IMPROVEMENT OF *PLEUROTUS FLORIDA* MUSHROOM PRODUCTION BY GAMMA IRRADIATION

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Abstract

Basidiospores of *Pleurotus florida* fungus were irradiated in a trial to improve their cultivation capabilities. Spore suspension was irradiated with gamma radiation at dose rates of 0.05, 0.15, 0.25, 0.35, 0.45, 0.55, 0.65, 0.75 and 1.0 kGy. Viable counts of spores decreased as the irradiation dose was increased. Number of survivors was reduced from 1.2×10^7 to 5.5×10^2 CFU/ml, when maximum irradiation dose was used. Different doses of gamma radiation improved radial growth when different sugar sources were used as compared with either non-irradiated culture or tissue culturing of the fungus. Gamma-irradiated culture (IC) showed high yield over both the non-irradiated and tissue cultured fungus and the highest biological efficiency obtained was 139.27 when irradiated at 1.0 kGy. However, Slight effect on yield over the tissue culture was noticed. On the other hand, irradiated culture with 0.25 kGy resulted in increased digestible protein content, which reached 21.363 % as compared with 19.557 and 14.856 % for the non-irradiated and the tissue culture, respectively.

INTRODUCTION

The oyster mushroom is the wide spread cultivated edible mushroom in Egypt (Mahmoud & El-Kattan, 1989) and is consumed as a good protein source (Chang, 1996). Geetha and Sivaprakasan (1996) stated that gamma radiation at dose rates of 2000, 2500 and 3000 rads induced significant increase in mycelial growth and yield of Pleurotus spp. Roy *et al.*, (2000) studied the effect of gamma radiation on the productivity of Agaricus bisporus and Pleurotus sajor-caju and revealed that a significant increase in yield of the resulting mushrooms was achieved by gamma radiation of the spawn.

Basidiospores of *Volvariella volvacea* has been studied with respect of their ploidy, using gamma radiation. Results has showed that there are two populations of *V. volvacea* spores determined by the survival curve: a relatively sensitive population and second population of approximately 10-fold greater resistance (Quaye, 1987).

Thus, Gamma irradiation was used in this study in a trial to improve the yield and protein content of *Pleurotus florida* mushroom.

MATERIALS AND METHODS

Preparation of fungal mycelium for mushroom production

Mature fruit bodies of *P. florida*, obtained from Mushroom Laboratory, Agricultural Research Center, Giza, Egypt, were used as a source of both a tissue cultured fungus and a spore suspension (Stamets, 1993). Ten tubes from the spore suspension were set aside and were used as a control.

Radiation D_{10} value (radiation dose required to inactivate of 90% of the spores of *P. florida*) was determined according to Anon. (1981). The irradiation process was carried out at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The irradiation facility used was: Gamma Cell 220 "product of Canada". The dose rate of this source was 1.5 Gy/min. at the time of experiment. In this method, 1.0 ml of the prepared spore suspension (10^6-10^7 spore/ml) was added to 9.0 ml of sterile saline solution (0.85 % NaCl) in test tubes. Three replicates were exposed to each of various doses of gamma radiation, *i.e.* 0.0, 0.05, 0.15, 0.25, 0.35, 0.45, 0.55, 0.65, 0.75 and 1.0 KGy. Immediately after irradiation, serial dilutions were made from each replicate and the count of survivors was enumerated on malt extract agar medium using pour plate technique (Koburger and Marth, 1984). The plates were incubated at 25°C for three days. The colonies were counted in each plate and the D_{10} value was calculated using the method explained by Anon .(1981).

The irradiated (IC) and non-irradiated cultures of *Pleurotus florida* were maintained on PDA medium and used for preparation of the sorghum spawn as described by Garcha (1981). Common black polyethylene bags of 3.0 kg wet substances capacity were used as mushroom beds. The wet and pasteurized rice straw was prepared according to Mahmoud and El-Kattan (1989) and was spawned at (5 % w/ w). The bags were pinned after inoculation and were incubated in the greenhouse at 25 $\pm$ 3°C in cultivated area during spawn run and fructification period, and relative humidity of 75-90% was maintained by spraying water. After completion of the spawn run period (15 days), the plastic were removed and the substrate surface was exposed to light and aeration. The relative humidity was slightly raised. Mushrooms were harvested within 7 days from exposure to light when fruiting bodies started to "curl"

up. Biological efficiency (B.E.) was calculated as Kg of fresh mushrooms harvested from 100 Kg of dry straw (Mahmoud and El-Kattan, 1989).

Ability of mycelial growth on different sources of sugar

Different sugar sources at a concentration of 20 g/L were tested for their effect on radial growth of the irradiated cultures of *P. florida* as compared with either the non-irradiated culture (control) or the tissue culture of the same fungus. Small discs (5 mm. in diameter) of the different *Pleurotus* cultures were transferred to Petri-dishes containing the tested sugars *i.e.* fructose, maltose, sucrose, mannitol, dextrose, and starch where the plates were incubated at 25°C. A set of 5 dishes was used for each particular treatment. Colony diameter was measured and recorded when any Petri-dish of the different treatments was completely covered with mycelial growth (90 mm.) (Chaudhuri and Sen, 1982).

Chemical analysis

Moisture content of mushroom samples was determined according to A.O.A.C (1995). Total nitrogen of mushroom was determined by micro-Kjeldahel method using boric acid as described by Lau (1982) and the digestible crude protein (N x 4.38) was calculated as mentioned by Crisan and Sands (1978).

RESULTS AND DISCUSSION

Viability of irradiated mushroom

Data in Table (1) and Figure (1) show that the viable counts of spores decreased as irradiation dose was increased. Irradiation dose of 0.05 kGy reduced the counts from 1.2×10^7 to 2.8×10^6 CFU/ml, indicating that this low irradiation dose reduced the counts by 76.7 %. Meanwhile, irradiation dose of 0.45 kGy reduced the counts by about 3 log cycles. The maximum irradiation dose used (1 kGy) reduced the viable spore counts from 1.2×10^7 to only 5.5×10^2 with a percent reduction in viability of about 99.99 % .

Table 1. Effect of different doses of gamma radiation on the viability of *Pleurotus florida* spores in physiological saline solution.

Irradiation doses (kGy)	No. of survivors	Reduction in viability, %
IC 0.0 kGy (control)	1.2×10^7	0.000
IC 0.5 kGy	2.8×10^6	76.667
IC 0.15 kGy	4.6×10^5	96.170
IC 0.25 kGy	1.5×10^5	98.750
IC 0.35 kGy	4.6×10^4	99.610
IC 0.45 kGy	1.8×10^4	99.850
IC 0.55 kGy	8.9×10^3	99.930
IC 0.65 kGy	4.8×10^3	99.960
IC 0.75 kGy	1.9×10^3	99.980
IC 1.00 kGy	5.5×10^2	99.995

D_{10} = 0.24 kGy r = - 0.97

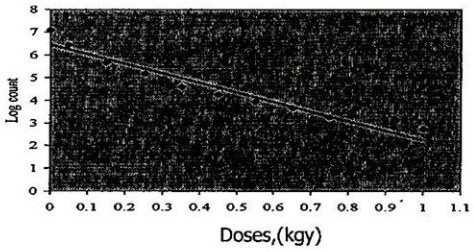

Fig 1. Survival response of *P. florida* to gamma radiation

Ability of mycelial growth on different sources of sugar

Table (2) shows the effect of different sugar sources on the radial growth (mm) of irradiated and non-irradiated cultures of *Pleurotus florida* for a maximum period of 8 days. The lowest radial growth for both control and IC was obtained on media containing fructose as a carbon source. On the other hand, the highest radial growth was obtained in media which contained starch, dextrose and sucrose as carbon sources. The highest maximum growth *i.e.* 90 mm was reached at 8 days when the spores were irradiated with 0.35 kGy and grown on either dextrose or starch media. Also, IC 0.75 kGy and grown on starch media gave maximum growth at 8 days. Results show positive increase in radial growth with increasing doses of gamma radiation up to certain limits with the different sugar sources. Non-irradiated culture showed the lowest radial growth on most sugar sources. These results are in agreement with those found by Dijkstra (1975).

Table 2. Radial growth (mm) of tissue, irradiated and non-irradiated Cultures of *Pleurotus florida* using different sugar sources.

Treatments	Source of sugars					
	Dextrose	*Fructose*	Maltose	Manitol	Starch	Sucrose
Tissue culture	76.8	37.3	53.8	78.5	87.2	87.2
IC 0.0 kGy (control)	71.8	47.3	49.0	68.7	76.2	58.2
IC 0.5 kGy	76.3	57.7	61.5	78.5	87.7	75.0
IC 0.15 kGy	71.2	55.5	51.5	76.8	88.5	89.0
IC 0.25 kGy	72.0	36.8	52.0	74.3	83.3	87.2
IC 0.35 kGy	90.0	59.8	80.0	87.2	90.0	89.2
IC 0.45 kGy	88.3	56.2	50.7	80.8	85.7	74.7
IC 0.55 kGy	75.7	51.8	56.0	85.8	88.2	85.0
IC 0.65 kGy	83.8	36.8	62.3	89.5	82.7	82.2
IC 0.75 kGy	89.0	54.0	54.2	85.7	90.0	87.2
IC 1.00 kGy	80.7	44.0	61.2	84.7	87.0	82.2

Production of mushroom

Table (3) shows that the tissue culture of *Pleurotus florida* yielded 5068.3g in ten flushes with a biological efficiency of 126.7. However, the non-irradiated culture (control) yielded 1347.32 g with a B.E. of only 33.68 in only three flushes. The highest yield of 5570.7g representing 139.2 biological efficiency was obtained from cultures irradiated with 1.00 KGy in only six flushes.

It can be seen from the data that gamma irradiation of *P. florida* spores reduced the flush number and consequently the time required for the production cycle. These results are in agreement with those found by Roy *et al.*, (2000), who proved that gamma radiation significantly increased yields of *Agaricus bisporus* and *Pleurotus sajor-caju.* Also, the shelf life of *Pleurotus sajor-caju* has been extended up to 9 days at 15°C as compared with only 6 days for the non-irradiated cultures.

Digestible protein content of *P. florida* mushroom reached 21.36 % in the cap of 0.25 KGy-IC, while it was 14.86 % in caps of tissue culture. It reached 12.06 and 5.47 % in the stems of the same samples, respectively.

It might be concluded that gamma radiation has increased the protein content of both cap and stem of *P. florida* mushroom compared to tissue culture technique usually used for commercial production.

It is clear from the results that the tissue culture gave higher mushroom yield than the non-irradiated spore culture conforming with Geetha and Sivaprakasan (1996). The highest radiation dose of 1.00 KGy gave highest yields with comparable protein content in a shorter production cycle. It seems that the few number of spores which escaped the lethal effect of the highest dose of gamma radiation i.e. 5.5×10^2 might have had better genetic characteristics (Roy *et al.*, 2000).

Finally, it could be concluded that gamma radiation of *P. florida* spores may have a positive effect on the economy of mushroom production because of not only the production cycles per season might be increased, but also the yield of such cycles one increased.

Table 3. Effect of gamma irradiation on total yield and digestible protein content of *Pleurotus florida*

Treatments	Total yield, g*	Cap, % as fresh weight	Total dry weight of cap	A	B	% moisture content		B.E	% digestible protein		No. flush
						Cap	Stem		Cap	Stem	
Tissue culture	5068.30	82.50	318.60	276.18	0.00	92.38	90.18	126.71	14.856	5.471	10
IC 0.0 kGy (control)	1347.32	72.86	89.27	0.00	-73.42	90.90	88.81	33.68	19.557	7.440	3
IC 0.05 kGy	4186.60	80.43	286.20	210.74	-17.40	91.50	90.45	104.67	18.504	11.779	7
IC 0.15 kGy	3279.90	84.49	282.94	143.44	-35.29	89.79	89.95	82.00	16.523	10.007	7
IC 0.25 kGy	867.50	79.01	83.00	-35.61	-82.88	87.89	83.07	21.69	21.363	12.064	4
IC 0.35 kGy	4006.70	85.39	276.78	197.38	-20.95	91.91	90.11	100.17	18.282	7.494	9
IC 0.45 kGy	4538.40	83.08	323.48	236.85	-10.46	91.42	91.90	113.46	18.441	8.450	7
IC 0.55 kGy	2283.50	73.06	194.40	69.48	-54.95	91.43	88.26	57.09	19.557	7.338	5
IC 0.65 kGy	2218.00	79.42	132.82	64.42	-56.24	92.46	90.26	55.45	18.335	9.884	7
IC 0.75 kGy	1056.20	78.45	92.98	-21.61	-79.16	88.53	81.84	26.41	16.954	7.869	3
IC 1.00 kGy	5570.70	73.27	239.19	313.47	9.91	94.14	93.01	139.27	16.514	5.899	6

* Yield fresh mushroom g / 4 Kg dry rice straw

A = % gain / loss over control

B = % gain / loss over mother culture

B.E: Biological efficiency, g fresh mushroom / 100 g dry substrate

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تحسين إنتاجية فطر عيش الغراب *Pleurotus florida* باستخدام أشعة جاما

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تـم تشـعيع معلـق جراثيم الفطر *Pleurotus florida* لتحسين القدرة الإنتاجية. وذلك باسـتخدام جـرعات مـن أشعة جاما بمقدار ٠,٠٥ و ٠,١٥ و ٠,٢٥ و ٠,٣٥ و ٠,٤٥ و ٠,٥٥ و ٠,٦٥ و ٠,٧٥ و ١,٠ كيلو جراى.

انخفضت حيوية الجراثيم بزيادة جرعة الإشعاع . ١,٢ x ١٠ ^{٧} للجراثيم الغير معاملة إلى ٥ر٥ x ١٠ ^{٢} خلية لكل ملليلتر من المعلق عند استخدام أعلى جرعة إشعاع. أدى تعرض الجراثيم للجرعات المختلفة من أشعة جاما إلى تحسين النمو الميسليومى للفطر علي مصادر مختلفة من السكر بالمقارنـة بالمزرعة الناتجة من جراثيم غير معاملة بالإشعاع أو المزرعة الناتجة من زراعة أنسجة الفطر.

لوحظ أن إنتاجية الفطر الناتج من جراثيم مشعة أعلى من إنتاجية الفطر الناتج من جراثيم غـير معاملة بالإشعاع أو الناتجة من الأنسجة الفطرية . ولقد تم الحصـول على أعلى كفاءة حيوية (١٣٦,٢٧) عـند تعريض جراثيم الفطر لأشعة جاما بمقدار ١ كيلو جراى، والذى كان أيضاً أعلى قليلاً من المحصول الناتج عن الأنسجة الفطرية. أما بالنسبة للبروتين فإن الثمار الناتجة من الجراثيم التى تعرضت لجرعة إشعاع مقدارها ٠,٢٥ كيلو جراى نتج عنها زيادة فى المحتوى البروتينى القابل للهضـم والـذى وصـل إلى ٢١,٣٦ % بالمقارنة بالثمار الناتجة من جراثيم غير معاملة أو الثمار الناتجة من زراعة الأنسجة والتى بلغت ١٩,٥٦ و ١٤,٨٦ % على التوالى.