledge on pretreatment would enhance precision of elimination to reduce competition.

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#### COMPETING INTEREST

Authors have declared that no competing interests exist.

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