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RESEARCH ARTICLE

COMPARATIVE EFFECT OF GAMMA IRRADIATED AND STEAM STERILIZED COMPOSTED 'WAWA' (*TRIPLOCHITON SCLEROXYLON*) SAWDUST ON THE GROWTH AND YIELD OF *PLEUROTUS OSTREATUS* (JACQ. EX. FR) KUMMER

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Abstract

Pretreatment of lignocellulosic materials is essential for bioconversion because of the various physical and chemical barriers that greatly inhibit their susceptibility to bioprocesses such as hydrolysis and fermentation. Composted wawa (*T. scleroxylon*) sawdust were subjected to physical pretreatment techniques of moist heat sterilization and gamma irradiation to evaluate their comparative effects on the growth and yield of *Pleurotus ostreatus*. Substrates were moist heat sterilized at 95-100^{o}C for 2.5 hours and irradiated with a CO_{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 and autoclaved similarly as stated above and their interactive effects measured. Economical yield, biological efficiency, total fresh weight and flush weights recorded from the various interactions, showed significant differences ($P<0.05$). Number of primordial, effective fruit bodies, stipe length and cap diameter represented growth attributes were also significantly different ($P<0.05$). Autoclaved spawn + steam sterilized sawdust (S + S) of 32 kGy set up recorded the greatest yield of 1779g of total fresh weight or economic yield and corresponding and biological efficiency of 68.4%. Steam spawn + non steam sterilized (S + nS) and irradiated spawn + non steam sterilized (I + nS) both recorded the lowest total fresh weight and biological efficiency of 0g, 0% respectively from the 0 kGy set up.

Key words: Gamma , irradiation, *Pleurotus ostreatus*, sawdust, steam, sterilization

Introduction

The genera *Pleurotus* (oyster mushroom) comprise of edible ligninolytic mushrooms capable of selective delignification of lignocellulosic farm residues (Cohen *et al.*, 2002, Obodai *et al.*, 2003, Kivaisi *et al.*, 2003, Poppe 2000, Poppe 2004), as a result of which the cellulose is exposed and can be utilized (Alborés *et al.*, 2006). There are various numbers of parameters affecting the growth and performance of oyster mushroom including substrate source, substrate quality, spawn, strain, compost, and complement (Royse *et al.*, 2004; Jafarpour *et al.*, 2010). Principal among these

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parameters is effective pasteurization of the substrates after composting to enhance complete mycelia colonization of the substrate.

According to Holiday *et al.* (2009), mushroom cultivation in more developed countries has evolved from an art into huge agri-business by way of the most innovative production technology and biotechnology available. It has therefore become imperative to employ the use of radiation technology to augment the existing technologies. Irradiation is a physical pretreatment for the effective disruption of lignocelluloses polymers thus making them more susceptible to microbial attack (Kumakura and Kaetsu, 1978; Al-Masri and Zarkawi, 1994; Al-Masri and Guenther, 1995; Betiku *et al*., 2005).

Gamma radiations have short wave length, high energy photons, and have deep penetrating power 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). Gamma rays come 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, 2002).

Because of the easy access to gamma radiation and the fact that mushroom production has become a serious agri-buisiness in Ghana, this experiment was carried out to compare the effect of these physical pretreatments; moist heat conventional method and gamma irradiation of sawdust (*T.scleroxylon*) on the growth and yield of *Pleurotus ostreatus.*

Materials and methods

Preparation of pure culture

Pure culture was obtained according to Mondal *et al.,* (2010) with modifications. Potato dextrose agar (PDA) culture or tissue culture planting method was used. The PDA media was prepared by using of 300 g peeled and sliced potato, 20 g dextrose, 30 g agar and 250 mg aspergine in one liter of water. About 5 ml of PDA mixture was poured in each test tube followed by plugging. The media was sterilized in an autoclave for 15 minutes at 121°C with 1.5 kg /cm^2 pressure. The sterilized PDA containing test tube was kept in slanting positions. The mushroom was thoroughly pre-washed in distilled water. A scalpel was then dipped in alcohol and flamed until it was red hot. Then it was cooled for 10 seconds. The cut was made length wise from the cap to down wards. Small piece of the internal tissue of the broken mushroom was cut and removed with a flamed needle. The needle with the tissue attached was then immediately inserted in to test tube slant and the tissue laid on the agar surface. The mouth of the test tube was flamed before the needle was inserted. The mouth of the test-tube was plugged with cotton plug. After 3 to 4 days, the tissue was covered with a white mycelium that was spread on the agar surface**.**

Preparation of spawn

Autoclaved. The stock culture substrate was prepared by using good quality sorghum grains and $CaCO_3$ and packed tightly in 25 x 18 cm polypropylene bag. These packets were sterilized in an autoclave for one hour at 121^{o}C and 1.5 kg/cm^2 atmospheric pressure and then these were kept 24 hours for cooling.

Irradiation. Sorghum grains were soaked overnight and irradiated at doses 0, 5, 10, 15, 20, 24 and 32 kGy at a dose rate of 1.6 kGy per hour in air. The absorbed dose was confirmed by ethanol-chlorobenzene (E.C.B) dosimetry. The same procedure was observed as described above. Then a piece of pure culture measured 1x1cm^2 was placed aseptically into the mouth of the each mother culture packet and the packets were placed in the growth chamber at 25±1°C in dark place. After 7 to 9 days the mother culture became white due to complete the mycelium running and then it was ready for inoculating spawn packets.

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

Substrate Preparation. The substrate consisted of wawa (*T. scleroxylon*) of 80-90%, 1-2% of $CaCO_3$ and 5-10% wheat bran. Moisture content was adjusted to 65-70% (Buswell, 1984). The mixture was mixed thoroughly, heaped to a height of about 1.5m and 1.5m base and covered with polythene and made to undergo fermentation for 28days. Turning was made every 4 days to ensure homogeneity.

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

Sterilization/Pasteurization. Bagged composted sawdust substrates were sterilized with moist heat at a temperature of 98-100 ^{o}C for 2.5 hours. Bagged composted sawdust substrates were subjected to 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 6 times.

Inoculation and incubation

The bags were inoculated with about 5g of spawn grains and so resulted in treatment permutations as follows.

Table 1. *Substrate compositions and their corresponding codes*

Substrate Code	Substrate Composition
S + S	Steamed spawn and Steamed sawdust
I + S	Irradiated spawn and Steamed sawdust
S + I	Steamed spawn and Irradiated sawdust
I + I	Irradiated spawn and Irradiated sawdust
S + n S	Steamed spawn and non-sterilized sawdust
I + n S	Irradiated spawn and non- sterilized sawdust

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

Cropping and harvesting

Fruit primordia were allowed to develop to complete fruiting bodies and were picked. Mushrooms were harvested by grasping the base of the stalk and pulling them by hand from the substrate, then were taken away and weighed the same day. The oyster mushrooms were harvested when the in–rolled margins of the basidiophores began to flatten (Tisdale *et al*., 2001). Humidity was kept as high as possible 80-85% by watering twice a day. Stipe length (length of cap base to end of stalk) and Average cap diameter = longest + shortest cap diameters/2 have been also calculated. Dates of each harvest were also recorded. Total number of flushes (flush number) produced per each bag was noted at the end of four weeks period. The distribution of the yield per flush was tabulated to observe changes in yield over the course of multiple flushes. Seven aspects of crop yield were evaluated according to some authors (Amin *et al*., 2008; Tisdale *et al*., 2001; Morais *et al*., 2000) as follows: (i) Mushroom size (MS). (ii) Biological efficiency (BE) = Weight of fresh mushrooms harvested (g) /dry substrate weight (g)] x100 (iii) flush number (iv) crop period (sum of incubation and fruiting periods) (v) Fresh weight. (vi) BY= [Weight of fresh mushrooms harvested (g) per dry substrate weight] and was expressed as kg fresh mushrooms/kg dry substrate weight. Also, economical or mushroom yield values were calculated as previous reported by Morais *et al*, (2000) as weight of fresh mushrooms harvested (g)/fresh substrate weight. The average MS was calculated as total fresh weight of mushrooms harvested divided by their total number of mushrooms. BY= [Weight of fresh mushrooms harvested (g) per dry substrate weight] and was expressed as g fresh mushrooms/kg dry substrate weight according to Amin *et al*. (2008). Average weight of individual mushrooms was determined as quotient of the total fresh weight mushrooms harvested by their total numbers according to Phillipoussis *et al*. (2001). Economical Yield (g/kg wet sawdust) = Total fresh weight of mushrooms. N.b- Dry weight of sawdust- 650g wet weight of sawdust- 1000 g/ 1 kg

Statistical Analysis

All experiments were performed in sextuplets (6). The data on mushroom size, mushroom yield,

and biological efficiency of *Pleurotus ostreatus*, cultivated on the pretreated sawdust of moist heat and gamma radiation were subjected to analyses of variance (one-way ANOVA) when significant differences were determined post test were made using Duncans multiple range test (DMRT).

Results and Discussions

Effect of pretreatment on lignocellulose content of sawdust

Pretreatment involves the alteration of biomass so that (enzymatic) hydrolysis of cellulose and hemi-cellulose can be achieved more rapidly and with greater yields. Possible goals include the removal of lignin and disruption of the crystalline structure of cellulose (Hubbe *et al.*, 2010; Mosier & Wyman, 2005; Lynd *et al*, 1991; Ghosh & Singh, 1993). Raw sawdust had parameter values lower than pretreated sawdust. For hemicelluloses, cellulose and lignin, raw sawdust recorded 8.11%, 37.62% and 16.85%. It has been reported that biodegradation of untreated natural lignocellulosic biomass is very slow, giving rise to the low extent of degradation, often under 20% (Fan *et al.*, 1980). This low rate and extent of conversion hinder the development of an economically feasible hydrolytic process (Betiku *et al.*, 2009).

Conversely, the low dosage 5 kGy recorded 10.89%, 39.24% and 17.56% .High dosage 24 kGy, 11.26%, 40.15% and 19.81%. Gamma irradiation affects these bonds and causes the van der Walls power to weaken, which results in the degradation of cellulose and increasing degradability of the cell wall constituents or depolymerizes and delignifies the fiber (Al-Masri & Zarkawi, 1994; Choi *et al.*, 2009). Additionally, with the breaking of hydrogen bonds, free radicals are produced and then the concentration of free radicals and also, the number of separated chains from cellulose (Fig.1.), increases with the increasing irradiation dose (Khan *et al.*, 2006; Byun *et al.*, 2008). Steam recorded 8.24%, 39.33% and 19.78%. (Table.1).which is suggestive of an increase in organic matter digestibility has been reported due to its cell wall degradation (Al-Masri & Guenther, 1995). These results were in accordance with the work of Eggeman & Ellander, (2005).

Table 2. *Effect of pretreatments of sawdust on lignocelluloses content on Air Dry Basis*

Sample	% Moisture	% D.M	% H.C (ADB)	% Cellulose (ADB)	% Lignin (ADB)	% Silica (ADB)
Steam	5.44	94.56	8.24	39.33	19.78	9.53
5 kGy	5.83	94.17	10.89	39.24	17.56	13.51
24 kGy	5.41	94.83	11.26	40.15	19.81	15.44
Raw	5.62	94.14	8.11	37.62	16.85	12.82

H.C- Hemicellulose D.M- Dry Matter

Yield and yield attributes of P.ostreatus cultivated on pretreated composted sawdust

The fruiting body is the fleshy edible part of fungi. Flushing (amount of fruit bodies produced per batch) was observed for a period of 8 weeks after incubation. The maximum number of mushrooms produced per flush was 748g from (S + I) of the 24 kGy set. The minimum number of mushrooms per flush was 0g from the (S + nI) of the 0 kGy set. Generally, production decreased with increasing flush numbers. This could be attributed to lignocelluloses depletion and accumulation of metabolites in the substrate (Kortei, 2011).

Total fresh weight/ Economical yield

The total fresh weight of mushrooms or economical yield is the proportion of fresh mushrooms to wet weight of substrate. It was recorded from 4 flushes of cropping period. There were statistically significant ($P<0.05$) variations in

the total fresh weights or economical yield different permutations among the different sets of experiments (Fig.1). The maximum total fresh weight was 1,779g recorded from the steam sterilized spawn and steam sterilized sawdust compost bag (S + S) of the 32 kGy set of experiment. The highest yield appeared to be due to comparatively better availability of nitrogen, carbon and minerals from this substrate (Shah *et al.*, 2004).

Table 3. *Effect of pretreatments of composted sawdust on the yield of P.ostreatus*

Substrate Code	Flush (g/kg wet sawdust)				Econ. Yield (g/kg wet wt)	B.E (%)	Bio Yield (kg/kg dry wt)	Radiation Dose (kGy)
	1st	2nd	3rd	4th				
S + nS	-	-	-	-	-	-	-	0
I + nS	-	-	-	-	-	-	-	
S + nI	12	-	-	-	12 a	0.46	0.005 a	
I + nI	5.5	-	-	-	5.5 a	0.21	0.055 a	
S + S	352	450	355	250	1407 d	54.1	0.54 d	5
I + S	531	316	210	248	1305 c	50.1	0.50 c	
S + I	594	355	413	180	1541 e	59.3	0.54 e	
I + I	568	356	386	184	1493 e	57.4	0.57 e	
S + S	438	292.5	292.5	234	1257 c	48.3	0.48 c	10
I + S	452.5	402.5	289	199	1343 c	51.7	0.52 cd	
S + I	595.5	395.5	295.5	240	1527 e	58.7	0.59 e	
I + I	510.5	394	333	226	1464 e	56.3	0.56 e	
S + S	425	249	204	181.5	1060 f	40.8	0.41 a	15
I + S	539	309.5	257.5	205	1311 c	50.4	0.50 c	
S + I	443	330.5	244	163.5	1181 b	45.4	0.45 b	
I + I	472	323	310.5	184	1290 c	49.6	0.50 c	
S + S	472.5	246	205.5	145	1069 f	41.1	0.41 a	20
I + S	399	321	138.5	236	1095 f	42.1	0.42 b	
S + I	497	278.5	262.5	143	1181 b	45.4	0.45 b	
I + I	434.5	361	261	172.5	1229 bc	47.3	0.47 c	
S + S	468.5	474	243.5	103	1289 c	49.5	0.50 c	24
I + S	384	560	202.5	68	1215 bc	46.7	0.47 c	
S + I	748	484.5	131.5	182	1546 e	59.4	0.59 e	
I + I	653.5	237.5	332.5	209	1432 de	55.0	0.55 e	
S + S	664	487.5	426	201	1779 g	68.4	0.68 e	32
I + S	552	406	351	196.5	1506 e	57.9	0.58 e	
S + I	574	504	345	190	1613 g	62.0	0.62 e	
I + I	639	324	281.5	147.5	1392 cd	53.5	0.54 d	

N.B nI= nS Means with same letters in a column are not significantly different (P> 0.05)

The minimum total fresh weight of mushrooms or mushroom yield was 0g recorded by permutations; steam sterilized spawn and non steam sterilized compost bag (S + nS) and irradiated spawn and non steam sterilized sawdust compost bag (I + nS).

For each set of experimental dosage, there was no significant difference (P> 0.05) between yields of the various permutations (Fig.1). Generally, irradiated sawdust compost bags produced comparable yields to other works by several researchers (Mshandete *et al.*, 2011; Mondal *et al.*, 2010; Baig *et al*., 2009).

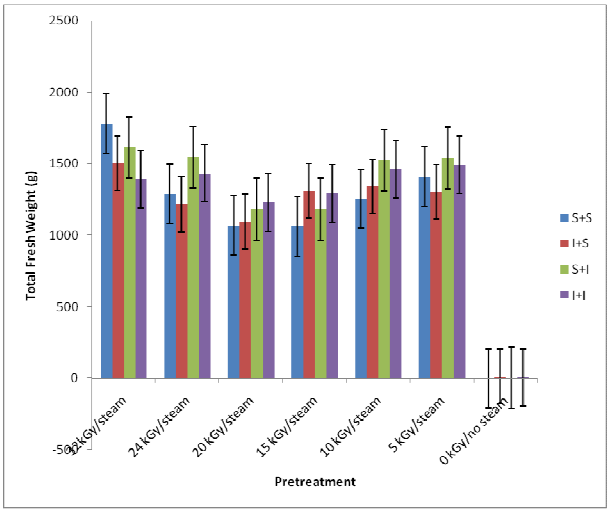

Figure1. *Comparative effect of pretreatment (Irradiation and steam) of sawdust on the yield of P. ostreatus*

Biological Efficiency, Bio-yield

The biological yield refers to the measure of total fresh weight to the dry weight of substrate. There were significant differences (P<0.05) in bio-yield with respect to the various treatments. Hence the biological efficiency is expressed as a percentage of the proportion. The maximum biological yield and efficiency (0.68 kg/kg of dry substrate weight and 68.4%) was recorded by steam sterilized spawn and steam sterilized sawdust compost bag (S + S) of the 32 kGy set of experiment. The minimum biological yield and efficiency (0g per flush, 0%) recorded by permutations; steam sterilized spawn and non steam sterilized compost bag (S + nS) and irradiated spawn and non steam sterilized sawdust compost bag (I + nS). The biological yield and efficiency of these substrates were within the range of works by several researchers (Obodai *et al.*, 2003; Mshandete *et al.*, 2011; Hasan *et al*., 2010).

Cropping Period

The cropping period for all the treatments was equal. Four weeks of incubation and six weeks of cropping.

Total number of primordia

There was significant variation (P<0.05) of the number of primordia recorded. The maximum number of primordia was 384 recorded by a combination of Irradiated spawn and irradiated composted sawdust (I + I) of the 24 kGy set. The minimum was 4 recorded by steamed spawn and non steamed composted sawdust bag (S + nS) of the 0 kGy set (Table 2). Thriving primordia ultimately becomes fruit bodies, if there is a balance of carbon to nitrogen (C: N) ratio (Hubbe *et al.*, 2010).

Total fruit bodies

The maximum fruit bodies recorded was 379 by irradiated spawn and irradiated composted sawdust (I + I) of the 32 kGy set. The minimum number of 0 fruit bodies was recorded by permutations; steam sterilized spawn and non steam sterilized compost bag (S + nS) and irradiated spawn and non steam sterilized sawdust compost bag (I + nS). There were significant differences (P<0.05) recorded. This result obtained for effective fruiting body might be due to the presence of glucose, fructose and trehalose in the substrate, as reported by Kitamoto *et al* (1995). Experimental findings by Poppe (1973) indicated that Indole Acetic Acid (IAA) increases the number of fruiting body of mushroom.

Cap diameter and Stipe length

The longest cap diameter and stipes (76mm, 67mm) respectively from the combination by irradiated spawn and irradiated composted sawdust (I + I) of the 24 kGy set. The shortest cap diameter and stipes (25mm, 21mm) respectively from irradiated spawn and non-irradiated composted sawdust (I + nI) of the 0 kGy (Table 4). There were significant differences (P<0.05) recorded. The ranges were in agreement with works (Raymond et al, 2013; Mshandete, 2010; Kortei, 2011; Owusu-Boateng & Dzogbefia, 2005; Ajonina & Tatah, 2012)

Mushroom size

According to researchers (Kurtzman, 2010; Reyes *et al*., 2009), interactions between environmental factors and nutrients in mushroom growth substrate have been reported to play important role in inducing formation of the fruiting bodies which results in mushroom size variations.

Table 4. *Effect of pretreatment of composted sawdust (T. scleroxylon) on the fruiting pattern of P.ostreatus*

Substrate Code	No.of primordia	No. of Fruit bodies	Average Stipe Length (mm)	Average Cap Diameter (mm)	Average Time b/n Flush Interval (days)	Mush-room Size	Dose (kGy)
S + n S	4 a	-	-	-	n.d	-	0
I + n S	12 a	-	-	-	n.d	-	
S + n I	10 a	8 a	41 ab	44 ab	n.d	1.5 b	
I + n I	9 a	9 a	21 a	25 a	n.d	0.6 a	
S + S	258 b	216 b	57 b	60 b	9 a	6.5 d	5
I + S	254 b	210 b	56 b	59 b	10 c	6.2 c	
S + I	340 d	297 c	60 c	68 bc	10 c	5.2 b	
I + I	349 d	302 c	60 c	66 b	9 a	4.9 c	
S + S	286 bc	231 b	58 b	58 b	13 e	5.4 b	10
I + S	280 bc	241 bc	59 b	60 b	13 e	5.6 b	
S + I	342 d	301 c	59 b	68 bc	12 de	5.1 b	
I + I	309 cd	256 bc	63 c	75 c	13 e	5.7 b	
S + S	259 b	259 bc	51 b	62 b	8 ab	4.1 a	15
I + S	272 b	198 b	53 b	64 b	9 b	6.6 cd	
S + I	307 cd	307 c	52 b	58 b	7 a	3.8 a	
I + I	288 c	214 b	56 b	60 b	9 b	6.0 c	
S + S	219 b	219 b	55 b	58 b	10 c	4.9 b	20
I + S	257 b	197 b	59 b	55 ab	11 d	5.6 b	
S + I	252 b	252 bc	57 b	57 ab	9 b	4.7 a	
I + I	229 b	181 b	57 b	56 ab	10 c	6.8 cd	
S + S	303 cd	223 b	50 ab	63 b	11 d	5.8 d	24
I + S	303 cd	320 c	67 cd	76 c	10.5d	3.8 a	
S + I	359 e	285 c	52 b	73 c	14 f	5.4 b	
I + I	384 e	324 d	56 b	66 b	10.5 d	4.4 a	
S + S	382 e	305 c	51 b	73 c	12.5 de	5.8 c	32
I + S	336 de	275 c	55 b	73 c	15 f	5.5 b	
S + I	373 e	279 c	49 ab	72 c	13 e	4.3 a	
I + I	325 d	379 e	43 ab	50 ab	12.5 de	3.7 a	

N.B nI= nS Means with same letters in a column are not significantly different (P> 0.05)

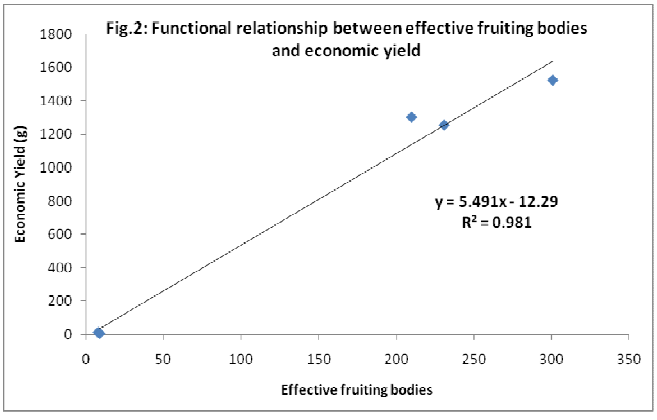

Figure 2. *Functional relationship between effective fruiting bodies and economic yield*

Maximum and minimum mushroom sizes of 6.8 and 0.6 were recorded by combination as in cap diameter and stipe lengths (Table 4). Mushroom sizes differed significantly (P<0.05) and were with the range reported by Raymond *et al*. (2013).

Relationship between yield attributes and economic yield

A positive linear relationship was observed between economic yield and effective fruiting body (Fig. 2). This suggests that economic yield is directly proportional to the number of effective fruiting body and more than 98 % (R^2= 0.981) of variation in the economic yield may be explained by variation of number of effective fruiting bodies harvested.

Conclusion

Technically, it was possible to use radiation doses of 5, 10, 15, 20, 24, and 32 kGy to effectively sterilize sorghum grains (*Sorghum bicolor*) for spawn production and for 'wawa'(*Triplochiton scleroxylon*) sawdust compost bag sterilization for the cultivation of *Pleurotus ostreatus.* Yields were comparable to moist heat sterilization. I would recommend the gamma irradiation technique to farmers and agribusinesses in countries that have access to gamma radiation facility.

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