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Comparative bioconversion of gamma irradiated and steam sterilized 'wawa' sawdust (*Triplochiton scleroxylon* L.) by mycelia of oyster mushrooms (*Pleurotus ostreatus* Jacq. Ex.Fr. Kummer)

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Abstract

Pleurotus ostreatus was cultivated on composted 'wawa' *(Triplochiton scleroxylon)* sawdust subjected to physical pretreatment techniques of moist heat sterilization and gamma irradiation to assess their comparative mycelial growth performance. Substrates were moist heat sterilized at either 95-100°C for 2.5 hours or gamma irradiated with a Cobalt 60 source at 0 kGy, 5 kGy, 10 kGy, 15 kGy, 20 kGy, 24 kGy and 32 kGy at a dose rate of 1.7 kGy/hour using the ethanol chlorobenzene (ECB) dosimetry. Sorghum grains for spawns were also irradiated at same doses (0 kGy, 5 kGy, 10 kGy, 15 kGy, 20 kGy, 24 kGy and 32 kGy) and steamed similarly and their interactive mycelia growth effects on pretreated sawdust measured. Final linear growth, colonizing time, rate of growth, primordial emergence time, density and percentage contamination were monitored for the four weeks at 28-32°C. The fastest time taken for full colonization was 22 days by irradiated (I+I) samples of the 15 kGy set up while the fastest rate of growth of 28.3 mm/day was recorded by irradiated sorghum and steamed compost bag (I +S) of the 15 kGy set up. There was no significant difference (P>0.05) in final linear growth range of 190.2- 248.8 mm recorded for all pretreated samples which also recorded very low (0-20%) contamination. Gamma irradiation was shown to be a good substitute and more efficient for sterilizing composted sawdust for mushroom cultivation and yield in the Ghanaian tropic conditions.

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Introduction

Mycelial biomass of a fungus consists of fine, thread-like hyphae, which grow extensively through the organic rich substrate of the ecosystem. This growth normally takes place under adequate environmental requirements such as temperature, pH, relative humidity and moisture in addition to nutrients to support the reproductive capacity in fungi (Petcharat, 1995; Sadaf *et al.*, 2005; Jonathan *et al.*, 2009). The mushroom mycelium has the ability to grow on a wide range of agricultural lignocellulosic waste, forest, litter, industrial and domestic waste materials (Canam *et al.*, 2013). The fungus accomplishes enzymatic degradation of the lignocellulosic portion of substrates by secreting saccharifying enzymes (cellulases, hemicellulases and xylanases) and oxidative (LiP, MnP and Laccases) (Hatakka, 2001; Periasamy and Natarajan, 2004; Weng and Chapple, 2010).

Mycelial cultivation has received great interest as an efficient method for industrial production of valuable metabolite. In the field of bioremediation, they could also be used to detoxify and ameliorate contaminated soils (Hirano *et al.*, 2000; Kubatova *et al.*, 2001). Various agro-industrial by-products have been tried as inexpensive growth substrates (Kortei, 2011; Oyetayo and Ariyo, 2013). The production of healthy mushrooms is largely dependent on the mycelia vigor, strain and its ability to outgrow its fungal or bacterial competitors as mycelium quality is directly linked to period of fruitification, number of fruiting bodies, the interval between flushes and basidiocarp size (OECD, 2005).

Besides its bioconversion of agro-lignocellulose to useful food, mushroom cultivation may assist in extenuating environmental pollution problems caused by the accumulation of recycled waste. The lucrativeness of mushroom cultivation is further enhanced by their low cost of production, since most

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of them can be cultivated on agro wastes or other industrial waste products. Furthermore, it could be enterpreneural for its potential to be fully explored in this Golden age of unemployment in Ghana.

Existing conventional technology available is the use of moist heat or steam for this process. But there are a number of disadvantages; it is usually very laborious, ineffective against all microbes, and lastly the throughput of the sterilization process is slow and few bags can be sterilized per unit time (Kortei, 2015). Mushroom cultivation is a buisiness venture in Ghana and so gamma irradiation technique would very beneficial.

Although various methods of pretreatment have been reported (Jeoh and Agblevor, 2001; Bigelow and Wyman, 2002) few reports exist on the use of gamma irradiation (Lam *et al.*, 2000; Martin and Thomsen, 2007). Gamma radiation, if used in high dosage on lignocellulosics, causes a decrease in cell wall constituents or depolymerizes and delignifies the fiber (Al-Masri and Zarkawi, 1994). An increase in organic matter digestibility has been reported due to cell wall degradation (Al-masri and Guenther, 1995). Gamma radiation has short wave length, high energy photons, and have deep penetrating power and so could serve both as a decontaminating agent and a hydrolytic agent (Gbedemah *et al.*, 1998; Mami *et al.*, 2013) for the bioconversion of lignocellulosic materials to expensive proteins per unit area (Kortei, 2011). The process may also be more environmentally friendly than the fuel wood for heating. Gamma rays from spontaneous disintegration of radioactive nuclides (Cobalt 60 or Cesium 137) as their energy source. During irradiation, the radioactive nuclides are pulled out of storage (water pool) into a chamber with concrete walls that keep any gamma rays from escaping (Park and Vestal, 2002).

Recent studies on the use of gamma radiation to sterilize lignocelluloses for mushroom cultivation proved successful (Gbedemah *et al.,* 1998; Kortei and Wiafe- Kwagyan, 2014) hence the need to further test the suitability of other dose ranges for mushroom cultivation. The objective of this paper was to evaluate the comparative efficiency of sterilizing composted sawdust with irradiation and moist heat techniques on the mycelia ramification of Pleurotus ostreatus and colonization of substrate for fruiting.

Materials and Methods

Determination of pH of substrate

This was done according to AOAC method (1990). Ten grams (10 g) of composted sawdust sample was weighed into a conical flask containing 10 ml distilled water and allowed to stand for 2 hours. A standard pH meter (3510 Jenway, U.K) was used to measure the pH of the suspension.

Daily compost temperature readings

Temperature readings within the compost were taken using a digital thermometer (Jenway, 332, U.K). Probe was inserted into the middle region of the compost pile and average of three (3) readings recorded in the mornings at 8.30 am.

Preparation of pure culture

Preparation was done according to a modified method of Narh *et al*., (2011). One week old pure cultures of *Pleurotus ostreatus* (Jacq. Ex. Fr) Kummer, strain EM-1, were obtained from the National Mycelium Bank at the CSIR- Food Research Institute in Ghana. Each of the bottled sterilized grains was aseptically inoculated with one (1) cm^2 of the one-week-old tissue culture of the experimental strain grown on Malt Extract Agar (OXOIDTM Ltd., Basingstoke Hampshire, England) using a flamed and cooled scalpel in a laminar flow hood. Thereafter, the spawns were incubated for 16-21days without illumination in an incubator (TuttlingtenTM WTC Binder, Germany) set at 28°C.

Preparation of spawn for steam sterilization and gamma irradiation

The spawns for steam sterilization were prepared using a modified method of spawn preparation outlined by Stamets and Chilton, (1983). Sorghum grains were obtained from the Madina Market in Accra, Ghana. One hundred and fifty grams (150 g) of sorghum grains were washed and steeped overnight in water. They were then thoroughly washed separately with tap water to ensure that dust and other particles had been removed, drained, tied in a wire mesh and steamed for 45 mins in an autoclave (Priorclave, Model PS/LAC/EH150, England) at 105°C to ensure that the steamed grains were cooked but intact since broken grains are more prone to contamination. Thereafter, the grains were air-dried to cool on a wooden frame with a wire mesh. To each grain lot, 3% (w/w) of calcium carbonate ($CaCO_3$) was added and thoroughly mixed manually. One hundred and fifty grams (150 g) aliquots of the grains were then weighed into transparent 25 x 18 cm heat resistant polypropylene bags plugged with cotton wool and covered with plain sheets of paper. The sheets were held in place with rubber bands. The bottled grains were sterilised in an autoclave (Priorclave, Model PS/LAC/EH150, England) at 121°C for 1hr.

Irradiation

Sorghum grains were soaked overnight and gamma irradiated at doses 0, 5, 10, 15, 20, 24 and 32 kGy at a dose rate of 1.7 kGy per hour in air. The absorbed dose was confirmed by ethanol-chlorobenzene (E.C.B) dosimetry. The same procedure was followed for bottling the grain as described above.

Thereafter, a piece of pure culture of *P.ostreatus* measured 1x1cm^2 was placed aseptically into the mouth of each mother culture packet and the packets were placed in the growth chamber at 25±1°C in the dark . After 7 to 9 days the mother culture became white due to complete the mycelium ramification and then it was ready for inoculating spawn packets.

Estimation of lignin, cellulose, hemicellulose and silica

Estimation of lignin cellulose, hemicellulose and silica was done by standard methods of AOAC, (1995).

Preparation of substrate

The substrate consisted of 'wawa' sawdust *(T. scleroxylon)* of 88%, 2% of $CaCO_3$ and 10% wheat bran. Moisture content was adjusted to 65% (Buswell, 1984). The mixture was mixed thoroughly, heaped to a height of about 1.5 m and 1.5 m base and covered with polythene and then made to undergo fermentation for 28 days at 30- 60°C. Turning was carried out every 4 days to ensure homogeneity.

Bagging

Composted sawdust was compressed into 0.18 m x 0.32 m heat resistant polyethylene bags. Each bag contained approximately 1kg of composted sawdust.

Sterilization/pasteurization

Bagged composted sawdust substrates were sterilized with moist heat at a temperature of 98-100°C for 2.5 hours. Bagged composted sawdust substrates were treated with gamma irradiation doses of 0 kGy, 5 kGy, 10 kGy, 15 kGy, 20 kGy, 24 kGy and 32 kGy 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 six times.

Inoculation and incubation

The bags were inoculated with about 5 g of spawn grains and so resulted in treatment permutations as follows: Autoclaved spawn and Steamed sawdust (A + S), Irradiated spawn and Steamed sawdust (I + S), Autoclaved spawn and Irradiated sawdust (A+I), Irradiated spawn and Irradiated sawdust (I+I), Autoclaved spawn and non-sterilized sawdust (A + nS/ nI), Irradiated spawn and non- sterilized sawdust, Irradiated spawn and non- sterilized sawdust (I + nS/ nI) . N.B. nS= nI

Compost bags were incubated at ambient temperature (28- 32°C) for the spawns to thicken for a period of 4 weeks.

Growth, rate of growth of mycelia and mycelia colonizing time

Mycelia growths were measured by weekly markings of longest and shortest growths on the compost bags and the average length was recorded. Average length = longest + shortest lengths/2. The rate of mycelia growth was then calculated as Average length/ time (Obodai *et al.*, 2003) Mycelia colonizing time was calculated as the time taken to undergo complete colonization of the substrate.

Mycelia density

The mycelia density was graded by colonizing vigor or intensity as described by Obodai *et al.*(2003).

Time for primordial appearance and number of contaminated bags

Time taken for the appearance of first batch of primordia was recorded. The number of contaminated bags were also recorded and expressed as:

$$\text{Contamination (\%)} = \frac{\text{Contaminated bags}}{\text{Total number of bags}} \times 100$$

Results and Discussion

Effect of moisture content and pH of sawdust during composting on the growth of P. ostreatus

The pH of sawdust during the composting period ranged from pH 6.81- 9.17 (Table 1) while that of pretreated substrates was pH 7.45- 8.83 (Table 2) which was generally neutral to alkaline. Also the moisture content of the composted sawdust samples ranged from 58.2- 65.8% (Table 1). The pretreated sawdust had moisture content values of 61.8- 64.1% (Table 2). These values were within the range of parameters required for growth and development of mycelia of Pleurotus ostreatus (OECD, 2005).

The temperature profile of sawdust during composting may cause a rise in temperature and accelerate the breakdown of proteins, fats, and complex carboydrates like cellulose and hemicelluloses thereby enhancing the growth of the mycelia. Initial temperatures increased from 32°C to 57°C prior to turning (Figure 1), but dropped to 44°C

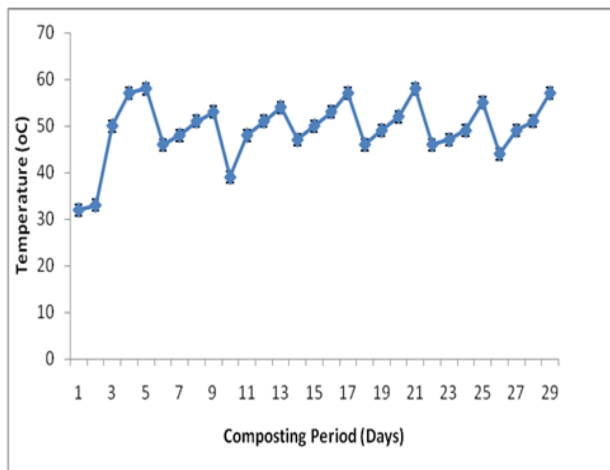

Figure 1. Temperature profile of composting sawdust with 4 days turning interval

after turning. This trend of temperature increases were observed throughout the period of composting. The succession of mesophilic and thermophilic microorganisms in composted material is connected with temperature changes and indicates the correct course of the composting process (Finstein *et al.*, 1986; Ishii *et al.*, 2000). High temperature (55–75°C) reached in the thermophilic phase of composting is a factor which decreases or completely reduces the number of pathogens (McGregor *et al.*, 1981). According to Strom (1985), the diversity and activity of microorganisms in composted material are affected by temperature, the degree of oxygenation and the availability of nutritive components. Thus microorganisms play the key role in the composting of organic matter and in the creation of humus (full-value fertilizer). However, it is important that the final product obtained is not only useful but also safe from the hygienic perspective (Wolna-Maruwka *et al.*, 2009).

Effect of pretreatment on cellulose, hemicellulose, lignin and silica

The biochemical parameters monitored over the period of decomposition and after pretreatment were lignin, cellulose, hemicelluloses and silica. The raw composted sawdust had the lowest values of 8.22, 40.16, 19.83 and 11.74% for hemicelluloses, cellulose, lignin and silica respectively (Table 3). Composted sawdust substrate treated with gamma radiation of 24 kGy had the highest indicated parameter values of 15.38, 42.33, 21.74 and 16.11% for hemicelluloses, cellulose, lignin and silica respectively (Table 3). The change in biochemical properties of composted sawdust could be attributed to the weakening of the intermolecular bonds of the polysaccharides by irradiation to make available the oligo, di and mono saccharides hence their increased in content. Ionizing radiation possesses the unique ability to initiate depolymerization and/or crosslinking reactions without the need to add toxic chemicals (Betiku *et al.*, 2009).

Table 1. Moisture content and pH value of sawdust recorded during a 28 days composting period at varying temperatures

Composting Time (Days)	Moisture Content (%)	pH
0	65.1^{b}	7.39^{ab}
4	60.3^{a}	8.54^{b}
8	58.2^{a}	6.81^{a}
12	61.1^{a}	8.60^{b}
16	65.8^{b}	8.75^{b}
20	64.9^{b}	9.17^{c}
24	59.7^{a}	8.93^{b}
28	62.8^{a}	8.85^{b}

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

Table 2. Effect of pretreatments (steam sterilization and irradiation) on the pH and moisture contents of composted sawdust *(T.sceroxylon)*

Pretreatment	Moisture Content (%)	pH
Steam	62.3^{a}	7.45^{a}
0 kGy	62.7^{a}	8.83^{b}
5 kGy	63.6^{a}	8.13^{b}
10 kGy	64.1^{a}	8.29^{b}
15 kGy	63.7^{a}	8.39^{b}
20 kGy	64.0^{a}	8.08^{b}
24 kGy	63.0^{a}	8.36^{b}
32 kGy	61.8^{a}	8.33^{b}

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

Mycelia growth

The spawn run period is calculated as the number of days from inoculation to complete colonization of the compost bag by the mycelium (Obodai and Vowortor, 2002). The various substrate formulations and their interactions had different nutrient compositions and so resulted in different growth responses as reported by other workers (Raper, 1978; Vetayasuporn, 2006; Kortei, 2008). On the average, three days after inoculation, observable whitish mycelia began to grow on the bagged substrates from the grains of inocula. The mycelia grew down the

Table 3. Effect of pretreatments of sawdust on the lignocelluloses content on Dry Matter Basis

Sample	% Moisture	% D.M	% H.C(DMB)	%Cellulose (DMB)	%Lignin (DMB)	%Silica (DMB)
Steam	5.44	94.56	8.71	41.59	20.92	10.08
5 kGy	5.83	94.17	11.56	41.67	18.65	14.34
24 kGy	5.41	94.83	15.38	42.33	21.74	16.11
Raw	5.52	94.13	8.22	40.16	19.83	11.74

DMB- Results are expressed on Dry Matter Basis
H.C- Hemicellulose
D.M- Dry Matter

substrates with the passage of time until colonization was fully or partially complete within an average of four weeks.

The linear growth of *P. ostreatus* mycelia down the substrates, was similar for all the growing time (weeks) thus showed no significant ($P<0.05$) difference for permutations of 5kGy, 10kGy, 15 kGy, 20 kGy, 24 kGy and 32 kGy with final linear growth range of 190.2- 248.8 mm (Table 4). However, a permutation of 0 kGy and non-steam sterilized substrates performed extremely poorly recording a final linear growth range of 12-15 mm (Table 4). This was statistically different ($P>0.05$) from all the treatment doses. This poor performance could be attributed to the presence of other competitive microorganisms such as *Aspergillus* spp., *Mucor racemosus, Rhizopus stolonifer, Fusarium oxysporum* etc (Kortei *et al*., 2015) which might have suppressed the development of the mycelia.

Colonizing time and rate of growth

The time taken to fully colonize the substrates and their rate of growth differed significantly ($P<0.05$). The fastest time taken was 22 days by (I+I) of the 15 kGy set up while the slowest time of 35 days was recorded and (A+S) of the 32 kGy set up (Table 4). The fastest rate of growth of 28.3 mm/day was recorded by (I +S) of the 15 kGy set up while the slowest rate of growth of 0.7 mm/day was recorded by (A+ nI) of the 0 kGy set up (Table 4). This observation could be attributed to the extent of depolymerisation of the polysaccharide substrates used and the hydrolysis of water molecules by the different gamma radiation doses as well as the steam. The time of colonizing and rate of growth of mycelia are directly related to nutrient availability in the substrate or inability to effectively utilize ligninocellulosic materials available. This is conjectured to have affected the growth and development of *P. ostreatus* mycelia as reported by other workers (Thomas *et al*., 1998; Wong *et al*., 2006; Kortei, 2008); also variation in chemical composition and C: N ratio as reported by Wong *et al*. (2006), may influence growth rate and colonization of substrate by mycelium of mushroom.

Primordia emergence

Time of primordial emergence is also related to the vigor of the mycelia. Thus weak mycelia resulted in delayed time of primordial emergence. Time of emergence differed significantly ($P<0.05$). However, it was positively correlated to the time taken to completely colonize the substrate. The time difference of complete colonization and primordial emergence was calculated to give the real time. The average time difference for primordial emergence was 0- 4 days after complete colonization of substrates. The fastest emergence time, was a difference of 0 day for substrates (A+I), (I+I) of 10 kGy set up, (A+I) of 15 kGy set up and (A+S) of 20 kGy set up. The time difference for emergence of primordia was within the same range as found by some workers (Obodai *et al*., 2003; Shah *et al*., 2004) who worked on the comparative study on the growth and yield of mushroom on different lignocellulosic by-products.

Mycelia density, percentage (%) contamination

Mycelia vigor is directly linked to optimal nutrients, pH, temperature and other physic-chemical properties of the substrate. They were in the range of very dense mycelia to no mycelia. These values were consistent with the percentage (%) contamination. Generally, all substrate combinations of the various dose experimental set up (5 kGy, 10 kGy, 15 kGy, 20 kGy, 24 kGy and 32 kGy) resulted in visually good mycelia growth which ultimately produced mushrooms. Conversely, the substrate combinations of the 0 kGy set up resulted in the poorest mycelia

Table 4. Effect of pretreatment of sawdust *(T. scleroxylon)* on mycelia growth of *P.ostreatus*

Dose (kGy) on Compost bag	Treatment Combination	Rate of mycelia Growth (mm/day)	Mycelia Colonizing time (Days)	Time taken till appearance of primordia (Days)	No. of Contaminated bags (%)	Total Linear Mycelia Growth (mm)	Mycelia Density
0	(A + n S)	1.5 ^{a}	n.d	n.d	100	12.0 ^{ab}	_
	(I + n S)	1.7 ^{a}	n.d	n.d	83.3	13.0 ^{a}	_
	(A + n I)	0.7 ^{a}	n.d	n.d	100	12.0 ^{ab}	_
	(I + n i)	1.4 ^{a}	n.d	n.d	90	15.0 ^{b}	_
5	(A + S)	19.5 ^{b}	33 ^{d}	34 ^{d}	0	230.5 ^{c}	+ + + +
	(I + S)	21.1 ^{b}	30 ^{c}	33 ^{d}	8.3	214.6 ^{c}	+ + +
	(A + I)	20.5 ^{b}	31 ^{cd}	33 ^{d}	0	212.8 ^{c}	+ + + +
	(I + I)	18.5 ^{b}	33 ^{d}	35 ^{d}	0	239.9 ^{c}	+ + + +
10	(A + S)	17.5 ^{b}	32 ^{d}	33 ^{d}	20	190.2 ^{c}	+ +
	(I + S)	19.3 ^{b}	31 ^{cd}	32 ^{d}	0	203.8 ^{c}	+ + + +
	(A + I)	21.6 ^{c}	29 ^{c}	29 ^{bc}	0	220.1 ^{c}	+ + + +
	(I + I)	24.3 ^{d}	28 ^{c}	28 ^{b}	16.7	241.6 ^{c}	+ + +
15	(A + S)	24.0 ^{d}	31 ^{cd}	32 ^{d}	16.7	231.0 ^{c}	+ + +
	(I + S)	28.3 ^{c}	24 ^{a}	25 ^{a}	0	248.1 ^{c}	+ + + +
	(A+ I)	23.0 ^{c}	26 ^{b}	26 ^{b}	0	246.7 ^{c}	+ + + +
	(I + I)	23.1 ^{c}	22 ^{a}	24 ^{a}	0	247.8 ^{c}	+ + + +
20	(A + S)	23.7 ^{cd}	30 ^{c}	30 ^{c}	0	233.0 ^{c}	+ + + +
	(I + S)	24.1 ^{d}	31 ^{cd}	29 ^{bc}	16.5	248.6 ^{c}	+ + +
	(A + I)	23.4 ^{c}	26 ^{b}	28 ^{b}	0	248.8 ^{c}	+ + + +
	(I + I)	22.9 ^{c}	28 ^{b}	29 ^{bc}	0	246.6 ^{c}	+ + + +
24	(A+ S)	20.4 ^{b}	35 ^{c}	37 ^{c}	0	213.5 ^{c}	+ + + +
	(I+ S)	20.0 ^{b}	34 ^{c}	36 ^{c}	8.3	202.8 ^{c}	+ + +
	(A+ I)	22.6 ^{c}	32 ^{d}	36 ^{c}	0	236.7 ^{c}	+ + + +
	(I + I)	21.9 ^{b}	33 ^{d}	34 ^{d}	0	231.0 ^{c}	+ + + +
32	(A +S)	20.3 ^{b}	27 ^{b}	29 ^{b}	0	211.6 ^{c}	+ + + +
	(I + S)	21.5 ^{bc}	27 ^{b}	28 ^{b}	16.67	230.1 ^{c}	+ + +
	(A + I)	19.6 ^{a}	29 ^{c}	31 ^{c}	0	243.9 ^{c}	+ + + +
	(I + I)	19.1 ^{a}	31 ^{cd}	33 ^{d}	0	240.0 ^{c}	+ + + +

Means with same letters in a column are not significantly different ($P<0.05$)
+ + + + - Very thick mycelia
+ + + - Thick mycelia
+ + - Poor mycelia growth
+ - Very poor mycelia growth
- - No mycelia
n.d - Not determined

growth and the highest number contaminations (Table 4). This presumably could be attributed to nutrient depletion and a buildup of temperature and other metabolites (Recycled Organics, 2003; Kortei, 2008) from the other microorganisms such as *Aspergillus, Cladosporium, Mucor, Neurospora, Paeciliomyces, Penicillium, Rhizopus* and *Trichoderma* (Obodai *et al*., 2010; Kortei *et al*., 2015).

Conclusion

The effect of gamma irradiation on the mycelia growth was apparently comparable to the steam method (conventional). Radiation dose of 15 kGy yielded better results in terms of all the parameters investigated (Total linear growth of mycelia, rate of growth of mycelia, colonizing time and percentage contamination) showed that irradiation is a good and efficient substitute for sterilizing composted sawdust bags for mushroom cultivation. In developing countries where gamma irradiation facilities are available, it will be technologically cheaper, practicable and more efficient to use gamma irradiation to sterilize and depolymerize lignocellulosic substrate for mushroom cultivation to on the whole, reduce the humdrum task involved in mushroom production.

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