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Short Research Article

Bioconversion of Low Quality Lignocellulosic Agricultural waste into Edible Protein by *Pleurotus djamor* (Rumph) Boedijn

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Abstract

The experiments were carried out at the Mushroom Research Laboratory, Department of Plant Pathology, Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.), India during 2010-2012. The culture of *Pleurotus djamor* was procured from Directorate of Mushroom Research, Solan and was maintained on PDA medium. Eight different substrates viz., wheat straw, corn cobs, lantana twigs, rice straw, poplar leaves, parthenium leaves, sugarcane bagasse, saw dust alone or wheat straw in combination with saw dust, wheat bran, cotton seed meal, urea, CAN, waste paper, lantana twigs and corn cobs were evaluated for the yield performance and biological efficiency (BE) of *P. djamor*. Among the various substrates Lantana twigs was found highly suitable with 75.23% BE for the cultivation of Pink oyster mushroom (*Pleurotus djamor*). Combination of wheat straw+wheat bran (9:1) and wheat straw+cotton seed meal (9:1) gave better yield with biological efficiency of 78.60% and 78.56%, respectively.

1. Introduction

A huge amount of lignocellulosic agricultural crop residues and agro-industrial by-products are annually generated, rich in organic compounds that are worthy of being recovered and transformed. Mushroom cultivation presents a worldwide expanded and economically important biotechnological industry that uses efficient solid-state-fermentation process of food protein recovery from lignocellulosic materials. On the surface of our planet, around 200 billion tons year^{-1} of organic matter are produced through the photosynthetic process (Zhang, 2008). However, majority of this organic matter is not directly edible by humans and animals and, in many cases, becomes a source of environmental problem. Moreover, today's society, in which there is a great demand for appropriate nutritional standards, is characterized by rising costs and often decreasing availability of raw materials together with much concern about environmental pollution (Laufenberg et al., 2003). Consequently, there is a considerable emphasis on recovery, recycling and upgrading of wastes. It is worth mentioning that only crop residues production is estimated to be about 4 billion tons per year, 75% originating from cereals (Lal, 2008).

Nevertheless, residues such us cereals straw, corn cobs, cotton stalks, various grasses and reed stems, maize and sorghum stover, sugarcane bagasse, corn husks, cottonseed and sunflower seed hulls, peanut shells, rice husks, waste paper, wood sawdust and chips, are some examples of residues and by-products that can be recovered and upgraded to higher value and useful products by chemical or biological processes (Wang, 1999; Fan et al., 2000; Webb et al., 2004). In fact, the chemical properties of such lignocellulosic agricultural residues make them a substrate of enormous biotechnological value. They can be converted by solid state fermentation (SSF) into various different value-added products including mushrooms. Commercial mushroom production, carried out in a large or small scale, is an efficient and relatively short biological process of food protein recovery from negative value lignocellulosic materials, utilizing the degrading capabilities of mushroom fungi .

Pleurotus spp. commonly known as oyster mushroom ranked third in the world mushroom production. Cultivation of the oyster mushroom has increased greatly throughout the world during the last few decades. Its popularity has been increasing due to its ease of cultivation, high yield potential and high nutritional and medicinal value. Furthermore, the abundant

agricultural wastes found countrywide offers opportunity for production that provides a more economical and environmental friendly disposal system. The use of fungi for the conversion of lignocellulose into food and feed rich in protein offers an alternative for developing unconventional source of proteins as food/feed. Yeast and algal protein require sophisticated techniques and heavy inputs where as the beauty of mushroom cultivation lies in its ability to grow on cheap lignocellulosic materials with minimum inputs and a high yield of valued food protein for direct human consumption. Pink oyster (*Pleurotus djamor*) is an extremely fast growing mushroom that fruits easily on a wide range of lignocellulosic substrates.

2. Materials and Methods

The experiments were carried out at the Mushroom Research Laboratory, Department of Plant Pathology, Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan (H.P) during 2010-2012. The culture of *Pleurotus djamor* was procured from Directorate of Mushroom Research, Solan and was maintained on PDA medium. Different agricultural and forestry waste materials viz., wheat straw, corn cobs, lantana twigs, rice straw, poplar leaves, parthenium leaves and twigs, sugarcane bagasse and sawdust (*Toona ciliata*) were used for the cultivation of *Pleurotus djamor*. The substrates were soaked in water for different periods depending on their ability to retain water. Moisture content of 60-70% with 6.5 pH was maintained in each case. Gypsum at the rate of 5% was added in each of the wet substrate. The ingredients were mixed thoroughly with hands which were then filled in polypropylene bags (one kg bag^{-1}) and tightly plugged with non-absorbent cotton. Sterilization was carried out at 22 psi for two hours. Five replicates of each treatment were kept throughout the cultivation studies. The pasteurized substrate was inoculated aseptically on the surface with freshly prepared spawn @ 2%. The spawned bags were kept at room temperature of 25+1°C and relative humidity of 85%. No fresh air was supplied during the incubation period. Water was sprayed on the walls and floor of the incubation room for maintaining the humidity. The bags were also sprayed twice a day using a foot sprayer with water. Time taken for complete mycelial run in each substrate was recorded. Observations were recorded on number of days taken for spawn run, initiation of primordia and total yield (upto 35-38 days). The spray of water was discontinued a day before the harvest of the fruiting bodies.

3. Results and Discussion

3.1. Evaluation of different substrates for cultivation of P. djamor

The perusal of data presented in Table 1 indicated that significantly minimum time for substrate colonization was recorded on parthenium (13.67 days) followed by wheat straw (14.67 days), corn cobs, lantana, sugarcane bagasse and rice straw which were statistically at par with one another. Among these, maximum time for complete colonization was taken on saw dust (18.33 days) followed by poplar leaves (17.67 days). After the complete colonization of the substrate blocks were kept for fruiting. It is obvious from the table that minimum time (18.33 days) for initiation was taken by wheat straw and lantana and this was statistically at par with each other.

On the contrary, maximum time (24 days) for initiation of primordia was observed on sugarcane bagasse that was statistically at par with poplar leaves and saw dust. Similarly, lantana supported maximum yield (225.7 g) of *P. djamor* followed by wheat straw (216.8 g) and parthenium (214.3 g). There was significant difference in yield on these substrates. The overall biological efficiency was maximum (75.23%) in lantana and minimum (29.33%) in saw dust.

3.2. Evaluation of combination of substrates for cultivation of P. djamor

The data regarding average spawn run of *P. djamor* on various combinations of substrates showed that wheat straw+wheat bran (9:1) supported fast growth and colonized the whole substrate within 10.33 days of inoculation followed by mixture of wheat straw+corn cobs (3:1) (10.67 days); wheat straw+cotton seed meal (9:1) (11 days); wheat straw+saw dust (3:1) (11.33 days) and wheat straw+waste paper (3:1) (11.33 days), where growth was significantly at par with one another. However, the fungus took maximum days for spawn run in wheat straw+urea (1%) (18.33 days) followed by wheat straw+CAN (0.5%) (16.67 days) and wheat straw+lantana

Table 1: Effect of different substrates on spawn run, primordia formation and yield of *Pleurotus djamor*

Treatments	Complete Spawn run (days)*	Primordia initiation (days)*	Yield (g 300 g^{-1} dry substrate)*	Biological efficiency (%)
Wheat straw	14.67	18.33	216.8	72.26
Corn cobs	15.67	19.67	185.0	61.67
Lantana twigs	15.67	18.33	225.7	75.23
Rice straw	15.67	21.00	207.7	69.23
Poplar leaves	17.67	23.67	114.7	38.23
Parthenium leaves	13.67	21.67	214.3	71.43
Sugarcane bagasse	15.67	24.00	98.90	32.96
Saw dust	18.33	23.33	88.00	29.33
Mean	15.87	21.25	168.88	
SEm±	0.60	0.57	0.47	
CD (p=0.05)	1.27	1.22	9.70	

*Average of three replications

(3:1) (12 days).

Similarly, data on time taken for primordia initiation of *P. djamor* revealed that significantly minimum time for initiation of primordia (12.67 days) was observed on mixture of wheat straw+corn cobs (3:1) followed by wheat straw+lantana (3:1) (13.33 days). Wheat straw+saw dust (3:1) and wheat straw+cotton seed meal (9:1) took maximum time of 15.67 days for primordia initiation and this was statistically at par with each other.

It is clear from the data depicted in Table 2 that treatment containing wheat straw+wheat bran (9:1) supported maximum yield (236.3 g) with a biological efficiency of 78.76% followed by wheat straw+cotton seed meal (9:1) which gave yield of 235.7 g with a biological efficiency of 78.56% and this was statistically at par with each other. Mixture of wheat straw+waste paper (3:1) gave yield of 123.6 g^{-1} 300 g whereas combination of wheat straw+lantana (3:1) gave yield of 117.5 g^{-1} 300 g dry substrate. Significantly much lower yield of 69.53 g was recorded on wheat straw+CAN (0.5%) followed by wheat straw+corn cobs (3:1) which gave an average yield of 104.7 g.

Most of the mushroom species possess the ability to degrade lignin, cellulose and hemicelluloses and to produce fruit bodies containing most of the essential amino acids, valuable vitamins, minerals and low energy carbohydrates. Pleurotus spp. have the potential to convert cheap cellulosics into valuable protein at a low cost. For the first time, obnoxious plant species like *Lantana camara* and Parthenium were used for the cultivation of *Pleurotus djamor*. For evaluating the substrate best suited for cutivation of *P. djamor*, different forest and agricultural wastes were tried for yield potential and biological efficiency. It was recorded that parthenium was colonized in minimum time of 13.67 days followed by wheat straw. Slowest spawn run was noticed with saw dust followed by poplar leaves. Minimum time for primordial initiation was observed in lantana and wheat straw which was at par with each other followed by corn cobs; whereas, maximum time was taken on sugarcane bagasse (24 days) followed by poplar leaves and saw dust which were statistically at par with each other.

After evaluating various substrates it was noticed that maximum yield and biological efficiency was obtained when Lantana twigs was used as substrate, which was followed by wheat straw, parthenium leaves, rice straw. Lowest yield and biological efficiency was recorded with saw dust followed by sugarcane bagasse. *Lantana camara* is a terrible weed, exerting huge detrimental effect on biodiversity. Its leaves and flowers contain toxins, lantadene A and B. As per review of Patel (2011), it acts as lignocellulosic substrate for cultivation of edible mushrooms. In addition to toxin, it contains bioactive ingredients exhibiting anticancer, antiulcerogenic, hypolipidemic and anti-inflammatory activity. Moreover, in our studies we make use of lantana twigs for substrate preparation which may not contain toxins.

More or less similar waste materials like sawdust or wood shavings (Block et al., 1959; Omari, 1974; Zadrazil, 1980), Paddy straw and wheat straw (Park et al., 1975; Jandaik and Kapoor, 1976; Kandaswamy and Ramaswamy, 1976; Zadrazil, 1976; Singh and Rajarathnam, 1977; Bhaskaran et al., 1978, Balazs and Szabo, 1979; Bano et al., 1979; Sivaprakasam and Kandaswamy, 1980; Stanek and Bisko, 1982; Ginterova et al., 1982; Delmas and Mamoun, 1983) and crushed corn cobs (Kostadinov and Stefanov, 1977; Sharma, 1984) have been used with varying success for the cultivation of various *Pleurotus* spp. It also appears that plant wastes can be utilized by several *Pleurotus* spp. but their nutritional availability to these species varies (Zadrazil, 1980).

The ability of different *Pleurotus* spp. to utilize such diverse waste materials may be due to their high saprophytic ability to decompose and utilize lignin containing materials (Nair, 1980). Paddy straw followed by wheat straw was found to give highest sporophore yield of *P. ostreatus* by Dubey (1999);

Table 2: Influence of substrate supplementation on yield of *Pleurotus djamor*

Treatments	Spawn run period (days)*	Primodia initiation stage (days)*	Yield (g 300 g^{-1} dry substrate)*	Biological efficiency (%)
Wheat straw+Saw dust (3:1)	11.33	15.67	96	32
Wheat straw+Wheat bran (9:1)	10.33	14.67	236.3	78.76
Wheat straw+Cotton seed meal (9:1)	11.00	15.67	235.7	78.56
Wheat straw+Urea (1%)	18.33	23.00	0.0	0.0
Wheat straw+CAN (0.5%)	16.67	22.67	69.53	23.17
Wheat straw+Waste paper (3:1)	11.33	13.67	123.6	41.2
Wheat straw+Lantana twigs (3:1)	12.00	13.33	117.5	39.16
Wheat straw+Corn cobs (3:1)	10.67	12.67	104.7	34.9
Mean	12.70	16.4	122.91	
SEm±	0.57	0.52	2.85	
CD (p=0.05)	1.22	1.11	6.04	

*Average of three replications

Hussain and Hussain (2004).

Utilization of substrates for oyster mushroom depends mainly on water holding capacity of the substrates, pH, air spaces in the particles. Oyster mushrooms can grow on various agricultural and domestic wastes such as wheat straw, soyabean straw, used tea leaves (Jain and Vyas, 2002 a,b, 2003). Growth and yield of *P. djamor* was poorest in saw dust, which are in conformity with the findings of Ponmurugon et al. (2007). Singh et al. (2009) also reported non-suitability of saw dust for *Pleurotus eous.*

To study the effect of supplementation on yield of *P. djamor*, different forest and agricultural wastes were tried in different combinations. It was recorded that wheat straw+wheat bran (9:1) was colonized in minimum time of incubation (10.33 days) followed by mixture of wheat straw+cotton seed meal (9:1). Slowest spawn run was noticed with wheat straw+urea (1%) followed by wheat straw+CAN (0.5%). The minimum time for initiation of fruiting bodies was observed on mixture of wheat straw+corn cobs (3:1) followed by mixture of wheat straw+lantana (3:1), whereas, maximum time was taken on wheat straw+urea (1%) followed by wheat straw+CAN (0.5%).

Maximum yield and biological efficiency was obtained from the treatment containing wheat straw+wheat bran (9:1) followed by wheat straw+cotton seed meal (9:1). Supplementation with a nitrogenous source is a key for higher yields in different combinations. Lowest yield and biological efficiency was recorded with wheat straw+CAN (0.5%) followed by wheat straw+saw dust (3:1). The results are in accordance with the findings of EI-Kattan et al. (1991). Although supplementations have been reported to enhance the yield, they have not been adopted by growers yet.

Pleurotus spp. could colonize and produce fruiting on pre-treated conifer (*Pinus spp*.) wood chips but they did not readily colonize non-pretreated conifer wood due to presence of inhibitory component (Croan, 2004). Good yield on saw dust was observed by Shah et al. (2004). Saw dust from nitrogen fixing trees mixed with 25% coconut residue was recorded to give efficient results in comparison to the use of saw dust alone (Iqbal et al., 2005). Saw dust substrate supplemented with manganese and soyabean was reported to give better yield than the use of saw dust alone (Esrada and Royse, 2007). Wheat straw+waste paper (3:1) gave an average yield of 123.6g. Similar type of observations have also been recorded by Baysal and Packer (2001). In conclusion, the treatment of lignocellulosic biomass with *Pleurotus djamor* offeres a promising means to convert low quality biomass into a high protein food. Among eight agrowastes applied in this study, five are viable substrates for mushroom cultivation alone or in combination.

4. Conclusion

Among the various substrates, Lantana twigs was found highly suitable with 75.23% BE for the cultivation of Pink oyster mushroom (*Pleurotus djamor*). Combination of wheat straw+wheat bran (9:1) and wheat straw+Cotton seed meal (9:1) gave better yield with biological efficiency of 78.60% and 78.56%, respectively.

5. Further Research

There is a need to conduct further research on the toxic/ allergic effect of Lantana twigs and Parthenium leaves on human beings, if any.

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