Journal of Advances in Microbiology

11(1): 1-8, 2018; Article no.JAMB.42270
ISSN: 2456-7116

Comparison of Growth and Development of *Pleurotus florida* against Wastes from Animal Origin

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Authors' contributions

This work was carried out in collaboration between all authors. Author Siddhant designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors Siddhant and OPU managed the analyses of the study. Author MK managed the literature searches and raw materials for the study. Author SS performed the statistical analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2018/42270
Editor(s):
(1) Pongsak Rattanachaikunsopon, Professor, Department of Biological Science, Faculty of Science, Ubon Ratchathani University, Thailand.
Reviewers:
(1) Yahaya Tajudeen, Federal University, Nigeria.
(2) Fatih Kalyoncu, Manisa Celal Bayar University, Turkey.
Complete Peer review History: http://www.sciencedomain.org/review-history/25085

Original Research Article

Received 26th March 2018
Accepted 5th June 2018
Published 11th June 2018

ABSTRACT

Two wastes from animal origin, viz., human hairs and egg shells were evaluated for different manifestations of white oyster mushroom, *Pleurotus florida*. The mushroom utilized both the substrates for their growth and sporophore formation. The mycelial growth was significantly (P=0.05) faster on egg shell (18 days) as compared to human hair (23 days). The crop of mushroom was harvested in four flushes where human hairs showed higher yield and biological efficiency of mushroom (165 gm, 33%) than egg shells (155 gm, 31%), respectively. In respect of yield parameters such as yield, biological efficiency, number of mushroom fruit bodies and average weight of sporophores, both the substrates were statistically at par to each other. Utilization of human hairs and egg shells by *P. florida* reveals a new strategy for mycoremediation of these wastes.

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Keywords: Animal wastes; biological efficiency; egg shell; human hair; Pleurotus florida; yield.

1. INTRODUCTION

The mushroom produces extra cellular enzymes which help in hydrolysing the complex organic substances into simpler forms those can be utilized and assimilated by them or release in surroundings. Generally, they utilize the substrates from plant origin i.e. lignocellulosic materials which comprise of three major groups of polymers i.e. cellulose, hemicelluloses and lignin [1] which represent the most abundant renewable organic matter on the earth. These substrates are used as a growing medium for oyster mushroom. These include waste from cereal [2], millet [3], Oil crop [4], Cotton [5], palm [6,7], pulses [8], vegetable and fruit [9], Beverage [10], sugarcane crop [11], wood and wood product [12], grasses [13], weed [14] and spices plant [15]. Bracket fungi belonging to order aphyllophorales were also mentioned for *Pleurotus* cultivation [16]. Among various mushrooms, *Pleurotus* sp is due most versatile, capable of colonizing and degrading a variety of lignocellulosic wastes into edible protein [17] and are considered suitable for bio-conversion of agro-waste in to food and feed in developing countries [18]. Apart from agro-wastes, few industrial wastes viz., paper residues [19], baby dipers [20], oxo-biodegradable plastic waste [21] etc. have also been tried for the growing of oyster mushroom. *Pleurotus* cultivation on animal wastes is little documented. Therefore, it was interesting to observe that how does the mushroom react when those substrates are given them as a food source. This investigation was conducted to know the efficiency of *Pleurotus florida* against animal wastes, such as human hairs and egg shells in respect of various parameters of mushroom production.

2. MATERIALS AND METHODS

2.1 Mushroom Culture

The pure culture of *Pleurotus florida* was obtained from the Mushroom Section of Plant Pathology Department, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur (U.P.) India. It was maintained on Potato Dextrose Agar medium (peeled, sliced and boiled potato, 200 g; dextrose, 20 g; agar, 20 g l^{-1}) by using serial subculture method [22].

2.2 Spawn Strategy

2.2.1 Spawn substrate

Wheat grain (*Triticum aestivum*) was used as a spawn substrate which was purchased from the seed market of Faizabad. The spawn was prepared by the conventional method [22].

2.2.2 Spawn dose

Inoculation of substrate was made with spawn of *P. florida* @ 15% w/w on dry weight basis under aseptic conditions.

2.3 Substrate Preparation

Human hair (HH) and egg shell (ES) were used as a growing medium for *Pleurotus florida*. The human hairs were collected from the Femina Beauty Care, Faizabad, India. It was shampooed to remove dust and traces of oil and then washed thoroughly with deionised water. Egg shells were collected from the egg market of Mahobara Bazar. To remove impurity and the interference material, these were also rinsed several times in deionised water and were then chopped in to small pieces. Both the substrates were then pasteurized in the solution of Formaldehyde (500 ppm) and Bavistin (75 ppm) separately for 18 hours as suggested by [23].

2.4 Method of Cultivation

Plastic bag technology was employed in this experiment. The beds were prepared from pasteurized substrate by multilayered (3) spawning following the procedure adopted by [16]. Several (6–8) holes were punched on the sides of the plastic bags to facilitate cross-sectional ventilation. Finally, a total of 4 polythene bags from each substrate type were incubated in cultivation room at 22-30°C temperature for spawn run. Once the substrates were colonized by mushroom mycelium, the mouth of bag was opened. The bags were irrigated as per requirement. The room was moistened to maintain the relative humidity in between 85-95 per cent. The fruit bodies of appropriate size were picked-up by gentle handling.

2.5 Concerning Data

2.5.1 Data regarding mushroom development and yield parameters

The growth and development of mushroom were monitored daily. The time lapsed in spawn run, fruit body initiation and maturation were recorded. Yield parameters, such as number of fruiting bodies, average weight of sporocarp, total fresh weight (g) of mushroom and biological efficiency were also recorded at harvest time. Four rounds of mushroom harvests were made across all substrate types in the course of the experiment. The biological efficiency (%) was calculated as follows:

$$\%BE = FWm/DWs * 100\%;$$

Where, BE is Biological Efficiency (%); FWm is total fresh weight (g) of mushroom yield across all flushes, and DWs is substrate dry weight (g).

2.5.2 Statistical analysis

Completely Randomized Design (CRD) was followed for the experiment. The data was statistically analysed by using the analysis of variance (ANOVA) at p=5%. The critical difference (CD) was worked out at five per cent probability level.

3. RESULTS

The results regarding various parameters of mushroom production are illustrated in Table 1 and Plate 1.

3.1 Mushroom Development

All the substrate showed mycelia colonization. The mycelial growth was significantly (P=0.05) faster on egg shell (18 days) as compared to human hair (23 days). Visual observations regarding mycelial characters also indicated that there was a compact mycelial growth with dense strand in case of egg shell. The interim period of Pin-head formation was observed following the invasion of substrates by mycelial growth. It occurred quickly in human hair (26 days), followed by egg shell (28 days). Although, egg shell took least time for spawn run, however, it showed delayed primordial initiation. The time required for maturity of fruiting bodies was observed 32 days and 33 days, respectively for human hair and egg shell.

3.2 Mushroom Yield

The crop of mushroom was harvested in four flushes where yield and biological efficiency ranged in between 155-165 gm, 31-33 % in both the animal wastes. Between them, human hair (165 gm, 33%) produced higher yield and biological efficiency over egg shell (155 gm, 31%), although, these were at par to each other.

3.3 Number and Average Weight of Sporocarp

Human hair and egg shell produced 27 and 25 mushroom sporocarps during the entire crop cycle respectively, which were found at par to each other. The weight per sporocarp was observed 6.11 gm and 6.20 gm for HH and ES, respectively without any significant difference.

4. DISCUSSION

The mushroom utilized both the substrate for their growth and sporophore formation which indicated that both of them have met the nutritional requirement desired for fungal growth. The variation in the number of days taken for a spawn to complete colonization of a given substrate is a function of the fungal strain, growth conditions and substrate type [24]. This variation could, in turn, be attributed to the variations in chemical composition and Carbon to Nitrogen ratio (C:N) of the substrates used [25].

Among animal wastes, highest sporophore yield was observed from human hair which might be due to utilization of keratin by *Pleurotus florida*. It is well established that major portion of human hair is keratin which is a fibrous and recalcitrant structural protein and is the third most abundant polymer after cellulose and chitin. Keratin is also the structural component of skin, feather, horns, hooves, nails, beaks, reptilian asteaderm and fish teeth and slime [26] which can be efficiently degraded by bacteria [27,28,29], actinomycetes [30,31] and fungi due to keratinase activity [32]. Fungal involvement in keratin degradation and keratinase production was shown by many workers [33,34,35,36,37,38]. Earlier, many ascomycetes [39] were reported for keratin degradation of feather [40,41] and hair alone [42] and in combinations. Although, there is a little exiting research on keratinase of basidiomycetes, there are few reports about keratinase production by genus *Pleurotus* spp. Recently, this activity was reported from white rot fungi, *P. pulmonarius* both *in vivo* and *in vitro*,

Table 1. Effect of animal wastes on various parameters of mushroom production

Substrate	Spawn run (Days)	Fruit body initiation (Days)	First harvest (Days)	Total yield from four flushes (gm/500 gm dry substrate)	Biological efficiency (%)	Total number of sporocarp	Average weight per sporocarp
Human hair	23	26	32	165	33	27	6.16
Egg shell	18	28	33	155	31	25	6.24
SE	**0.57**	**-**	**-**	**8.82**	**1.76**	**0.92**	**0.37**
CD (P=0.05)	**1.41**	**-**	**-**	**21.61**	**4.32**	**2.25**	**0.97**

Average of four replications

(A) **(B)**

Plate 1. Effect of human hair (A) and egg shell (B) on cropping of *Pleurotus florida* (Strain- P1)

using hair as a substrate [43]. For the growing of *Pleurotus* spp., paddy straw is found most common and efficient substrate [44] which generally yield 85-95% fresh mushrooms. Comparatively lower yield in hair substrate was probably due to presence of numerous disulfide bonds (S-S) [45,46] which are considered to be responsible for the stability of keratin and its resistance to chemical agents and enzymatic lysis (proteases).

Egg shell showed compact mycelial growth in our experiment. It might be due to their Ca and organic content i.e. shell membrane which favour the vegetative growth of mushroom. Earlier, egg shell was found suitable as a supplement for the mycelial growth of seven different species of *Hericium* [47]. The egg shell comprises calcified shell and shell membrane including inner and outer membrane. It contains about 95% calcium carbonate. 0.3 % phosphorus, 0.3% magnesium and trace of Na, P, Zn, Mn, Fe and Cu. The importance of calcium to the growth of fungi was already reported by several workers [48]. [49] found best mycelia growth when media containing all the essential macro nutrients including calcium while media without Ca gave lowest mycelial dry weight. Egg shell also produced mushroom sporophores. The delay in primordial development was because of the fact that initiation of fruit bodies only occurs when the substrate mycelium has attained a certain threshold density. [50] reported agricultural lime and egg shell powder yielded higher biological efficiency, and improved calcium level in *P. ostreatus*. [51] investigated the effect of addition of egg shell to saw dust substrate to evaluate the growth of *Flammulina velutipes*. Although, the egg shell did not improve the quality of mushroom in their experiment, it significantly increased the yield of mushroom fruit body.

5. CONCLUSION

Human hairs and egg shells are considered waste materials which are commonly disposed off in landfills without any pretreatment, therefore, its accumulation causes many environmental problems. In our investigation, *Pleurotus florida* utilized both the aforesaid wastes for its growth and fructification which revealed a new strategy for remediating sites contaminated by these wastes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/25085