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Evaluation of Mycelia Growth, Morphology and Yield for Low Dose Gamma Irradiated Grey Oyster Mushroom *Pleurotus sajor-caju*

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ABSTRACT: Mycelium growth and yield of irradiated grey oyster mushroom *Pleurotus sajor-caju* by gamma rays was investigated due to effects of irradiation. In order to establish the effect, mycelium of *P. sajor-caju* was irradiated by gamma rays at dose 0.1 to 0.6 kGy with dose rate 0.227 Gy sec^{-1} at the radiation facility in Malaysian Nuclear Agency. The radiation effects were evaluated on growth rate of irradiated mycelia, induction of different mycelia types, colonization period on substrate, size of fruit bodies and mushroom yields. The results shown that growth rate of irradiated mycelium were slightly slower than the control and decreased as the dose increased. Irradiation was found can induced more fruit bodies with no significant different on size of fruit bodies. The mushroom yield represented by BE of irradiated mycelium is higher than control and increased as the dose increased. Irradiation was found sufficient to increase the yield of mushroom and it is directly to support local mushroom industry.

KEYWORDS: gamma rays, radiation effects, irradiated mycelium, growth rate, yield.

1 INTRODUCTION

The Malaysian mushrooms industry is new and small. However it is growing to cater for increasing local demand as food sources as well as new sources of wealth for farmers [5]. The most common species cultivated is *Pleurotus sajor-caju* [7] as this species can grow well at temperature between 21 to 29 C [1]. According to [3], Malaysia imported about 90% of mushroom mainly from countries such as China, Thailand and Japan to meet the local needs from 2001 to 2004.

Despite an increasing demand, local growers face many challenges such as unavailability of quality seed, high percentage of contamination, and low mushroom yields [4]. Therefore, new mushrooms varieties strains with high yield and novel character, such as fast colonization and disease resistant are needed to support the development and sustainability of the industry. Several methods can be applied to develop new mushrooms varieties strains such as mutation induction by chemical and radiation [1].

In this works, development of new strain using gamma rays radiation induced mutation method was studied. The aim of this study is to evaluate growth performance of irradiated mycelium and the mushrooms yield. The scopes of this work covers on irradiation, sub-culturing of mushroom mycelia, mushroom seed preparation of selected irradiated mycelia, cultivation of mushroom, characterization of mycelia colonization on substrate over the period of mycelia growing, morphology of fruit bodies and the mushrooms yield.

2 Materials And Methods

2.1 Mycelium Culture

The mushrooms strain used in this study was *Pleurotus sajor-caju* strains ATCC 32078 which was purchased from American Type Culture Collection (ATCC) and deposited at Sterifeed Mushrooms Culture Collection Centre, Malaysian Nuclear Agency under the purchased agreement for non-commercial application.

2.2 Sample Preparation and Irradiation

Selected mycelia of *P. sajor-caju* were cultured onto potato dextrose agar (PDA) in petri dishes, incubated for 10 days and irradiated. The irradiation of *P. sajor-caju* mycelia was carried out at the Malaysian Nuclear Agency's Biobeam GM 800 gamma irradiator which use Cesium-137 as gamma radiation source at a dose rate of 0.227Gy sec^{-1}. Samples of mycelia were exposed to gamma rays at different dose levels; 0 (control), 0.1, 0.2, 0.4 and 0.6 kGy respectively. These doses were defined as low dose irradiation. The data for survival percentage of irradiated mycelium were collected from observation on the growth of mycelium for 12 days. Culture that showed no growing activity during this period was considered as not survives.

2.3 Liquid Seed Preparation

The irradiated mycelia were subcultured onto PDA petri dishes. The growth of the subcultured of irradiated mycelia on new PDA petri dishes were observed every two days for a period of two weeks. The growth performances of the mycelia were determined by measuring the radial diameter of mycelia growth on PDA. In addition, types of mycelia obtained as the result of irradiation were classified into three groups i.e. mycelia only, a mixed of mycelia and primordia, and primordia only. For each dose, the mixed mycelia and primordia type with faster grows was selected to be used for liquid seed preparation for mushroom cultivation.

2.4 Mushroom Cultivation

Mushrooms cultivation substrates consisting mixture of saw dust, limestone powder and rice bran, with 70% moisture content were prepared. Substrates were packed into plastic bags with an average weight of 0.7 kg per bag. Substrates bag were sterilized using autoclave at 121 °C for 1 hrs. In the cultivation process, 20 ml mature mushroom seed was inoculated into each substrate bag. After inoculation, the substrates were incubated on the racks in the incubation room at ambient temperature. The incubation process was allowed into a certain period until the substrate bags log were fully grown and covered by mycelia. The growth of mycelia on the bag log substrate were monitored periodically and recorded every week.

2.5 Mushroom Fruiting

The bags containing fully grown and thick mycelia were opened for fruiting process. It took three to five days for fruit bodies of the mushrooms to emerge from the substrate. The fruit bodies were harvested and the size and weight of each fresh mushrooms fruit bodies were recorded. The size of mushroom fruit body was determined by measuring the diameter of mushroom pileus.

3 Results And Discussion

3.1 Growth Rate of Irradiated Mycelium on PDA

The growth rate of irradiated *P. sajor-caju* mycelium on PDA is shown in Table 1. The effect of radiation on growth rate of the irradiated mycelium was clearly observed after 6^{th} days. The growth rate of irradiated mycelia was slower than control and decreased as the irradiation dose increased. In 12 days incubation period, control mycelium has fully grown, covering the PDA surface whereas the irradiated mycelium has not.

Table 1. Growth rate of irradiated P. sajor-caju mycelium on PDA

Dose (kGy)	Average of mycelium diameter (cm)/day±S.D.					
	2	4	6	8	10	12
0 (control)	0.86±0.06	1.6±0.10	2.85±0.09	4.49±0.08	6.66±0.13	8.42±0.05
0.1	0.75±0.11	1.3±0.13	1.83±0.26	3.63±0.53	5.11±1.17	6.38±1.07
0.2	0.72±0.12	1.27±0.27	1.82±0.42	3.15±0.79	4.21±1.38	5.93±1.45
0.4	0.69±0.11	1.11±0.15	1.49±0.22	1.89±0.28	2.48±0.32	3.79±0.36
0.6	0.62±010	0.77±0.20	1.23±0.48	1.80±0.92	2.47±1.16	3.31±1.37

Incubation was carried out for 12 days at 25-27°C
Values are means S.D. ± of replicate

3.2 GROWTH RATE OF IRRADIATED MYCELIUM ON CULTIVATION SUBSTRATE

The results of growth rate and colonization period of irradiated mycelium *P. sajor-caju* on substrate are shown in Table 2. The growth rate of irradiated mycelium slightly lower than control and decreased as the irradiation dose increased. Irradiation can cause injury to mycelium cell, therefore at certain dose of irradiation mycelium is unable to grow [2].

The growth rate of mycelium could effect the colonization period of mycelium on substrate. Mycelium with fast growths rate will colonize the substrate in shorter period. Results on colonization periods of mycelium on substrate are shown in Table 2. The colonization periods of irradiated mycelium on substrate took longer time compared to control mycelia. It implies that, irradiation had an effect on growth rate of mycelium on cultivation substrate.

Table 2. Growth rate and colonization period of irradiated mycelium P. sajor-caju

Dose (kGy)	Growth rate (cm/ day±S.D.)	Colonization period (weeks)
0	0.60±0.07	6-7
0.1	0.37±0.02	10-11
0.2	0.30±0.03	10-11
0.4	0.23±0.03	11-12
0.6	0.16±0.02	12-13

3.3 MUSHROOM MORPHOLOGY

Mushroom fruit bodies were classified into 4 classes of morphology, i.e straight, curly, mixed of straight and curly, and stunted [6]. The number of mixed straight and curly fruit body has increased from 33.3% for control to 40 %, 50 %, 45.8 % and 67.6 % when the doses of irradiation is increased to 0.1 kGy, 0.2 kGy, 0.4 kGy and 0.6 kGy respectively. Mushroom fruit bodies with morphology mixed straight and curly was increased compared than control and it was increased when the dose increased. The morphology of break type mushroom was only observed at irradiation dose of 0.4 kGy (Fig.1).

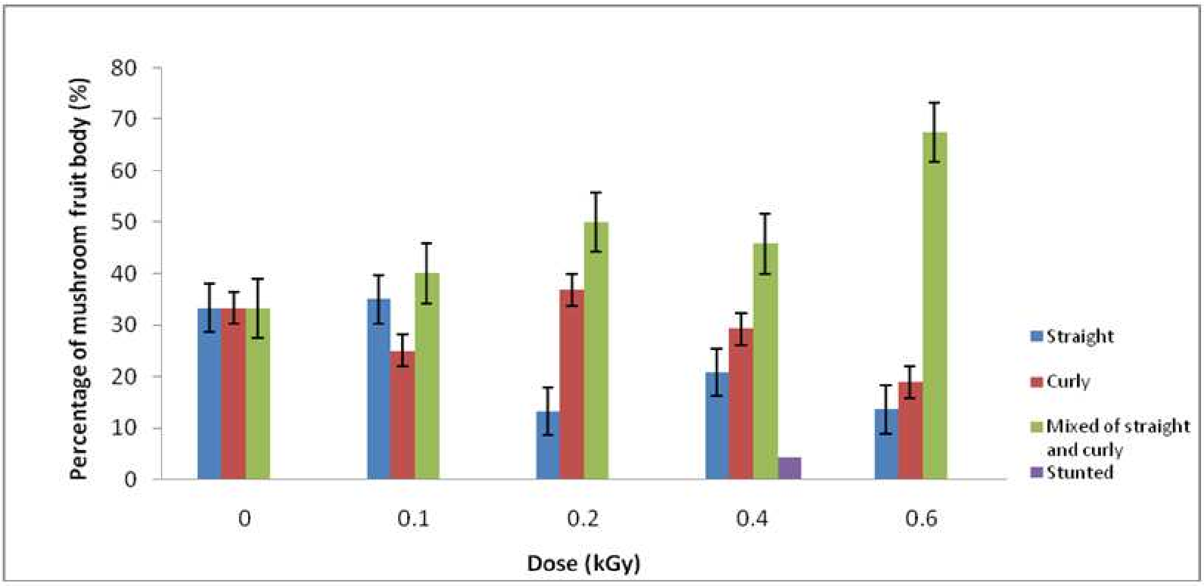

Fig.1 Percentage of morphology fruiting bodies for different doses of irradiation

3.4 MUSHROOM YIELD

The results of weight and size mushroom fruit body is shown in Fig.2, where the average weight of mushroom fruit bodies obtained from irradiated mycelium is slightly higher compared to the control. The size of mushroom fruit body from irradiated mycelium is lower at 0.1 and 0.6 kGy and higher at 0.2 and 0.4 kGy than to the control mycelia. Turkey Test ($P<0.05$) on the weight and size of mushrooms fruit body obtained from the cultivation of irradiated mycelia at doses 0.1 kGy, 0.2 kGy, 0.4 kGy and 0.6 kGy shown no significant different compared to control However irradiation can induce more formation of fruit body and it increased as the dose of irradiation increased (Table 3).

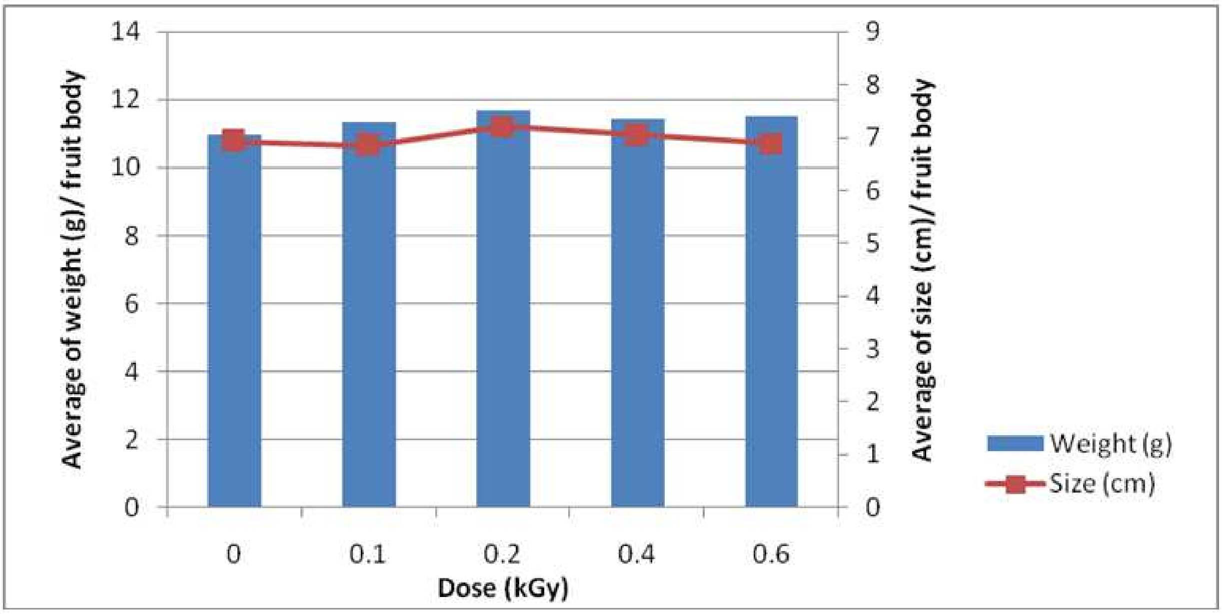

Fig.2 Graph average of weight and size mushroom fruit body for each dos of irradiation

Table 3. Average of number fruit body and mushroom weight per bag for each dos of irradiation

Dose (kGy)	Number of bag was produce fruit body	Number of fruit body/ bag	Mushroom weight (g)/ bag
0 (control)	40	4	48.52^{a}
0.1	40	6	70.72^{b}
0.2	40	7	79.91^{b}
0.4	40	7	77.27^{b}
0.6	40	8	83.61^{b}

Means with small font shown has not significant different at Tukey test (P<0.05)

Average mushroom yield, calculated as Biological Efficiency (BE) increased as the dose of irradiation increased i.e 23.10%, 33.68%, 38.05%, 36.80 % and 39.82% for doses of 0 (control), 0.1 kGy, 0.2 kGy, 0.4 kGy and 0.6 kGy respectively. The results obtained in this work provide some interesting findings. The consequence of radiation also affects fruiting body and yield of the mushroom. The benefit of radiation was obvious with the result shown biological efficiency (BE) of irradiated mycelium of *P. sajor-caju* is higher than control and it was increased when the dose is increased.

4 CONCLUSION

Low dose irradiation of 0.1 to 0.6 kGy was found sufficient to develop new strain of mushroom with high yield charater and it can directly support to local mushrooms production. This approach can also be applied to other species of mushroom to expand mushroom industry in Malaysia.

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