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Effect of mutagens on *Pleurotus eous* APK1 for biomass improvement

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Abstract

Investigations on the oyster mushroom *Pleurotus eous* APK1 were conducted to enhance the yield performance by the process mutagenesis . The wild strain of *P. eous* APK1 was mutated by physical and chemical mutagens such as UV radiation with two different time intervals (15 and 30 minutes) and ethidium bromide (200 µg/ml concentration). The mother spawn was developed for the wild and mutant strains using sorghum grains and subsequently cultivated in paddy straw by poly propylene bag method. Among the three mutant strains, UV_{30} has grown rapidly but biomass content was significantly equal to wild strain. Other two mutants UV_{15} and $EtBr_{200}$ had slightly increased in their biomass when compared to the wild strain, particularly the UV_{15} mutant yielded high biomass in a single harvest. The morphological characteristics, growth rate and high yield of UV mutant strains, proved that the UV irradiation could be used as an effective physical mutagen for strain improvement.

Keywords : Morphological characterization, mushroom cultivation, mutagenesis, *Pleurotus eous*, strain improvement.

INTRODUCTION

Mushroom is an emerging high value crop with great opportunities for enterprise diversification and income generation. They are becoming increasingly important component of human diets worldwide, due to their nutritional (Bernas *et al*., 2006; Barros *et al*., 2008) and medicinal characteristics (Jedinak *et al*., 2010; Nunes *et al*., 2012). More than 2000 species of edible mushrooms are known, out of which only few species have been cultivated commercially (Patil, 2012). Among the various types of edible mushrooms, *Pleurotus* spp (oyster mushroom) have become more popular and widely cultivated throughout the world, particularly in Asia and Europe. *Pleurotus* occupies the third place in the world's production of edible mushrooms, after *Agaricus bisporus* and *Lentinula edodes*. Its popularity has been increasing due to their favourable organoleptic and medicinal properties, vigorous growth and undemanding cultivation conditions. It can be cultivated on log, weeds, wastes and a wide variety of agroforestry (by-) products for the production of food, feed, enzymes, medicinal compounds or for waste degradation and detoxification (Gregori *et al*., 2007; Akinyele *et al*., 2012). It is not surprising that the demand for *Pleurotus* is increasing significantly due to population growth, market expansions, changing of consumer behaviour and developments. However, the existing productivity cannot cope with the increasing demand due to lower productivity of the existing strains.

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To increase the productivity of the existing strains of *Pleurotus* several techniques have been developed. Strain improvement is one of the successful approaches used to improve the strains for higher yield and quality. Strain improvement is usually done by mutating the microorganism by techniques like classical mutagenesis, which involves exposing the microbe to physical mutagens such as X-rays, Y -rays, UV rays and chemical mutagens such as NTG (Nitrosoguanidine), BU (5'-bromouracil), EMS (ethyl methyl sulfonate), sodium nitrite, etc. Chemical mutagenesis usually results in a single nucleotide substitution in the target genome, whereas physical mutagenesis induces errors in DNA replication (ultraviolet light), deletions and translocations (ionizing radiation). Mutation may induce one or more change in characteristic of the mutated organisms. The characteristic changes may occur at the gene level which will be passed on to the next generation. Mutation is generally applied to the mushrooms to obtain better quality traits and productivity. The desired mutant characteristic can be economically beneficial for example resistant to pathogen, higher yield, etc. (Carlile and Watkinson, 1994; Ira Djajanegara and Harsoyo, 2008).

Mutagenesis on *Pleurotus* mushroom has been widely studied by many investigators for higher yield and induction of desirable characters like sporelessness, white colour of the basidiocarp, etc.. Imbernon and Labarere (1989) applied UV irradiation and chemical treatment for stimulation of sporeless mutants in

Pleurotus ostreatus and *Pleurotus pulmonarius*. Ravishanker *et al.* (2006) used UV Light and gamma rays for strain improvement in *P. ostreatus*. Strain improvement of *P. ostreatus* PO-2, PO-6 and PO-7 for lower spore count and colour of the sporophore was studied by Sharma and Sharma (2014).

However, reports on the strain improvement of *Pleurotus eous* for yield performance through classical mutagenesis are scanty. The present article deals with enhancement of the yield of indigenous *Pleurotus eous* APK1 strain by random mutagenesis using ultra-violet (UV) irradiation and ethidium bromide treatment.

MATERIALS AND METHODS

Culture collection and maintenance

The pure culture of *P. eous* APK1 in the form of spawn was collected from Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India. The mycelium of *P. eous* APK1 from the spawn was transferred in to the sterile potato dextrose agar (PDA) plates under aseptic conditions and incubated for 5 days at 28±2°C to attain sufficient growth.

The pure culture of *P. eous* APK1 was maintained on PDA slants at 4°C for subsequent uses and sub-cultured bi-monthly.

Production of spawn for wild *P. eous* APK1

Sorghum whole grains were used as a substrate for the spawn production of wild *P. eous* APK1 strain. The grains were washed thrice with running tap water to remove the dust particles, chaffy and damaged grains. Then the grains were soaked in water for 6-8 hours, drained and half cooked for 15 to 20 minutes to ensure that the grains were cooked but intact (broken grains are more prone to contamination). Then they were spread over a blotting paper to remove the excess water. The grains were then added with 3 per cent (w/w) of calcium carbonate (to reduce the stickiness of grains and to neutralize the pH of the grains) and thoroughly mixed manually. Aliquots of the grains were filled in 100 ml narrow mouthed glucose bottles the mouth was plugged with non-absorbent cotton and covered with brown wrapper. The bottled grains were then sterilized in an autoclave at 121°C and 15 lbs for 15 minutes. After sterilization the bottles were allowed to cool under room temperature. Each of the bottled sterile grains were aseptically inoculated with the mycelial disks (1cm of actively growing mycelium) of *P. eous* APK1 wild strain grown on potato dextrose agar using a flamed and cooled scalpel in a laminar flow hood. After that, the spawns were incubated at room temperature for 15-20 days without any light and mycelia running on the substrate were recorded at three days interval (Dinesh Babu and Subhasree, 2010; Narh *et al.*, 2011). An uninoculated control was maintained to rule out experimental errors. After the completion of spawn running, the master spawn of wild *P. eous* APK1 strain was divided into two parts and used for cultivation and induction of mutation respectively.

Production of mutants

P. eous APK1 mutant strains were produced by following the method of Adebayo *et al.*, (2012) with minor modifications. The actively growing mycelium (5 days old) of wild *P. eous* APK1 strain from the spawn was first made into suspension with sterile physiological saline (0.85% sodium chloride) and mutated using physical (UV radiation) and chemical mutagens (ethidium bromide). The mycelial suspension was exposed to ultraviolet radiations (210nm) for two variable time periods (15 and 30 minutes) at the distance of 10 cm from the UV source for physical mutation. The mycelial suspension was treated with 10 ml of ethidium bromide (EtBr) at the final concentration of 200µg/ml and kept at 37°C in an orbital shaker for 30 minutes for inducing chemical mutation. After mutagenesis, spawn was prepared for the UV (UV_{15}, UV_{30}) and ethidium bromide ($EtBr_{200}$) mutant strains as described for the method of production of wild strain spawn production.

Cultivation of mushroom

Preparation of mushroom bed

Mushroom beds for the wild and mutant strains of *P. eous* APK1 were prepared with paddy straw substrate following the method of Patil (2012). All the instruments were sterilized with dilute solution of potassium permanganate and alcohol. Sun dried paddy straw was obtained from agricultural field of local farmers in Tiruchirappalli and chopped into small pieces (2-3 cm long). Afterwards, they were soaked in water for about 6-8 hours under weight to moisten it and excess water was drained off. The substrate was steam sterilized in an autoclave at 121°C, 15 lbs pressure for 20 minutes, cooled to ambient temperature and excess of water was drained out to moisture 70% (Zadrazil, 1978; Ingale and Ramteke, 2010). Polythene bags in the size of 30 x 60 cm were filled with sterilized substrates and multi layered technique was adopted for spawning. Each bag was filled with 1 kg of dry substrate and the spawn of wild and mutant *P. eous* APK1 strain were added individually at the rate of 2% of the wet weight basis of substrate. The mouth of the polythene bags was tied and incubated in a cool shady room with the relative humidity of 65 – 70% and sufficient light and ventilation. Small holes were made on the sides of the polythene bags (without disturbing the beds) for aeration. During cropping, the bags were watered thrice a day using a hand sprayer. The mass of substrate was kept undisturbed for 20 days for the appearance of fruiting bodies.

Fruiting and harvesting

The mushroom beds of wild and mutant strains were observed for the formation of pinheads and fruitbodies during (basidiocarps) incubation. When pin heads grew into mature fruit bodies, harvesting was carried out with sharp edged scalpel. Spraying with water was discontinued a day before the harvest of the fruit bodies. The mushroom beds were maintained up to the harvest of the third flush and a small layer of substrate was scrapped off from all the side of the beds after each harvest.

Characteristic features of *P. eous* APK1 wild and mutant strains

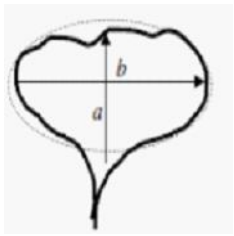
Yield determination

Total weight of *P. eous* APK1 wild and mutant strains were determined by measuring the weight of all the fruit bodies harvested from 1st, 2nd and 3rd flush (Patil, 2012). Then the mushrooms were cleaned, sun dried and preserved in polythene bags for further use.

Morphological features

The morphological characteristics such as colour, stipe length and its diameter, pileus length and its breadth, thickness of pileus, number of carpophores and weight of individual carpophore of the wild and mutant strains of *P. eous* APK1 were recorded.

Area of fruit body



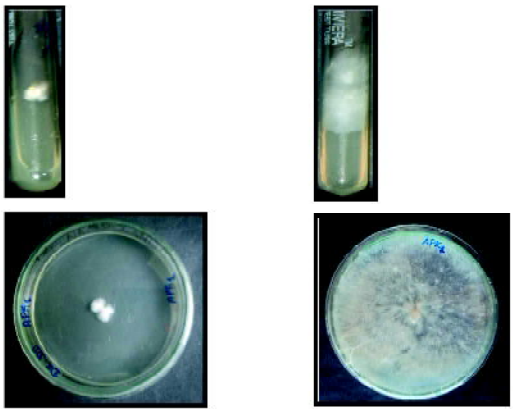
The size of the fruit body was determined by measuring in two directions from the maximum expanded portion of the pileus. Thus the area of the fruiting body was calculated by considering the projected area as an ellipse and using the following formula

Area of fruiting body = $\pi \times a \times b$

where a = minor diameter and b = major diameter of the fruit body at its maximum expanded portion.

Table 1. Morphological characteristics of wild and mutant strains of *P. eous* APK1

S. No.	Morphological characters (before and after harvesting)	*P. eous* APK1			
		Wild	Mutant		
			UV_{15}	UV_{30}	$EtBr_{200}$
1.	**Fruiting bundle**				
a.	Colour - Early stage	Pinkish	Pinkish	Pinkish	Pinkish
	Matured stage	Creamy white	Creamy white	Creamy white	Creamy white
b.	Number of carpophores	24	30	14	13
2.	**Individual basidiocarp**				
a.	Pileus – Length (cm)	6.6	7.2	7.3	7.3
	Breadth (cm)	9.16	8	10	8.5
	Thickness (cm)	0.2	0.2	0.25	0.2
b.	Stipe – Length (cm)	1	1.5	0.9	1.75
	Diameter (cm)	0.83	1	0.5	1
c.	Area of fruiting body (cm^2)	190	181	229.4	195
d.	Weight of the individual carpophores (g)	15	13.6	28.3	15



a. After 1 day incubation b. After 3 days incubation

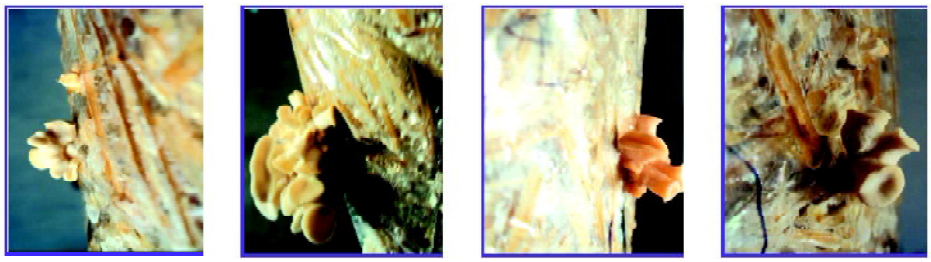
Fig 1. Pure mycelial growth of wild *P. eous* APK1strain

a. Before harvesting b. After harvesting

Fig 5. Shrinked carpophore of UV_{30} mutant strain of *P. eous* APK1

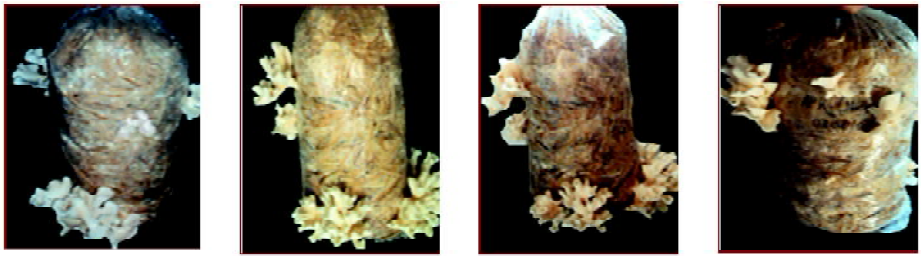
a. Control b. Wild Mutant c.UV_{15} Mutant d.UV_{30} Mutant e.$EtBr_{200}$

Fig 2. Mother spawn of wild and mutant strains of *P. eous* APK1



a. Wild b.UV_{15} Mutant c. UV_{30} Mutant d.$EtBr_{200}$ Mutant

Fig 3. Pinhead formation of *P. eous* APK1 wild and mutant strains



a. Wild b.UV_{15} Mutant c. UV_{30} Mutant d. $EtBr_{200}$ Mutant

Fig 4. Fruiting bodies of wild and mutant strains of *P. eous* APK1

RESULTS AND DISCUSSION

Growth characteristics of wild *P. eous* APK1

By using the simple plating technique, the culture of wild *P. eous* APK1 strain from the spawn was inoculated on to the potato dextrose agar medium for the experimental use. The mycelia started their growth after a day of incubation and fully grown pure white mycelium was appeared over potato dextrose agar medium after three days of incubation (Fig. 1). Similar observation was found for *P. eous* APK1 strain procured from Tamilnadu Rice Research Institute (TRRI), Aduthurai. The strain attained pure white mycelial growth on potato dextrose agar on the third day of incubation (Dinesh Babu and Subhasree, 2010).

Deployment of Spawn of wild and mutant strains with sorghum grains

The spawn of wild *P. eous* APK1 strain was prepared by inoculating the mycelial disks of *P. eous* APK1 in to the sterile sorghum grains. The mycelium took 18 to 20 days to complete the process of growth spread over the spawn in the bottle. After 20 days, the spawn bottles appeared pure white in colour due to the impenetrable mycelial growth (Fig. 2). Similar to the wild strain, spawn were prepared for the mutant strains by inoculating the UV and ethidium bromide treated mycelial suspension into the sterile sorghum grains. The UV mutants (UV_{15} and UV_{30}) took 15-18 days to complete the process of growth of mycelium in the sorghum grains; where as the mycelium of ethidium bromide mutant ($EtBr_{200}$) completed the process of growth through the grains within 16-20 days (Fig. 2). Although no significant difference was noted in the number of days taken for the complete development of spawn among the four strains (wild and mutant) tested, fast colonization was achieved in the UV_{15} mutant strain. *Pleurotus* species usually colonize spawn medium completely within 20 days of incubation which was evidenced from the results of several investigators. Dinesh Babu and Subhasree (2010) reported that the complete mycelial growth of *P. eous* APK1 strain was observed in about 2 weeks when the wheat grains were used as a spawn substrate. Tan (1981) reported that the spawn development took 3 weeks in their study with *Pleurotus* species. Hence it is suggested that rate of colonization varied depending on many factors including inherent genetic differences within strains.

Cultivation of mushroom

The wild and mutant strains of *P. eous* APK1were cultivated by polypropylene bag method using paddy straw as the substrate. Spawn running in mushroom beds of all strains took about 2-3 weeks after inoculation of mother spawn. These results agree with the findings of Dinesh Babu and Subhasree (2010). They observed that the mycelia of *P. eous* APK1 spread over the paddy straw after 15 days of spawing.

Traditionally, *Pleurotus* mushrooms are largely cultivated using straws of paddy and wheat due to their abundance and cellulose content. They can also be cultivated using a number of agro-wastes including ragi straw, stalks and leaves of maize, sunflower, cotton, sugarcane bagasse, etc. In the present study, paddy straw was used as a substrate for the cultivation of *P. eous* APK1 based on availability and efficiency. Dinesh Babu and Subhasree (2010) cultivated *P. eous* APK1 using different agro wastes such as paddy straw, sorghum stem, 'varagu' straw and sugarcane trash ,and reported that the maximum yield was obtained with paddy straw (1190g/kg of substrate). Nallathambi and Marimuthu (1993) also reported that the paddy straw was the most suitable substrate for the cultivation of *Pleurotus* species.

Pinhead formation

Pinhead formation is the second stage of mycelial growth during the cultivation of mushroom. In the wild strain of *P. eous* APK1 pinhead formation was observed after 20 days of spawn running, where as in the mutant strains (UV and EtBr) they were formed after 15 days of spawn running, which was comparatively faster than the wild strain (Fig. 3). Generally during mushroom cultivation pinheads like structures appear 6-7 days after completion of the spawn running as reported by Ahmad (1986) and Shah *et al.* (2004). They have also reported that *Pleurotus* sp. (*P. ostreatus*) completed spawn running in 17-20 days over different substrates and pinheads formation occurred in 23-27 days.

Fruit bodies formation

Formation of fruit bodies is the third and final stage during the process of cultivation of mushroom. The fruit bodies of all the strains developed 3-5 days after pinheads formation or 20-25 days after spawning (Fig. 4). These findings are in conformity with Quimio (1976, 1978) and Shah *et al.* (2004) who reported that the fruit bodies of *Pleurotus* sp. (*P. ostreatus*) developed after ,appeared 3-4 weeks of spawning.

Yield of *P. eous* APK1 mushroom

The matured fruiting bodies of wild and mutant strains of *P. eous* APK1 mushroom were harvested in three flushes. The comparatively more yield was obtained in the first flush than the second and third flush. The yield of the *P. eous* APK1 was significantly high in UV_{15} mutant (830 g/kg) followed by $EtBr_{200}$ mutant strain (625 g/kg) and UV_{30} mutant (580 g/kg) which was comparatively higher than the wild *P. eous* APK1 strain (515 g/kg). These results suggest that the yield of mutant strains was notably higher than the wild *P. eous* APK1 strain. Though the yield of the wild *P. eous* APK1 strain was comparatively less than the results recorded

by Dinesh Babu and Subhasree (2010), the yield was increased when the wild strain was subjected for mutagenesis.

Morphological features

The colour of the fruit bodies of the wild *P. eous* APK1 was pinkish in early stage and creamy white in the matured stage. Similar morphology was recorded in all the three mutant strains. The total number of carpophores of the UV_{15} mutated strain of *P. eous* APK1 was higher than the wild and other mutant strains. But the weight of the individual carpophores was higher in the UV_{30} mutant strain (28.3g) than the wild strain (15g) and other mutants. The length, breadth, thickness of the pileus and area of the fruit body were fairly high in the UV_{30} mutant strain when compared with the wild strain (Table 1). However, the length and diameter of the stipe was high in the $EtBr_{200}$ mutant strain rather than the wild strain. A special feature was observed in UV_{30} mutant strain of *P. eous* APK1, which grew more rapidly than the other mutant strains and also it lost its moisture content within 24 hours. The edges of the fruit bodies were shrinked and their colour also changed from creamy white to slight yellow within a day (Fig. 5).

In the present study, the UV mutant strains (UV_{15} and UV_{30}) of *P. eous* APK1 revealed high yield, fast growth rate and high number of carpophores. Similarly *P. pulmonarius* LAU 09 strain mutated with UV irradiation showed yield improvement over the wild (parent) strains, especially at 90 minutes exposure (Adebayo *et al.*, 2012). Ultra violet radiation (UV light) has been reported as one of the best physical methods for strain improvement for better yield performance (Kang *et al.*, 1999). The use of UV light to obtain the mutants of fungal strain is better than that of X-rays, because of its simplicity, inexpensive and readily available of germicidal UV lamp. Hence, in the present study UV irradiation was used as the physical mutagen.

CONCLUSION

Although cultivation of oyster mushroom, *Pleurotus* with various agrowastes has been frequently documented, few reports are available regarding the cultivation and strain improvement of *P. eous* for high yield. In the present study, an attempt has been made to develop an improved strain of *P. eous* APK1 for high productivity and nutrition composition. All the physical and chained mutant strains exhibited faster growth than the wild type. The mutant UV_{15} was found as a significant mutant strain because of its high growth rate, increased number of carpophores and yield. The purpose of the study was to increase the biomass of wild *P. eous* APK1 strain through mutagenesis and that was achieved in the case of UV_{15} mutated strain.

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REFERENCES

Adebayo, E.A., Oloke, J.K., Bordoloi, A.K., Barooah, M. and Bora, T.C. 2012. Improvement of *Pleurotus pulmonarius* Lau 09 through mutation for yield performance. *World J. Microbiol. Biotechnol.*, 29: 1-13.

Ahmad, I. 1986. *Some studies on oyster mushroom* (*Pleurotus* spp.) *on waste material of corn industry*. M.Sc. dissertation, University of. Agriculture, Faisalabad, Pakistan.

Akinyele, J.B., Fakoya, S. and Adetuyi, C.F. 2012. Anti-growth factors associated with *Pleurotus ostreatus* in a submerged liquid fermentation. *Malaysian J. Microbiol.*, 8: 135-140.

Barros, L., Cruz, T., Baptista, P., Estevinho, L.M. and Ferreira, I.C.F.R. 2008. Wild and commercial mushrooms as source of nutrients and nutraceuticals. *Food Chem. Toxicol.*, 46: 2742-2747.

Bernas, E., Jaworska, G. and Lisiewska, Z. 2006. Edible mushrooms as a source of valuable nutritive constituents. *Acta Sci. Pol. Technol. Aliment.*, 1: 5-20.

Carlile, M.J. and Watkinson, S.C. 1994. *The fungi*. Academic Press, London.

Dinesh Babu and Subhasree. 2010. Valuing the suitable agro-industrial wastes for cultivation of *P. platypus* and *P. eous*. *Advances in Biological Research,* 4: 207-210.

Gregori, A., Svagelj, M. and Pohleven, J. 2007. Cultivation techniques and medicinal properties of *Pleurotus* spp. *Food Technol. Biotech.*, 45: 238-249.

Imbernon, M. and Labarere, J. 1989. Selection of sporeless or poorly spored induced mutants from *P. pulmonarius* and selective breeding. In: Mushroom science XII (part I): *Proceedings of the 12th International congress on the science and cultivation of edible fungi*. pp. 109-123.

Ingale, A. and Ramteke, A. 2010. Studies on cultivation and biological efficiency of mushrooms grown on different agro-residues. *Innovat. Rom. Food Biotechnol.*, 6: 25-28.

Ira Djajanegara and Harsoyo. 2008. White oyster mushroom (*Pleurotus florida*) mutant with altered antioxidant contents. *Biotropia.*, 15: 65-7.

Jedinak, A., Dudhgaonkar, S., Jiang, J., Sandusky, G. and Sliva, D. 2010. *Pleurotus ostreatus* inhibits colitis-related colon carcinogenesis in mice. *Int. J. Mol. Med.*, 26: 643-650.

Kang, S.W., Ko, E.H., Lee, J.S. and Kim, S.W. 1999. Overproduction of beta-glucosidase by *Aspergillus niger* mutant from lignocellulosic biomass. *Biotechnol. Lett.* 21: 647-650.

Nallathambi, P. and Marimuthu, T. 1993. *Pleurotus platypus*: a potent oyster mushroom for organic recycling of agricultural wastes. *Mushroom Res.*, 2: 75-78.

Narh, D.L., Obodai, M., Baka, D. and Dzomeku, M. 2011. The efficacy of sorghum and millet grains in spawn production and carpophore formation of *Pleurotus ostreatus* (Jacq. Ex. Fr) Kummer. *International Food Research Journal.*, 18: 1143-1148.

Nunes, M.D., da Luz, J.M.R., Paes, S.A., Ribeiro, J.J.O., da Silva, M.C.S. and Kasuya, M.C.M. 2012. Nitrogen supplementation on the productivity and the chemical composition of oyster mushroom. *Journal of Food Research.*, 1: 113-119.

Patil, S.S. 2012. Cultivation of *Pleurotus sajor-caju* on different agro wastes. *Sci. Res. Rept.*, 2: 225-228.

Quimio, T.H. 1976. Cultivation *Ganoderma* the "*Pleurotus*-way". *Mushroom Newsletter for Tropics*, 6: 12-130.

Quimio, T.H. 1978. Indoor cultivation of *Pleurotus ostreatus*. *Philippines Agriculturist.*, 61: 253-262.

Ravishanker, S., Pandey, M., Tewari, R.P. and Krishna, V. 2006. Development of sporeless/low sporing strains of *Pleurotus* through mutation. *World J. Microb. Biot.*, 22: 1021-1025.

Sharma, R. and Sharma, B.M. 2014. Strain improvement in *Pleurotus ostreatus* using UV light and ethyl methyl sulfonate as mutagens. *African. J. Microbiol. Res.*, 8: 432-436.

Shah, Z.A., Ashraf, M. and Ishtiaq Ch, M. 2004. Comparative study on cultivation and yield performance of oyster mushroom (*Pleurotus ostreatus*) on different substrates (wheat straw, leaves, saw dust). *Pakistan Nutrition.*, 3: 158-160.

Tan, K.K. 1981. Cotton waste is a fungus (*Pleurotus*) good substrate for cultivation of *Pleurotus ostreatus*: the oyster mushroom. *Mush. Sci.*, 11: 705-710.

Zadrazil, F. 1978. Cultivation of *Pleurotus*. In: Edi Chang, S.T. and Hayes, W.A. (Ed.), *The biology and cultivation of edible Mushrooms*. Academic press, New York. pp 521-538.