Int.J.Curr.Microbiol.App.Sci (2023) *12*(10): 111-120

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 12 Number 10 (2023)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2023.1210.014

Possibility of Evolving High Yielding Varieties of Oyster Mushroom through Anastomosis and Mutation

Sumaiyya Pathan[1*], Jeetu Narware[2*] and Aniruddha G. Deshmukh[3]

[1]Department of Plant Pathology, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli (M.H.), India
[2]Department of Mycology and Plant Pathology, Institute of Agricultural sciences, BHU (U.P.), India
[3]Department of Plant Pathology, Post Graduate Institute, Akola (M.H.), India

**Corresponding author*

Keywords

H. ulmarius, *P. sajor-caju*, *P. florida*, *P. citrinopileatus*, Oyster mushroom

Article Info

Received:
18 August 2023
Accepted:
20 September 2023
Available Online:
10 October 2023

A B S T R A C T

High production and good quality are always the principal goals for agriculturally important crop. Oyster mushroom is the choicest edible species cultivated in various region of the world. The yield performance improvement was carried out through hyphal anastomosis of four oyster mushroom *viz.*, *Pleurotus sajor-caju* (Ps), *P. citrinopleatus* (Pc), *P. florida* (Pf) and *Hypsizygus ulmaius* (Hu) with each other and by physical mutation that comprised the exposure of mushroom culture to UV radiation and by chemical mutation by Ethyl Methyl Sulfonate (EMS). Date of spawning, days required for spawn run, days required for pinhead initiation, total yield/ bed, biological efficiency was evaluated. Out of six crosses, the cross between Ps and Pc recorded the maximum biological efficiency. In respect of effect of UV radiation maximum biological efficiency (98.27%) was observed in Ps when its mycelium was exposed to UV radiation for a period of 30 minutes. EMS had negative effect on mycelial growth of Pc and Ps whereas it had positive effect on mycelial growth of Pf at three concentrations (0.003, 0.004 and 0.005%). The results suggested that the exposure to UV radiation for 30, 4, 5, and 10 minutes respectively was effective in enhancing the biological efficiency of the mushrooms under study. EMS favored mycelial growth of Hu except at a concentration of 0.005 per cent.

Introduction

Mushrooms have long been a part of the human diet and used as both foods and medicine. They are the fruiting bodies of filamentous fungi that grow above the ground (Agarwal *et al.*, 2021). The most cultivated mushrooms in the world are *Agaricus bisporus*, *Lentinula edodes*, *Pleurotus* spp., *Auricularia auricula-judae*, *Volvariella volvacea*, and *Flammulina velutipes*. On the other hand, the most famous wild mushrooms are *Boletus edulis*, *Cantharellus* spp., *Craterellus cornucopioides*, *Morchella* spp., and *Marasmius oreades* (Dimopoulou *et al.*, 2022). The genus *Pleurotus*,

familiar as oyster mushroom, belongs to phylum Ascomycota and Basidiomycota. *Pleurotus* is the second largest grown mushroom globally after shiitake (Barh *et al.,* 2019). They are the fruiting bodies of filamentous fungi that grow above the ground (Agarwal *et al.,* 2021). About 40 species of the genus *Pleurotus* are under cultivation and among the mushrooms cultivated all over the world, oyster mushrooms rank third in world production Tesfaw *et al.,* (2015). China is currently the leading producer of cultivated edible mushrooms worldwide accounting for approximately 73 percent of the world's total mushroom production (Dimopoulou *et al.,* 2022). Apart from China (88% share in oyster mushroom cultivation), South Korea, Japan, Italy, Taiwan, Thailand and Philippines are the other major producers of oyster mushrooms.

In India, many species of oyster mushroom such as *P. sajor-caju, P. florida, P. citrinopileatus* etc are mainly cultivated in the states of Orissa, Karnataka, Tamil Nadu, Maharashtra, Andhra Pradesh, Madhya Pradesh, West Bengal and most of the North Eastern states. Indian mushroom production scenario is negligible amounting only to 50,000 tonnes per annum as compared to the world production of 55 lakh tonnes (Banik *et al.,* 2010).

The development and improvement in the mushroom strains stands to be an active topic for continuous research in the field of mushroom cultivation. Strain improvement generally includes the parameters such as higher yield, better nutritional quality, colour and development of sporeless mutants. In order to overcome these constraints strain improvement has been carried out using the techniques *viz*. protoplast fusion (Das *et al.,* 1995). Dikaryon mating (Larraya *et al.,* 2001). and interspecific hybridization (Jaswal *et al.,* 2013). Mutation has long been exploited in crop breeding programs in order to improve both quality and productivity.

Various methods of inducing mutation include chemical, biological, and physical agents such as ultraviolet (UV) and gamma irradiations (Fahad *et al.,* 2009). Chemical mutagens e.g., EMS, MMS and NTG have been used to induce desirable characters like sporelessness and white colour of the basidiocarp in *P. ostreatus* (Mukherjee *et al.,* 1986). Therefore, the present investigation was undertaken to explore the possibility of developing a high yielding strain/s of oyster mushrooms with better biological efficiency through hyphal anastomosis and mutation by using physical (UV light) and chemical Ethyl Methyl Sulfonate (EMS) mutagens.

Materials and Methods

The present study was conducted at the Department of Plant Pathology, College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri (M.S.). The pure cultures of four oyster mushrooms (*Pleurotus sajor- caju, P. citrinopileatus, P. florida and Hypsizygus ulmarius*) available in the department of Plant Pathology, College of Agriculture, Dapoli were used for the study.

Augmentation of pure culture

The cultures of four oyster mushrooms required for the study were grown on potato dextrose agar (PDA) medium. The pH of PDA medium was maintained at 7 whenever it was prepared. Freshly prepared potato dextrose agar (PDA) slants were used to maintain the cultures by frequent sub culturing the pure cultures of all the four species of oyster mushroom. The slants with profuse mycelial growth were preserved in refrigerator for further studies.

Mycelial anastomosis

All the four parent cultures were grown separately on PDA and after their profuse mycelial growth on the medium; their mycelial bits (5 mm) were cut with a sterilized cork borer in laminar air flow. Then two such bits were transferred in Petri plates containing fresh sterile medium in such a way that each plate contained a single bit of two parent cultures. Desired distance was maintained between the two bits to provide sufficient space for the

growth of each culture. When the mycelia of both the cultures fused together, the bits of the mycelium in fusion zone were cut by sterilized cork borer and transferred to fresh PDA medium. The crosses obtained by this method were designated as mentioned below.

The pure cultures of the crosses obtained were maintained in PDA slants for further use.

Mutation

Physical mutation

Physical mutation comprises the exposure of mushroom culture to UV radiation which is a commonly use physical mutagen. In order to study the effect of this mutagen on mushroom cultures, the mycelial bits of four mushroom cultures under study were aseptically transferred to sterilized solidified PDA medium in Petri plates.

After 48 hours of growth the actively growing cultures were exposed to UV radiation in laminar air flow cabinet by keeping open plate below the UV tube for different exposure timings *viz*.1, 2, 3, 4, 5, 10, 15 and 30 minutes. The laminar air flow chamber was covered with black cloth sheets to avoid photo reactivation. Three replications per treatment were maintained. Such treated plates were incubated at 25±2^{o}C temperature and observed daily. After proper mycelial growth, the irradiated cultures were used for spawn preparation.

Chemical mutation

Ethyl Methyl Sulfonate (EMS) was used as chemical mutagen. Required quantity of PDA medium was prepared by following standard procedure. EMS solution was prepared by dissolving 1 μl, 2 μl, 3 μl, 4 μl and 5 μl quantity of EMS in 100 ml distilled sterile water so as to get desired concentration of 0.001per cent, 0.002 per cent, 0.003 per cent, 0.004 per cent and 0.005 per cent. The mycelial bits of the four parent cultures were dipped in the solution of each concentration separately for 1 hr. Such treated bits were then transferred to Petri plates containing solid PDA medium. Untreated bits inoculated in PDA served as control. Three replications were maintained per treatment. The inoculated plates were observed daily. Observations on colony diameter were recorded on the 8thday of inoculation. After mycelial growth, the same cultures were used for spawn preparation.

Spawn preparation and Cultivation of oyster mushroom

The spawn of oyster mushroom was prepared on wheat grains. Inoculated flasks were incubated at 25 ± 2^{o}C for 16-18 days. The fresh spawn with profuse mycelial growth was used for further experiments.

Spawn of parent cultures, anastomosed culture and mutant culture was prepared Sanitation of incubation and cropping room was done. Cultivation of oyster mushroom was carried on paddy straw substrate. Mushroom beds were then stacked on iron shelves in spawn run room.

Complete darkness and temperature around 25-30^{o}C were maintained in this room till completion of spawn run. Beds with full white mycelial growth were opened and transferred to the cropping room on wooden hangers. In cropping room, about 25-30^{o}C temperature and 85-90 per cent relative humidity was maintained. The humidity in the cropping room was maintained within a range of 80-85 per cent with the help mist blowers which were run for 5-10 minutes 2-3 times a day.

Date of spawning, days required for spawn run, days required for pinhead initiation, total yield/ bed, biological efficiency was evaluated.

The data recorded were statistically analyzed and the differences in the treatments were tested for their significance as per the methods suggested by Gomez and Gomez (1984). In the experiment on anastomosis, CRD design was used whereas for analyzing data related to mutation, FCRD design was used.

Results and Discussion

It is revealed from the data presented in table 2 that all the treatments were statistically significant. Maximum biological efficiency (75.80%; 379.00g) was recorded in the treatment T2 (cross between Ps x Pc) which was significantly superior to rest of the treatments. It was followed by T1(73.33%; 366.67g), T9(71.76% - *P. citrinopileatus*) and T10 (69.46 % - *H. ulmarius*). The treatments T3, T7, T8, were statistically at par with each other. The treatment T8 was statistically at par with T4 which was at par with T6.

The results of present study indicate that, the cross between Ps x Pc was the best among the crosses as well as the sole cultures. The treatments T9 (*P. citrinopileatus* – 71.7%) and T10*(H. ulmarius*-69.46%) were superior to their cross with each other. The least BE (57.33%) was recorded in T5 (cross of Pf X Hu).

In *P. sajor-caju*, mycelial growth decreased as the time of exposure to UV radiations increased from 1 minute to 15 minutes of exposure. However, at 30 minutes exposure the mycelial growth slightly increased and it was same as that of 5 minutes exposed plates. Full growth (9 cm) was observed in control (table 3). The mycelial growth of *P. florida* exhibited an erratic pattern. It was maximum (6 cm) after exposure to UV radiations for 1 min. It gradually decreased to 3.7 and 3.2 cm at the subsequent exposure periods but it increased to 4 cm at 4 min exposure time. It increased further to 4.6 cm at 5 minutes and then suddenly decreased to 2.6 cm at 10 min. There was no any definite pattern in the mycelial growth. In case of *P. citrinopileatus*, the mycelial growth increased from 7.7 to 8 cm up to 4 min exposure and then suddenly decreased to 5.9 cm at the subsequent exposure time. It reached 6.8 cm at 10 min and then gradually decreased to 6.1 cm in further exposures. The mycelial growth of *H. ulmarius* was also manifested in erratic manner. But the least growth of this fungus (3.5 cm) was recorded after exposure to UV radiations for 30 minutes. On the basis of the results of this experiment it can be summarized that exposure to UV radiation has negative effect on mycelial growth of all the four species of oyster mushrooms under study.

It is revealed from the results presented in table 3 that among the sub treatments T8 (72.5g; 58.00%) recorded significantly higher yield as compared to rest of treatments followed by T4 (64.02g; 51.20%). Treatment T4 was at par with T5 (61.22g; 48.90%) and T6 (59.40g; 47.50%). Treatment T6 was also statistically at par with T9 (55.74g; 44.50%), T3 (54.50g; 43.60%) and T2 (53.61g; 42.80%). The treatment T2 was at par with T7 (52.19g; 41.70%) and T1 (51.82g; 41.40%). These results suggest that the exposure to UV radiation for 30, 4, 5, and 10 minutes respectively was effective in enhancing the biological efficiency of the mushrooms under study.

It is clear from the results presented in table 4 that the highest average yield and biological efficiency (74.54g; 59.63%) was recorded in *Pleurotus florida* which was significantly superior to rest of the treatments. It was followed by *P. sajor-caju* (60.19g, 48.15%) and *P. citrinopileatus* (43.48g; 54.34%) which were statistically at par with each other. The least yield and BE were recorded in *H. ulmarius* (Fig.1).

In respect of interaction of main and sub treatments, it is revealed from data presented in table 4 shows significant results. The maximum biological efficiency (98.27%; 122.83g) was recorded by *P. sajor-caju* when the mycelium was exposed to UV radiation for a period of 30 minutes. It was statistically superior to rest of the interactions, followed by *P. florida* when the mycelium of this mushroom was exposed to UV radiation for 10 minutes (76.13%; 95.17g). The BE of *P. florida* exposed to UV radiation for 10 minutes was found at par with *P. florida* exposed to UV radiation for 5 minutes(67.47 %; 84.33).

P. citrinopileatus recorded maximum biological efficiency of 70.80 percent when its mycelium was exposed to UV radiation for 4 minutes. Maximum

biological efficiency of *H. ulmarius* (57.47%) was recorded in 5 min exposure but as compared to other mushrooms the biological efficiency of this mushroom was very low. Effect of Ethyl Methyl Sulfonate (EMS) on mycelial growth of oyster mushrooms:

This experiment was conducted to study the effect of Ethyl methyl sulfonate (EMS) mutagen on mycelial growth of oyster mushrooms. Four species of oyster mushroom viz., *P.sajor-caju, P.florida, P.citrinopileatus and H. ulmarius* were treated with different concentrations of EMS *viz*. 0.001, 0.002, 0.003, 0.004 and 0.005 per cent. Effect of different concentration of EMS on mycelial growth of oyster mushroom was evaluated and results are presented in Table 4 (Fig.1). Results in Table 5 revealed that the colony diameter of *P. sajor-caju,* at 0.004 per cent concentration of EMS was same as that in control while other treatments had slightly negative effect on its mycelial growth.

In *P. florida,* maximum colony diameter (8.5 cm) was recorded in treatment T3 (0.003). In T 4 and T5 the colony diameter was 8.2 cm. It was equal in T1 and T6 (8.1 cm). Minimum mycelial growth was observed at T2 (8 cm). On the basis of these results it can be concluded that 0.003 per cent, 0.004 per cent and 0.005 per cent concentration has positive effect on mycelial growth of *P.florida.*

In case of *P. citrinopileatus* maximum growth was observed at T6 (8.5 cm) which was followed by T2 (8.4 cm), T1, T3 (8.3 cm), T5 (8.2 cm), and T4 (8.1 cm) respectively. Minimum growth was observed at 0.004 per cent EMS. All the treatments had negative effect on the mycelial growth of *P. citrinopileatus.*

However, in *H.ulmarius*, poor growth was observed at T5 (7.9 cm). Growth in T2 and T6was numerically at par while other treatments had superior mycelial growth. All the treatments had positive effect on mycelium of *H. ulmarius* except T5. The nutrients found in edible mushrooms include sugars (sucrose, xylose, rhamnose, mannose, and fructose), amino acids (glutamic, aspartic, glutamate, methionine, and cysteine), proteins, fatty acids (linoleic, stearic, palmitic, adrenic, and nervonic acid), vitamins (folate, riboflavin, ascorbic acid niacin, thiamine, ergocalciferol, and cyanocobalamin) mineral contents (Ca, Mg, K, P, Na, Fe, Cu, Zn, Cd, and Mo) and phenolic compounds (gallic acid, caffeic acid, protocatechuic acid, p-coumaric acid, p-hydroxybenzoic acid and pyrogallol). Because they are low in calories and fat and high in dietary fiber, mushrooms are regarded as healthy foods (Dawadi *et al.,* 2022). In the present study, an attempt was made to know whether the crosses of four oyster mushrooms viz. *P. sajor-caju*, *P. florida, P. citrinopileatus* and *H. ulmarius* obtained through anastomosis have better yield potential than the sole cultures. It is also, to understand, whether the UV radiation and chemical mutagen EMS bring about desirable changes in DNA of the cultures which leads to enhancement in biological efficiency of the mushrooms under study. In the experiment on the effect of mycelial anastomosis on biological efficiency of oyster mushrooms it was observed that maximum BE (75.80 %) was recorded in the treatment T2 (cross between Ps x Pc) which was followed by T1 (Ps x Pf). Both the treatments were significantly superior to rest of the treatments.

Abella *et al.,* (1994) conducted studies on hyphal anastomosis of three *Pleurotus* spp (*P. sajor-caju*, *P. ostreatus* and *P. cystidiosus*) by dual culture technique. They found that, hyphal combinations were significantly superior to parent cultures in terms of number of basidiocarps, length of stipe and productivity period. The results of the present study are in agreement with those of Abella *et al.,* (1994).

The results of present indicate that, *P. citrinopileatus* is a better former than *P. sajor-caju*. The higher yield potential of Pc has been transferred to the progeny and the fleshiness of the fruit body which is a character of *P. sajor- caju* has also been inherited by the progeny. Hence the cross has inherited the two characters i.e., higher yield from one parent and fleshy fruit body from the other parent.

Table.1 Crosses obtained by anastomosis

Parent culture	Parent culture	Designation of the anastomosed culture
***P. sajor-caju*= Ps**	*P. florida* = Pf	Ps x Pf
***P. sajor-caju*= Ps**	*P. citrinopileatus* = Pc	Ps x Pc
***P. sajor-caju*= Ps**	*H. ulmarius = Hu*	Ps x Hu
***P. florida* = Pf**	*P. citrinopileatus* = Pc	Pf x Pc
***P. florida* = Pf**	*H. ulmarius = Hu*	Pf x Hu
***P. citrinopileatus* =Pc**	*H. ulmarius = Hu*	Pc x Hu

Table.2 Effect of mycelial anastomosis on biological efficiency of oyster mushrooms

Sr. no.	Species	Spawn run Period (days)	Pinhead formation (days after opening of bag)	Yield per 500g dry substrate (g)	E (%)
T1	*Ps X Pf*	22	5	366.67	73.33
T2	*Ps X Pc*	22	5	379.00	75.80
T3	*Ps X Hu*	22	5	325.00	65.00
T4	*Pf X Pc*	22	3	316.33	63.26
T5	*Pf X Hu*	28	12	286.67	57.33
T6	*Pc X Hu*	22	3	313.00	62.60
T7	*P. sajor caju*	22	5	324.67	64.93
T8	*P. florida*	21	5	322.83	64.56
T9	*P. citrinopileats*	21	3	358.83	71.76
T10	*H. ulmarius*	28	4	347.33	69.46
	SE = 1.94				
	CD 5% = 5.73				
	CD 1 % = 7.81				

Table.3 Effect of UV radiation on colony diameter (cm) of oyster mushrooms

UV light exposure (minutes)	Mean colony diameter (cm)			
	P. sajor-caju	***P. florida***	***P. citrinopileatus***	***H. ulmarius***
1 min	8.1	6.0	7.7	4.7
2 min	8.1	3.7	7.8	4.1
3 min	7.7	3.2	7.9	4.8
4 min	6.9	4.0	8.0	3.9
5 min	6.6	4.6	5.9	4.1
10 min	6.2	2.6	6.8	4.7
15 min	6.1	3.3	6.2	4.1
30 minu	6.6	4.8	6.1	3.5
Control	9.0	9.0	9.0	9.0

Table.4 Effect of UV radiation on biological efficiency of oyster mushrooms

Sub Treatments	Main Treatments (oyster mushroom species- M)								Mean Yield (T) (g)	Mean BE (%)
	M 1 *P. sajor-caju*		M 2 *P. florida*		M 3 *P.citrinopileatus*		M 4 *H. ulmarius*			
	Yield	BE	Yield	BE	Yield	BE	Yield	BE		
T1 (1min.)	55.83	44.67	64.33	51.47	55.17	44.13	47.17	37.73	51.82	41.45
T2 (2min.)	54.00	43.20	69.50	55.60	55.83	44.67	52.50	42.00	53.61	42.89
T3 (3min.)	52.83	42.27	71.50	57.20	58.17	46.53	53.00	42.40	54.50	43.60
T4 (4min.)	46.67	37.33	79.83	63.87	88.50	70.80	61.17	48.93	64.02	51.21
T5 (5min.)	42.67	34.13	84.33	67.47	71.17	56.93	71.83	57.47	61.22	48.97
T6 (10min.)	45.83	36.67	95.17	76.13	59.00	47.20	55.83	44.67	59.40	47.52
T7 (15min)	64.33	51.47	55.83	44.67	53.17	42.53	53.33	42.67	52.19	41.75
T8 (30min.)	122.83	98.27	82.17	65.73	53.17	42.53	42.83	34.27	72.50	58.00
T9 (control)	56.67	45.33	68.17	54.53	63.33	50.67	51.50	41.20	55.74	44.59
mean	60.19	48.15	74.54	59.63	61.94	49.56	54.35	43.48	58.34	46.66
	Mushrooms	**Treatments**	**Interaction**							
SEm	1.09	1.63	**3.27**							
CD1%	**4.06475**	**6.09**	**12.19**							

Fig.1 Effect of mycelial anastomosis on biological efficiency of oyster mushrooms

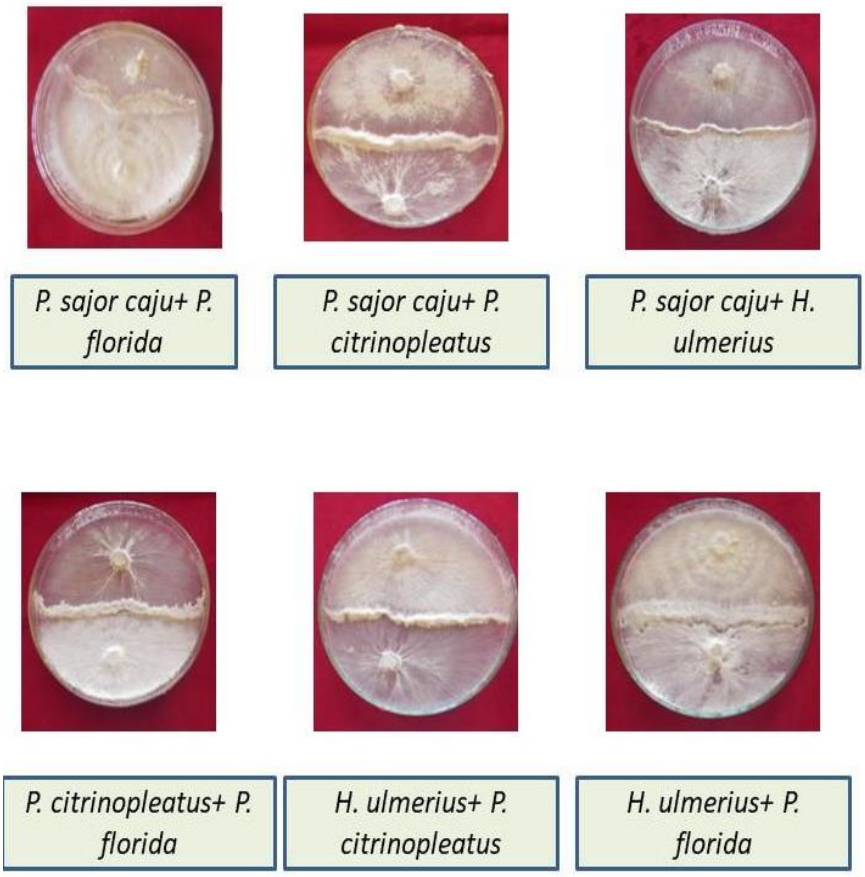

Table.5 Effect of Ethyl Methyl Sulfonate on colony diameter (cm) of oyster mushrooms

Ethyl Methyl Sulfonate (concentration)	Mean colony diameter (cm)			
	P. sajor-caju	***P. florida***	***P. citrinopileatus***	***H. ulmarius***
T1-0.001%	8.2	8.1	8.3	8.6
T2-0.002%	8.4	8.0	8.4	8.0
T3-0.003%	8.2	8.5	8.3	8.5
T4-0.004%	9.0	8.2	8.1	8.6
T5-0.005%	8.6	8.2	8.2	7.9
T6-Control	9.0	8.1	8.5	8.0

Hence this cross fulfilled the objectives of the present study. But the results of this study differ with those of Abella *et al.,* (1994) in terms of the spawn run period as the progeny took 22 days spawn run period as that of Ps. The results of this study are in conformity with those of Dhitaphichit *et al.,* (2005) and who reported that the cross exhibited combination of the characteristics of both the parental strains. Experiment conducted by Adebayo *et al.,* (2013) on improving yield performance of *Pleurotus pulmonarius* through hyphal anastomosis with *P. sapidus* and *P. ostreatus* revealed improvement in yield potential of the crosses. This is in concurrence with present results in respect of only two crosses Ps X Pc and Ps x Pf out of the six crosses obtained.

The results of the experiment on effect of UV radiation on biological efficiency of mushrooms imply that maximum biological efficiency (98.27 %) was recorded in *P. Sajor-caju* when the mycelium was exposed to UV radiation for a period of 30 minutes and it was followed by *P. florida* (76.13%) when the mycelium of this mushroom was exposed to UV radiation for 10 minutes. Biological efficiency of *H. ulmarius mushroom* was very low as compared to other mushrooms. These results are in accordance with those of Al-Qurainy and Khan (2009) who found that the best mycelial growth of *P. columbinus* was observed at twenty minutes irradiation period. Sharma *et al.,* (2014) found that, exposure of *P. ostreatus* mycelium to UV radiation for a period of 10 minutes and 15 minutes favours mycelial growth. The results of present study are in contradiction with the results of Sharma *et al.,* (2014) Effect of chemical mutagen EMS on mycelial growth of the four mushroom species reveled that, in case of Pc and Ps all the treatments had negative effect on the mycelial growth. But in respect of Pf, 0.003, 0.004, and 0.005 per cent concentration had positive effect on mycelial growth. Similarly, EMS had positive effect on mycelial growth of Hu except at a concentration of 0.005 per cent. These results are contradictory with those of Lee *et al.,* (2011) and Sharma *et al.,* (2014) who reported that, higher concentration of chemical mutagen (<1%) hampered mycelial growth of the mushrooms they studied. Teimoori *et al.,* (2014) also reported that EMS (1.5%) was detrimental for the mycelial development.

Out of six crosses of oyster mushrooms viz., *P. sajor caju*, *P. florida*, *P. citrinopileatus* and *H. ulmarius* were obtained through anastomosis. Out of which, the cross between *P. sajor caju* and *P. citrinopileatus* recorded the maximum biological efficiency. The cross between Pf and Hu was poor performer with the least biological efficiency of 57.33 per cent. In case of the crosses of *P. sajor-caju* and *P citrinopileatus*, with other mushrooms, maximum morphological characters of fruiting bodies were like to those of Ps or Pc. This indicates that these two mushrooms have dominant genes which are definitely passed on to the progeny.

In respect of effect of UV radiation maximum biological efficiency (98.27%) was observed in *P. sajor-caju* when its mycelium was exposed to UV

radiation for a period of 30 minutes.

EMS had negative effect on mycelial growth of Pc and Ps whereas it had positive effect on mycelial growth of Pf at three concentrations (0.003, 0.004 and 0.005 per cent).EMS favored mycelial growth of Hu except at a concentration of 0.005 per cent.

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How to cite this article:

Sumaiyya Pathan, Jeetu Narware and Aniruddha G. Deshmukh. 2023. Possibility of Evolving High Yielding Varieties of Oyster Mushroom through Anastomosis and Mutation. *Int.J.Curr.Microbiol.App.Sci.* 12(10): 111-120. **doi:** https://doi.org/10.20546/ijcmas.2023.1210.014