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Perusal of Pleurotus Djamor Growth by Using Different Substrates and Evaluate the Amount of Protein Content.

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ABSTRACT:

Pleurotus djamor is commonly called a pink oyster mushroom. The flavor of the pink oyster mushroom has been described as meaty and fishy. It is a species of fungus in the family Pleurotaceae. It was originally named Agaricus djamor. The present study describes the cultivation of pink oyster mushrooms with the utilization of vegetable waste (cabbage, cauliflower and radish leaves) in combination with agro waste (paddy straw and sugarcane bagasse) as substrate. Different ratios of both the substrates were used for the cultivation. When cultivation was carried out on vegetable waste alone, there was absence of mycelium spread and fructification. However, the combination of 50% vegetable waste and 50% paddy straw supported significant growth. It also shows high protein content during the estimation process. Thus the study implies that vegetable waste can prove to be a potent substrate for cultivation of pink oyster mushrooms with high protein content.

Keywords: spawn, pink oyster mushroom, vegetable waste, agro waste, protein, biuret method.

INTRODUCTION:

Mushrooms are one of the most loved food not only for its exotic taste but also for the benefits with which it comes. It can be consumed in various forms like fresh, pickled, dried, powdered, canned etc. Its farming has picked up a fast pace among contemporary entrepreneurs owing to its nutritional and medicinal benefits and low cost input with high output. Mushrooms are a fleshy fungi (Basidiomycota, Agaricomycetes) having a stem, cap and gills underneath the cap [1]. Because of its medicinal and nutritional benefits, mushrooms have long been valued as a significant dietary item. They are regarded as a good source of carbohydrates and protein. More than 3000 species of mushrooms have been shown to be edible, yet only ten of them are commercially grown. *Agaricus bisporus* is the most widely farmed mushroom in the world. It is followed by *Pleurotus* species, which account for around 27% of all cultivated mushrooms and include 5 to 6 different species [2]. From ancient times, mushrooms are looked upon as a delicacy for human consumption. The importance of consumption of mushrooms is based upon its nutritional benefits on human health. The exotic flavor, fleshy texture, richness of the mushroom makes it one of the most desirable food item for human consumption. Mushrooms are rich source of proteins polyunsaturated fatty acids (PUFA) and many other vital nutrients essential for human body with the advantage of having a low calorific value. Oyster Mushroom (*Pleurotus* sp.) also known as Dhingri in India is a macro lignocellulolytic fungus belonging to basidiomycetes [3]. Cultivation of edible mushrooms with agricultural and agro-industrial residues as substrate is an efficient and economically reliable technology for converting these materials into a valuable protein rich food and a cash crop of commercial interest [4]. It is considered to be unique based upon its ability of rapid mycelial growth and ability to muster its food by secreting some degrading enzymes. It also has a distinctive fruiting body. Tropical and temperate regions provide suitable environmental conditions which favour the growth of *Pleurotus djamor*. The various substrates that can be used for production of *Pleurotus djamor* are vegetable waste (cabbage, cauliflower and radish leaves) in combination with agro waste (paddy straw and sugarcane bagasse). The choice of substrate play an important role in the production of mushroom. It can invariably influence the growth characteristics and yield [5]. There is an increase in demand for production of *Pleurotus djamor* due to its medicinal properties which can play a crucial role in saving people from some life threatening diseases. Few medicinal properties of *Pleurotus djamor* include anticancer, immune modulator effect, anti inflammatory activites [6]. Microbial technology can help in large scale recycling of agro waste in India [7]. An alternative way of use of agricultural residues/wastes is in the use of the organic material in mushroom production [8]. The following study enlightens the aspect of the effect of different substrates on the yield of *Pleurotus djamor.*

MATERIALS AND METHODS:

Sterilization Procedure:

All the apparatus, culture media, glasswares were sterilized using autoclave at 121ºC at 15 psi pressure for 1 hour. The culture room was cleaned with detergent followed by disinfection using 70 % ethyl alcohol. The inoculation procedures were carried out in the laminar air flow cabinet under strictly sterile conditions. UV was switched on 15 minutes prior to the inoculation [9].

Preparation of Potato Dextrose Agar media (PDA):

250 grams of potatoes were taken, washed, peeled, sliced then they were subjected to boiling to make it soft, then it was filtered using a muslin cloth. Water was added to make 1000 ml media and the mixture was stirred then 18 gm of Agar was added followed by addition of 20g of dextrose and it was heated with continuous stirring for 45 minutes. Then the media was kept for sterilization in the autoclave at 121º C at 15 psi pressure for 1 hour after plugging the mouth of the conical flask having the media with non absorbent cotton and covering with brown paper. After sterilization the media was allowed to cool down then poured into test tubes to make slants allowing the organism to proliferate maximally [9].

Preparation of Pleurotus djamor Culture:

Single hyphal tip method was followed for purification and maintainence of *Pleurotus djamor*. The media used for culturing *Pleurotus djamor* was potato dextrose agar (PDA). The growth took place on sterilized petridishes having potato dextrose agar media for 7 to 10 days. After 7 to 10 days of incubation, microscopic observation of single branched hyphae was done at low power (10X) of the microscope. PDA slants were then made and the culture was transferred to the slants followed by incubation at room temperature (25º C) for 7 days. Further subculturing was done again and stored for future use [9].

Spawn Production:

Jowar grains were selected, soaked in water overnight followed by washing. Then they were subjected to boiling for 15 mins. It was taken care that the grains did not split during boiling. Excess water was drained by placing the grains in a thin layer on a wire net. Then the grains were cooled to at 25 ºC 1.0 percent of gypsum ($CaSO_4$) and 0.2 percent of calcium carbonate was then mixed with the grains. 500ml glass bottles were taken and the grains were filled upto 150mm in three replicates. Non absorbent cotton was used to plug in the bottles to avoid contamination, followed by wrapping with butter paper. Then the bottles were kept for sterilization in the autoclave at 121ºC at 15psi for 2 hours. Then the culture of *Pleurotus djamor* which was prepared and kept previously was inoculated in these bottles. The bottles were then incubated at 25 ºC [10].

Substrate Preparation:

Different substrates were chosen for this experiment. Vegetable waste like cabbage, cauliflower, radish leaves were used alone as well as in combination with paddy straw and sugarcane bagasse as substrate. These substrates were used in different ratios for the cultivation. The straws were chopped into small pieces. Following the chopping the small pieces were placed in water to get soaked for the next 18 hours. Carbendazim (8gm/100ml water) and formalin (120ml/100ml water) was added to it. Then the excess water was drained and mixing of the substrates nicely was done followed by packaging in 18X25 cm polypropylene bags [10].

Spawning:

The polypropylene bags were kept in dark condition with 5 to 7 holes made in it for proper aeration. The crop room temperature was maintained at 25ºC and relative humidity at 85 percent. After completion of the spawn run the polypropylene bags were opened to let sporophore formation happen. Sporophore formation requires fresh air and release of carbon dioxide happened so proper ventilation was maintained in the cropping room [10].

Sporophore Production:

After 5 to 7 days the fruiting bodies started to appear. After 3 to 4 days of pinhead initiation the sporophores were harvested. Harvesting was done by twisting at the base. Care was taken that no broken pieces were left because they could have lead to rotting in the remaining flushes. Falling of direct sunlight on the fruiting bodies should be avoided, water spraying should be done three times a day to maintain the relative humidity at 85%. Number of fruiting bodies decrease gradually, after about 3 flushes they hardly appear. Turning of spawn block runs was done to let the lower surface also produce fruiting bodies after two flushes. To prevent insect infestations certain insecticides like malathion (0.1%) was used [10].

Harvesting:

The sporophores were harvested after maturity. The sporophores were irrigated before the harvesting to keep it fresh. The yield and biological efficiency was compared with respect to different substrates for 6 weeks of harvest period. Proper ventilation was maintained in the cropping room throughout .

Protein Estimation Using Biuret method:

For performing the estimation of protein content from the extracted mushroom sample the protein that was chosen as standard was bovine serum albumin. 0.2 to 1 ml aliquotes of the standard protein solution was pipetted out into a series of test tubes. The test tubes were labelled. The volume in all the test tubes was made upto 1ml by adding required amount of distilled water. Test tube containing only distilled water was labelled as blank. 3ml biuret reagent was added to all the test tubes, followed by incubation at 37 ºC for 10 minutes. Then absorbance was read at 540 nm using a calorimeter, the standard graph was plotted taking the concentration of protein (µg/ml) on X axis and Optical density (nm) on Y axis. Then from the standard graph the concentration of protein was calculated [11].

RESULTS AND DISCUSSION:

Satpal reported the combination of both paddy straw and wheat straw yielded better results when compared to either of the two when used alone for the cultivation of *Pleurotus djamor*, so the results obtained in our study are in accordance with the above mentioned findings [10]. The use of wheat straw and paddy straw in a combination served better yields as compared to using different substrates alone [3]. The cultivation of *Pleurotus* yielded best results when suitable supplements were used along with the substrates than any one substrate alone [6]. In the present study the substrate with paddy straw, vegetable waste alone and in a combination are compared for the best suitable substrate for cultivation of *Pleurotus djamor* it was concluded based on the results obtained that when paddy straw and vegetable waste are used in 1:1 ratio it is considered the most suitable as it resulted in a greater average yield, biological efficiency and a greater protein concentration. It was seen that when a combination of both vegetable waste and paddy straw was used the number of fruiting bodies was 10 which was higher than when compared to using vegetable waste or paddy straw alone for the cultivation and the days required for spawning, pinhead formation, for fruiting body formation was comparatively lesser compare to when these substrates were used alone for the cultivation. The maximum time was taken for spawning, pinhead formation, fruiting body formation happened when only vegetable waste was used for cultivation, followed by paddy straw taking little lesser time for the generation of the same parameters being discussed indicating that when vegetable waste and paddy straw are used in a combination we get our results faster and better. The average yield and biological efficiency was also maximum when a combination of both vegetable waste and paddy straw was taken, followed by little lesser average yield and biological efficiency was found when only paddy straw was used and the least average yield and biological efficiency was found when only vegetable waste was used for the cultivation of *Pleurotus djamor.*

Table 1: Days for completion of spawn running, pinhead formation and fruiting body formation on different substrates.

Substrates	**Days of completion of spawn running**	**Days for pinhead formation**	**Days of fruiting body formation**	**Number of fruiting bodies**
100% Paddy straw (PS)	30	35	40	08
100% Vegetable waste (VW)	50	60	65	03
50%PS + 50% VW	25	30	34	10

Table 2: Weight, average yield and biological efficiency of different substrates

Substrates	**Weight of each substrate (gm)**	**Average yield (gm)**	**Biological efficiency (%)**
100% Paddy straw (PS)	500	50	33.33
100% Vegetable waste (VW)	500	15	10
50% PS + 50% VW	500	60	40

Dry weight of substrate - 150gm

Formulae : BE (%) = $\frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$

Table 3: Protein estimation by biuret's method.

Substrates	**Protein concentration**
100% Paddy straw (PS)	700 mg/ml
100% Vegetable waste (VW)	300 mg/ml
50%PS + 50% VW	800 mg/ml

Graph 3: Based on table 3, indicating protein concentration after estimation by biuret method.

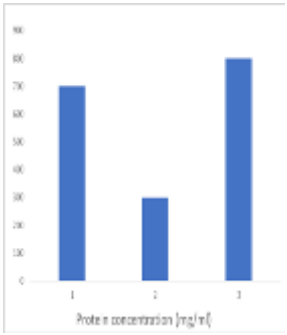
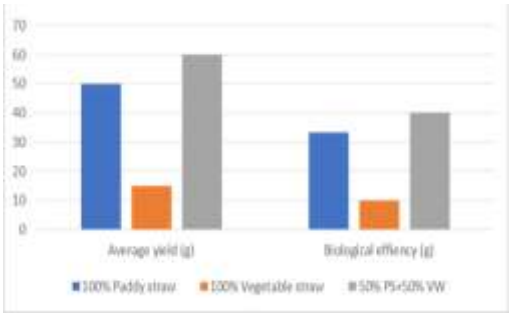
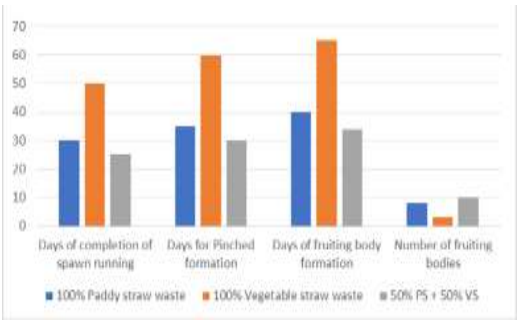


Fig 1: Estimation of protein by biuret method

Graph 1: Based on Table 1 Values, indicating days of completion of spawn running, pinhead formation, fruiting body formation and number of fruiting bodies.



Graph 2: Based on Table 2 values, indicating Average yield and Biological Efficiency



CONCLUSION:

The experiment was done to check the effect of different substrates individually and in combination on the yield of *Pleurotus djamor*. The extracted mushroom sample grown on vegetable waste alone and a combination of both vegetable waste and paddy straw was used to estimate the total protein content using biuret method. The standard graph was plotted taking concentration of protein (µg/ml) on X axis and Optical density (nm) on Y axis, followed by calculations based on the graph. The concentration of protein was found to be 800 (µg/ml) when both vegetable waste and paddy straw were used in a combination which was comparatively higher than when vegetable waste or paddy straw was used alone. Thus it can be concluded that a combination of both vegetable waste and paddy straw in 1:1 ratio is the best suited for cultivation of *Pleurotus djamor,* followed by usage of only paddy straw and only vegetable waste as a choice based on efficiency of these as substrates for the cultivation.

Future Perspectives:

Pleurotus djamor is a protein rich delicacy, which can be cultivated utilizing vegetable waste. This reaps double benefits, on one hand it is resulting in reduction of environmental pollution and on the other hand it is serving as protein rich supplements for people suffering from malnutrition. It can serve as nutraceutical due to its benefits on human health in the near future.

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