Effect of substrates on Biochemicals of *Hipsizygous Ulmaris* Pleurotus ostreatus spp.

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***Abstract*: In this present investigation we have taken Hipsizygous .Hipsizygous species are cultivated around the world because of their short life cycle, rich inmineral content and medicinal compounds. This Mushroom has gained importance only in the last decade. It is being cultivated in Many countries in the subtropical and temperate zones. The earliest record of Pleurotus cultivation in India is of Bano and Srivastava [1962]. The different species of Pleurotus grow within a temperature range 15 to 30°C. Pleurotus spp. Can be grown on agriculture waste materials . The study was conducted to evaluate various biochemical's of mushroom from the dried powder of *Hipsizygous Ulmaris*, which were harvested from different substrates such as Maize, Wheat straw, Paddy straw, Soybeanand Blackgram mix, all Substrates Mixed, Sugarcane Baggasse, Jowar substrates. The maximum amount of protein was observed in paddy straw, whereas, the minimum amount of protein was observed in wheat straw.**

***Keywords*: Biochemicals, Oyster Mushroom, Substrates.**

Introduction:
The word mushroom actually refers to the fungi that is seen with the naked eyes and that is pickedby other Organisms and sometimes used as food. In a broad sense, Mushroom is a macro fungus with a distinctive fruiting body, which can be either epigeous or hypogenous and large enough tobe seen with naked eye and to be picked by hand (Chang and Mills, 1992). Mushrooms are not only basidiomycetes; they can also be ascomycetes, grow underground, have a non-fleshy textureand could be inedible. All The poisonous and the non-poisonous fungi that can be seen with the naked eye and can be picked with the hand are described as mushrooms. Nutritional value of mushrooms lies between that of meat and vegetables. The rich source of proteins, vitamins and minerals and low in fat content (2-8%) unique chemicals constitution of mushrooms makes them low calorie food 8 and choice diet for those suffering from hypertension, arthrosclerosis, diabetes,obesity etc. (Subramanian, 1995). Mushrooms normally contain 19-35% protein. Mushroom proteins contain all the essential amino acids and are especially rich in lysine and leucine, which are lacking in most staple cereal food. The low total fat content, and High proportion of polyunsaturated fatty acids (72-85%) relative to total fatty acids, is considered a significant contribution to the health value to mushrooms. The widespread malnutrition coupled with increasing protein requirement in the Country has necessitated the search for new and alternative means to meet the protein requirement of the country and one of the alternative ways is the production of mushroom which is noted for its high protein content.

Substrates:
Agricultural wastes are rich in various types of nutrients and their disposal is difficult to manage as excess of nutrients in them can cause leaching left in the field, as a compost. Mostly they are disposed of by means of incineration which causes pollution. Hence, there is always a high demandfor discovering an agricultural waste management method which is cost effective and contributesless to environmental pollution. Mushroom cultivation on agricultural wastes fulfils these requirements. Agricultural wastes are rich in lignin cellulosic components which are difficult to break down, but can effectively be done mushroom cultivation. Mushrooms are fleshy fungi, sporebearing fruiting bodies which are produced above ground on soil. They often refer to the fruiting body of the gill fungi, which do not contain chlorophyll like green plants and as a result cannot manufacture food by their own. They are very nutritious products that can be generated from lignocellulose waste materials. The bioconversion of agricultural wastes into a value added productis a good means of their use. The property of edible mushroom fungi to convert complex organic compounds into simpler ones is used to transform the useless agricultural waste into valuable products.

Materials and methods:
Oyster Mushroom Cultivation Process:
The process for the cultivation of oyster mushrooms is simple because its cultivation does not lenditself to complex scientific procedures. Farmers can cultivate the fungi with less supervision. Thecultivation of the fungi relies on the interaction of a particular set of physical, chemical and biological factors (Rangel et al., 2006). The important areas to note in the cultivation of oyster mushrooms in order to balance the three factors include substrate selection, composting, sterilization of substrates, bagging of substrate, spawning / inoculation, incubation, fruiting. The agro-climatic conditions in our country especially in the North Indian States are conducive for mushroom cultivation when the Temperature is 15-30° and relative humidity is 70-8-%. The production decreases during peak periods of winter. Cultivation of Oyster Mushroom requires:

- Wooden racks
- Polythene bags (polypropylene bags)
- Plastic bags
- Pleurotus spawn (Hipsizygous Ulmaris)

- Formaldehyde (25ml/15l water)• Bavistin (8gm/15lwater)Preparation of mushroom beds:

1. Climate and other conditions

Pleurotus spp. Is one of the choice edible mushrooms which can be cultivated in the tropics. It is cultivated in many countries and has gained importance in the last decade. Different species of Pleurotus are suited for growing within temperatures up to 28-30°C, Although it produces faster and produces larger mushrooms at 25°C during the cooler season of the year.(i.e. winter season).

2. Substrates

Like other mushrooms, Pleurotus species can be grown on various agricultural waste materials using different technologies. They grow well on lignocellulos materials, converting them into digestible and protein rich. Substances for animal feeds. The pleurotus Spp. Can be grown on various agricultural wastes. I.e. Maize, wheat straw, paddy straw, soybean and blackgram, mixedsubstrate, Sugarcane bagasse, and Jowar. The substrates used in every region may depend on availability of agricultural wastes (substrates).

3. Preparation of substrate – Sterilization/ Pasteurization

Sterilization at 100° C (pasteurization) is more acceptable because the cost is lower (the steamer may only be an ordinary large capacity casserole or a drum and substrates thus steamedare less susceptible to contamination. The substrate is steamed for 2-3 hours, depending on thevolume and size of the bags. When using a lower temperature (60-70°C) as in room or bulk pasteurization, the substrates whether in bulk or already packed in bags are steamed for at least6-8 hours.

Chemical sterilization technique (DMR, Solan 1987).

Ninety litres of water are taken in a rust proof drum (preferably of galvanized sheet) of 200 liters of capacity. Ten kg of wheat straw is slowly steeped in water. In another plastic bucket carbendazim 50% WP (75.ppm) . Bavistin 7.5gm and 125 ml formaldehyde (37-40%) is dissolvedslowly poured on already soaked wheat straw. Straw is pressed and covered with a polythene sheetafter 15-18hours straw is taken out and excess water drained.

4. Inoculation / spawning

Spawning is carried out aseptically, preferably using the same transfer chamber or the sameinoculation room as is used in spawns preparation. Grain or sawdust spawn is commonly used to inoculate the substrate in bags. With grain spawn, the bottle is shaken to separate the seeds colonized with the white mycelium. After lifting the plug and flaming the mouthof the bottle, a few spawn grains (about 1to 2 tsp.) are poured into the substrate bag. Boththe plug of the spawn and the plug of the compost bag are replaced and the next bags are then inoculated. The newly inoculated bags are slightly tilted to distribute the grains evenlyin the shoulder area of the bag around the neck.

5. Incubation:

The spawned compost bags are kept in a dark room until the mycelium has fully penetratedto the bottom of the substrate. In 20 to 30 days, depending upon the substrate/substrate combination, the substrate appears white, due to the growth of the mycelium. The Bags were kept for an additional week before they are opened to check that the mycelium is mature enough to fruit. Most strains of the mushroom form primordial after 3 to 4 weeksof mycelia growth. The bags are opened to initiate fruiting, inside a mushroom House.

- ❖ The agro wastes were collected from local farms/places and were used for filling the bags.
- ❖ The substrates were chopped in pieces and soaked in water overnight to moisten it and excess water was drained off
- ❖ After soaking the substrates were chemically sterilized. The polythene bags of the size 35 x 45cm were filled with 2kg dried substrates and added at the rate of 2% of the wet weight wet basis of substrates. Pinning of bags was done for proper aeration. After inoculation the bags were kept in a room where the temperature and humidity were maintained around 25°C and 80 to 90% humidity respectively with sufficient light and ventilation for 20 days. The spawn run was completed within 16 days. The polythene bags were tear-off following the spawn run. Formation of fruit bodies was evident within 3-4 days after removal of poly bags. The beds were maintainedup to the harvest of the third flush, which was completed in 35 days after spawning.

6. Fruiting:

Fruiting requires an appropriate temperature range (20-28°C), ventilation, light, moisture and humidity (80-95%). To provide moisture, daily watering of the substrate is required but, excessive watering should be avoided. If the temperature inside the room rises to morethan 30°C, a light water mist should be used to lower the temperature. Approximately 3-4days after opening the bags, Mushroom primordial will begin to form. If the substrate is not fully colonized, the onset of fruiting is likely to be delayed. The harvesting ofMushroom takes place within20-24 days from the time form bed preparation. While harvesting the Mushroom, they are to be grasped by the stalk and gently twisted and pulled.A knife should not be used. If kept in a refrigerator or in a cool place the Mushrooms can remain fresh for up to 3-6 days.

7. Drying:

The drying of mushrooms was done using shade dry. It is the best method for preserving flavor and potency. It's important to have a right environment to do this drying procedure.A good spot has been selected for drying mushrooms to make sure it's Protected from moisture, insects, and animals. Some airflow was allowed to pass. The Mushrooms were sliced into ½-inch pieces or, depending on the shape, and size of the Mushrooms. The Mushrooms were placed in a big tray, and covered with a white cotton cloth taking care not to stack them on top of each other. The Mushrooms were checked few times throughoutthe day. For complete drying of mushrooms it take 2-3 days. Depending on the environment, there's a good chance for mushrooms to get fully dry. After the drying of Mushrooms, they were grinded into fine powder for further Biochemical analysis procedure.

Biochemical analysis of Mushroom.

The various biochemical analysis was carried out from the dried powder of *Hipsizygous Ulmaris,*which were harvested from different substrates such as Maize, Wheat straw, Paddy straw, Soybeanand Blackgram mix, all Substrates Mixed, Sugarcane Baggasse, Jowar substrates were used. The following biochemical parameters analyzed by using standard procedures like Quantitative Estimation of Total Protein by Lowery et al., 1951, Quantitative estimation of total carbohydrate by Dubois et al., 1956 and extraction of Lipid analysis by Sato and Murata, 1988. Quantitative estimation of free amino acids (Jeyaraman,1981).

Quantitative Estimation of Total Protein by Lowery et al., (1951)

Lowery's method is a colorimetric method to determine the protein contents of the given sample.CHEMICALS:
REAGENT A: 2% sodium carbonate (anyhydrous) in 0.1 N NaOH dissolve in 100ml distilled water, weight accurately 29% Sodium Carbonate and dissolve in a little volume of 0.1N NaOH and then raise the volume of solution to 100 ml with 0.1 N NaOH.
REAGENT B: 0.5 % CaSO4-5H2O in 1% Na-K Tartarate. Dissolve 1gm of Na-k tartarate in a little volume of distilled water and raise the volume to 100ml distilled water. Dissolve 0.5gm of CuSO4-5H2O in this solution.
REAGENT C: Mix 49ml of reagent A , 1ml of reagent B, just before use.
REAGENT D: Dilute commercially available, folin phenol reagent 1:2 distilled water.STANDARD PROTIEN SOLUTION:
Dissolve 20mg (0.02gm) of Bovine serum albumin and raise then volume to 100ml distilled water(200ml/ml BSA).
PROCEDURE FOR PROTIEN ESTIMATION:
5gm of Mushroom powder was taken with 50ml of 0.1N NaOH and boiled for 30 minutes. The solution was cooled, at room temperature and centrifuged at 1000g by a DSC-200T, centrifuge content was measured according to Lowery's method.
Pipette out 0.2, 0.4, 0.6, 0.8 and 0.1 ml of working standard into a series of test tubes. Pipette out0.1ml and 0.2ml of sample extract into other test tubes. Make up the volume to 1ml in all test tubes. A tube with 1ml of water serves as blank. Add 5ml of reagent C to each tube including blank. Mix well allow to stand for 10minutes. Then add 5ml of reagent D. Mix well, incubate at room temperature in dark for 30minutes. Blue colour is developed. The reading was taken at 660nm colorimetric method.

Quantitative estimation of total carbohydrate by Dubois et al., (1956)

The total soluble carbohydrate content was determined according to the method of Dubois et al

1.0 ml of sample was mixed with 1.0 ml phenol solution and added 5.0 ml of 96% sulphuric acidto each tube and shake well. Incubated in boiling water bath for 20 minutes, after which the absorbance was read at 490 nm against a reagent blank. The analysis was performed in triplicatesand the results were expressed as mg/g.

Extraction of Lipid analysis by Sato and Murata, (1988).

100mg of fresh mushroom sample was homogenized in a pestle and mortar with extraction solventand filtered through filter paper. The moisture in the filtrate was removed with sodium sulphate ina vortex mixer. Then it was taken in a pre-weighed bottle and dried with a steam of nitrogen. Thedried extract was weighed the total lipids were estimated by subtracting the initial weight from thefinal weight. The dried extract was weighed and the total lipids were estimated by subtracting theinitial weight from the final weight. The amount of total lipids was expressed as mg/g fresh weight.

Quantitative estimation of free amino acids (Jeyaraman,1981)

One hundred mg of mushroom sample was taken and ground with 80 per cent ethanol in a pestle and mortar and homogenized. The homogenate was centrifuged at 15,000 g. One ml of the supernatant was diluted to 5 ml (1:4 conc.) With distilled water, one ml of ninhydrin reagent was added and kept in a water bath for 15 min. Then the tubes were cooled and one ml of 50 percent ethanol was added. The purple colour developed was measured in Spectronic-20 at 450 nm. Standard graph was made using a mixture of alanine, aspartic acid, tryptophan, proline and lysine.The result was recorded as as mg/g of the samples.

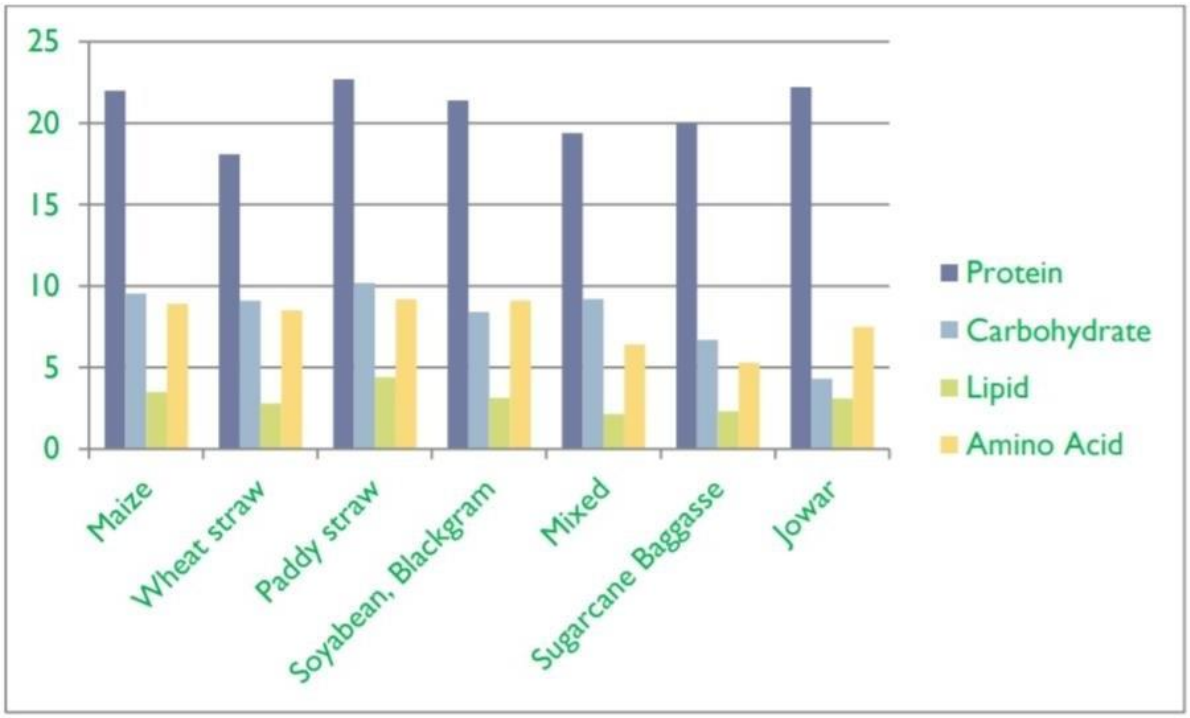
RESULTS & DISCUSSION:

Mushrooms are protein rich ecofriendly food and it is cultivable initially as an empirical process.But the scientific understanding of mushroom cultivation will help in improving the cultivation technology. Rajini Bisaria et al., (1987) cultivated oyster mushroom on different agro wastes likepaddy straw, wheat straw etc and they have reported maximum yield with wheat straw. Singh et al. (1995) suggested the use of Sugarcane trash for the production of oyster mushroom. The widerange of plant waste that have been reported include, paddy straw, Sugarcane baggasse, wheat, maize, rice straw etc. and does not require costly processing method and enrichment material (Mondal, 2010; Stanley, 2011). Mushrooms with their pleasant flavour, texture and high productivity per unit area have been recognized as an exceptional food source to alleviate malnutrition in developing countries. Karuppuraj et al., (2014) reported that the yield improvementof P. florida on unexplored locally available lignocellulosic materials such as paddy straw, wheat straw was used. The growth of mushrooms on wheat straw and other substrates, the paddy straw was considered as the best substrate in terms of relative digestibility and nutrient status (Calzada,et al., 1987). In the present investigation, the maximum content of protein (22.7 mg/g), carbohydrate (10.2 mg/g), amino acid (9.2 mg/g) and lipid (4.4 mg/g) was observed in paddy strawused as a substrate. The moderate contents of protein (22.2 mg/g) and amino acid (7.5 mg/g), observed in Jowar used as a substrate and carbohydrate (6.7 mg/g) observed sugarcane bagasse used as a substrates The minimum content of protein (18.1mg/g), was observed in Wheat straw, Carbohydrate (4.3 mg/g), was observed in Jowar substrate, lipid (2.3 mg/g) was recorded in Sugarcane bagasse and amino acid (5.3 mg/g) was observed in sugarcane bagasse used as a substrate.

a. Effect of various substrates on Biochemicals of mushroom.

Substrates	Protein	Carbohydrates	Lipids	Amino acids
Maize	22.0	9.53	3.5	8.9
Wheat	18.1	9.1	2.8	8.5
Paddy straw	22.7	10.2	4.4	9.2
Soybean Blackgram	21.4	8.4	3.13	9.1
Mixed	19.4	9.2	2.15	6.4
Sugarcane baggase	20.0	6.7	2.3	5.3
Jowar	22.2	4.3	3.1	7.5

Graphical representation of above table .a.



REFERENCES:

[1] Bano, Z. and Srivastava, H.C (1962). Studies On the cultivation of Pleurotus species On paddy straw. Food Science 11: 36-38

[2] Calzada, J.F., Deleon, R., De arriola, M.C., and Rala, C. 1987. Growth of mushroom on wheat straw and pulp strain selection, Biological wastes, 30: 217 – 226. Chang ST, Miles PG (1992). Mushroom biology – A new decipline. Mycologist, 6: 64-65.

[3] Dubios, M., Gilles, K.A., Hamilaton, J.K., Robers, P.A., and Smith, F. 1956.Colorimetricmethods for determination of sugar and related substances. Anal. Chem.., 28: 350-356.

[4] Jayaraman, J. 1981 Calorimetric estimation of amino acid In: Laboratory Manual in Biochemistry, Wiley Eastern Ltd., New Delhi.

[5] Karuppuraj, V., S.Chandra Sekarenthiran and K. Perumal. 2014. Yield improvement of Pleurotus florida fruiting bodies from locally available unexplored lignocellulosic Substrates. Int. J. Curr. Microbiol. Appl. Sci., 3(10): pp. 985-990.

[6] Lowery, O.H., Rosebrough, N.J. and Randall, R.J. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.

[7] Mondal, S.R., Rehana, M.J., Noman, M.S., Adhikary, S.K. 2010. Comparative study on Growth and yield performance of oyster mushroom (Pleurotus florida) on different Substrates. J. Bangladesh Agri. Univ., 8(2): 213 -220 .

[8] Rajini Bisaria, Madan, M. and Bisaria, V.S. 1987. Biological efficiency and Nutritive valueof Pleurotus sajor-caju cultivated on different agro wastes. Biol. Wastes, 19: 239-255.

[9] Rangel DEN, Fernandes EKK, Dettenmaier SJ, Roberts DW (2010) Thermotolerance of germlings and mycelium of the insect-pathogenic fungus Metarhizium spp. And mycelialrecovery after heat stress. J Basic Microb 50:344–350.

[10] Sato, N. and Murata, N. 1988. Membrane lipids:In methods in Enzymology (Eds.), Packer,L. and Glazer, A.N.,167: 251-259.

[11] Singh, A.K., Swasthi, S.K., and Bharat Raj. 1995. Utilization of sugarcane trash [Dried leaves) for the production of Oyster mushroom Pleurotus florida Mushroom Res., 4: 35- 38.

[12] Subramanian CV. Mushrooms: Beauty diversity relevance. Current Science, 1995; 69(12):986-998.