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- A REVIEW

BIOREMEDIATION OF ENVIRONMENTAL AND AGRICULTURAL LIGNOCELLULOSIC WASTE THROUGH MUSHROOM CULTIVATION

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ABSTRACT

India is an agricultural nation and thus also rich in agricultural waste. Large amount of lignocellulosic waste and other types of such wastes are produced primarily through the activities of the agricultural, forest and food processing units. Various strains of mushrooms including oyster and shiitake can be used as a tool in process of bioremediation in which hazardous wastes are biologically converted to harmless compounds. Pleurotus species can easily colonize and degrade large varieties of waste e.g., rice husk, waste paper, corn cobs, saw dust, wood chips, sugarcane, bagasse, cotton waste, cotton seed hull, chicken and horse manure etc. Substrate preparation, inoculation, incubation, and production conditions depend on the mushroom species to be cultivated. These cultivated mushrooms are rich in essential nutrients such as carbohydrates, proteins, vitamins, minerals and several kinds of amino acids. Mushroom production in India has been estimated at 48000 tonnes per annum. It is mainly cultivated on the hills as it requires low temperature for its growth; however with the advent of modern cultivation technology it is now possible to cultivate this mushroom seasonally under uncontrolled conditions and throughout the year by employing environmentally controlled conditions. In the last ten years, large numbers of commercials units have been built by the entrepreneurs/farmers throughout the country for the production of button mushrooms. Various institutes in India are engaged in finding new technologies for growth of these agricultural macro fungi. Bioconversion of lignocellulosic agro-residues through mushroom cultivation offers the potential for converting these residues into protein rich palatable food. The technology can also limit land and air pollution associated with burning agricultural waste into the environment.

Key words: Lignocellulosic waste, Bioremediation, Mushrooms, Cultivation.

INTRODUCTION

Mushroom is a fleshy, spore bearing fruiting body of fungus. Like all fungi, mushrooms are not plants and do not undergo photosynthesis. These macro fungi can be

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used as a tool in the process of bioremediation in which hazardous wastes are converted into harmless compounds. Cultivation of mushrooms can be used in recycling of certain agricultural and environmental wastes such as those released from industries. Cultivation of edible mushrooms with agricultural residues, such as rice and wheat straw, is a value added process in which otherwise considered wastes are converted into human food. More than 2000 species of mushrooms exist in nature but approximately 22 species are intensively cultivated utilizing particular substrate and environmental conditions[1].

Mushrooms are unique biota which assembles their food by secreting degrading enzymes and decompose the complex food materials present in the biomass which then generate simpler compounds. These substrates are usually by-products from industry, households and agriculture which are usually considered as wastes. Mushroom cultivation on such wastes can prevent earth from consequently causing environmental pollution and health hazards. India being an agricultural nation producing enormous amount of waste in agro-industry and wood industry. Using 25% of the yearly volume of cereal straws can produce approximately 317 million metric tons (317 billion Kg) of fresh mushroom per year[2].

Mushroom production can generate equitable economic growth that has already had an impact at national and regional levels. The conversion of waste into edible mushrooms has been named non-green revolution by many researchers[3,4]. However, mushroom science is a relatively new applied science and mushroom industry is still small, need to investigate and explore. This review article deals with cultivation of various edible mushrooms on various types of wastes.

Mushrooms are cultivated on variety of substrates: The mushroom cultivation is a highly efficient method of disposing off agricultural residues as well as producing nutritious food[5]. The *Pleurotus* species also called as “Oyster mushrooms” or “Dhingri” now ranks second among the cultivated mushrooms in the world[6].

Cultivation of edible mushrooms is an art. It requires patience and clear knowledge of humidity and temperature. Mushrooms are grown on a variety of substrates. It has been recognized as a high potential converter of cheap cellulose into valuable protein[7].

There are two stages to mushroom growth : Mycelium growth and growth of fruiting body. Growing or cultivation of edible mushrooms is done in well sterilized place, adequate humidity during fruiting phase as well as during mycelia growth is very necessary. High concentration of CO_2 during mycelial growth and low concentration is required during formation of fruiting bodies. When CO_2 concentration in the growing bags exceeds 600 ppm, the stalk elongates and pileus donot develop normally[6]. Oxygen is required in more concentration for fruiting body initiation. Mild light is necessary during development of fruiting bodies. Mycelia formation occurs in dark while fruiting stages require 80-210 Lux of light. Temperature also plays an important role in cultivation of any mushroom strain , low temperature (10-28^{o}C) is required during fruiting bodies formation while little higher (20-25^{o}C) temperature is necessary for mycelia formation.

The main parameter used to evaluate mushroom yield is called biological efficiency (BE). It mainly depends on type of substrate used and conditions provided for cultivation of any edible mushroom species.

$$\text{BE (\%)} = \frac{\text{Total fresh wt. of mushrooms}}{\text{Dry wt. of the initial substrate}} \times 100$$

Various experiments were carried out using different types of wastes as substrates and then its Biological Efficiency is evaluated. Several distinct methodologies have been tested in various experimental studies aimed to increase yield, including differences in composting, pasteurization, kinds of substrates and supplements used.

According to worldwide survey, about 90 kinds of waste have been proven to be useful for oyster mushroom growth. Growing oyster mushroom on such agricultural waste gives satisfaction and lead to production of food and income. Oyster mushroom is popular mushroom due to its nutritional, medicinal and potential commercial value. As agricultural wastes are easily accessible and quite free sources, re-use of them could be considered as a breakthrough in production management[8].

Cultivation of the oyster mushroom, *Pleurotus* sajor-caju, on rice and wheat straw without nutrient supplementation was investigated[9].

Seven different type of substrates viz. Mango, Jackfruit, Coconut, Jam, Kadom, Mahogony, Shiris sawdust with wheat bran and $CaCO_3$ were evaluated for cultivation of *Pleurotus* mushrooms. The maximum biological yield per substrate was obtained as: Mango sawdust > Mahogony > Shiris > Kadom > Jam > Jackfruit > Coconut sawdust. The lowest yield was observed in Coconut sawdust (83 g). Cost benefit analysis revealed that the Mango sawdust and Shiris sawdust were promising substrates for the growing of Oyster Mushroom (*Pleurotus flabellatus*)[10].

Evaluation of combination usage of substrates as wood chips, boll, sugarbeet pellet pulp and palm fiber has been done along with supplements including wheat bran, rice bran, soya cake powder, soya cake powder + rice bran and carrot pulp for growth of *Pleurotus* species. Results revealed that boll, sugarbeet pellet pulp and palm fiber, which is abundant in bran, showed better growth period, fruiting body weight, yield and biological efficiency than wood chips[11]. Among mushrooms, *Pleurotus* (Oyster mushrooms) can make use of the largest variety of waste substrates with its fast mycelial growth and its multilateral enzyme system that can biodegrades nearly all types of available wastes. In Malaysia, Oyster mushroom is a popular mushroom due to its nutritional, medicinal and potential commercial value. This fungus is cultivated on sawdust and rice husk. In this study, the efficiency of cultivating oyster mushroom was assessed using palm oil mesocarp fibre as a substrate. This study revealed that palm oil mesocarp fibre could be used as a substrate for the production of mushroom just as other types of ligocelluloses materials[12].

Shiitake mushroom is traditionally cultivated on the shi tree (*Castanopsis cuspidate*) or wood logs in Japan. Scarcity of the shi tree has necessitated a search for alternative substrates for shiitake cultivation. Large amounts of freely available sawdust from different trees offer a potential alternative substrate source for mushroom cultivation in the tropics. It was cultivated on sawdust from the woody plants Babla (*Acacia nilotica*), Champa (*Michelia champaca*), Garzon (*Dipterocarpus alatus*), Ipil-ipil (*Leucaena glauca*), Jackfruit (*Artocarpus heterophyllus*), Mango (*Mangifera indica*), Raintree (*Albizia saman*), Segun (*Tectona grandis*), Shimul (*Bombax ceiba*), Shisoo (*Dalbergia sissoo*) or mixtures of sawdust from all of the trees with equal ratio or rice straw to determine growth and fruiting characteristics. Cultivation on Jackfruit resulted in significantly faster mycelial growth compared to other substrates. This study was undertaken to evaluate growth and yield of shiitake mushroom on locally available sawdust of different trees[13].

In Ghana, due to increase in production of the grain in the country, various problems associated with environmental pollution occur due to improper waste disposal methods. To avoid it cultivation of edible mushrooms using rice straw as substrate is done. Studies and reports are prepared on varying pretreatments and substrate formulations of rice straw (*Oryza sativa*) and 'wawa' sawdust (*Triplochiton scleroxylon*) as substrate for cultivation of *Pleurotus ostreatus*[14]. The study has been laid in which biodegradation of cowpea shells is done by cultivating *Pleurotus* spp. which further upgrade its nutritional values and digestibility for its use as ruminants feed[15]. Various cellulosic wastes and local agricultural wastes including wheat straw, soyabean straw and bran of rice, when used as culture media prove to be great substrate for cultivation of *Pleurotus eryngii*[16].

Pleurotus pulmonarius shows significantly higher colonization rate on cotton waste and cassava peel. As there is high availability of cassava in Nigeria, process of biodegradation of cassava peel which is otherwise considered as 'waste' through mushroom cultivation, can be used on large scale[17]. Bioconversion of lignocellulosic biomass by *Pleurotus* sajor-caju offers a promising way to convert low quality biomass into an improved human food[18].

In Mexico, huge amount of coffee pulp is discharged causing insalubrious condition and pollution in nearby rivers. Coffee pulp has also been exposed to a variety of microorganisms naturally occurring in the environment. To reduce such pollution, this coffee pulp can be used in production of Oyster mushrooms (*Pleurotus*)[19].

Study was done to describe the possibility of growth of *Pleurotus ostreatus* species under two different ecological regions (Peshawar and Swat of Khyber Pukhtoonkhwa). The study concluded that these areas of Pakistan are suitable for the cultivation of *Pleurotus ostreatus*[20].

Colak *et al.*, prepared two compost based wheat straw and waste tea leaves using wheat bran, ammonium nitrate, urea, molasses and gypsum as an activator and cultivated *Agaricus bisporus*[4]. This outdoor composting process took 28 and 35 days for wheat straw and waste tea leaves. If indoor composting is done it took 7 days. Composts were spawned with mycelium of *Agaricus bisporus*and filled in plastic bags. Chalk is also added to give pH of 7.5-8. Temperature and humidity are maintained according to Hays and Shandilya[21].

In Jaipur, Sanganeri handemade pulp, paper and cardboard industries were selected, there discharged effluent and solid sludge were utilized for cultivation of *Pleurotus florida.* 1.25 mL/L of 40% formalin solution (v/v) and 0.75 g/L (w/v) of antifungal bavistin were added to slurry prepared through solid sludge collected. Cultivated basidiocarps showed normal morphology of stipe and pileus[22].

Experiments were carried out in Egypt, in order to investigate the possibility of replacing peat casing soil by waste paper in mushroom production. *Agaricus* mushrooms were cultivated in plastic boxes or plastic bags. Humidity and CO_2 content were frequently measured. The casing is prepared by uncomposted shredded paper, composted paper and composted paper with nitrogen addition. Data revealed that uncomposted shredded paper was not suitable for mushroom production. Composting waste paper with the addition of nitrogen to lower C/N ratio gave promising results to be used as casing soil in mushroom production[12].

The effect of varying pre-treatments and substrate formulations of rice straw and sawdust of *Triplochiton scleroxylon*on the yield and biological efficiency (BE) of *Pleurotus ostreatus* strain EM-1 was studied. Bag culture method was adopted. Biological efficiency and suitability of substrates for cultivation of *P. ostreatus* in descending order is rice straw with composted sawdust with additives > Composted sawdust with additives > Rice straw only > Rice straw with fresh sawdust and additives. Pre-treatment and substrate formulation can improve yield of *P. ostreatus strain* EM-1 when chopped rice straw and sawdust of *T. scleroxylon* are used as substrates[14].

Hernandez *et al.,* used washed pangola grass (*Digitaria decumbens*) supplemented with coffee dregs as substrate in proportions of 70% and 30%, respectively for cultivating *P. ostreatus.* The composting process was carried out in boxes with or without ventilation. The experiment was divided in 5 treatments: T1 – control (out of box and mixed once a day during five days), T2 – without aeration and without mixture, T3 – with aeration and without mixture, T4 – without aeration and with mixture, T5 – with aeration and mixture. Soon after 5 days of the experiment, *P. ostreatus* matrixes were inoculated into the substrate until its complete colonization. The results showed the BE was lower in composts without mixture and aeration[23].

Castro *et al.,* evaluated the yield of *Pleurotus* sajor – caju using the residue of cotton textile processing as substrate. Two kinds of supplements were added to the cotton residue: Treatment 1: Bran, Treatment 2: Bran and bean straw. The material composting was performed during 10 days and, afterwards submitted to pasteurization until reaching the temperature of 60^{o}C; the process lasted 24 hr. Next, inoculation and incubation were performed during six weeks. It was observed that both treatments resulted in satisfactory BE (55.76% in T1 and 55.39% in T2), thus cotton textile residue being an excellent alternative for *P.* sajor- caju cultivation[24].

Mane *et al.,* grew *P.* sajor-caju in several agro-industrial residues: cotton processing residue, wheat straw, soy straw, pea stalk and peanut stalk. Residues are washed and pasteurized at 80^{o}C for 24 hrs. After, substrates are cooled and inoculated with fungus. Composts were stored inside polythene bags and incubated for 15 days at 27^{o}C. The best results were obtained when using cotton residues, pea stalk and wheat straw as substrates[1].

Bernardi *et al.,* used elephant grass as substrate and supplemented with tannery leather sawdust for *P. ostreatus* cultivation. Supplementation was in concentration of 0, 5, 10, 15 and 20% in relation to the wet mass of elephant grass. The compost was inserted into glass bottles and then inoculum was added. Bottles were sealed, sterilized twice for 40 min at 121^{o}C, incubated at 26^{o}C for 37 days. Then, bottles are kept into incubation room for 60 days more. Only treatment supplemented with 0% and 5% of tannery residue obtained fruitification with BE of 76 and 64% respectively. Substrates with more than 5% of supplementation were not colonized by the fungi[25].

P. sajor-caju and *P. ostreatus* were grown in banana tree straw, supplemented with rice bran (5%) by Bonatti *et al.*, The substrate was inserted into sealed polythene containers and submitted to sterilization for 1.5 hour at 121°C. Containers were cooled and inoculation is done. Incubation is done for 20 days at 25°C. "Fruitfication" was then induced, followed by additional 40 days of incubation. Moisture, fat, carbohydrate. Ash, protein and raw fiber analyses were performed. *P.* sajor-caju presented higher Biological efficiency (7.51%) than *P. ostreatus* (6.34%)[26].

Pedra and Marino evaluated *Pleurotus* spp. yield using coconut bark supplemented with rice and wheat bran. There experiment consisted of six treatments: T1: coconut sawdust (100%), T2: coconut sawdust (80%) and rice bran (20%), T3: coconut sawdust (80%) and wheat bran (40%), T4: coconut sawdust (60%) and wheat bran (20%), T5: coconut sawdust (60%) and rice bran (40%), T6: coconut sawdust (60%) and wheat bran (40%). The composts were stowed inside sealed containers and then sterilized twice for 40 minutes at 121°C. The substrate was cooled and inoculated. Incubation was done for 30 days at 25°C. The substrate was not colonized by fungus in T1, but treatments T4, T5 and T6 presented BE values of 14.32, 15.69 and 15.61% respectively, concluding that the addition of coconut bark supplementation to the substrate favors the development of the mushrooms[27].

Akyuz and Yildiz grew *P. eryngii* using soy straw and wheat straw as substrates. The experiment was divided into three treatments: T1: soy straw + wheat straw, T2: bean straw, T3: wheat straw, All of them were supplemented with rice bran in proportions of 5 and 10%. The experimental period was 100 days. Treatment 1 presented the most satisfactory BE (93%), with supplementation of 5% of rice bran. Treatment 3 showed the lowest BE (7%) with 10% rice bran supplementation[16].

Weeds were also used as substrates for *P. ostreatus* cultivation. The following species were used : *Leontis species* (Lamiaceae), *Sidaacuta* (Malvaceae), *Parthenium argentatum* (Asteraceae), *Ageratum conyzoides* (Asteraceae), *Cassia sophera* (Caesalpiniaceae), *Tephrosia purpurea* (Papilionaceae) and *Lantana camara* (Verbenaceae). The plants were completely sun dried, sectioned into small pieces and soaked in water and subsequently excess water was drained. Each species was inoculated with or without supplementing with wheat straw and afterwards submitted to incubation. *Leontis*spp. supplemented with wheat straw showed *P.ostreatus* yield (1.30 Kg/Kg). It was concluded that use of weeds is an efficient method for cultivation of edible mushrooms[15].

Motato *et al.,* grew *P. djamor* using leaves, stalk and fruit of the banana tree (*Musa paradisiaca*) and jequitiba (*Cariniana pyriformis*) sawdust according to seven treatments: T1: sawdust (100%), T2: leaves (100%), T3 : stalk (100%), T4: sawdust + stalk (50/50), T5: sawdust + leaves (50/50), T6: sawdust + fruit (50/50), T7 : sawdust + leaves + stalk + fruit (25% each). It was reported that fungal growth was more successful in T2, with a BE of 24.1%[28].

Alexandrino *et al.,* aimed to verify the production of lignocellulolytic enzymes by *P. ostreatus.* Dry, ground, and orange residue was used as substrate for the cultivation[29].

Sales-campos grew *P. ostreatus* in wood (*Simar oubaamara*) and (*Ochrom apiramidale*) sawdust, in agro industrial crushed sugarcane (*Saccharum officinarium*) and in stem of pupunheira palm tree (*Bactris gasipaes* kunth), all of the residues occurring in the Amazon region. All treatments obtained high BE[30].

CONCLUSION

Mushroom is an attractive crop to cultivate in developing countries for many reasons, one of the most charming is that they can decompose organic matter and can grow on agro-industrial residues, leaves, sawdust, fruit peels and industrial effluents according to availability. These edible fungi have capacity of synthesizing hemicellulose and lignin degrading lignocellulytic enzymes, can easily degrade and decompose agricultural wastes. Edible fungi are also used in the pre-treatment of byproducts employed in the feeding of ruminant animals, in order to make them viable for consumption. The studies and practical applications showed the viability of the use of several byproducts as substrates for edible mushroom cultivation. These substrates include elephant grass, coast- cross, crushed sugar cane, processing residues (cotton, paper, olive, oil, tannage), sawdust, straws (wheat, soy, banana tree, corn, bean) and stalks (banana tree, pea, peanut). Several kinds of materials are used as supplementation and highest biological efficiency was obtained with wheat straw and rice bran.

Mushroom cultivation at all stages is cost efficient and require very little in the way of technology. It was observed that edible fungi also degrade most of the organic matter used as substrate, thus representing a very useful way to eliminate toxic products from the environment, by turning them into nutritional source. Cultivating edible mushrooms represents a promising alternative for small producers because of low cost labor and easily available raw material. The market for mushrooms continues to grow due to interest in their culinary, nutritional, health benefits and potential for use in waste management.

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