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Comparative effect of gamma irradiated and steam sterilized sorghum grains (Sorghum bicolor) for spawn production of Pleurotus ostreatus (Jacq. Ex. Fr) Kummer.

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A B S T R A C T

The efficacy of gamma irradiation and moist heat sterilization of sorghum grains (Sorghum bicolor) for the production of spawns in heat resistant polypropylene bags were assessed. Both methods were tested single (moist heat or irradiated) and combined (moist heat and irradiated). The mycelium colonizing time, rate of colonizing, percentage contamination and mycelia density were parameters investigated. The best treatment was the set of experiment which was steamed and irradiated at a dose of 25 kGy (S+I) which produced a growth rate of 11.8 mm/day, colonized completely in 7 days and had a very thick mycelia density without (0%). The slowest treatment was the set of experiment of non- irradiated sorghum treatment (nI) which produced a mycelium growth rate of 10.0 mm/day, used 13 days to complete colonization and also produced a poor mycelia density owing to high (80%) contamination. The results of this experiment clearly demonstrated that gamma radiations could be used as a substitute of steam sterilization for sorghum spawn production for the cultivation of oyster mushrooms (Pleurotus ostreatus).

Key words: *Mycelium, spawn, gamma radiation, Sorghum bicolor, Pleurotus ostreatus*

Introduction

Mushrooms have become attractive as a functional food and are important as a source for the development of drugs and nutraceuticals (Islam and Ohga, 2012; Chang, 1999), especially antioxidants (Yen and Hung, 2000; Ferreira et al, 2007) and antimicrobial compounds (Barros et al, 2004). Alternative or substitute mushroom products are mycelia which are used as food and food-flavoring material, and also for the formulation of nutraceuticals and functional foods (Weng, 2003) Mushroom spawn is the network of fungal cells growing on grain substrate. Mushrooms are grown from hyphae (threadlike filaments) that become interwoven into mycelium and propagated on a base of steam sterilized cereal grain usually sorghum, rye or millet (Royse, 2003). This mycelium impregnated cereal grain is called spawn and is used to inoculate mushroom substrate (Royse, 2003). Spawn quality is counted the most important part in mushroom production (Mohammadi-Goltapeh and Purjam, 2003) as failure to achieve a satisfactory harvest may often be traced to unsatisfactory spawn used (Chang, 2009). Environmental factors such as temperature, oxygen, carbon dioxide, humidity, light and pH have been reported to have a significant effect on mycelia growth during spawn preparation (Nwanze et al, 2005; O.E.C.D, 2005).

Mycelial cultivation has received great interest as an efficient method for industrial production of valuable metabolites. In the field of bioremediation, they could be used to detoxify and ameliorate contaminated soils (Hirano et al, 2000; Kubatova et al, 2001) and various agro-industrial by-products have been tried as inexpensive growth substrates (Hatvani, 2001; Fang and Zhong, 2002) and some of these wastes are chopped rice straw, sawdust, water hyacinth leaves, used tea leaves, cotton wastes and lotus seed husks (Chang, 2009; Kortei, 2011). Successful utilization of agro-wastes for both mycelia and sporophore formation of macro fungi, supplies the nutrients needed by these fungi to convert them to protein-rich palatable food (Lakshmi, 2013). In most laboratories, cereal grains such as wheat (Elhami and Ansari, 2008; Chang, 2009; Stanley,

2010), rye (Chang, 2009), sorghum (Chang, 2009; Stanley, 2010), rice (Oei, 1996), millet (Oei, 1996; Elhami and Ansari, 2008; Stanley, 2010) and white maize (Stanley, 2010) are used as mother spawn.

In Ghana, Pleurotus ostreatus (Jacq. Ex. Fr) Kummer, strain EM-1, is the most cultivated mushroom (Obodai and Johnson, 2002). The spawns of this mushroom has been prepared using moist heat sterilized sorghum grains. It is inferred that gamma irradiation could be used to prepare spawn. Gamma irradiation is a physical pretreatment for the effective decontaminating and a hydrolytic agent (Gbedemah et al, 1998; Mami et al, 2013; Kortei et al, 2014). Gamma radiations have short wave length, high energy photons, and have deep penetrating power so could serve both as a decontaminating. 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 and Vestal, 2002).

This paper seeks to compare the effect of gamma irradiation and moist heat sterilization of sorghum grains for spawn preparation.

Materials and Methods

Pure culture

One-week-old pure tissue 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 $1cm^2$ of the one-week-old tissue culture of the experimental strain grown on Malt Extract Agar (OXOID^{TM} Ltd., Basingstoke Hampshire, England) using a flamed and cooled scalpel in a laminar flow hood. Thereafter, the spawns were incubated for 16-21 days without illumination in an incubator (Tuttlingten^{TM} WTC Binder, Germany) set at 28°C

Spawn preparation

The spawns were prepared using a modified form of the method of spawn preparation outlined by Narh et al, (2011). The cereal grains used was sorghum obtained from the Madina Market in Accra, Ghana. The grains were separately 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^{o}C to ensure that the steamed grains were cooked but intact. Broken grains are more prone to contamination. Thereafter, they were air-dried to cool on a wooden frame with a wire mesh. To the sorghum grain, 3 percent (w/w) of calcium carbonate ($CaCO_3$) was added and thoroughly mixed manually.

Moist heat sterilization

About 265g of grains were packed into bottles and then transferred into transparent heat resistant polypropylene bags (24cm x 38 cm) and then plugged with cotton wool and covered with plain sheets. The sheets were held in place with rubber bands (Plate 1). The grains were sterilized in an autoclave (Priorclave, Model PS/LAC/EH150, England) at 121^{o}C for 1hr.

Irradiation

Sorghum grains were soaked overnight and packaged as described above and then irradiated at doses 0, 5, 10, 15, 20, 25 and 32 kGy at a dose rate of 1.7 kGy per hour in air from a cobalt 60 source (SLL 515, Hungary). Doses were confirmed using the ethanol-chlorobenzene (ECB) dosimetry system at the Radiation Technology Centre of the Ghana Atomic Energy Commission, Accra, Ghana.

Table 1. Treatments used in experiment

Code	Interpretation
S + A	Steamed and autoclaved
S + I	Steamed and irradiated
I + I	Irradiated

Each treatment was repeated as gamma radiation dose varied. Each set of experiment, the treatment were replicated three times.

pH determination
According to AOAC, (1995).
Moisture Content determination
According to AOAC, (1995)

Growth and rate of growth of mycelia

Mycelia growths were measured by weekly markings of longest and shortest growths on the compost bags, then average length taken. Average length= longest + shortest lengths/2. The rate of mycelia growth was then calculated as Average length/ time.

Mycelia density and colonizing time

The mycelia density was graded by colonizing vigor or intensity (Obodai et al, 2003). Mycelia colonizing time was calculated as the time taken to undergo complete colonization.

Number of contaminated bags

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

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

Results and Discussions

The mycelium of P. ostreatus grew optimally at a temperature of 23-28^{o}C and pH of 5-6 (Table 2). Thus the ability of the mycelia to tolerate this temperature and pH range of 3- 10 enabled them to flourish in Soghum bicolor in the tropics (Fasidi, 2008). Studies by some workers (Zadrzil, 1978; Nwokoye et al, 2010; Kortei et al, unpublished) established that the spread of mycelial growth of Pleurotus spp was related to the temperature of the substrate. Mycelial development forms the vegetative growth phase of mushroom growing and temperature is highly important since it is directly related to the growth and adaptability as well as the quantity of the quality of fruiting-bodies produced (Kufordzi and Fasidi, 2009).

Moisture content (%) and pH

Moisture content of the sorghum grains ranged between 17.85±0.64 - 19.28±1.50 % (Table 2). Low moisture content below a critical level (< 30%), would decrease activities of microorganisms by restricting the motility and make them dormant (Hubbe et al, 2010). 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 (Tiquia et al, 1996; Dougherty, 1998). It has been observed that where the excess water sets at the bottom of the substrate, the mycelia colonizes the substrate just to the level of the water. A higher contamination rate by bacteria has also been observed where there is excess moisture. Hence, a moisture content ranging between 30- 40% would be appropriate when cereal grains such as sorghum is being used as substrate for EM-1 spawn production (Plate 1).

The pH of the substrates ranged between 5.18±0.05 - 6.71±0.04. Generally, there were significant differences ($P<0.05$) between the treatments. Fully irradiated (I + I) sorghum grains had comparatively higher pH values (5.48±0.05 - 6.51±0.04) than sorghum grains of fully moist heated (S+A) and partially moist heated and irradiated (S +I) which recorded 5.18±0.05- 5.85±0.05 and 5.36±0.04 - 5.88±0.05 respectively (Table 2). Some scientists stated that optimum pH ranges are mainly related to different species, strains, enzymatic systems, important vitamin entry in the cell, mineral capture, and surface metabolic reactions (Hung and Trappe, 1983; Barros et al, 2006). The pH values obtained were within the optimal pH range for growth of P. ostreatus (Narh et al, 2011), hence their ability to support good mycelia growth. High pH tends to suppress the growth as well as antagonize certain weed fungi in compost thus reducing competition for the mushroom (Kadiri, 1994). On the contrary, non pretreated sorghum grains recorded values of range 5.88±0.05 - 6.71±0.04 which was not within the optimal range of mycelia growth, might have caused their abysmal performance. These results are in agreement with other researchers (Narh et al, 2011; Kortei, 2015 unpublished).

Rate of growth

The number of days from inoculation to the total colonization of a substrate is related to the mycelia growth rate on the substrate. A faster growth rate results in a corresponding reduction in the days required for complete colonization of the substrate by the mycelia (Mottaghi, 2006; Narh et al, 2011). The rate of growth of mycelium was recorded after mycelium inoculants grew down the sorghum grains after passage of time and resulted in varied responses (Figs.1-7) and (Table.1). The fastest rate of mycelia growth was 0.71 cm/day recorded by a single treatment of gamma radiation of 15 kGy (I+I) on soaked raw sorghum. The various treatments under the 5, 10, 15, 20, 25 and 32 kGy sets of experiment showed no significant differences ($P>0.05$) (Table 1). This might be due to the fact that at 5 kGy dose level and beyond, there was effective decontamination of the sorghum grains which might have reduced the number of competitive microorganisms. According to Elhami and Ansari, (2008), the combined effect of metabolic activities and substrate oxygen concentration might be

responsible for maximum mycelia growth. The results obtained were in agreement with Fan et al, (2000), recorded 0.97 cm/day mycelia growth on sorghum. The slowest mycelium growth rate of 0.3 cm/day was recorded by raw non-irradiated and non autoclaved sorghum grains (nI) (Fig.1). This slow rate of growth of mycelium could be attributed to oxygen depletion and certain metabolites produced by competitor microorganisms. Respiration rate is directly related to O2 concentration of substrate (Mehravaran, 1993).

Table 2. Effect of pretreatment (gamma radiation and moist heat) on the physical characteristics of sorghum grains (Sorghum bicolor).

Dose (kGy)	Treatment	Moisture content (%)	pH	Temperature (^{o}C)
0	nS + nA	18.85 ± 0.85	6.71 ± 0.04	24.1 ± 1.50
	S + nI	19.28 ± 1.50	5.88 ± 0.05	23.9 ± 1.50
	nI + nI	19.00 ± 1.20	6.51 ± 0.04	23.3 ± 1.50
5	S + A	18.69 ± 0.85	5.84 ± 0.05	24.1 ± 1.50
	S + I	18.34 ± 0.85	5.69 ± 0.05	24.2 ± 1.50
	I + I	18.53 ± 0.86	5.48 ± 0.05	22.3 ± 1.40
10	S + A	17.94 ± 0.64	5.18 ± 0.05	27.1 ± 1.80
	S + I	18.21 ± 0.78	5.71 ± 0.05	24.4 ± 1.50
	I + I	19.01 ± 1.20	5.98 ± 0.04	27.5 ± 1.80
15	S + A	17.89 ± 0.64	5.85 ± 0.05	24.5 ± 1.50
	S + I	18.93 ± 0.88	5.70 ± 0.05	24.4 ± 1.50
	I + I	18.27 ± 0.33	6.21 ± 0.06	24.1 ± 1.50
20	S + A	18.56 ± 0.45	5.64 ± 0.05	24.0 ± 1.50
	S + I	18.42 ± 0.82	5.43 ± 0.04	24.6 ± 1.50
	I + I	18.96 ± 0.88	5.88 ± 0.05	24.2 ± 1.50
25	S + A	18.84 ± 0.64	5.47 ± 0.04	24.5 ± 1.50
	S + I	19.00 ± 1.20	5.36 ± 0.04	23.6 ± 1.40
	I + I	18.77 ± 0.87	6.47 ± 0.06	23.9 ± 1.50
32	S + A	18.85 ± 0.64	5.76 ± 0.05	21.7 ± 1.30
	S + I	18.30 ± 0.33	5.73 ± 0.05	21.5 ± 1.30
	I + I	17.85 ± 0.64	6.08 ± 0.04	22.4 ± 1.30

Results are averages of 3 ± standard deviation

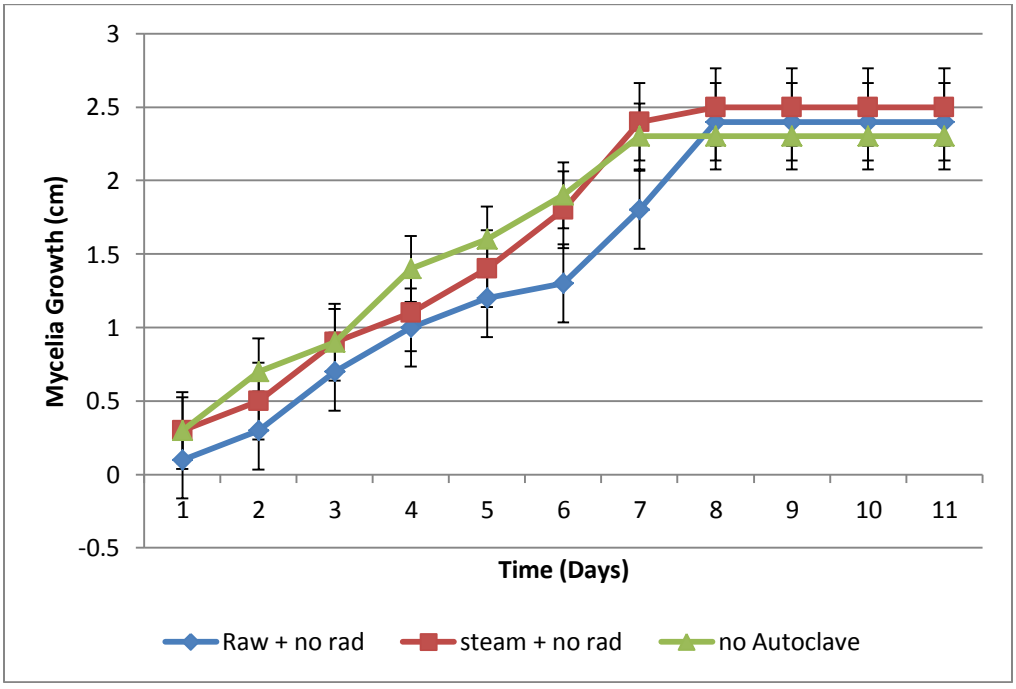

Figure 1. The effect of non autoclaved and non- irradiated (0 kGy) sorghum grains on the daily mycelia growth of P.ostreatus strain EM-1.

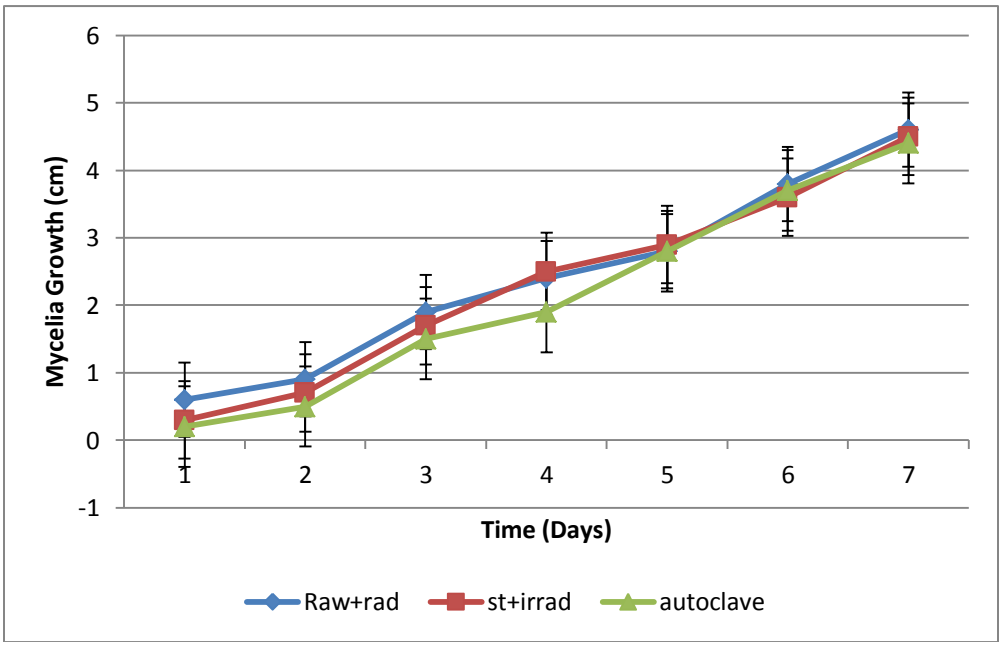

Figure 2. The effect of autoclaved and irradiated (5kGy) sorghum grains (raw and steamed) on the daily mycelia growth of P.ostreatus strain EM-1.

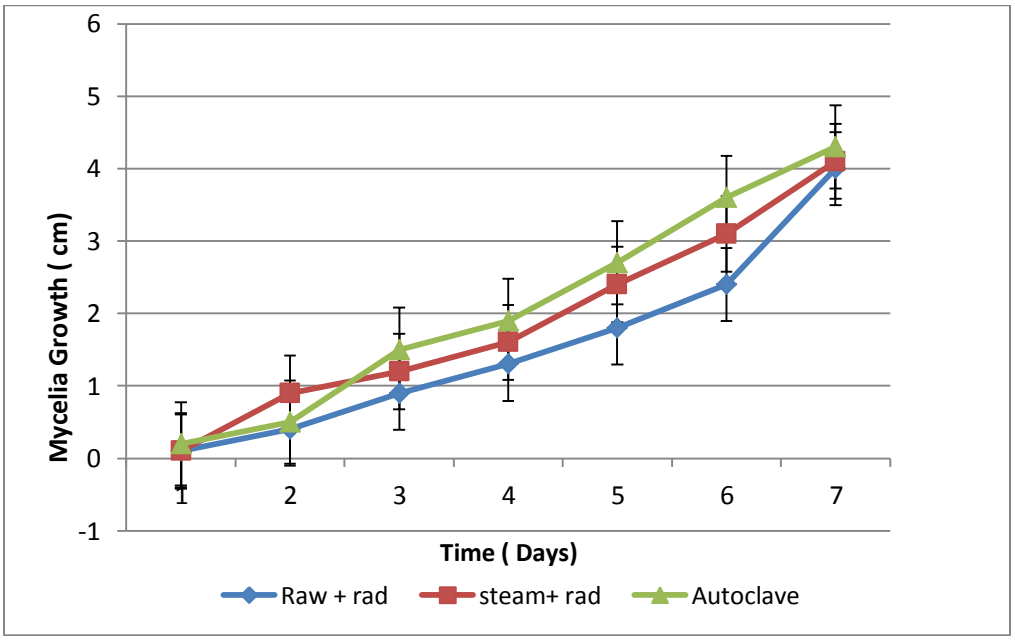

Figure 3. The effect of autoclaved and irradiated (10 kGy) sorghum grains (raw and steamed) on the daily mycelia growth of P.ostreatus strain EM-1

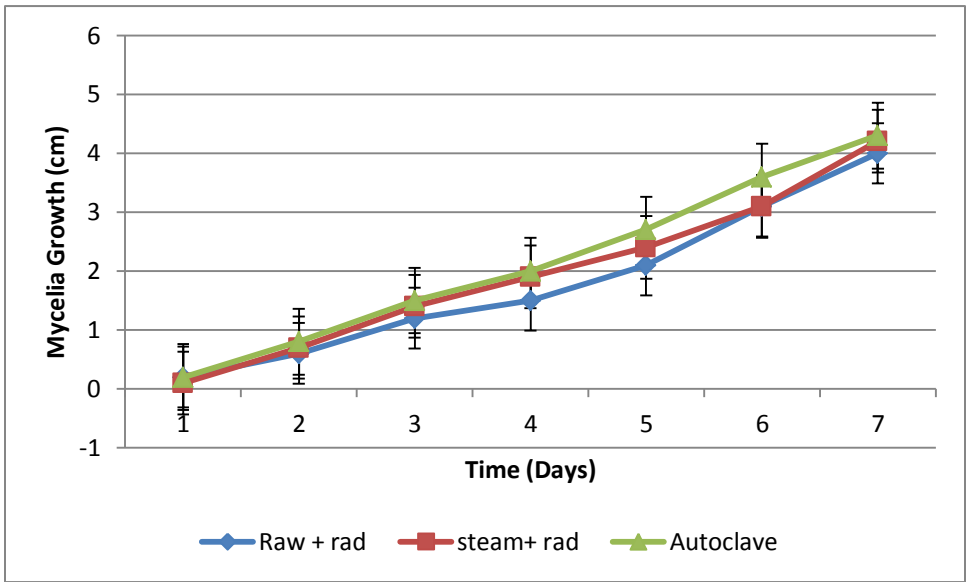

Figure 4. The effect of autoclaved and irradiated (15 kGy) sorghum grains on the daily mycelia growth of P.ostreatus strain EM-1.

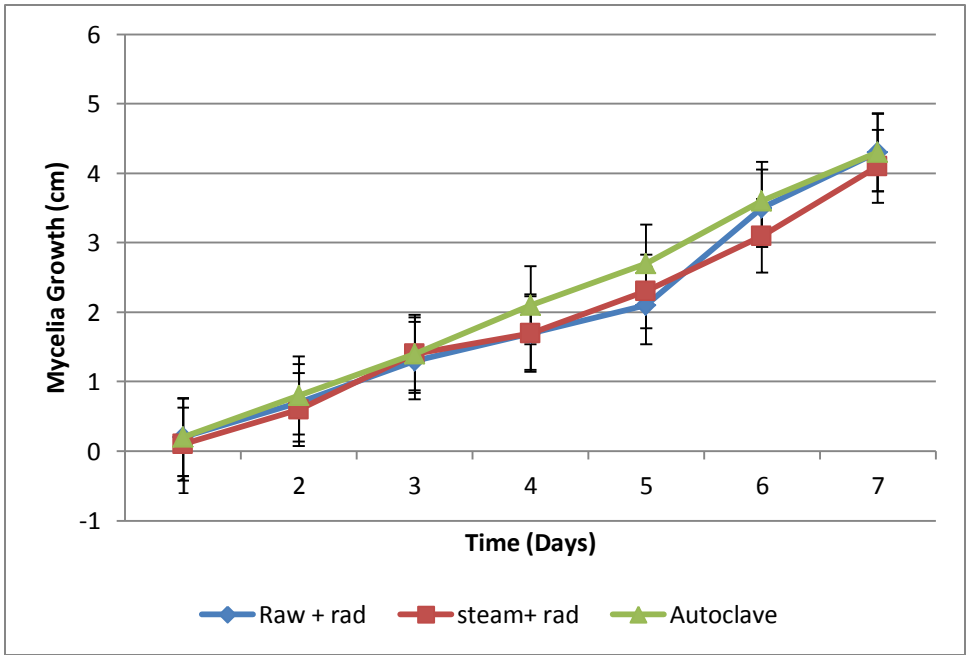

Figure 5. The effect of autoclaved and irradiated (20 kGy) sorghum grains on the daily mycelia growth of P.ostreatus strain EM-1.

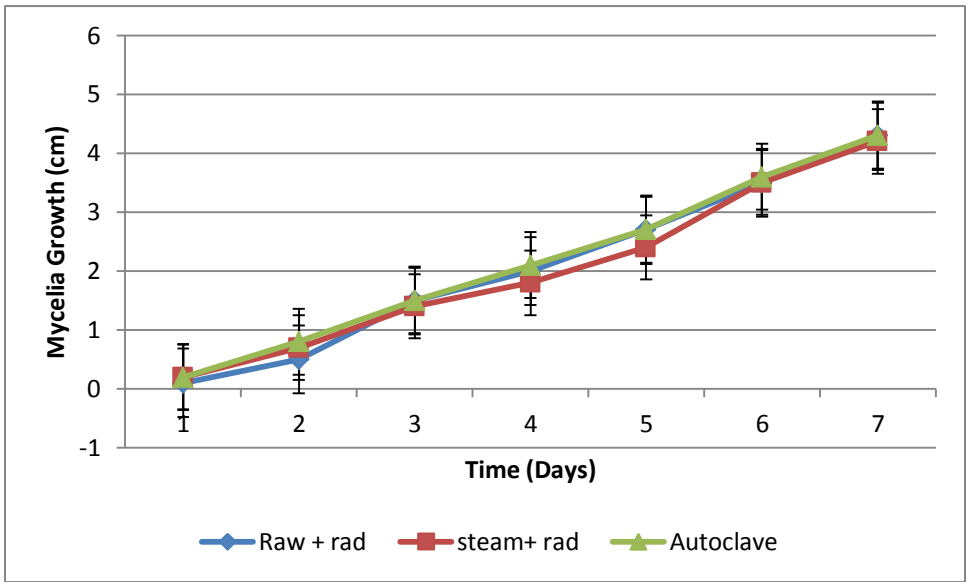

Figure 6. The effect of autoclaved and irradiated (25 kGy) sorghum grains on the daily mycelia growth of P.ostreatus strain EM-1.

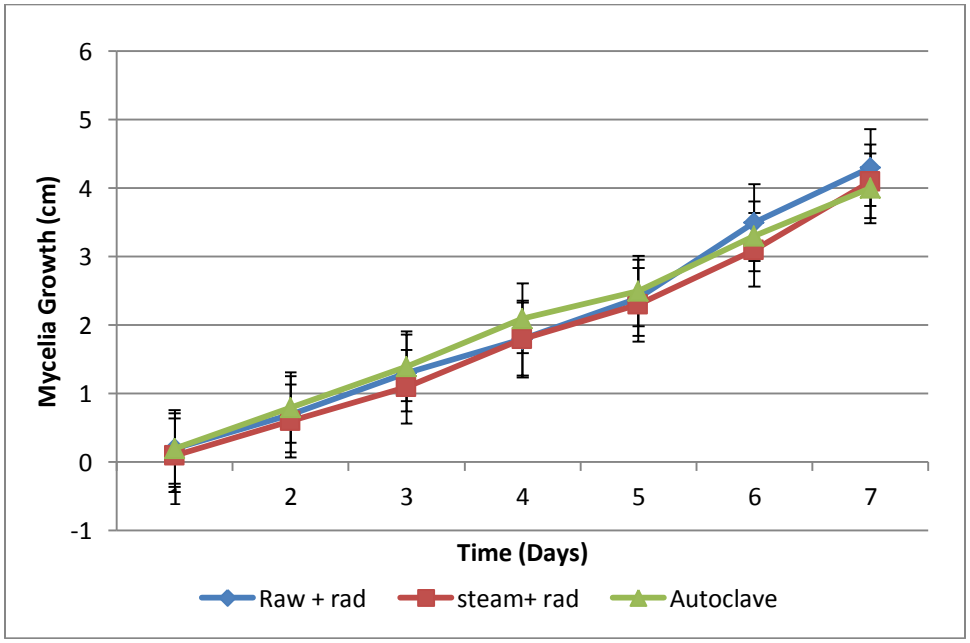

Figure 7. The effect of autoclaved and irradiated (32 kGy) sorghum grains on the daily mycelia growth of P.ostreatus strain EM-1.

Time taken for complete colonization

Spawn running requires high humidity (80- 90%) and high temperature (25- 30°C) for the vegetative growth (Buah et al, 2010). The shortest time of an average of 7 days was observed for the complete colonization of sorghum by P. ostreatus was attained by treatment combinations which involved irradiation at 5 kGy to 32 kGy (Figs. 2- 7). The time taken for complete colonization was not significantly different ($P>0.05$) within this dose range. The longest time of 11 days for complete colonization was observed for the non-pretreated sorghum (nS+nA), (S+nI) and (nI+nI). Growth halted on the 8th day and rate was insignificant due to competition from contaminants which resulted in prolonged colonization time (Fig. 1).

Radiation treatment is known to affect the molecular size of polysaccharides, whether it is applied to a solution or in the solid state, although the decrease in molecular size is larger when the polysaccharide is in solution (Jumel et al, 1996) and cause a break up of polysaccharides into smaller units (Bouchard et al, 2006) for assimilation which speed up growth of mycelia. The values obtained were comparable to works of Fan et al, (2000) who reported 9 days as fastest time for spawn run

on sorghum grains at 24^{o}C temperature. Stanley and Awi- Awaadu, (2010) also recorded a slightly higher value of 10 days as fastest colonization time for completion for P. pulmoniarius on white maize. The longest time of 12 days was recorded for non moist heated (nA + nA) likewise non irradiated (nI + nI). In terms of time for complete colonization, there was no significant difference (P>0.05).

Contamination and Mycelium Density

Contamination of P. ostreatus spawn was apparent in non pretreated sorghum substrates which ranged between 50-80% (Table 1). Also, pretreated sorghum substrates of 5 kGy recorded less contaminations of between 5- 10%. Contaminations appear in the spawn after a deficient sterilization of the sorghum grains of cereal which act as substrate (Kortei et al, 2015, unpublished data). This circumstance permits these contaminants to compete successfully with P. ostreatus for space and nutrients (Internet, 2004). Several fungi have been found to produce a range of antibiotics, each produced under specific conditions which either deter or inhibit some fungal competitors.

Among the various treatments, there were no notable differences in the density of mycelia (Table 2). The mycelia were dense on all the treatments except for non pretreated (non steam or non irradiated). According to I.A.E.A/RAS, (2001) and Bouchard et al., (2001), gamma radiation and moist heat are effective depolymerization agents for the breakdown of polysaccharides into simplest units of carbon, hydrogen and oxygen which were utilized by the mycelia. Results obtained were in agreement with Narh et al, (2011) who observed no difference in mycelia density when they used moist heat to produce P. ostreatus strain EM-1 spawn on millet and sorghum.

Table 3. Influence of pretreatment (Irradiation and steaming) of sorghum (Sorghum bicolor) on mycelia growth during spawn run period

Dose (kGy)	Treatment	Av. Rate of growth (cm/Day)	Time for Complete Colonization (Days)	Contamina-tion (%)	Mycelia Density
0	No stm + no autoclave	0.3c	11b	60cg	+
	Stm + non irrad.	0.3c	10b	50bf	+
	Non Irradiated	0.3c	11b	80cg	+
5	Stm + autoclave	0.68ab	7a	5de	+
	Stm + irrad	0.7ab	7a	10f	+ +
	Irradiated	0.7ab	7a	0	+ + +
10	Stm + autoclave	0.65ab	7a	5de	+ +
	Stm + irrad.	0.66ab	7a	0	+ + +
	Irradiated	0.68ab	7a	0	+ + +
15	Stm + autoclave	0.63ab	7a	0	+ + +
	Stm + irrad.	0.68ab	7a	0	+ + +
	Irradiated	0.71ab	7a	0	+ + +
20	Stm + autoclave	0.68ab	7a	0	+ + +
	Stm + irrad	0.66ab	7a	0	+ + +
	Irradiated	0.68ab	7a	0	+ + +
25	Stm + autoclave	0.70ab	7a	0	+ + +
	Stm + irrad	0.66ab	7a	0	+ + +
	Irradiated	0.70ab	7a	0	+ + +
32	Stm + autoclave	0.70ab	7a	0	+ + +
	Stm + irrad	0.66ab	7a	0	+ + +
	irradiated	0.63ab	7a	0	+ + +

Means with same letters in a column are not significantly different (P> 0.05)
Degree of mycelial density when the mycelia fully colonises the substrate:
+ poor running growth,
++ mycelium grows throughout the whole bag but is not uniformly white,
+++ mycelium grows throughout the whole bag and is uniformly white

Plate 1. Gamma irradiated raw sorghum soaked overnight

Plate 2. Complete mycelia colonization of gamma irradiated sorghum

Conclusion

These criteria investigated (colonizing time, rate of growth, mycelia density and percentage contaminations) show that irradiation is a good and efficient substitute for sterilizing sorghum grains for spawn production which is used in mushroom cultivation industry in Ghana

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