EFFECTS OF GAMMA-IRRADIATION ON THE MYCELIA OF SOME *PLEUROTUS ERYNGII* STRAINS GROWN *IN VITRO*

EFECTE ALE IRADIERII GAMMA ASUPRA MICELIILOR UNOR TULPINI DE *PLEUROTUS ERYNGII* CULTIVATE *IN VITRO*

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Abstract. *This work focuses on evaluating the effects of applying low doses of Gamma radiation on the mycelia of some Pleurotus eryngii (King Oyster) strains. Mycelia grown on agarized media PDA and MEA were irradiated with doses ranging from 50 to 1500 Gy. Subcultures were obtained by transferring irradiated and non-irradiated mycelia to fresh PDA, MEA solid media and PDB, MEB liquid media (submerged culture). The cultural characteristics and growth rates of irradiated and non-irradiated subcultured mycelia were verified – after the first passage. Higher doses caused the mycelia to slow down and reduce the amount of biomass obtained. The dose of 200 Gy applied to the mycelia of the three strains of P.eryngii verified, in some cases, resulted better than the lower doses 50 Gy, 100 Gy, 150 Gy.*
Key words: Gamma radiation, *Pleurotus eryngii*, mycelial biomass

Rezumat. *Lucrarea îşi propune să evalueze efectul aplicării unor doze slabe de radiații Gamma asupra miceliului unor tulpini de Pleurotus eryngii (King Oyster). Miceliile cultivate pe medii agarizate PDA şi MEA au fost iradiate cu doze cuprinse între 50 şi 1500 Gy. Subculturi au fost obținute prin transferul miceliilor iradiate pe medii proaspete PDA, MEA şi în medii lichide PDB, MEB (cultură submersă). S-au verificat caracterele culturale şi ratele de creştere ale miceliilor iradiate şi neiradiate subcultivate - primul pasaj. Dozele mai mari au determinat încetinirea creşterii miceliului şi reducerea cantității de biomasă obținută. Doza de 200 Gy aplicată miceliilor celor 3 tulpini de P.eryngii verificate a determinat, în unele cazuri, rezultate mai bune decât dozele mai mici 50 Gy, 100 Gy, 150 Gy.*
Cuvinte cheie: Radiații Gamma, *Pleurotus eryngii*, biomasă miceliană

INTRODUCTION

The application of Gamma radiation technology has proven useful in many fields, including agro-horticultural and medical biotechnologies. An example is obtaining organisms with improved characteristics either through mutagenesis or by stimulating the production of bioactive compounds of medical/pharmaceutical

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importance. Studies on edible and/or medicinal mushroom species have shown the positive effects of applying low doses of Gamma radiation on mycelium and carpophores. Gamma-irradiation with doses up to 2.0 kGy has, in some cases, increased the production of mushrooms and their quality by increasing the shelf life and content of bioactive compounds useful in different biotechnological applications (Akram *et al*., 2012; Kim *et al*., 2012; Rashid *et al*., 2016).

Our experiments were conducted to evaluate the effects of low-dose Gamma radiation on the mycelia of some *Pleurotus eryngii* (King oyster) strains, a relatively new species for the assortment grown in Romania (Zăgrean *et al*., 2016). The characteristics of irradiated mycelia grown *in vitro* on agarized media and in submerged cultures are presented.

MATERIAL AND METHOD

Biological material: 3 strains of *P. eryngii* (RDIVFG collection) PeM-39, PeM-41, PeM-45. Two irradiation tests were performed independently. Pure dikaryotic mycelium cultures were grown in Petri dishes onto agar medium, PDA (test 1) and MEA (test 2), with an initial pH of 6.5. After incubation the mycelia were Gamma-irradiated at IRASM/IFIN-HH Măgurele (Co-60 source) at different dose levels: 0 (control), 50, 100, 200, 500, 1000 Gy (test1) and 0, 50, 100, 150, 200, 300, 1500 Gy (test 2). The next day, circular fragments (5 mm) from irradiated mycelia - the active growth area - were transferred simultaneously in Petri dishes onto PDA/MEA media and into 500 ml vials with liquid media - 400 ml PDB (experiment 1) and, respectively, 300 ml MEB (experiment 2), both with an initial pH of 6.5. Incubation of cultures was performed at 27-28℃, with shaking at 100 rpm. Measurements of mycelial extension onto solid medium were performed at 14 days after inoculation with irradiated and non-irradiated mycelium. The submerged cultures were incubated for 27 days (test 1) and 21 days (test 2). Then they were filtered and lyophilized (test 1), respectively dried in the oven (test 2) and the obtained biomass was weighed. The experiments were performed in a randomized design, with 3 replicates/treatment/variant, and the results were expressed as mean values ± SD.

RESULTS AND DISCUSSIONS

In our first experiment, in all three *P. eryngii* strains tested, the amount of biomass obtained by growing non-irradiated mycelium (control) in submerged culture (PDB medium) exceeded that obtained in any of the variants with irradiated mycelium (Figure 1). The non-irradiated strain PeM-39/0 Gy produced a freeze-dried mycelial biomass amount of 1.2097 g (mean/replicate), higher than that obtained by the other two strains with non-irradiated mycelium, PeM-41/0 Gy (1.0813 g) and PeM-45/0 Gy (1.1015 g).

As shown in the graph in figure 1, the increase of the irradiation dose decreased the amount of biomass produced by the irradiated mycelia upon the first transfer to fresh medium. In the case of the PeM-41 strain the mass of the harvested and dried mycelium decreased progressively, inversely proportional to the value of the applied gamma radiation dose. Not the same thing happened in the other two strains, where irradiated mycelia with doses of 100 Gy and 200 Gy each produced more biomass than in the variants irradiated with 50 Gy.

Note that in these two strains, PeM-39 and PeM-45, the amount of biomass produced by irradiated mycelia at the dose of 200 Gy is higher, compared to the values associated with the lower irradiation doses, respectively 50 Gy and 100 Gy. These results may be correlated with those obtained by Rashid *et al*. (2016) in the study of mycelium irradiated by *Pleurotus sajor-caju,* from which they obtained cultures on bags with the highest yield of mushroom harvest at the dose of 200 Gy.

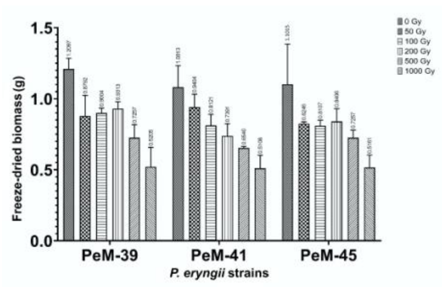

Fig. 1 Fungal biomass obtained in submerged cultures (test 1)

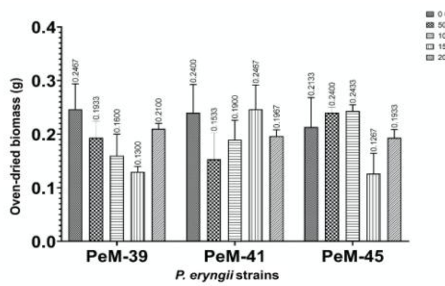

Fig. 2 Fungal biomass obtained in submerged cultures (test 2)

Analyzing the data from experiment 2, regarding the amount of *P. eryngii* oven-dried fungal biomass obtained from subcultures in MEB liquid medium (first transfer), it is observed that in this trial, the non-irradiated control samples produced more biomass than most irradiated probes (fig. 2). Thus, in the PeM-39 strain, from the non-irradiated control (0 Gy), an average of 0.2467 g/replicate dried fungal biomass was obtained, exceeding all variants with irradiated mycelium. In PeM-41, in the case of samples irradiated with 150 Gy (0.2467 g), the quantity of dry biomass exceeded that of the non-irradiated control (0.2400 g). In the PeM-45 strain, the control variant (0.2133 g) was overtaken by two irradiated variants: 50 Gy (0.2400 g) and 100 Gy (0.2433 g) respectively.

As in experiment 1, the results obtained by applying the irradiation dose of 200 Gy are to be noted. The amount of biomass obtained from the mycelium of the PeM-39 strain following irradiation at this dose (0.2100 g) exceeds that obtained by applying smaller doses, namely 50 Gy (0.1933 g), 100 Gy (0.1600 g) and 150 Gy (0.1300 g). In the PeM-41 strain, 200 Gy (0.1967 g) irradiated samples yielded more biomass than 50 Gy (0.1533 g) and 100 Gy (0.1900 g) irradiated samples. In PeM-45 strain, mycelia irradiated with 200 Gy (0.1933 g) produced more biomass than those irradiated with 150 Gy (0.1267 g).

In the case of *P. eryngii* cultures grown on solid MEA medium, colony radius measurements were made at 14 days after inoculation. The statistical interpretation of the results regarding the combined influence of the strain and the dose of gamma irradiation on the growth of the mycelium is shown in table 1.

Table 1

Combined influence of strain and irradiation dose on *P. eryngii* mycelium growth (MEA medium, pH 6.5, 14th-day incubation at 27-28 °C)

No.	Strain/Dose (Gy)	Mycelium growth			
		Mean radius of the colony (mm)	%	Difference (+/- mm)	Significance
1	PeM-39/0 (Ctrl)	35.25	100.00	-	-
2	PeM-39 / 50	33.63	95.40	-1.63	-
3	PeM-39 / 100	33.08	93.04	-2.17	-
4	PeM-39 / 150	31.46	89.24	-3.79	-
5	PeM-39 / 200	34.37	97.50	-0.88	-
6	PeM-39 / 300	29.04	82.38	-6.21	00
7	PeM-41 / 0	37.16	105.41	+1.91	-
8	PeM-41 / 50	37.29	105.78	+2.04	-
9	PeM-41 / 100	36.79	104.37	+1.54	-
10	PeM-41 / 150	28.63	81.21	-6.62	00
11	PeM-41 / 200	32.54	92.31	-2.71	-
12	PeM-41 / 300	30.83	86.46	-4.42	0
13	PeM-45 / 0	34.45	97.63	-0.80	-
14	PeM-45 / 50	32.92	93.39	-2.33	-
15	PeM-45 / 100	32.63	92.53	-2.62	-
16	PeM-45 / 150	31.96	90.66	-3.29	-
17	PeM-45 / 200	33.58	95.26	-1.67	-
18	PeM-45 / 300	25.04	71.03	-10.21	000
	DL 5%			4.18	
	DL 1%			5.53	
	DL 0.1%			7.25	

Taking as a control the variant represented by the non-irradiated PeM-39 strain, it turns out that it was only surpassed by the PeM-41 strain in the non-radiated (0 Gy) variants and irradiated with 50 Gy and 100 Gy with the insignificant differences of 1.91 mm, 2.04 mm and 1.54 mm respectively.

The rest of the variants had slower growths compared to the control PeM-39, statistically insignificant with the exception of 4 variants: PeM-39/300 Gy, PeM-41/150 Gy, PeM-41/300 Gy and PeM-45/300 Gy. All the three verified strains showed at 300 Gy smaller growth than the non-irradiated control, with statistically assured values also in the negative sense. Thus, in PeM-39/300 Gy and PeM-41/150 Gy the differences from the control are distinctly significant in the negative sense, having values lower than the non-irradiated PeM-39 by -6.21 mm, respectively -6.62 mm. PeM-45/300 showed the slowest growth in the test, with -10.21 mm smaller than the control, a very significant negative result.

The dose of 200 Gy is highlighted by the small differences recorded between the control and each of the three strains irradiated with this dose. In the case of PeM-39 (-0.88 mm) and PeM-45 (-1.67 mm), these differences are the smallest, compared to the differences corresponding to the other doses applied to these strains.

In Table 2 it is shown that irrespective of the applied irradiation dose, the PeM-41 strain showed a significant faster growth by 1.07 mm than the PeM-39 strain (control) and the PeM-45 strain had a slower growth than the control with a difference of -1.04 mm, also significant.

Table 2

Influence of the strain on the growth of irradiated and non-irradiated mycelium by *P. eryngii* (MEA medium, pH 6.5, 14th-day incubation at 27-28 ° C)

Strain	Mycelium growth			
	Mean radius of the colony (mm)	%	Difference (+/- mm)	Significance
PeM-39 (Ctrl)	32.80	100.00	-	-
PeM-41	33.87	103.26	+1.07	*
PeM-45	31.76	96.82	-1.04	0

DL 5% 0.96
DL 1% 1.27
DL 0.1% 1.67

In table 3 it is shown that in the three verified strains the irradiation produced the decrease of the growth rate of mycelium. At 50 Gy the decrease in the growth rate of mycelium was insignificant. Doses of 100 Gy, 150 Gy, 200 Gy and 300 Gy had a statistically assured negative influence.

Table 3

Influence of irradiation dose on the growth of *P. eryngii* mycelium (MEA medium, pH 6.5, 14th-day incubation at 27-28 ° C)

Irradiation dose (Gy)	Mycelium growth			
	Mean radius of the colony (mm)	%	Difference (+/- mm)	Significance
0	35.62	100.00	-	-
50	34.61	97.16	-1.10	-
100	34.17	95.92	-1.45	0
150	30.68	86.13	-4.94	000
200	33.49	94.02	-2.13	00
300	28.30	79.44	-7.32	000

DL 5% 1.37
DL 1% 1.80
DL 0.1% 2.37

The largest differences from the non-irradiated control strain were recorded in the case of 150 Gy and 300 Gy doses, with values of -4.94 mm, respectively -7.32 mm, both being very significant in the negative sense. Note the dose of 200 Gy, at which the verified mycelia showed faster growths than those irradiated with a lower dose, respectively 150 Gy.

CONCLUSIONS

1. In our first experiment, in all three *P. eryngii* strains tested, the amount of biomass obtained by growing non-irradiated mycelium (0 Gy) in submerged culture (PDB) exceeded that obtained in any of the variants with irradiated mycelium (50, 100, 200, 500, 1000 Gy). In the second experiment, with irradiated (50, 100, 150, 200, 300, 1500 Gy) mycelia subcultured in MEB liquid medium, the non-irradiated control samples produced more biomass than most irradiated probes, with few exceptions: PeM-41/150 Gy, PeM-45/50 Gy, PeM-45/100 Gy.

2. The non-irradiated strain PeM-39 produced, in both tests, an amount of mycelial biomass higher than the other two strains, PeM-41/0 Gy, PeM-45/0 Gy.

3. The growth performances of non-irradiated mycelia (control): the PeM-41 strain showed the fastest growth onto agar media, with the colony radius measuring 37.16 mm after 14 days of incubation (MEA medium), followed by PeM-39 with 35.25 mm and PeM-45, with 34.45 mm.

4. The effect of the dose of 200 Gy, compared with the lower doses of 50, 100, 150 Gy was manifested in the first passage of irradiated mycelia by higher growth rates onto agar media in the case of PeM-39 and PeM-45 strains.

5. In these two strains, PeM-39 and PeM-45, the amount of biomass produced in submerged culture (PDB, 1^{st} test) by irradiated mycelia at the dose of 200 Gy was higher, compared to the values associated with the lower irradiation doses, respectively 50 and 100 Gy. Similar results were recorded in the 2^{nd} test, with PeM-39 and PeM-41 strains. This could indicate that the recovery processes following irradiation occur faster and more efficiently in mycelia treated with this dose (200 Gy), compared to the others.

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