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INCREASING THE NUTRITIONAL QUALITY OF *PLEUROTUS ERYNGII* BY GAMMA IRRADIATION OF LIVING MYCELIUM

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Abstract

Pleurotus eryngii, known as oyster mushroom, is a commercially cultivated edible mushroom, widely appreciated for its good taste and texture. In this study, we investigated the influence of low doses of gamma irradiation (between 100 and 300 Gy) applied to the living mycelium of two strains of P. eryngii (PeM39 and PeM41), in order to enhance the synthesis of bioactive metabolites with nutritional values. For this purpose, we determined the content of polyphenols, flavonoids, proteins and carbohydrates in ethanol extracts (70%) of P. eryngii fruiting bodies obtained from irradiated mycelium. The results showed that, in the fruiting bodies of both strains, the 300 Gy dose was optimal for stimulating an increase in phenols, flavonoid and carbohydrate content, and the 200 Gy dose was optimal for increasing protein content. Thus, gamma-irradiation treatment of mycelial inoculum may be an effective tool for stimulating the synthesis of secondary metabolites with antioxidant and nutritional properties in P. eryngii.

Key words*: Fruiting bodies, gamma irradiation, mycelium, metabolites synthesis, Pleurotus eryngii.*

INTRODUCTION

Pleurotus eryngii (also known as king oyster mushroom) is an edible mushroom cultivated widely in many regions of the world. *P. eryngii* is reportedly rich in protein, carbohydrates, unsaturated fatty acids, vitamins and other nutrients and is low in fat, making it a high-quality, low-calorie food. In addition, *P. eryngii* is also a rich source of the disaccharide, trehalose (Reis et al., 2012). Polysaccharides from *P. eryngii* have a variety of biological activities, including anti-oxidant, anti-hyperlipidaemia, antitumor, bacteriostatic and immune-regulatory (Zhang et al., 2020). Phenolic compounds are mushroom antioxidants which are strong radical scavengers and free radical inhibitors and phytonutrients (Michalak, 2006). Gamma irradiations are physically induced stresses that has been considered as the rapid and fast method to enhance the quality and quantity of plant characteristics (Patil et al., 2018). Bioactive compounds such as phenols and flavonoids can be induced under proper dose of radiation. Both polyphenols and flavonoids are chemical compounds, synthesized by the body, involved in antioxidant defence (Pelcaru et al., 2021). Plants exposed to gamma radiation produce various defence and antioxidant enzymes, many of which produce secondary metabolites that activate the induction of oxidative stress conditions. Gamma irradiation exerts its effects by inducing oxidative stress in the cells which increases the total phenol due to the release of phenolic compounds from glycosides components (Patil et al., 2018). Gamma irradiation is considered to be a physically induced stress on living organisms or cells. Radiation treatment can be a much faster way to quantitatively improve the chemical synthesis of antioxidant compounds that may play a role in defending irradiated tissue (Charbaji and Nabulsi, 1999). Most research uses gamma radiation as a conditioning agent for harvested mushrooms or their extracts to increase nutrient or antioxidant levels by breaking down existing compounds (Kim et al., 2009; Fernandes et al., 2012). In this study, we used gamma irradiation on the living mycelium of two isolates of *P. eryngii* (PeM39 and PeM41) as a tool to stimulate the

production of biologically active metabolites in the fruiting bodies.

MATERIALS AND METHODS

Mushroom material. *Pleurotus eryngii* used in this study were purchased from the Research and Development Institute for Vegetable and Floriculture Vidra and consisted of two isolates named PeM39 and PeM41. Fruiting bodies of the two isolates were obtained from living mycelium irradiated at different irradiation doses.

Irradiation of the mycelium of *P. eryngii*. The irradiation was performed by using a ^{60}Co research irradiator GC-5000 (B.R.I.T. Mumbai, India) located in IRASM Radiation Processing Department of "Horia Hulubei" National Institute of Physics and Nuclear Engineering (Romania). Mycelial samples were acutely exposed to gamma rays at following average doses: 0 (control), 100, 200, and 300 Gy (Gray) respectively, at a dose rate of 0.8 Gy/s. An alanine dosimetry system was used for dose evaluation. The reference material for the doses is water. Irradiation temperature, as measured inside the irradiation chamber, was in the range of 27–28^{0}C.

Mycelium cultivation and mushroom production. The irradiated mycelium of the two *P. eryngii* isolates was grown at the Vidra Institute on a substrate composed of a mixture of sawdust, straw and corn stalks enriched with nitrogen-rich additives. The first carpophores appeared after about 10-15 days and after another few more days the mushrooms were harvested.

Preparation of *P. eryngii* extracts. The dried mushrooms were shredded using a blender. 50 ml of ethanol (95^0) was added to 5 g of powder of the two *P. eryngii* isolates. Rotary evaporator (Büchi, Model R-205V, Merck USA) was used to remove the solvents of the filtrate. The residues obtained were weighed and dissolved in 70% ethanol. The final concentration of the extracts was 100 mg / ml.

Total phenols content. The concentration of phenolic compounds in ethanol extracts of mushrooms was determined by using the Folin-Ciocâlteau (Sigma-Aldrich) method (Singleton, 1999). This is based on the reduction of the Folin-Ciocalteu reagent by phenolic compounds, which will form chromogens that can be detected spectrophotometrically at OD= 765 nm. For the determination of phenols, a calibration curve was carried out starting from standard solutions of Gallic acid. The total phenol content in the ethanol extracts was calculated based on the regression equation (y = 0.001x + 0.0041; R = 0.9999) obtained for different Gallic acid concentrations (50 - 500 mg/l) and expressed in milligrams of Gallic acid equivalents per liter extract (mg GAE/l). Both the samples and the reference substance were worked in triplicate, and their average absorbance value was calculated.

Total flavonoid content. The flavonoid content in ethanol extracts of *P. eryngii* isolates obtained from irradiated mycelium was quantified according to the colorimetric method of aluminum chloride, described by Piyanete et al., 2009. To achieve the reaction, 150 µl AlCl3 10% solution was added over the sample/ standard quercetin solution, and the mixtures was incubated at room temperature for 5 min., after which 200 µl of 1M sodium hydroxide solution was added sequentially. The standard curve was made of quercetin and comprised 5 dilutions (0.1, 0.5, 1.0, 1.5 and 2.5 mg/mL), starting from a stock solution of 1 mg/mL in 80% methanol. The absorbance was read at 510 nm, and the flavonoid content was calculated based on the regression equation: y = 0.3604x + 0.0269; R = 0.9534 and expressed in mg of quercetin equivalents (QE) per mL of extract.

Total proteins content. Total proteins content was quantified according to Bradford method (1976). The method is based on the use of Coomasie Blue G dye as reaction substrate. For determination of total proteins content a standard curve was made starting from standard BSA (bovine serum albumin) solutions (5 – 50 µg/ml). The reaction mixtures (Bradford reagent with samples or standard solutions of BSA) were measured spectrophotometrically at a wavelength of 595 nm. The total protein content was calculated based on the regression equation: y = 0.0025x + 0.2233; R = 0.8099. The results obtained were expressed in µg BSA/ml.

Total carbohydrates content. The total amount of carbohydrates was estimated using phenol-sulfuric acid method (Dubois et al., 1956) with a few modifications and proceeded

with a curve where glucose concentrations ranging from 10 to 80 µg. After sample preparation, aliquots of 0.2 to 1.5 mL were pipetted into test tubes and the volume was filled until 2.0 mL with 0.8 mL of 5% (w/w) phenol and 5 mL of sulfuric acid. The tubes were then shaken and left to stand for 30 minutes for further reading in a spectrophotometer (Eppendorf UV-VIS) at 490 nm. The total sugar content in the tested samples was calculated based on the regression equation: y = 0.0061x + 0.1014; R = 0.9987. Results were expressed in µg glucose /ml.

Statistical Analysis. All experiments were performed in three replicates. Results were expressed as mean values (SEM) and standard deviation (±SD). The graphics were plotted by using Microsoft Office Excel 2010.

RESULTS AND DISCUSSIONS

Two *Pleurotus eryngii* isolates, PeM 31 and PeM 41, grown from mycelium irradiated at 100, 200 and 300 Gy, were harvested and analysed for total phenols, flavonoids, proteins and total sugars (carbohydrates). Analyses were performed on 70% ethanol extracts from the fruiting bodies of the two isolates.

Quantitative estimation of total phenol content. Phenols are important constituents of plants which are responsible for scavenging the free radical due to the presence of hydroxyl groups and it directly responsible for the anti-oxidative potential (Sharififar et al., 2009). The estimation of total phenolic content was expressed in terms of Gallic acid equivalent in milligram (mg) per litre. The calibration curve obtained from y equation (y = 0.001x + 0.0041; R = 0.9999) was used to determine the total phenol content (Figure 1). High phenol content values were found in both isolates of *P. eryngii* grown from mycelium irradiated at 200 and 300 Gy. Compared to the controls (PeM39 = 217.9±1.53 mg GAE/l and PeM41= 216.9±0.58 mg GAE/l, respectively), isolates from mycelia irradiated at 300 Gy showed the highest phenol concentrations: 312.23±2.91 mg GAE/l in *P. eryngii* PeM39 extracts and 237.96±1.53 mg GAE/l in *P. eryngii* PeM41 extracts. (Figures 1 and 2, Table 1).

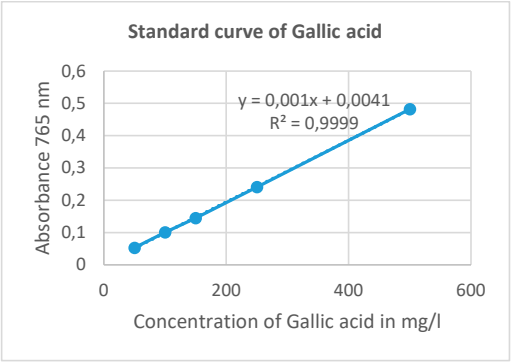

Figure 1. Gallic acid standard curve and the regression equation

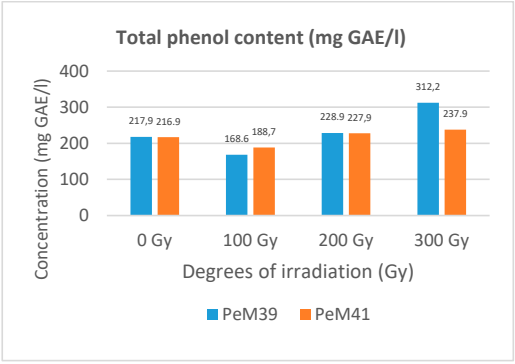

Figure 2. Total phenol content (mg GAE/l) in *P. eryngii* PeM39 and PeM41 from irradiated mycelium. Data represent the mean ± SD, $n = 3$

Estimation of total flavonoid content

Flavonoids are secondary metabolite having antioxidant properties (Wong and Chye, 2009). The total flavonoid content was expressed in terms of quercetin equivalent in mg/ ml. The calibration curve obtained from y equation (y = 0.3604x + 0.0269; R = 0.9534) was used to determine flavonoid content as shown in Figure 3. The total flavonoid content of the various extract of *P. eryngii* from gamma irradiated mycelium are represented in Figure 4 and Table 1. In this case, both isolates of *P. eryngii* grown from mycelium irradiated at 200 and 300 Gy showed the highest flavonoids concentrations in comparison to the controls (PeM39 = 1.53±0.01 mg QE/ml and PeM41 = 1.63±0.06 mg QE/ml). Irradiation dose of 300 Gy showed the highest amounts of content 2.15±0.09 mg QE/ml in PeM39 samples and 2.51±0.09 mg QE/ml in PeM41 samples.

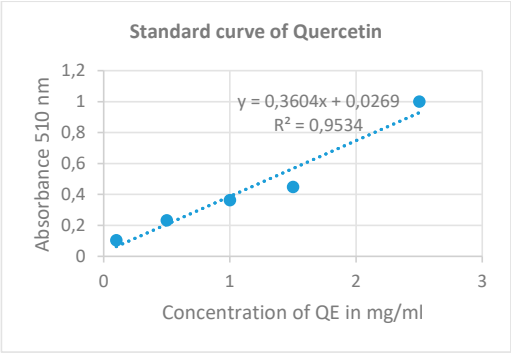

Figure 3. Standard curve of Quercetin and the regression equation

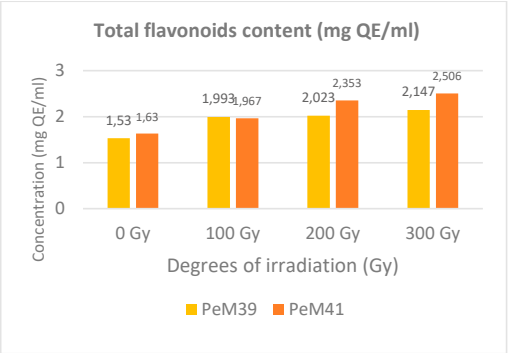

Figure 4. Total flavonoid content (mg QE / ml) in *P. eryngii* isolates from irradiated mycelium. Data represent the mean ± SD, $n = 3$

Estimation of total proteins content

The total protein content was calculated based on the regression equation: y = 0.0025x + 0.2233; R = 0.8099. The results obtained were expressed in µg BSA/ml (Figure 5). High protein content values were found in both isolates of *P. eryngii* grown from mycelium irradiated at 100 and 200 Gy. Compared to the controls, 14.08±3.44 mg µg BSA /ml in PeM39 extracts and 17.28±2.2 µg BSA /ml in PeM41 extracts, isolates from mycelia irradiated at 200 Gy showed the highest proteins values, 33.68±4.61 µg BSA /ml in *P. eryngii* PeM39 extracts and 21.68±0.2 µg BSA /ml in *P. eryngii* PeM41 extracts. (Figure 6, Table 1).

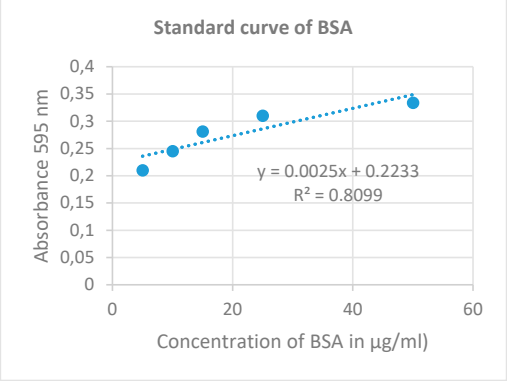

Figure 5. Standard curve of BSA and the regression equation

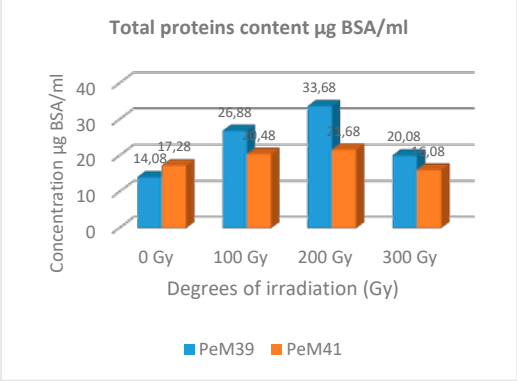

Figure 6. Total protein content (µg BSA / ml) in samples of *P. eryngii* isolates grown from irradiated mycelium. Data represent the mean ± SD, $n = 3$

Estimation of total carbohydrates

The concentration of total carbohydrates content in the tested samples was calculated based on the regression equation: y = 0.0061x + 0.1014; R = 0.9987 (Figure 7). Results were expressed in µg glucose /ml. In this study, the 92±6.53 µg glucose /ml and in PeM41 with 66.27±5.17 µg glucose /ml as compared to controls which are 51.41±5.79 µg glucose /ml for PeM39 and 56.16±1.25 µg glucose /ml for PeM41 (Figure 8 and Table 1). It was found that both isolates from mycelium irradiated at highest total carbohydrates values were found in both isolates of *P. eryngii* grown from mycelium irradiated at 300 Gy. At this dose, highest values of total carbohydrates were found in PeM39 with 63.

100 Gy, the carbohydrate concentration decreased compared to the controls, and increased with increasing irradiation at 200 Gy and 300 Gy respectively.

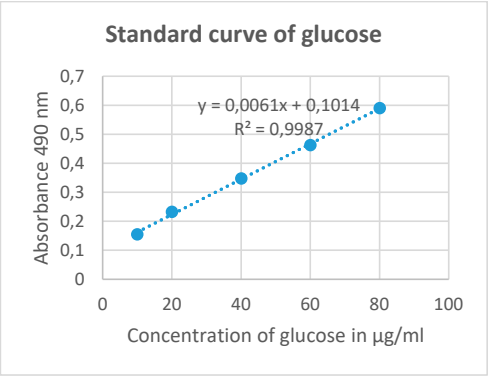

Figure 7. Standard curve of Glucose and the regression equation

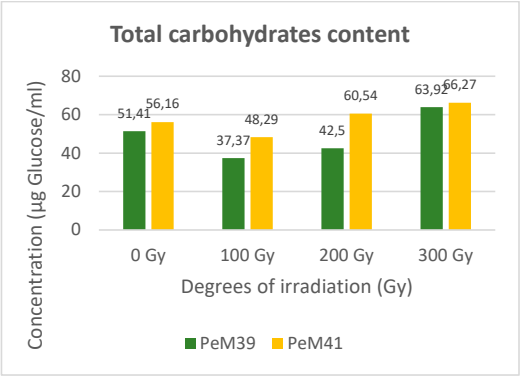

Figure 8. Total carbohydrate content (µg Glucose / ml) in *P. eryngii* isolates grown from irradiated mycelium. Data represent the mean ± SD, $n = 3$

Table 1 shows the data obtained on the total content of phenols, flavonoids, proteins and carbohydrates in ethanol extracts of *P. eryngii* PeM 39 and *P. eryngii* PeM 41 obtained from fruiting bodies results from mycelia subjected to different degrees of irradiation.

These data reveal that the total phenol content decreased in both isolates originating from fungal mycelium irradiated at 100 Gy and then increased from 200 Gy to 300 Gy.

This indicates that there is an accumulation of phenolic compounds in the body of the fungus with increasing irradiation doses. The same situation was found for the carbohydrate content. Initially, in both isolates grown from the fungal mycelium irradiated at 100 Gy the carbohydrate content decreased significantly, and then there was an increase in carbohydrate content with increasing irradiation doses.

Estimation of total flavonoid content showed that flavonoids accumulate in the fungus with increasing irradiation levels, which has been observed in both isolates.

It was also observed that the irradiation doses of 100 Gy and 200 Gy, to which the mycelium of the two isolates were subjected, were optimal for the accumulation of higher amounts of protein in the body of the fungus.

Table 1. Total phenols, flavonoids, proteins and carbohydrates content in fruiting bodies of *P. eryngii* isolates from gamma irradiated mycelium

Sample	Irradiation dose (Gray)	Total phenols (mg GAE /l)	Total flavonoid (mg QE /ml)	Proteins (µg BSA /ml)	Carbohydrate (µg Glucose /ml)
***P. eryngii* PeM 39**	**0**	217.90±1.53	1.53±0.01	14.08±3.44	51.41±5.79
	100	168.57±1.33	1.99±0.05	26.88±1.00	37.37±4.47
	200	228.90± 5.03	2.02±0.03	**33.68±4.61**	42.50±0.92
	300	**312.23±2.91**	**2.15±0.09**	20.08±0.64	**63.92±6.53**
Sample	**Irradiation dose (Gray)**	**Total phenols (mg GAE /l)**	**Total flavonoid (mg QE /ml)**	**Proteins (µg BSA /ml)**	**Carbohydrate (µg Glucose /ml)**
***P. eryngii* PeM 41**	**0**	216.90±0.58	1.63±0.06	17.28±2.20	56.16±1.25
	100	188.92±4.51	1.97±0.03	20.48±0.60	48.29±1.77
	200	227.91±8.39	2.35±0.22	**21.68±0.20**	60.54±4.52
	300	**237.96±1.53**	**2.51±0.09**	16.08±1.43	**66.27±5.17**

Data represent the mean ± SD, $n = 3$

CONCLUSIONS

As a result of these studies, it can be concluded that the increase in irradiation at doses ranging from 200 to 300 Gy, which was subjected to the mycelium from which the fruiting bodies resulted, leads to an accumulation of phenolic compounds, flavonoids and carbohydrates. Protein accumulation occurred only in isolates grown from mycelium irradiated at doses lower than 300 Gy. To our knowledge, this is the first work showing the use of sub lethal doses of gamma irradiation as a treatment of *P. eryngii* living mycelium for increasing synthesis of biologically active metabolites with antioxidant and nutritional properties in fruiting bodies of the fungus.

REFERENCES

Bradford, M. M., (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein – dye biding. *Analytical Biochemistry*, 72 (1–2). 248–254.

Charbaji, T.; Nabulsi, I., (1999). Effect of Low Doses of Gamma Irradiation on in Vitro Growth of Grapevine. *Plant. Cell Tissue Organ. Cult*., 57. 129–132.

Dubois, M. K., Gilles, J. K, Hamilton J. K., Rebers, P. A., Smith F., (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem*., 28. 350-356.

Fernandes, A., Antonio, A. L., Oliveira, M. B., Martins, A., & Ferreira, I. C., (2012). Effect of gamma and electron beam irradiation on the physico-chemical and nutritional properties of mushrooms: a review. *Food Chem*., 135(2). 641–650.

Kim, J. H., Sung, N. Y., Kwon, S. K., Srinivasan, P., Song, B. S., Choi, J. I., et al. (2009). Gamma-Irradiation improves the color and antioxidant properties of Chaga mushroom (*Inonotus obliquus*) extract. *J. Med. Food*, 12(6). 1343–1347.

Michalak A. (2006). Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Pol J Environ Stud* 15:523–530.

Patil AS, Suryavanshi P, Fulzele D., (2018). Evaluation of Effect of Gamma Radiation on Total Phenolic Content, Flavonoid and Antioxidant Activity of *In Vitro* Callus Culture of *Artemisia annua*. *Nat Prod Chem Res* 6: 345.

Pelcaru C.F., Ene M., Petrache A.M., Negrut D.N., (2021). Low Doses of Gamma Irradiation Stimulate Synthesis of Bioactive Compounds with Antioxidant Activity in *Fomes fomentarius* Living Mycelium. *Applied Sciences* 11(9):1-12.

Piyanete C, Meechai P, Nakbanpotecc W., (2009). Sci. Antioxidant activities and phenolic contents of extracts from *Salvinia molesta* and *Eichornia crassipes*. *Res J Biol*.; 4:1113-1117.

Reis F.S., Barros L., Martins A., Ferreira I.C.F.R., (2012). Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: an inter- species comparative study, *Food Chem. Toxicol*. 50 (2); 191–197.

Sharififar F, Dehghn-Nudeh G, Mirtajaldini M., (2009). Major flavonoids with antioxidant activity from *Teucrium polium* L. *Food Chemistry* 112: 885-888.

Singleton V. L., Orthofer R., Lamuela-Raventos R. M., Lester P., (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Meth. Enzymol*; 299, 152-178.

Wong J.Y,, Chye F.Y., (2009). Antioxidant properties of selected tropical wild edible mushrooms. *Journal of Food Composition and Analysis* 22 : 269-277.

Zhang, B., Li, Y., Zhang, F., Linhardt, R. J., et al. (2020). Extraction, structure and bioactivities of the polysaccharides from *Pleurotus eryngii*: A review. *International Journal of Biological Macromolecules*, Vol. 150 : 1342 – 1347.