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RESEARCH ARTICLE

Is UV irradiation mutagenesis an appropriate approach for producing oyster mushroom thermotolerance strains?: Preliminary investigation

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Abstract

The threat of different abiotic unfavorable factors to mushroom production necessitates the search for superior strains currently in use in the industry. The most appropriate technology for this aim appears to be mutagenesis. The aim of this study is to examine the effects of physical mutagen (irradiation) on the evolution of oyster mushroom strains with a particular emphasis on heat tolerance. TUFC30034, TUFC100146, TUFC33106, TUFC34869, TUFC30044, TUFC12458, and TUFC30088, *Pleurotus ostreatus* fungal cultures, were studied at (25, 30, and 35°C) temperatures based on colony morphology and mycelial growth rate. The findings revealed that different strains are sensitive to heat stress in different ways. In comparison to the other strains, TUFC 30034 and TUFC 30044 demonstrated higher heat tolerance. TUFC 34869 had a texture that was somewhat tolerating, regularly shaped, and compact. The candidate strain (TUFC34869) was also grown on a sawdust/rice bran medium to look at yield indices such as mycelium running, pinhead initiation, 1st harvest, and total yield. Due to its performance at various temperatures as well as vegetative features, TUFC 34869 was chosen for further mutation testing. After being incubated at (25, 30, and 35°C), three monosporic mutants were isolated from UV-irradiated cultures, although they demonstrated retarded mycelial growth as compared to their parental strain at 35°C. A more comprehensive study strategy is required, as well as testing the effects of UV on a larger number of *Pleurotus* strains.

Keywords: Heat stress; Mutation; Mycelium growth; Oyster mushroom; UV-C light.

1. Introduction

The Mushrooms are distinct edible macrofungi that belong to the Basidiomycota phylum (Sundari *et al.*, 2018; WA *et al.*, 2022). They offer a range of nutrients, such as proteins, vitamins, and minerals (Mirończuk-Chodakowska *et al.*, 2019), in addition to having an impact on the environment and the economy (He *et al.*, 2018). As organic biocontrol agents, they can also be employed to protect plants (Latz *et al.*, 2018). They are therefore more in demand throughout most of the world. Even though there are over 300 genera of mushrooms, very few of them are economically cultivated (Thongklang *et al.*, 2014). Production and breeding improvements for mushrooms are fairly restricted when compared to other food crops (Sonnenberg *et al.*, 2017). Few studies on mushroom breeding have been conducted in Egypt, and further research is urgently required to create and acquire new strains with novel traits. One of the main abiotic factors that restrict the growth of edible mushrooms is heat stress (Chang and Miles,

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2004; Yan *et al.,* 2020). It causes damage to proteins and cell membranes, which affects the yield and quality of fruiting bodies. *Pleurotus* grows best at a temperature of 25°C (Xiu-ming *et al.,* 2019), however different strains species have diverse sensitivities to heat stress (Ancín-Azpilicueta *et al.,* 2012).

Pleurotus is a large genus with several edible species (Barth *et al.,* 2019). It is the world's second most extensively cultivated mushroom (Hoa and Wang, 2015). Since not all mushroom producers have ideal climatic control, *Pleuotus* high-quality basidiocarps are typically accessible in Egypt throughout the winter. When the temperature rises to 30°C or more, instability in yield and quality can be clearly observed. Fully controlled growing rooms are energy intensive and expensive, hence commercial farming relies on partially controlled rooms, and some farmers raise mushrooms in open fields, as they do in China and many other developing countries (Lei *et al.,* 2019). For several days, heat stress can limit mycelial growth, hinder fruiting, and degrade the quality of mushrooms (Chang and Miles, 2004). Their thermotolerance needs to be increased to make oyster mushrooms more appropriate for industrial production.

Despite the regular documentation of oyster mushroom production using a variety of agrowastes, there are few data on *Pleurotus* strain enhancement for high temperatures. Mutagenesis is a well-established method for developing enhanced or novel types with desirable characteristics (Teimoori *et al.,* 2014; Suprasanna *et al.,* 2017). Among the several approaches, radiation may be a very promising mutagenesis technique. Many researchers have looked into it; nevertheless, further research is needed to develop superior mushroom strains with desirable traits such as heat tolerance.

In certain studies, it has been shown that chemical mutagens are more effective than physical mutagens, but in other studies, it has been shown that the opposite is true (Chandra *et al.,* 2011). The goal of this study is to examine the effects of physical mutagen on evolving oyster mushroom strains while taking into account their heat stability tolerance.

2. Material and methods

2.1. Fungal cultures resource

P. ostreatus strains were collected from the Fungus/Mushroom Resource and Research Center at The Faculty of Agriculture, Tottori University, Japan. All strains were obtained in the form of mycelium on 2% malt extract agar (MEA) slants.

2.2. Subculturing

2 percent MEA (1 liter of distilled water, 20 g of agar, and 20 g of malt extract) were applied for all laboratory culture preparations and strains' vegetative growth assessments. The test tubes were filled with medium and autoclaved for an additional 30 minutes after the MEA had been autoclaved at 121°C for 10 minutes. Tubes were slanted and left to solidify. Afterward, they were infected with 6 mm diameter agar discs containing *P.ostreatus* mycelium under aseptic conditions (Saskiawan *et al.,* 2016). Slants were maintained at 4°C for usage after being incubated at 25°C for 7–10 days to allow for complete mycelial growth. 6 mm mycelial discs from the subculturing slants were aseptically infected into sterilized Petri plates that had been filled with 2 percent MEA (Fig. 1). Once they had reached complete mycelial growth (7-10) days, they were used to make basidiocarps (Fig. 2).

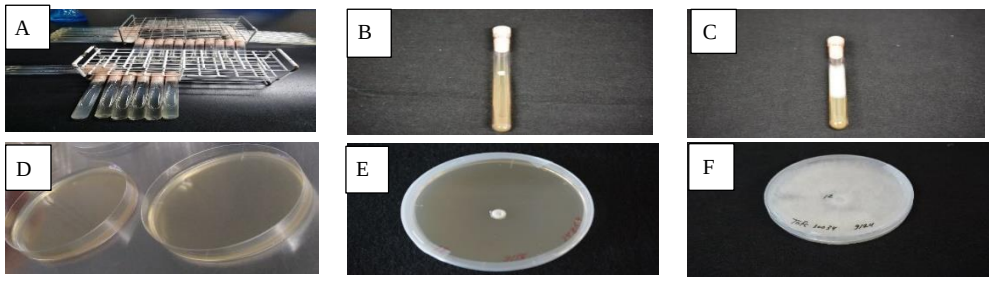

Figure 1. Subculturing; **A:** 2% MEA slants preparation, **B:** mycelium inoculation into slants, **C:** Full mycelial growth after 7 days incubation, **D:** 2%MEA Petri-plates preparation, **E:** mycelial disks inoculation from previously prepared slants into plates, **F:** Full mycelial growth after 7 days incubation.

2.3. Experimental design

The work was divided into three distinct steps; Heat sensitivity testing, Radiosensitivity testing, and mutagenic induction.

2.3.1. Heat sensitivity testing

To choose the best strain for mutation, seven Pleurotus ostreatus fungal cultures were examined: TUFC30044, TUFC100146, TUFC33106, TUFC34869, TUFC30034, TUFC12458, and TUFC30088. On a 2% MEA medium, fresh mycelia were inoculated and cultivated for 10 days at various temperatures (25, 30, and 35°C) in the dark. Based on mycelial growth rate (colony diameter), and colony morphology (descriptive data of colony shape and thickness), the candidate strain was chosen.

The thermotolerance assessment was carried out using the following experimental design: a factorial experiment with (21) treatments was examined using a randomized complete block design (RCBD) with three repetitions (7 strains X 3 temperatures). Each replication contained 9 Petri plates, with 25°C serving as the control.

2.3.1.1. Basidiocarps formation of the candidate strain

The candidate strain's basidiocarps were developed on a substrate with a sawdust/rice bran ratio of 3:1 (v/v). The moisture content of the substrate was between 65 and 70 percent (Hamed *et al.*, 2021). High-density polyethylene bags were used to package 300g of the substrate (SE-25ES, Sakato Sangyo Co. Ltd., Japan). To aerate the substrate mass, four holes were punched into it (Fig. 2. E). The bags were then sealed with a heat sealer and autoclaved for an hour at 121°C. After sterilization, the plastic bags were allowed to cool to room temperature. Each substrate culture received one fully prepared Petri plate for inoculation (an agar plug containing mycelia). Inoculated bags were kept in the incubation room at 25°C in the dark for 18 to 21 days to produce enough growth (Rashad *et al.*, 2019). After that, bags were moved to the growing room until fruiting bodies developed.

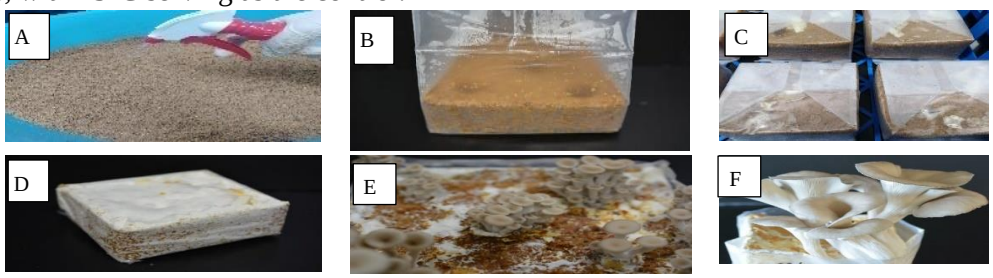

Figure 2. Basidiocarbs production; **A:** Sawdust/rice bran culture preparation, **B:** Sealed bags filled with the culture, **C:** Bags were autoclaved and inoculated by P ostreatus mycelium, **D:** Full mycelial growth after 18 days incubation, **E:** Pinheads formation, **F:** Basidiocarps started to mature.

To help with the creation of pinheads, they were opened and a small layer of the substrate was scraped from the surface of the beds (Riffiani *et al.*, 2019). Cultures were watered twice daily throughout cropping using a hand sprayer. When pinheads grew into mature fruiting bodies, harvesting was carried out by grasping the stipe and gently pulling or twisting the fruiting body from the substrate into mature fruiting bodies. (Fig. 2).

2.3.2. Radiosensitivity test

The following procedures were utilized to conduct this test on the candidate parent strain chosen for further mutation experimentation:

- Only mature basidiocarps taken from the candidate parent strain's first flush were used to make spore prints (Fig 3. A).
- To make multispore suspensions, spores from spore prints were streaked into sterile distilled water using an inoculating needle. Based on the hemocytometer count (Fig. 3. B) and the likelihood of fungal colony development following streaking of suspension onto a 2 percent MEA petri dish after incubation at 25°C for 3 to 5 days, six dilution levels (undiluted, 10^2 to 10^6 spores/mL) were compared and the optimum multispore dilution level was chosen for UV irradiation (Baral *et al.*, 2018). (Fig.3.D)
- To determine the UV dose that reduces spore survival to 5 to15%, uncovered Petri plates containing the optimal spore suspension onto a 2 percent MEA were subjected to a UV-C lamp (15 watts = 250 nm, Philips, Japan) giving eight treatments (0, 1m, 1m & the 30s, 2m & 30s, 3, 4, and 5 min). The experiment was performed with a completely randomized design (CRD) and three duplicates of each treatment were used.

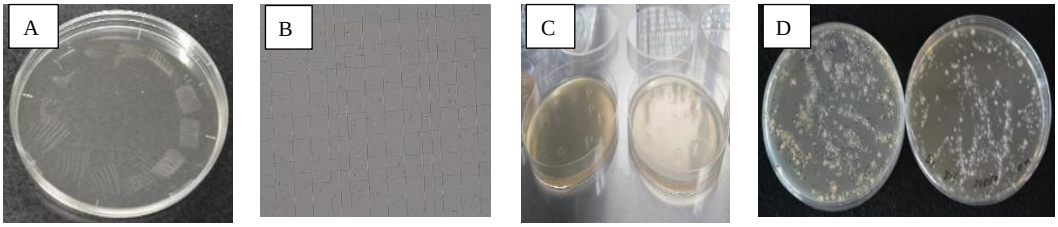

Figure 3. Spore suspension; **A:** Spore print, **B:** Spore concentration determined by Hematocytometer under a compound microscope, **C:** Spores suspended on 2% MEA Petri-dishes. **D**: Suspended plates after 3-5 days.

2.3.3. Production of mutants under heat stress

- The candidate strain was tested at different temperatures (25, 30, and 35 °C) after receiving the recommended dose of ultraviolet light. Compared to the control (irradiation-free), each treatment had three replicates.
- From UV-irradiated cultures, three monosporic mutant colonies (Single cultures) were isolated. Using a sterile cork borer, they were inserted into the middle of freshly manufactured 2 % MEA. The cultures were then compared to their parent strain while being incubated at 35°C in the dark. Microscopic investigation was used to assure monosporic cultures (Fig. 4).

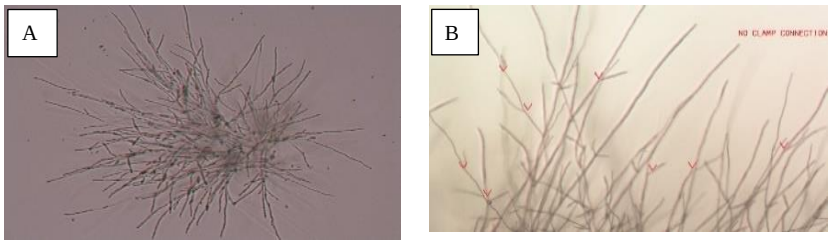

Figure 4. A: Single colony 10x magnification**, B:** single colony under 20x magnification.

2.4. Data analysis

The SAS 16 program used a one-way analysis of variance (ANOVA) to analyze the data that were produced. Duncan's test was used to determine whether there were significant differences between groups (p 0.05). To compare the variations between the mutant and parent strains, the T-test was performed (Gomez and Gomez, 1984).

3. Results and discussion

In particular for non-industrial production where temperature control is not possible, breeding or producing edible mushroom cultivars adapted to high temperatures can ensure fruit body quality and productivity. In this assay, an effort has been made to develop a thermotolerant strain of *Pleurotus ostreatus*. We primarily marked the heat sensitivity of seven strains as the mutation experiment's starting point. We used the mycelium growth rate to quantify the heat sensitivity of strains (Tables 1, 2). We further used the observations of hypha thickness (Table 3) and shape (Table 4). The way that the strains' mycelial growth reacted to being exposed to various temperatures differed. Our results back up the assertions made by (Lu, 2014; Das, 2016; Wang *et al.*, 2017) that different germplasms have different levels of heat tolerance.

Similar conclusions have been drawn from research on the reactions of *P. ostreatus* mycelia to heat stress. Heat stress decreased *Pleurotus pulmonarius*'s mycelial development and slowed lipid peroxidation, according to Liu et al., 2019's paper. Yan et al 2020's study likewise found a connection between increased glycolysis and mitochondrial dysfunction and Mycelia's negative response to heat stress. Accumulating ROS, decreased oxygen consumption, and ATP synthesis may also be signs of the cause of mycelial growth suppression under high-temperature stress, according to Lei et al. 2019's article. In our investigation, the mycelial growth rates of each strain were measured at3,5,7 and 10 days.

Table 1. Mycelial colony diameter (mm) in the seven tested *Pleurotus* strains as affected by different temperatures (25, 30, 35°C) after 3 and 5 days of incubation.

	Mycelium Colony diameter (mm)			Mycelium Colony diameter (mm)		
	3 Days			5 Days		
Strain/Temp.	25°C	30°C	35°C	25°C	30°C	35°C
30034	28.27±0.15c	30.96±0.41c	18.24±0.47a	69.06±0.36b	68.57±0.14b	29.88±0.16a
100146	32.55±0.24b	35.05±0.03a	8.340±0.50g	68.00±056b	66.25±0.55c	10.00±0.34e
33106	26.20±0.14d	22.98±0.35e	9.290±0.35f	51.42±0.11f	45.52±0.04f	11.16±0.21e
34869	25.44±0.03d	30.00±0.56d	12.96±0.92c	60.00±0.38c	61.96±0.64d	21.00±0.41c
30044	25.55±0.96d	26.39±0.36f	15.96±0.40b	53.68±0.25e	51.78±0.54e	26.24±0.45b
12458	23.17±0.09e	28.37±0.20e	10.30±0.04e	73.13±0.01a	76.57±0.01a	15.61±0.86d
30088	34.49±0.13a	33.33±0.25b	11.31±0.25d	72.48±0.36a	69.61±0.00b	16.14±0.62d

Data are means of three replicates ± values represents standard deviation of the mean. Mean followed by same letters in the same column are not significantly different.

The majority of the strains had high growth rates at 25°C and 30°C after 10 days of mycelial growth. On the other hand, all of the strains under study displayed a reduction in the rate of mycelium growth at the highest temperature examined (35°C). Additionally, we noticed that the examined strains' mycelial development diameters varied to some extent (Table 1,2)

Like our results, (Liu *et al.*, 2020) examined the sensitivity of *Pleurotus* spp. mycelium to the temperature at 25,30, and or 35°C and found that the temperature was maximal at 25-30°C, whereas a temperature of 35°C was detrimental to

mycelial growth, indicating significant variations in the strains' tolerance to high temperature. When the temperature was increased to 35°C, the mycelial growth rate in all strains reduced (Li *et al.*, 2010).

Table 2. Mycelial colony diameter (mm) in the seven tested *Pleurotus* strains as affected by different temperatures (25,30, 35°C) after 7, and 10 days of incubation.

	Mycelium Colony diameter (mm)			Mycelium Colony diameter (mm)		
	3 Days			5 Days		
Strain/Temp.	25°C	30°C	35°C	25°C	30°C	35°C
30034	75.14±0.59c	72.58±0.31c	37.81±0.15a	83.28±0.08a	82.67±0.10a	52.57±0.07a
100146	78.12±0.55b	78.97±0.50b	15.11±0.62f	81.73±0.09c	82.88±0.30a	19.16±0.06g
33106	75.88±0.06c	71.14±0.60c	12.81±0.16g	83.24±0.49a	77.26±0.73b	20.88±0.12f
34869	78.29±0.49b	78.47±0.52b	29.36±0.34c	83.24±0.24a	82.78±0.50a	45.05±0.02c
30044	70.32±0.18d	68.10±0.25d	33.25±0.07b	83.30±0.47a	82.75±0.04a	50.00±0.27b
12458	82.00±0.01a	82.91±0.31a	19.18±0.16e	82.32±0.41b	82.73±0.24a	21.77±0.25e
30088	81.46±0.30a	81.27±0.13a	23.41±0.36d	83.04±0.21a	82.68±0.23a	36.77±0.46d

Data are means of three replicates ± values represents standard deviation of the mean. Mean followed by same letters in the same column are not significantly different.

Table 3. Different strains' mycelium thickness in the different temperatures.

Strain/Temp.	25°C	30°C	35°C
30034	C	C	C
100146	Th	Th	Th
33106	C	C	C
34869	SC	SC	SC
30044	C	C	C
12458	Th	Th	Th
30088	C	C	C

C: Compact, SC: Somewhat compact, Th: Thin.

Table 4. Different strains' mycelium shape in different temperatures.

Strain/Temp.	25°C	30°C	35°C
30044	R	R	R
100146	R	R	IR
33106	R	R	R
34869	R	R	R
30034	R	R	R
12458	R	R	IR
30088	R	R	IR

R: Regular, IR: Irregular

In the current study, after 10 days of culture, the relative mycelial growth rate (percent of maximum) varied across strains TUFC 30034 and TUFC 100146, ranging from 23.44 percent to 63.12 percent. The TUFC 30034 and TUFC 30044 strains were the only ones that sustain maximal rates at the 35°C test temperature. They were more thermotolerant than every strain tested. They were predominantly eliminated from the mutation experiment as a result. Strains TUFC 100146 and TUFC33106, in contrast, exhibited the lowest mycelial growth rates at the same temperature. The average colony diameters of the two strains previously mentioned were 19.16 mm and 19.16 mm, respectively, at 35°C.

Figure 5. A: Compact mycelium, **B:** Somewhat compact mycelium, **C:** Thin mycelium.

We also noted that their mycelium's hyphae are very sensitive to temperature variations. These two strains were also excluded from the mutation experiment due to their high heat sensitivity and the fact that they displayed thin texture and inconsistent morphological shape when exposed to the highest temperature (35°C). In addition, due to the heat stress, TUFC 30088's form was erratic. The temperature of 35°C, which is regarded to be fatal for the majority of common strains, only partially killed TUFC 34869 (Fu *et al.*, 2014). It had a substantial thickness and was frequently chapped. As a result, it was selected as a potential strain for the mutation experiment. An additional production test was conducted for strain TUFC 34869. (Table 5).

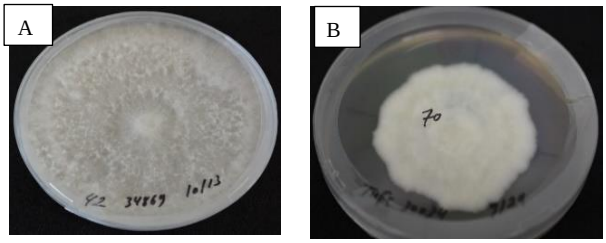

Figure 6. A: Regular shaped mycelium, **B:** Irregular shaped mycelium

Table 5. growth and yield parameters of *Pleurotus* (34869) per 300 g substrate

Mycelium running (days)	Pinhead initiation (days)	1st harvest (days)	Total yield (g)	Biological efficiency (BE) (%)
14.00±1.00	7.00±1.00	12±0.50	190.00±6.72	95.00±2.25

*Data are means of three replicates ± values represents standard deviation of the mean. BE calculated as fresh weight of mushroom/dry weight of substrate *100.*

The multispore dilution level of 10^{3} was discovered to be the best for plating spore cultures under UV light to induce mutation in *Pleurotus* (TUFC 34869). A lethal dose (LD) of 90% was found at 2 min based on the association between the number of surviving colonies and exposure duration, UV dose reduces spore survival to levels of 5 to 10% (Table 6).

The experiment treatments stopped at 5 minutes which showed no spores survival (Fig. 8). To create mutants under heat stress, a heat tolerance experiment (25, 30, 35°C) was modified to the TUFC 34568 multi-spores that had been exposed to radiation for 2 minutes (Fig. 7).

Table 6. Effect of UV irradiation time on the number of colonies of *Pleurotus* TUFC 34869

Time	Number of colonies
0 min	674.10±0.19[a]
1 min	485.80±2.87[b]
1 min&30s	240.00±0.67[c]
2m	34.500±0.17[d]
2 min&30s	5.7800±0.84[e]
3 min	1.7400±0.52[f]
4 min	0.3300±0.19[f]
5 min	0.0000±0.00[f]

Means were separated using Duncan's Multiple Range Test and means followed by the same letter(s) are not significantly different at a 0.05 probability level.

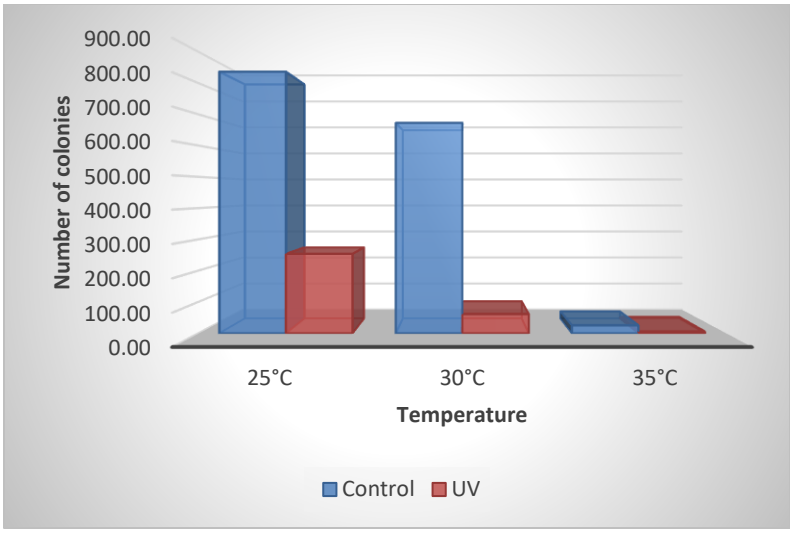

Figure 7. Effect of UV irradiation on the *Pleurotus* TUFC 34869 number of colonies under different temperature.

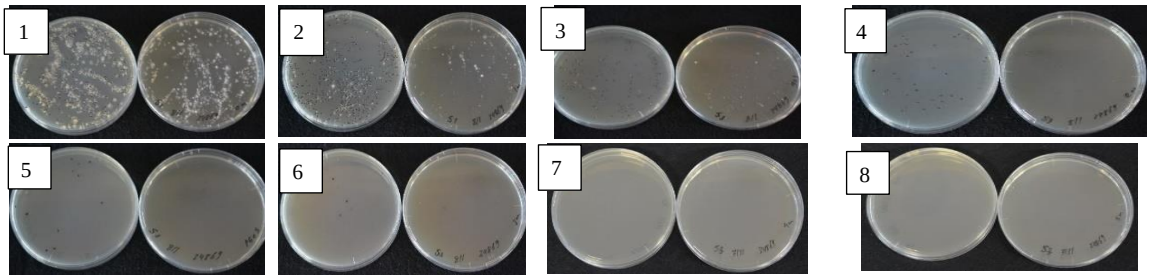

Figure 8. Irradiated TUFC 34869 after 7 days inoculation 1 to 8 represents 8 Irradiation times (0 min, 1 min,1 min & 30 Seconds, 2 min, 2 min & 30 seconds, 3 min, 4 min, 5 min).

Exposure of spores to UV light resulted in retarded cottony growth of mycelium when they were subjected to the highest temperature of 35°C (Fig. 9). In deep contrast to our results regarding the negative effect of UV rays regarding heat tolerance, UV mutation of the spores in many investigations proved to be effective in the development of sporless strains (Pandey *et al.*, 2010), higher productivity (Dhanasekaran *et al.*, 2013), strains of higher nutritional value (Majolagbe *et al.*, 2013). Compared to the study sizes of some other investigators, we only investigated a relatively smaller number of strains. We hypothesized that if the UV irradiation had a major impact on the development of heat-tolerant strains, at least some isolated spores should have been positive for this mutation. We think further investigations of a larger number of strains should be done with the same strategy.

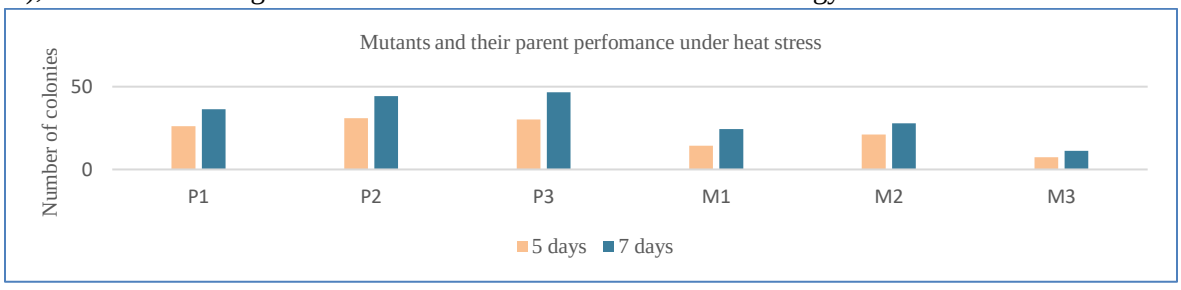

Figure 9. Monosporic cultures isolated after 35°C heat stress. P= Parent strain (control), M= Mutant

4. Conclusion

We confirmed in our trial that the various strains respond to heat differently. The *P. ostreatus* TUFC 34869 strain is also quite vulnerable to UV exposure. The *Pleurotus* spores' mutation, however, did not produce the expected outcomes. Due to the relatively small number of strains used in this study, more research utilizing our method and a greater variety of *Pleurotus* strains assessing the effects of UV should be done. We were able to validate these findings because we ran the experiment more than three times, and we used the published data as a starting point for further research.

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Authors' Contributions

All authors are contributed in this research.

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Institutional Review Board Statement

All Institutional Review Board Statements are confirmed and approved.

Data Availability Statement

Data presented in this study are available on fair request from the respective author.

Ethics Approval and Consent to Participate

Not applicable

Consent for Publication

Not applicable.

Conflicts of Interest

The authors disclosed no conflict of interest starting from the conduct of the study, data analysis, and writing until the publication of this research work.

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