INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
21–0902/2022/27–1–53–59
DOI: 10.17957/IJAB/15.1898
http://www.fspublishers.org

Full Length Article

Effect of Different Doses of Acute Gamma Radiation on the Cultivation of *Volvariella volvacea*

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Received 30 September 2021; Accepted 26 November; Published 30 January 2022

Abstract

In mushroom cultivation, acute gamma radiation can be used and studied to improve the production of its fruiting body. Therefore, the objective of this study was to investigate the effect of gamma radiation on the mycelial growth and production of the fruiting body of *Volvariella volvacea* (Bull.) Singer. The mycelia were exposed to gamma radiation at various doses: 0 (control), 300, 600, 900, 1200 and 1500 Gy. The irradiated mycelial growth rate was recorded on potato dextrose agar (PDA) and spawning substrate. The spawn was then produced by inoculating the treated mycelia on paddy straw and subsequently cultivated on an empty fruit bunch to compare the production. The result showed no significant difference in mycelial growth rate on wheat, the number of mushrooms, and mushroom weight between control and treatments of 300, 600 and 900 Gy. However, there was the presence of hairy structures on the fruiting bodies of *V. volvacea* in all spawns with irradiated mycelia and the production of clustered fruiting bodies from spawns treated with 1200 Gy observed. The effect of treatments on the texture of fruiting bodies was insignificant. Although acute gamma radiation did not significantly increase the production of *V. volvacea* compared to the control treatment, there were morphological changes observed on the *V. volvacea* fruiting body which can be studied further.

Keywords: Gamma radiation; Irradiation; Mycelium; Oil palm empty fruit bunch; Spawn

Introduction

Volvariella volvacea (Bull.) Singer belongs to the *Volvariella* genus from the Pluteacea family, is an edible cultivated mushroom and commonly known as paddy straw mushroom or Chinese mushroom (Chang 1977; Chen *et al*. 2019). *Volvariella volvacea* is widely grown in tropical and subtropical climates like China, Thailand, Philippines, and Malaysia (Bao *et al.* 2013). Due to *V. volvacea* qualities of having a nice texture and aroma, pleasant flavour, fast growth rate, and easy cultivation technology, this mushroom became a popular choice amongst consumers and growers (Ahlawat and Tewari 2007; Thiribhuvanamala *et al.* 2012; He *et al.* 2018). Besides that, the demand and cultivation practices of *V. volvacea* have increased worldwide, given their rich protein content of 19 to 35% of protein on a dry weight basis with all essential amino acids required by the human body (Kumud *et al.* 2014). Furthermore, *Volvariella volvacea* additionally has significant pharmaceutical values, such as immunosuppressive proteins, anti-tumor polysaccharides, and immunomodulatory lectins (Liu *et al.* 2011). Therefore, the cultivation of *V. volvacea* in Malaysia could promote societal livelihood through economic, nutritional, and medicinal values (Marshall and Nair 2009; Rosmiza *et al.* 2016).

The cultivation of *V. volvacea* is increasingly popular worldwide because of factors like prevailing external climatic conditions, short growing times, low input requirements, and since agricultural waste is highly available, only a small investment is needed (Ahlawat and Tewari 2007; Rosmiza *et al.* 2016). There are several factors that affect the growth and production of *V. volvacea*, such as spawn production, a substrate for the mushroom bed, and conditions during cultivation like temperature, relative humidity, and hydrogen potential (Miles and Chang 2004). Moreover, Ukoima *et al*. (2009) stated that using an empty fruit bunch (EFB) as the substrate could increase the production of *V. volvacea* and reduce biomass waste in the environment. The highest possible production of *V. volvacea* is obtainable through high-quality mycelium and substrate in the spawn preparation. Therefore, improving the quality of the mycelium enhances the quality of the spawn, which in turn might result in high production of the mushroom.

To cite this paper: Fisol FN, S Abdullah, A Mohamad, MTM Mohamed, A Misran, S Salim, B Azhar (2022). Effect of different doses of acute gamma radiation on the cultivation of *Volvariella volvacea* (Bull.) singer. *Intl J Agric Biol* 27:53–59

In recent studies, the application of mutation techniques using acute gamma radiation on the mycelium was suggested to improve mushroom production by developing new varieties of mushroom species (Rashid *et al.* 2014). Irradiation refers to a process of exposing a substance to ionizing radiation from a variety of different sources, such as electron beams and gamma rays (Akram and Kwon 2010; Fernandes *et al.* 2012). Gamma rays have been reported as an efficient ionizing radiation technique and one of the most convenient methods in performing radiation-induced mutation for developing new varieties as it can cause mutation over a wide spectrum (Nakagawa 2009; Noordin *et al.* 2014). Whether spontaneous or induced, the mutations are crucial for genetic variability, which can improve crop yield (Manjaya 2009). Furthermore, Ibrahim *et al.* (2017) showed the exposure of gamma irradiation on the mycelium of *L. edodes*, proving that irradiation can affect the mycelial growth of the mushroom due to the alteration of the genetic compatibility of these mycelia. In addition, gamma irradiation is usually applied as an improved post-harvest technology on fresh mushrooms (Akram *et al.* 2012). However, there were no reported trials on acute gamma radiation used to the mycelia of *V. volvacea* during the spawn preparation. Therefore, this study aimed to determine the optimal doses of radiation exposure for improving the growth and production of *V. volvacea*.

Materials and Methods

Irradiation treatment

The PDA culture of *V. volvacea* with complete mycelial inoculation was irradiated using Biobeam GM 8000 Gamma Irradiator with Cesium-137 (Cs-137) as radioactive sources. Mycelia samples were exposed at six different low doses of irradiation, *i.e*., 0 (control), 300, 600, 900, 1200 and 1500 Gy at a rate of 12.5 Gy min^{-1}. Each treatment contained three samples of irradiated mycelia. The irradiated mycelia for each treatment were sub-cultured on the new PDA. All the subcultures were next incubated at room temperature, RT (25 ± 2°C). The growth of mycelium on PDA was measured by drawing two perpendiculars of a straight line at the back of each Petri dish. The measurement unit used was in millimetres (mm). The mycelial growth on PDA was recorded every day for seven days. The growth rates were determined by fitting the linear growth function, following the formula of $y = k_r x + c$ (where, k_r is the growth rate, y is the distance covered by mycelium, and x is the respective time) and expressed in mm day^{-1} (Koutrotsios and Zervakis 2014).

Inoculation of irradiated mycelia on wheat

The selected mycelia grown from each treatment were inoculated on a wheat substrate prepared in test tubes with dimensions of 60 × 25 mm. The samples were incubated at room temperature, RT (25 ± 2°C). The growth of mycelium along the test tubes was observed at two-day intervals, with the growth rate of mycelium being determined. Moreover, the growth of mycelium on the wheat substrate was recorded by measuring the mycelia running down along the test tube. The growth of mycelia was observed and recorded in two-day intervals, and the growth rate was determined.

Preparation of spawn and mushroom bed

Spawn's preparation: The method of preparation of the spawn followed Miles and Chang (2004) with a slight modification. First of all, the paddy straw was soaked in water for about four hours. The paddy straw was then washed and left to remove excess water. After that, the paddy straw was cut into a shorter length (5–7 cm). Subsequently, it was mixed with 1% calcium carbonate, $CaCO_3$. Then, the paddy straw was transferred into a polypropylene plastic bag and covered with a cap. Each treatment consists of four replicates with 100 g of paddy straw for each bag. The prepared spawn bags were autoclaved at 121°C for 60 min and left to cool at room temperature (25 ± 2°C). About 10 to 15 grains covered with mycelium from each irradiation dose were transferred into the prepared spawn bag. This process was done under a sterilized condition. The inoculated spawn bags were left at room temperature (25 ± 2°C) until the spawn run for each treatment was completed.

Mushroom bed preparation: The empty fruit bunch (EFB) was prepared and used as the substrate for the mushroom bed. The EFB was composted for nine days, covered by canvas, and watered at three-day intervals. The pH of the EFB was adjusted using calcium carbonate, $CaCO_3$ to achieve pH 6–8. After nine days, the EFB was watered until reaching 70–80% moisture and was feet-pressed to remove excess water and reduce the air gap in the bed to ensure more compact mycelial growth. The EFB was then arranged in a basket with a measurement of 45 × 35 cm. Hundreds of grams of treated spawn from each treatment were used for one mushroom bed. There were four replicates of mushroom beds for each treatment. All the mushroom beds were covered with black polythene sheets for seven days. After seven days, the polythene cover was removed to prepare the polypipe into a dome shape before the polythene sheet was lifted using polypipe as the support.

Determination of *V. volvacea* production and texture analysis

Production of the fruiting body: After 15 days from the spawning process, the first flush of *V. volvacea* was observed and harvested. The mushrooms were harvested at the button stage. The numbers and weight of the mushrooms for each treatment were recorded for each harvest time or flush.

Texture analysis: Five replicates of the button stage of *V. volvacea* weighing in the range of 25 ± 2 g from each treatment were chosen. The texture analysis was done using an

Instron Texture Analyzer to compare the firmness of the mushroom.

Statistical analysis

The raw data were processed using Microsoft Excel 2016, and all the statistical tests included Analysis of Variance (ANOVA), and Duncan Multiple Range Test, which is for multiple mean comparisons, were performed using Statistical Analysis System (SAS) 9.4 Software.

Results

Growth of *V. volvacea* mycelia on PDA and wheat grains

Mycelia growth on PDA: All treatments showed thick white cottony mycelia before light orange formation began to appear as early as day 6. The time taken for spawn run for mycelia irradiated with 1500 Gy was longer, reaching nine days compared to other treatments, which were only seven days. Besides that, there was no noticeable difference in mycelia colour and growth characteristic between all treatments (Fig. 1). However, the treatments of 600 and 900 Gy resulted in significantly higher growth rates of *V. volvacea* mycelia, which are 11.78 ± 0.10 mm day^{-1} and 11.85 ± 0.13 mm day^{-1}, respectively to control (10.72 ± 0.16 mm/day) and other treatments (Fig. 2).

Mycelia growth on wheat: There was no significant difference ($P > 0.05$) in the mean growth rate of mycelia irradiated with 300, 600 and 900 Gy (9.57 ± 0.20, 9.82 ± 0.16, and 9.83 ± 0.17 mm day^{-1}, respectively) compared to 0 Gy which at 9.70 ± 0.16 mm day^{-1} (Fig. 3). Even though the mycelia irradiated with 1200 and 1500 Gy had slower growth rates (8.62 ± 0.20 and 8.23 ± 0.09 mm day^{-1}, respectively), there were no noticeable in the colour of mycelia and no mortality of mycelia observed (Fig. 4).

Evaluation of *V. volvacea* production for each treatment

Harvesting periods: The cultivation period is the time between the spawning and production of the first flush of mushrooms. The spawn with mycelium irradiated at 1500 Gy took the longest time to produce a fruiting body compared to other treatments. The spawn with 300, 600 and 900 Gy irradiated mycelia showed a shorter time for fruiting body production compared to control and spawn with 1200 Gy irradiated mycelia (Table 1).

Physical appearance: This study resulted in no noticeable differences in the colour appearance of the fruiting body of *V. volvacea* produced between all treatments. However, there was a noticeable presence of hairy structure on the fruiting body produced by all spawn with irradiated mycelia (Table 1). In addition, the fruiting body produced from spawn treated with 1200 Gy irradiated mycelia showed a clustered form.

Number and weight of fruiting body: The mean number of mushrooms produced by 300, 600 and 900 Gy showed no significant difference ($P > 0.05$) to control except for treatments 1200 and 1500 Gy (Table 2). The mean weight of the fruiting body showed no significant difference between control treatment and spawned treated with 300, 600, 900 and 1200 Gy. However, spawn with 1500 Gy irradiated mycelia showed very poor production of *V. volvacea*, with only one out of four replicates of the mushroom bed producing *V. volvacea* fruiting body. The other beds of 1500 Gy spawn showed stunted growth of fruiting bodies, which only grows until the pinhead stage.

The texture of fruiting body: The mean texture (firmness) of the fruiting body of *V. volvacea* showed no significant difference ($P > 0.05$) between all treatments (Table 2).

Discussion

Results obtained from this study suggested that the mycelial growth rate of *V. volvacea* on PDA and wheat was significantly affected by gamma irradiation. The growth rate of *V. volvacea* mycelia increased from control (0 Gy) until 900 Gy before it started showing a decreasing mycelial growth on PDA. On the other hand, on the wheat substrate, the mycelia irradiated with 1200 and 1500 Gy showed a significantly slower growth rate compared to others. The growth rate of a mycelium depends on the formation of clamps, which enables the exchange of genes and extension to more areas as compatible mated mycelia was observed, showing a faster growth (Kothe 2001; Rosnina *et al*. 2016). Ibrahim *et al.* (2017) study on *Lentinula edodes* that were exposed to a higher dose of gamma irradiation showed decreasing numbers of clamp connections compared to control. The study indicates that there are genetic compatibility changes between individual hyphal cells within the irradiated *L. edodes* mycelia. In short, exposure to higher doses of gamma radiation could decrease the number of clamps, thus reducing mycelial growth. This also caused the possibility of sterile mycelia being selected during the subculture process to increase.

Moreover, Beejan and Nowbuth (2009) studied the gamma irradiation on a different strain of *Pleurotus* species resulted in no definitive trend alterations observed in mycelia colonization rates on media with the increasing doses of gamma radiation. They concluded that irradiation could improve the *Pleurotus* spp. strains, and at certain strains under different doses, the mushroom yield can be enhanced. Therefore, the mycelial growth on the PDA and substrate can be different depending on the mushroom species and types of substrate used.

The clustered fruiting body of *V. volvacea* at the button stage is perhaps induced by gamma-ray mutagenesis. According to Nie *et al.* (2017), all the abnormalities in the morphology of *V. volvacea* produced compared to control can indicate mutation. The gamma radiation could modify some physiological characteristics and thus create new mutants. This could help produce higher amounts of

Table 1: The evaluation of *V. volvacea* characteristics produced from each treatment

Dose (Gy)	Cultivation period (Four replicates of mushroom's bed)	Characteristic of the fruiting body at button stage
0 (control)	18 days	i) White colour ii) No hairy structure presence
300	15 days	i) White colour ii) Presence of hairy structure at the base of the fruiting body
600	15 days	i) White colour ii) Presence of hairy structure at the base and the surface of fruiting body
900	15 days	i) White colour ii) Presence of hairy structure at the base and the surface of fruiting body
1200	18 days	i) White colour ii) Presence of hairy structure at the base and the surface of fruiting body iii) Most fruiting bodies produced in clustered form
1500	24 days (only one mushroom's bed produce the fruiting body)	i) White colour ii) Presence of hairy structure at the base and the surface of fruiting body

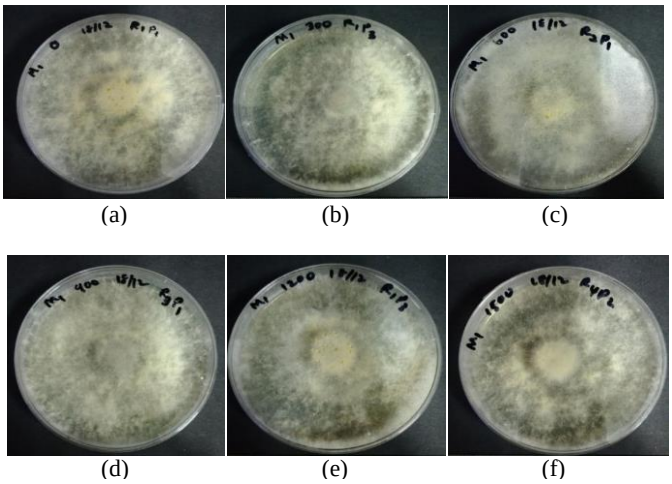

Fig. 1: The mycelial growth on PDA at day 7 for each irradiation doses; (**a**) 0, (**b**) 300, (**c**) 600, (**d**) 900, (**e**) 1200 and (**f**) 1500 Gy

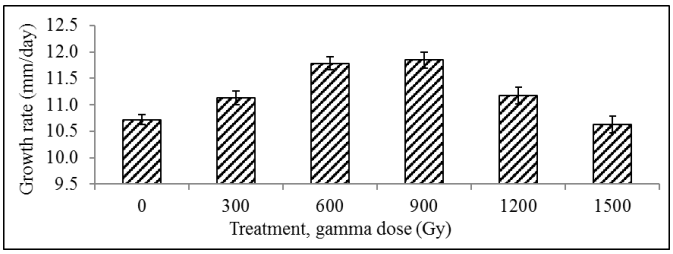

Fig. 2: The mean growth rate of mycelia (mm/day ± S.E) on PDA for each dose of irradiation throughout seven days

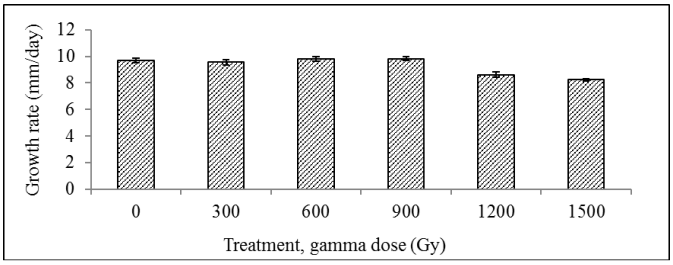

Fig. 3: The growth rate of mycelia (mm/day ± S.E) on wheat for each dose of irradiation for 12 days

essential metabolites, developing agriculturally and economically significant varieties besides potentially increasing productivity (Noordin *et al.* 2014). In this study, only spawn colonized with 1500 Gy irradiated mycelia showed very poor production, while other treatments showed no significant difference to control treatment.

Table 2: Mean number, weight (g), and texture of fruiting body for each treatment

Treatment (Gy)	Number ± SE	Weight ± SE (g)	Texture ± SE (N)
0	6.00 ± 1.22^{a}	105.38 ± 26.82^{ab}	13.75 ± 0.55^{a}
300	6.75 ± 1.25^{a}	104.00 ± 22.58^{ab}	13.04 ± 0.22^{a}
600	7.75 ± 1.31^{a}	140.25 ± 21.43^{a}	12.41 ± 0.10^{a}
900	8.00 ± 0.58^{a}	162.88 ± 10.54^{a}	12.25 ± 0.33^{a}
1200	2.75 ± 0.48^{b}	43.88 ± 16.81^{bc}	12.48 ± 0.84^{a}
1500	0.25 ± 0.25^{b}	2.50 ± 2.50^{c}	Nd

Nd is not determined. The same superscript letter for each value in the same column showed no significant difference ($P > 0.05$) between the treatments

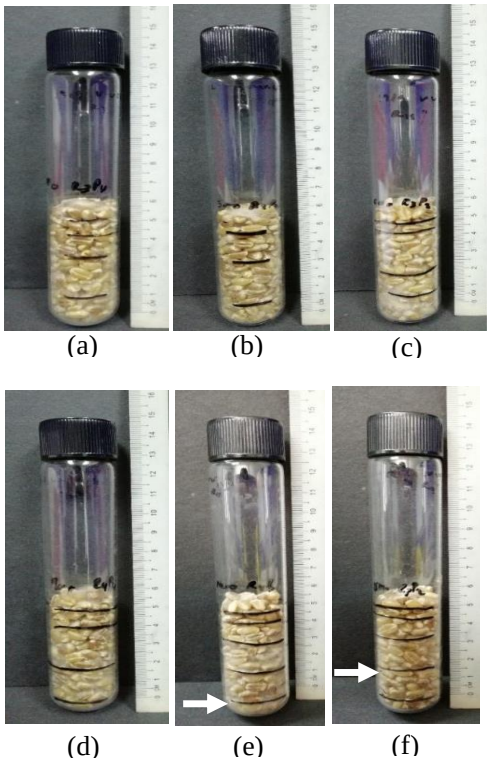

Fig. 4: The mycelial growth on wheat at Day 12 for each irradiation dose; (**a**) 0, (**b**) 300, (**c**) 600, (**d**) 900, (**e**) 1200 and (**f**) 1500 Gy. The mycelia completely covered the wheat at Day 12 for treatments 0, 300, 600 and 900 Gy. The mycelial growth of treatments 1200 and 1500 Gy at Day 12 showed the arrow in Fig. 4 (e) and (f)

Therefore, any increase in the induction of gamma radiation on mycelium more than 1500 Gy possibly shows no mushroom production. Apart from that, Lee *et al.* (2000) investigated the gamma irradiation exposure on *P. ostreatus* mycelia at 2000 Gy, which showed a reduction in mycelial growth compared to mycelia irradiated at 1000 Gy. However, the study concluded that the mycelia irradiated at 2000 Gy also able to produce a fruiting body.

Besides, Ramchander *et al.* (2015) stated that the nature of mutation could be decided by determining the correct radiation doses. Therefore, a mutation can happen to the mycelial strain at certain radiation doses, causing no production of fruiting bodies of *V. volvacea.* The texture analysis of a mushroom indicates that the mushroom has high firmness. However, this study concluded that acute gamma irradiation does not affect the firmness of the fruiting body. Other than that, Shrivastava (2006) also showed a similar result for oyster mushrooms where there was no significant difference in the texture of mushrooms between all treatments during storage. However, Hou *et al.* (2018) indicate that gamma irradiation (0.8 kGy) on the fruiting body of *V. volvacea* helps in maintaining their good quality and firmness for seven days storage period under 16 ± 0.5°C compared to other irradiation doses. Another study was done by Xiong *et al.* (2009) on gamma irradiation of *Pleurotus nebrodensis* at 1200 and 2000 Gy, which reduced fruiting bodies firmness by 45 and 62%, respectively. Meanwhile, the reduction percentage of the fruiting body firmness control group (non-irradiated) was 58%. Although the study on gamma irradiation showed a significant effect on the mushroom morphology, the IAEA (1992) reported that irradiation with an average dose of

10000 Gy shows no toxicological hazards and introduces no specific microbial or nutritional problems. Therefore, the irradiation method can be implemented in mushroom production for commercial purposes.

Conclusion

Acute gamma radiation on mycelia showed a significant effect on the spawning rate of *V. volvacea*. Further study on the effect of acute gamma radiation on nutrient content and physiological changes in fruiting bodies of *V. volvacea* is highly recommended. It is hoped that this study will stimulate improvement in current mushroom cultivation methods, thereby contributing to the production and quality of the *V. volvacea* simultaneously.

Acknowledgments

All the work was carried out by facilities provided at the Faculty of Agriculture, Universiti Putra Malaysia and Malaysian Nuclear Agency. This work was supported by Malaysian Higher Education (MOHE) for the FRGS grants (FRGS/1/2016/WAB01/UPM/02/15. We are thankful to Mohamad Yuzaidi Azmi from the Department of Agriculture (DOA) Padang Terap, Kedah, for his willingness to participate in this study.

Author's Contributions

FNF performed the methodology, collected and analyzed the result. Also, FNF wrote the manuscript. SA, AM, designed the conceptual ideas and interpreted the results. SA, SS and BA participated and revised the manuscript. Apart from that, SA, MTMM, AM participated and supervised the project. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All authors declare no conflicts of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable.

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