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Biological efficiency and quantification of biochemical substances in Shiitake mushroom cultivated on different agricultural wastes in India

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Lentinula edodes or Shiitake is a macro-fungus which produces brown edible and medicinal fruiting bodies. The Shiitake mushroom is the second most important edible mushroom in the world from the standpoint of production just after *Agaricus bisporus*. In an effort to understand the different constituents of agricultural lignocellulosic residues and the impact of different substrates on mushroom yield, it was cultivated on different agro-wastes viz. wheat straw, pulses wastes, banana leaves, cotton wastes, sugarcane bagasse and corn stalks to determine the effect of these agro wastes on yield, crude protein, crude fiber and ash content. Wheat straw showed highest yield and biological efficiency. Cotton wastes had maximum amount of fiber and ash but mushroom produced on pulses wastes had maximum crude protein. Maximum crude fiber content and ash was found in the fruiting bodies grown on cotton wastes.

Key words: Biochemical analysis, biodegrader, *Lentinula edodes*, substrates

INTRODUCTION

Protein is one of the most important nutrients in food, being particularly important for building body tissues. Mushrooms with protein content ranging from 3-7% when fresh, to 25-40% when dry, can play an important role in enriching human diets when meat sources are limited. As a dietary source of protein, mushrooms are superior to most fruits and vegetables with the exception of beans and peas (Anderson, 2008). Mushrooms are grown on some organic substrates, mostly waste materials from farms, plantations or factories. These otherwise useless by-products can therefore be recycled to produce value-added mushrooms. According to the Food and Agriculture Organization (2004) about 20 million tons of usable agricultural wastes are discarded each year. India is an agriculture based country in which various cheap agricultural wastes like rice straw, wheat straw, saw-dusts of various trees etc (Kaur and Lakhanpal, 1995) are easily available and can be utilized for the production of *Lentinula edodes*. This mushroom can be successfully cultivated on several crop residues like paddy straw, cotton waste, wheat straw (Ashrafuzzaman *et al., 2009)* and sugarcane industrial waste (Ivan *et al., 2003*). For a long time, this mushroom has been valued for its unique taste and flavour and as a medicinal tonic (Wasser, 2002). *Lentinula edodes* or Shiitake is the first medicinal macro-fungus to enter the realm of modern biotechnology. According to a Chinese folk fare, it is capable of generating stamina, curing colds, improving the circulation and lowering blood pressure. Recently, it has been found that it contains many biologically active substances and is effective in lowering serum cholesterol levels and possessing antitumor and antiviral activities (Chang and Miles, 2004). It is also believed to prevent premature aging. A number of products prepared from *L. edodes* are sold throughout the world as dietary supplements (DS). The market value of mushroom DS products worldwide is estimated at US $ 6 billion per year. The worldwide production of this mushroom is more than 1,564,000 MT with almost 88.8 per cent (1,397,000 MT) produced in China (Chang,2007). Many brands of this mushroom product are sold in the market. Modern researches on this mushroom's biology, biochemistry pharmacology and therapeutics have

provided a firm basis for the market of *Lentinula* products. This mushroom functions as a nature's recycler, which can convert lignocellulosic wastes in to protein rich food. Cultivation of edible mushroom like *Lentinula edodes, Volvariella volvacea* and *Pleurotus sajor-caju* is one of the most economically-viable processes for the bioconversion of many types of lignocellulosic wastes (Buswell *et al.*, 1996). Although mechanized cultivation is providing adequate amount of food, yet the actual problem is to provide sufficient amount of protein for constantly increasing world population. So the production of this mushroom is going to narrow down this protein gap. Understanding of the different utilization of diverse constituents of agricultural lignocellulosic residues and the impact of different substrates on mushroom yield will be invaluable for the effective bioconversion of locally available agricultural wastes and for mushroom diversification in India where the respective mushroom market is largely dominated by *Agaricus* and *Pleurotus* species (Sharma *et al., 2006*). Keeping this in view the present study has been done to find out the effect of different substrates on yield and biochemical analysis of substrates and fruiting bodies of mushroom.

MATERIALS AND METHODS

Culture isolation and maintenance

Lentinula edodes strain L1 was obtained from the Mushroom Research and Training Centre (MRTC), Pantnagar. It was maintained on PDA (potato dextrose agar) at 25°C.

Spawn and substrate preparation

The spawn was prepared on wheat grains according to the methodology of (Garcha, 1994). The substrates used for the experiment were agricultural wastes like wheat straw (WS), banana leaves (BL), cotton wastes (CW), corn stalks (CS), sugarcane bagasse (SB) and pulses straw (PS), all enriched with 10 per cent wheat bran. The substrate mixture was filled only 3/4 the capacity, in 2 kg capacity polypropylene bags. Five replications per substrate were kept. The neck of the bags were plugged with non-absorbent cotton and sterilized at 121°C for 90 minutes. After cooling, the bags were inoculated with the fungus. The bags were kept in the crop room at relative humidity of 80-85 %, at 25°C temperature in the dark for 60-70 days for complete spawn run. After the spawn run, slitting was done and relative humidity of 80-90 % was maintained by sprinkling water.

Mycelium growth rate test

All the substrates were appropriately moistened (60-65%) and filled uniformly in test tubes. The tubes were leveled and plugged with non absorbent cotton and sterilized for 1.5 at 121°C (Pande and Tewari, 1990). After cooling, the tubes were inoculated with three agar plugs cut from a growing colony in a Potato Dextrose Agar (PDA) containing Betri dishes and incubated at 25°C. Five replications for each treatment were kept. Linear growth was measured at an interval of 24hs.. Mycelial running rate (MRR) on each substrate was estimated on the basis of the ratio between the total distance covered by the mycelium and the time needed for growth to occur: MRR= ($cm \cdot day^{-1}$)

Biological efficiency

Fruiting bodies were harvested after maturity. Numbers of well-developed fruiting bodies were recorded. Yield and biological efficiency were calculated using the following formula: Biological efficiency (%) = Fresh weight of fruit body / Dry weight of substrate x 100

Biochemical analysis of substrates and fruiting bodies

Colonized substrates were subjected to proximate analysis at different stages of mushroom development viz. before inoculation, after spawn running and after termination. After harvesting, mushrooms collected from six different substrates were also analyzed for crude protein (N X 6.25), crude fiber and ash contents (AOAC, 1990).

Statistical analysis

All the data were subjected to analysis of variance (ANOVA) technique and all possible interactions were calculated. Duncan's Multiple Range (DMR) test was applied to separate significant differences among different strains and substrate means (Duncan, 1955).

RESULTS AND DISCUSSION

Mushrooms depend on substrates for nutrition and the substrate is normally a source of lignocellulosic materials which support growth, development and fruiting of mushroom (Chang and Miles, 2004). Sawdust is the most popular basal ingredient used in substrates to produce shiitake (Miller and gong, 1987; Palomo *et. al., 1993*). Other basal ingredients can include straw and corn cobs, or their mixtures. Regardless of the main ingredient used, starch-based supplements such as wheat bran, rice bran, millet, rye or corn, can be added at 10 to 40% of dry weight to the main ingredient (Ivan *et al.*, 2003; Royse, 1996).

Table. 1: Biological efficiency and yield attributes of Shiitake mushrooms grown on different substrates

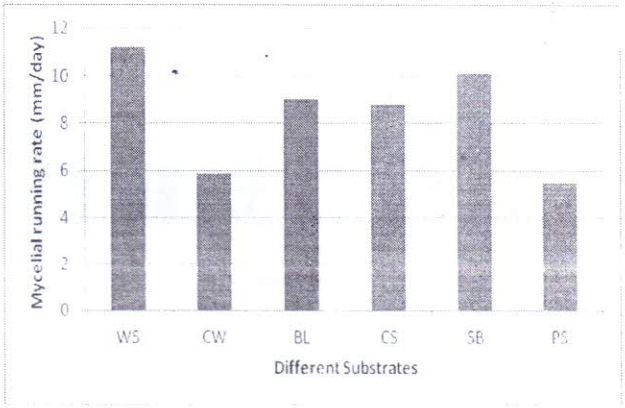
Substrates	Numbers of fruiting bodies	Yield (g)	Biological efficiency (%)
Wheat straw	11	80.4	45.9
Corn stalks	3	23.2	9.9
Banana leaves	5	19.0	8.14
Cotton waste	7	56.6	24.2
Sugarcane baggasse	9	77.4	33.2
Pulses straw	3	20.3	5.8
CD (P=0.01)		10.3	

Table. 2: Crude protein, ash, moisture and crude fiber percentage in the fruiting bodies of Shiitake mushrooms harvested from different substrates

Substrates	Crude Protein (%)	Ash (%)	Moisture (%)	Crude fiber (%)
Wheat straw	28.57	2.43	89.90	7.88
Cotton waste	34.17	10.80	89.30	11.90
Banana leaves	23.92	5.93	88.60	7.34
Corn stalks	21.77	1.53	88.20	7.04
Sugarcane bagasse	30.51	0.96	86.00	7.22
Pulses straw	20.25	2.05	88.10	8.14
LSD	0.95	0.54	2.22	0.81

Mycelial running rate (MRR) in test tubes

Remarkable differences were recorded in mycelia running rates of the fungus on different substrates in the test tubes. It ranged from 4.8 to 10.6 mm/day (Fig.1). The highest MRR was recorded for wheat straw substrate followed by sugarcane bagasse. The presence of the right proportion of alpha-cellulose, hemi-cellulose, pectin and lignin was the probable cause of higher rate of mycelium running in wheat straw. Suitable C:N ratio might be responsible for the higher mycelial growth. The capacity of mushroom to grow on lingo-cellulosic substrates is related to the vigour of its mycelium (Permana *et al.*, 2004).



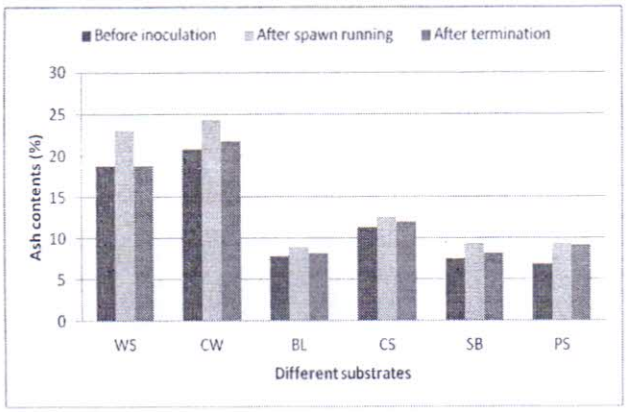
Abbreviations : WS, Wheat straw; CW Cotton wastes; BL, Banana leaves; CS, Corn stalks; SB, Sugarcane bagasse; PS, Pulses straw

Fig.1: Linear extension rates of *Lentinula edodes* strain L1 on different substrates (mean of five replications)

Biological efficiency and yield

The number of fruiting bodies varied with different substrates (Table 1). The maximum number of fruiting bodies developed on wheat straw substrate followed by sugarcane bagasse. Cotton wastes and pulses straw substrates gave lowest number of fruiting bodies. Earlier workers also found that wheat straw substrate gave highest yield and biological efficiency and is a most suitable substrate for production *L. edodes* (Kovassne and Kovacs, 2000; Philippoussis *et al.*, 2003). It was predetermined that substrates containing glucose, fructose and trehalose produced the highest number of primordia while those containing glycerol, xylose, sucrose and fructose produced abnormal fruiting bodies. The product of cellulolytic action of the fungus was simple and soluble carbohydrates and the end products being glucose was absorbed by the fungal mycelium for growth

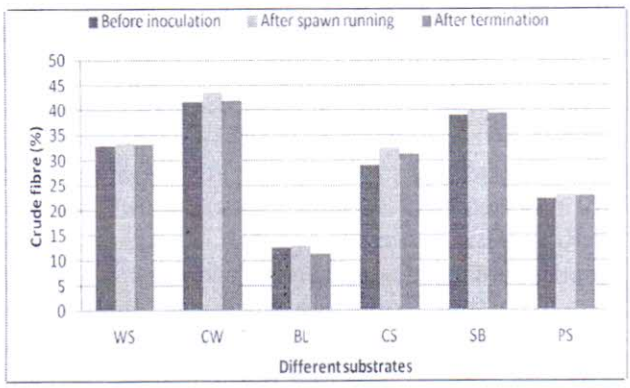
and energy. Therefore, cellulose rich organic substrates are good for the cultivation of mushroom (Gerrits and Muller, 1965, Quimio, 1987). Among the substrates tested, wheat straw had maximum amount of cellulose and supported highest production of mushrooms. It has been reported previously that wheat straw contains 44-55 per cent cellulose, 25-35 per cent hemicelluloses and 15-20 per cent lignin (Ramasamy and Kandaswamy, 1976). High cellulose content resulted in enhanced cellulose enzyme production and increased yield of mushroom (Ramasamy and Kandaswamy, 1976).



Abbreviations : WS, Wheat straw; CW,Cotton wastes; BL, Banana leaves; CS, Corn stalks; SB, Sugarcane bagasse; PS, Pulses straw.

Fig. 2: Ash contents in the substrates at different growth stages of the mushroom

In a similar manner, other substrates like cotton wastes and banana leaves have high lignin and phenol content decreased the activity of the enzyme, hence slow growth and low yield.

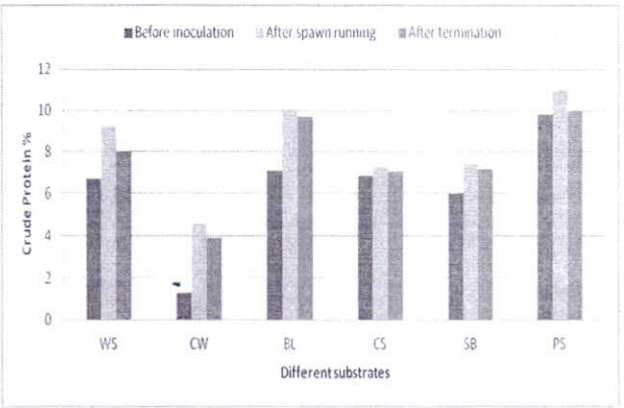


Abbreviations : WS, Wheat straw; CW,Cotton wastes; BL, Banana leaves; CS, Corn stalks; SB, Sugarcane bagasse; PS, Pulses straw.

Fig. 3: Crude fiber percentage in the substrates at different growth stages of the mushroom

Biochemical analysis of substrates and fruiting bodies

Biochemical analysis for the crude protein, crude fiber and ash content in the substrates showed variations at different growth stages of Shiitake mushroom which showed that different phases of mushroom growth had significant effect on the biochemical makeup of the substrates and *vice-versa*. As evident from the results, of the six substrates tested, cotton waste was richest substrate in proteins, ash contents and fibers (Figs.2, 3 and 4). Amount of ash content signifies the amount of silica present in the mushrooms. Different substrates contained different amount of ash contents during the three growth stages of mushroom. Least amount of ash was recorded



Abbreviations : WS, Wheat straw; CW,Cotton wastes; BL, Banana leaves; CS, Corn stalks; SB, Sugarcane bagasse; PS, Pulses straw.

Fig. 4: Crude protein percentage contents in the substrates at different growth stages of the mushroom

before inoculation which subsequently increased after spawn running and ultimately decreased after harvesting of the fruiting bodies (Fig. 2). The highest crude fiber was found in cotton waste and lowest percentage of the fiber was found in the banana leaves (Fig. 3). The mean values of the protein percentage of the substrates showed that highest protein was present in the pulses wastes (Fig. 4). The data for the crude protein percentage showed that protein percentage increased after the spawn running of the mushroom (11.02%) which was 9.85% before inoculation of spawn, but that percentage was decreased after the termination of the fruiting bodies and became (9.97%). The results of biochemical analysis of the agricultural wastes used for the cultivation of mushroom showed

high protein content in substrates after the inoculation with mushroom spawn as compared to the protein content before inoculation. Similarly, crude fiber and ash contents were also high in agricultural wastes inoculated by fungus as compared to non-inoculated ones. Different scientists reported that, the fungal growth on different agro-wastes enhances their nutritional values (Hernandez *et al.* 2011). The increase in the fiber, ash and protein content of the substrates after the treatment with mushroom spawn may be due to secretion of certain extracellular enzymes which are involved in the breakdown and subsequent metabolism of the substrates. Biological treatment of agricultural wastes with fungi increased the nutritional values of these treated substrates and these substrates can also be used as animals feed (Belewu and Banjo, 1990).

The biochemical analysis of the fruiting bodies obtained showed that, the fruiting bodies harvested from different substrates had different crude protein, ash contents. The ash percentage and crude fiber were highest in the fruiting bodies harvested from cotton waste (Table 2). This signifies that the nutritive values of mushrooms also depend on the composition of their substrates. Dry matter of Shiitake mushrooms contained large amounts of carbohydrates i.e. 5.8 g/100 g fresh weight. *L. edodes* proved to be an especially good source of dietary fibers (3.3 g/100 g fresh weight) and crude fat, ash and protein contents varied from 0.31-2.09 g/100 g fresh weight. Amino acid concentrations in *L. edodes* were 15.24 per cent in caps and 11.35 per cent in stipes (Mattila *et al.*, 2002). Shiitake mushroom acts as a biodegrader, reduces environmental pollution and produce beautiful and flavorful brown mushrooms and if only one-fourth of the world's annual yield of cereal straw (2.325 million tonnes) was used to grow mushrooms, about 377.8 million tonnes of fresh mushrooms could be produced and such an amount would provide 4.103 million people with 250 g of fresh mushroom daily (Madan *et al.*, 1994; Jandaik Goyal, 1995). The cultivation of mushrooms by utilising cereal straw and pulses wastes can solve the problem of protein deficient food of developing countries and also generate employment. In the conclusion it can be stated that Shiitake mushroom can be grown on the wide variety of agricultural wastes available in India. Cotton waste, wheat straw, pulses wastes and banana leaves are the good substrates for the mushroom cultivation. These waste materials are also abundantly and cheaply available in the country. The treatment of the substrates with *L.edodes* enhanced the amount of protein, fiber and ash contents and they can be used for feeding animals. The mushroom can be consumed directly either as a balance diet or as health food (Dietary supplements) and can also be used in canned form. Shiitake cultivation and consumption both appeal to health-conscious populations, vegetarians and peoples facing protein malnutrition problems for its attributes of high nutrition, quality protein, essential amino acids, low calorie content and health benefits. In India where Shiitake mushrooms are not a traditional food, demand for mushrooms such as Shiitake depends on the continuous effort of mushroom producers to educate consumers.

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