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CULTIVATION OF *PLEUROTUS FLORIDA* BY STANDARD PROTOCOL AND STUDY OF THE TEMPORAL CHANGES IN TOTAL PROTEIN CONTENTS OF THIS OYSTER MUSHROOM RELATIVE TO GROWTH OF ITS FRUITING BODY

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ABSTRACT
Proteins are the essential components of protoplasm, forming the physical basis of life. Proteins are very important for growth and repair of the body and are of prime biological importance. Their acute deficiency in the food causes retardation of the physical and mental growth. Deficiency of proteins causes PEM (Protein Energy Malnutrition) diseases in infants like marasmus and kwashiorkar. Mushrooms are rich in protein and constitute a valuable source of supplementary food. Use of mushrooms can contribute positively in facing the challenge of world-wide food shortage, originating with rapidly expanding human population. *Pleurotus florida* (locally known as the Dhingri mushroom) is an edible mushroom having excellent flavour and taste. The present work deals with the cultivation of *Pleurotus florida* by standard protocol and study of the temporal changes in overall protein contents with respect to growth of the mushroom. Biochemical analyses show the gradual decrease in protein contents per unit mass from lower growth stages of mushroom to higher ones over time and its reasons. This change is attributed to the increase in intercellular spaces and water contents in between these spaces over time, thereby reducing the number of cells per unit area irrespective of overall increase in size and number of cells in the fruiting body.

Keywords- Dhingri mushroom, PEM, *Pleurotus florida*, Protein, Temporal gene expression

INTRODUCTION:

Proteins occupy a central position in the architecture and functioning of living matter. They are intimately connected with all phases of chemical and physical activity, that constitute the life of the cell (White, Handler and Smith, 1964). They are therefore, essential to cell structure and function. Proteins are the essential components of protoplasm, forming the physical basis of life. The proteins with catalytic activity (enzymes) are largely responsible for determining the phenotype or properties of a cell in a particular environment. Thus, the tremendous diversity of living system is essentially due to this organic compound. The total hereditary material of the cell or genotype dictates which type of protein the cell can produce. In fact, the proteins have built into their structure the information that instructs them in "what to do" (catalytic activity), "where to go" in the cell (intracellular organization) and "when and how to perform" (control of function through interaction of proteins with other proteins, small activators or inhibitors). The proteins are therefore referred as body builders. Proteins perform a great variety of functions. Proteins are very important for growth and repair of the

body and are of prime biological importance. Their acute deficiency in the food causes retardation of the physical and mental growth. Deficiency of proteins causes PEM (Protein Energy Malnutrition) diseases in infants like marasmus and kwashiorkar**.** Main sources of animal proteins are: fish (16.5%), meat (22%), eggs (11.9%), milk, etc. And main sources of plant proteins are: pulses, soyabean, peas (5.2%), beans, cereals, etc. Soyabean is regarded as the best source of plant proteins. These are traditional sources of proteins. Mushrooms are higher fungi (macrofungi), belonging to the class Ascomycetes (e.g. *Morchella, Tuber,* etc.) and Basidiomycetes (e.g. *Agaricus, Auricularia, Tremella,* etc.). They are characterized by having heterotrophic mode of nutrition. They may be edible or non-edible. According to Chang and Hayes (1978), edible mushroom refers to both epigeous and hypogeous fruiting bodies of macroscopic fungi that are already commercially cultivated or grown in half culture process or implemented under controlled conditions. Atkins (1983) and Bel (1976) have described following five different groups of edible mushroom. These are:

1. Those which grow on fresh or almost fresh residue, e.g. *Pleurotus* and *Lentius*.
2. Those which grow on slightly composted material, e.g. *Volvariella* and *Coprinus*.
3. Those which grow on well composted material, e.g. *Agaricus*.
4. Those that grow on soil and humus, e.g. *Morchella*.
5. Mycorrizal fungi, e.g. *Boletus* and *Cantharellus*.

Mushrooms are rich in protein and constitute a valuable source of supplementary food. Use of mushrooms can contribute positively in facing the challenge of world-wide food shortage, originating with rapidly expanding human population at the rate of more than 2 lakh per day. In developed countries, the average consumption of animal protein is about 31 kg per capita per year, whereas it is only 4 kg in India. In India, the plant proteins are more widely used than the animal proteins. Mushrooms are the richest source of vegetable proteins. They contain 21-30% proteins on dry weight basis. Thus the percentage of proteins in mushrooms is much higher than in cereals, pulses, fruits or vegetables. The proteins of mushrooms contain all essential amino acids and their quantity is higher than in the egg. There is also a good amount of lysine amino acid in mushrooms (about 550 mg/g). Generally, mushroom contains 85-90 per cent water of its dry matter. However, amount of water is greatly influenced by relative humidity and temperature during growth and storage. Besides, mushrooms contain sufficient quantities of mineral elements, such as Ca, Na, P and K. They also contain folic acid and are as such very good source of iron. They contain vitamins B, C, D and K, which are not distorted during ripening, freezing or canning process. On the other hand, they contain very little fats (0.35-0.65% dry weight) and starches (0.02% dry weight). Thus mushrooms make an excellent food for diabetic and heart patients. Mushrooms have attracted a large number of workers as these have been recognized as plants of great economic importance- as medicine, food, etc. This has resulted into accumulation of abundant literature. Besides work on taxonomy; edible and non- edible

nature; genetics and conservation, biochemical analyses have also attracted attention of many workers. Bano (1976), Anderson and Fellers (1942), and Khanna and Gharcha (1981) have contributed to the detail information about food value of mushrooms. Chang (1980), Hussain (2001), Tewari (1986), Nagaratna and Mallesha (2007) and Oei (1996) have contributed to different methodologies of mushroom cultivation with special reference to developing nations. The studies of Fasidi, Ekuere and Usukama (1993); Okhuoya, Isikhuemhen and Evan (1998); Onuoha, Ukaulon and Onuoha (2009); Khan, Kausar and Ali (1981); Krishnamurthy (1981); Kurtzman (1976); Shah, Ashraf and Ishtiaq (2004); and Zardazil (1978) focus upon different aspects of different species and varieties of *Pleurotus*. The most recent work of Somashekhar, Reddy and Vedamurthy (2010) focuses on the methods of spawn inoculation for better yield of mushroom, *Plerotus florida*. In India, mushroom cultivation started long before a century, as the *Volvariella valvacea* was cultivated on paddy straw. Therefore, this mushroom is also known as the paddy straw mushroom. In 1950s, an attempt was made to cultivate mushroom in Coimbatore (Thomas *et al.*, 1943). In 1962, *Pleurotus flabellatus* (*Dhingri coroyester*) was successfully cultivated in Mysore. Besides many attempts, its cultivation could not be popularized upto the late 1960s. For the first time an attempt was made for artificial cultivation of *A. bisporus* at Solan (Himachal Pradesh) where synthetic compost preparation technology was developed, by using horse dung and wheat straw. Rapid development took place at this centre. Modern Spawn Laboratory and Air Conditioned Cropping rooms were constituted under the guidance of an expert from Food and Agricultural Organizations (FAO). From 1974, a coordinated scheme was launched at Solan, Bangalore, Ludhiana and New Delhi. FAO deputed its experts for improving the cultivation technology. Dr. W.A. Hayes came to India, who recommended for incorporation of molasses and brewer's grain in the preparation of synthetic compost. This increased the mushroom yield. In 1977, State Department of Horticulture (H.P.) launched a project of Rs. 1.27 crore, under which a Central Mother Unit (CMU) for bulk pasteurization of compost and casing soil was established. CMU supplies about 80 tonnes of pasteurized compost per month to growers in Solan, Shimla and Sirmur districts (Sohi, 1988). During 1966-70 mushroom cultivation was introduced in Kashmir Valley, where by the end of 1975, the number of growers increased to 90. This took up its cultivation as cottage industry in Srinagar and Jammu region. In 1974, Uttar Pradesh Department of Agriculture (UPDA) started mushroom cultivation on exploratory trial at Vivekanand Parvatiya Krishi Anushandhan (VPKA), Almora. U.P. Govt. also sanctioned a project for mushroom cultivation to the Department of Botany, Kumaun University, Nainital. At Almora Centre, two crops in a year are raised (i.e. in February-April and September-November) in natural conditions. The compost is prepared from agro-wastes i.e. straw of wheat, barley and oat and dehulled corn cobs, grasses, fresh leaves, etc. Since 1983, a large number of growers started mushroom cultivation during winter around Delhi, Chandigarh and some districts of Haryana (e.g. Sonepat, Rohtak, Karnal) and Punjab (e.g. Ferozpur, Patiala, Ludhiana and Jalandhar). In Bhiwani district of Haryana, mushroom cultivation is gaining much popularity. Under the guidance of specialists of

Krishi Gyan Kendra and scientists of Haryana Agriculture University (Hissar) the farmers in villages Tagrana and Bamla have undertaken cultivation of mushrooms. These villages have been declared as mushroom villages.

Since mushrooms have a very short life, it should reach to consumers within a short time or immediately canned. This will lead to proper marketing of mushrooms. Improper care for marketing results in the increase in price. Different technologies for cultivation and processing of mushrooms have been developed at CFTRI (Central Food Technological Research Institute), Hyderabad, RRL (Regional Research Laboratory), Jammu and NBRI (National Botanical Research Institute), Lucknow. RRL and NBRI are distributing mushroom spawns in rural areas for mass cultivation. CFTRI has developed technique for processing and drying mushrooms.

Pleurotus is one of the important mushrooms gaining popularity in recent years. It is found growing naturally on dead organic materials rich in cellulose. Its several species are edible such as *P. sajor-caju, P. sapidus, P. flabellatus, P. ostriatus, P. corticatus, P. florida,* etc. These species can be cultured successfully on various agricultural, domestic, industrial and forestry waste materials. It is very versatile in nature as far as substrate preference and growth are concerned. However, it can be grown on paddy straw, gunny bags, rice husk, copped *Parthenium* stem, etc. *Pleurotus florida* is commonly known as White Oyster mushroom due to resemblance of its mature fruiting body with shell of Oyster (a mollusc) and white colour. In India, it is locally known as the Dhingri mushroom. It is one of the species of mushrooms widely cultivated at many KVK (Krishi Vigyan Kendra) centres of Indian Council of Agricultural Research (ICAR) all across India. It is generally grown whole of the year except in the summer season. It can be grown on a variety of substrates. Its cultivation does not require much space. It has comparatively smaller generation time. Its cultivation does not require much financial input.

Spatiotemporal gene expression is the activation of genes within specific tissues of an organism at specific times during development. Gene activation patterns vary widely in complexity. Some are straightforward and static, such as the pattern of tubulin, which is expressed in all cells at all times in life. Some, on the other hand, are extraordinarily intricate and difficult to predict and model, with expression fluctuating wildly from minute to minute or from cell to cell. Spatiotemporal variation plays a key role in generating the diversity of cell types found in developed organisms; since the identity of a cell is specified by the collection of genes actively expressed within that cell, if gene expression was uniform spatially and temporally, there could be at most one kind of cell. What causes spatial and temporal differences in the expression of a single gene? Because current expression patterns depend strictly on previous expression patterns, there is a regressive problem of explaining what caused the first differences in gene expression. The process by which uniform gene expression becomes spatially and temporally differential is known as symmetry breaking. The amount of proteins in a living organism is not constant throughout life. Rather both qualitative and quantitative changes occur in the protein contents. This is due to time based differential expression of genes, i.e., temporal gene expression. It is possible that the protein

which is present in a cell at a certain stage (or stages) of life, may not be present at other stage (or stages). Similarly, the same protein may show quantitative differences at different stages of life.

The present work was carried out to grow a locally grown edible mushroom in lab by standard protocols and to estimate its protein contents (in term of quantity) at different stages of growth using different biochemical techniques so that the trend in the pattern of overall expression of genes in terms of overall quantity of proteins translated relative to different growth stages could be known, i.e., the temporal changes in protein contents relative to growth of the mushroom under study could be established.

MATERIALS AND METHODS

A. Site selection

Holy Cross- Krishi Vigyan Kendra, Canary Hill, Hazaribag is an institution of ICAR, devoted to the welfare of the farmers as it is providing much scientific information and high quality nurseries to them. Besides this, it is also cultivating edible mushrooms. It has a Mushroom Training Unit and a Mushroom Spawn Unit with all facilities required for mushroom culture. Therefore, this site was selected for cultivation and production of mushrooms for my work.

C. Species selection

At Holy Cross- Krishi Vigyan Kendra, Canary Hill, Hazaribag three species of mushrooms are grown. These are: *Pleurotus florida,*, *Agaricus bispora*, and *Pleurotus sajor-caju*. Pleurotus is one of the important mushrooms gaining popularity in recent years. It is found growing naturally on dead organic materials rich in cellulose. Its several species are edible such as *P. sajor-caju, P. sapidus, P. flabellatus, P. ostriatus, P. corticatus, P. florida*, etc. These species can be cultured successfully on various agricultural, domestic, industrial and forestry waste materials. It is very versatile in nature as far as substrate preference and growth are concerned. However, it can be grown on paddy straw, gunny bags, rice husk, copped *Parthenium* stem, etc.

B. Cultivation of *Pleurotus florida*

The given method of cultivation of *Pleurotus florida* is based on the training provided to the farmers or those interested in mushroom culture at the Mushroom Training Unit and the Mushroom Spawn Unit of Holy Cross-Krishi Vigyan Kendra, Canary Hill, Hazaribagh, Jharkhand.

1. Preparation of Culture Medium

The culture medium contains all the nutrients for microbial growth. Culture media may be of solid or liquid types. Potato-Dextrose-Agar (PDA) is a solid medium specifically suitable for growth and maintenance of common fungi under artificial environmental conditions.

For preparing PDA medium, 50 g of boiled and peeled potatoes are taken. The potatoes are mashed in 100 ml of water and boiled. The solution is filtered with a cheese cloth to obtain potato extract in a beaker. 5 g dextrose is added and the volume of the solution is made upto 250 ml by adding additional 150 ml of water. The pH is measured and adjusted to 7.5. The mixture is warmed in a water bath up to 60^{o}C. 5 g Agar is added and dissolved slowly. The final mixture is boiled till the culture becomes transparent. The culture medium is then autoclaved at 15 lb/inch^{2} pressure for 10min. After autoclaving, the medium is brought inside the pre-disinfected laminar flow chamber for media plate preparation.

2. Obtaining Pure Culture

Sterilized and cooled PDA medium is poured into sterile Petri dishes and when solidified they were inoculated by a piece of tissue or spore(s) of mushroom inside the laminar flow chamber. Petri dishes are incubated at suitable temperature (here 20-25^{o}C) for growth of hyphae (Chang and Li, 1982). After one week, fungal growth is found covering the whole surface of mediun in the Petri dish.

3. Preparation of Spawn

Spawn is a fungal growth impregnated with mycelial fragments of mushroom which serves as inoculum for mushroom cultivation. Many substrates are used for spawn making either alone or in combinations. For the selection of substrates to be used in making spawn, care is taken for cost and availability of raw materials and mycelia growth on it as well.

For preparing spawn, wheat grains are used. The wheat grains are boiled in water until they swelled and soaked in water for 5-10min. Water is decanted and 2 percent lime (calcium carbonate) is mixed. Then, grains are transferred into glass tubes. The tubes are plugged with cotton and autoclaved at 121^{o}C for 30min and cooled down to 30-40^{o}C. The grains are inoculated with pure culture of mushroom and incubated at suitable temperature for proper infestation of mycelium for its use as spawn (Chang and Li, 1982). Krishi Vigyan Kendras (KVKs) from all around India provide spawns to the farmers for free of cost or at a very minimal price for mushroom farming.

4. Substrate Preparation for Cultivation

The mushroom is grown on plant straw. Since paddy straw is easily available and cheap, it is widely used. Paddy straw used should be fresh and well dried. Paddy straw is chopped into 3-5 cm pieces and soaked in fresh water for 8-16 hours. Excess water from straw is drained off by spreading it on filter paper followed by heat treatment. Heat treatment of substrate results in minimizing contamination problem and gives higher and almost constant yields. It can be done by pasteurization. For pasteurization, water is boiled in a wide mouth container such as tub or drum. The wet substrate is filled in side gunny bags. The filled bag is dipped in hot water of 80- 85^{o}C for about 10-15 minutes. To avoid floating, it should be pressed with some heavy material or with the help of a wooden piece. After pasteurization, excess hot water is drained off from container.

5. Spawning and Spawn Running

Inoculation by spawn of prepared substrate for mushroom cultivation is known as spawning. When the pasteurized substrate has cooled down to room temperature, it is ready for filling and spawning. At this stage, substrate moisture content is about 70%. Polythene bags (35 x 50 cm) are used for its cultivation. One 500 ml bottle spawn (200- 250g) can be used for 10- 12kg wet straw (3 bags). Spawning can be done in layer spawning or through simple spawning.

In case of layer spawning, substrate is filled in bag, pressed to a depth of 8-10 cm and seeded with a handful of spawn by spreading above it. Similarly, 2nd and 3rd layers of substrate are put and simultaneously after spawning, the bags are closed. In case of simple spawning, pasteurized straw is mixed with 2% spawn and filled in bags. After that it is gently pressed, and the bags are sealed for spawn running (development). Spawned bags are stacked on racks in neat and clean place, in closed position. Temperature at 25-35^{o}C and humidity at 70-85% is maintained by spraying water twice a day on walls and floor. It takes

15- 20 days when bags get fully covered with white mycelium.

6. Cropping and Harvesting

After 20-22 days, when bags are fully impregnated with white mycelium, they are transferred into cropping room and the polythene covers are removed. The open blocks are kept on racks about 20 cm apart with gap of 50-60 cm between two shelves. Mushrooms are grown in a temperature range of 20-33°C. Relative humidity is maintained by spraying water twice a day on the walls and floor of the room. A light spray of water is given on blocks as soon as the small pin heads appeared. Once pinheads are 2-3 cm big a little heavier watering is done on blocks and watering of blocks is stopped to allow them to grow. Mushrooms are plucked before they shed spores to get quality harvest. After 1st flush of harvest, 0.5 to 1 cm outer layer of the block is scrapped. This helps to initiate 2nd flush which appear after 10 days.

After harvesting they are packed in perforated (5-6 small holes) polythene bags to keep them fresh. It loses freshness after about 6 hours, which can be minimized by keeping them in refrigerator.

B. Measurement of size

The diameter of the pileus or discs of fruiting body from five different stages- from pin-head stage up to the stage before they shed spores was measured across the edges having maximum distance.

C. Determination of moisture content

Soon after harvesting, the moisture content was determined by measuring the weight of a single fruiting body disc from each sample before and after the removal of water by evaporation. Slow drying by evaporation was performed for each sample in the incubator at 30°C.Moisture content was determined by following formula for each sample:

$$\% \text{ Moisture} = M_{initial} - M_{dried} / M_{initial} * 100$$

Here, $M_{initial}$ and M_{dried} are the mass of sample before and after drying respectively. To obtain an accurate measurement of the moisture content of material, evaporation method is necessary to remove all water molecules.

D. Determination of total protein content

We have used Biuret method for determination of proteins in aqueous sample (Burtis and Ashwood, 2006). Biuret method is one of the simplest methods for protein estimation. This method is sensitive to the amino acid composition of the protein. Its sensitivity is moderately constant from protein to protein and because of its simple procedure and quick result, it is widely used for protein estimation in crude extract over a large range of concentration. The assay is based on copper ion binding to peptide bonds of protein under alkaline conditions giving a violet or purple colour. In this reaction Cu++ reacts with the peptide bond and reduces to Cu+ ions resulting in purple- violet coloured complex. The intensity of the charge- transfer absorption bond resulting from the copper-protein complex is linearly proportional to the mass of the protein present in the solution. In simple words, the number of peptide bonds present in a sample governs the colour intensity of the sample. Colour intensity of this product is measured at 540nm by a colorimeter. The absorbance value is directly proportional to protein concentration in the sample (Beer- Lambert Law).

1. Preparation of Biuret Reagent

Despite its name, the reagent does not in fact contain biuret ($(H_2N\text{-}CO\text{-})_2NH$). The test is so named because it also gives a positive reaction to the peptide-like bonds in the biuret

molecule. For preparing Biuret reagent, 3g of copper sulphate ($CuSO_4.5H_2O$) and 9g of sodium potassium tartarate was dissolved in 500 ml of 0.2 mol/litre sodium hydroxide; 5g of potassium iodide was added and made up to 1 litre with 0.2 mol/litre sodium hydroxide.

2. Preparation of Standard BSA (Bovine Serum Albumin)

1g of BSA was weighed and dissolved in a little amount of distilled water in a volumetric flask and made the volume to 100ml.

3. Preparation of Mushroom Extract

5g of each fresh sample was crushed separately using pestle and mortar in 10ml of distilled water. The homogenate of each sample was then filtered through a muslin cloth and centrifuged. The supernatant of each sample was taken for the quantitative estimation of proteins. Note that we have not taken dry mushroom after removing its water content, rather we have taken fresh mushrooms immediately after harvesting from the thallus growing over straw-bags.

4. Estimation Procedure

0.2, 0.4, 0.6, 0.8 and 1 ml of working standard BSA was pipetted out in to the series of labeled test tubes. The volume was made up to 1 ml in all the test tubes by adding distilled water. 1 ml of each sample was pipetted out in other test tubes and labelled. A tube with 1 ml of distilled water served as the blank. 3 ml of Biuret reagent was added to all the test tubes including the "blank". The contents of the tubes was mixed by vortexing/shaking the tubes and warmed at 37°C for 10 min. Now the contents were cooled and recorded the absorbance at 540 nm against blank using colorimeter. Then the standard curve was plotted by taking concentration of proteins along X-axis and absorbance at 540 nm along Y-axis. Then from this standard curve the concentration of proteins in the samples was calculated.

E. **Temporary mounting of transverse section of pileus or disc of the fruiting body**

The pileus or discs of the fruiting bodies of mushrooms at pin- head stage and stage before they shed spores were plucked. Each pileus was observed from the lower side under a high magnification lens. Transverse section (T.S.) of each pileus was cut and stained with safranin (or can also be stained with fast green) and mounted in glycerine to study.

RESULTS:

1. Size and moisture content

Pleurotus florida exhibits variations in size, moisture content, cellular organizations and protein contents with respect to growth. As given in table-1, moisture content is higher in the latter stages in comparison to the lower ones. Also, the size of the fruiting body increases with growth. Graph-1 and graph-2 show the pattern of respective changes in the size and the moisture content of the fruiting body relative to growth. Both of these show a positive linear relationship between size and moisture content with growth of mushroom.

2. Protein contents

In contrast, as given in table-4 and graph-4, total protein contents per unit biomass show a linear decrease relative to growth of mushroom. Protein contents per unit biomass decrease with growth of *Pleurotus florida*. The sharp increase in moisture content with increase in size (table-1, graph-2) may be regarded as one of the reasons causing gradual dilution of proteins relative to mushroom growth.

3. T.S. of Pileus

Externally, the upper surface of pileus of fruiting body seemed tougher than the lower

surface. The pileus at the pin- head stage seemed tougher than at the stage of fruiting bodies before they shed spores and the latter was spongier than the former. The pileus, on the underside, was found to bear many lamellae or gills which hang down vertically and extend almost radially from the stipe (stalk) to the margin of the pileus. The gills were found to be more conspicuous in the stage of fruiting bodies before they shed spores than in the pin- head stage. Transverse sections of pileus of both stages showed peculiar differences in their cellular organizations. The cells in the slide of pin-head stage were found to be round and smaller in size, densely packed with very few intercellular spaces and more in number per unit area (or volume). On the other hand, the cells in the stage of fruiting bodies before they shed spores were found to be elongated and larger in size, loosely packed with a large number of intercellular spaces per unit area (or volume) which impart sponginess to the pileus.

DISCUSSION:

An animal or plant starts its life as a single cell— a fertilized egg. During development, this cell divides repeatedly to produce many different cells in a final pattern of spectacular complexity and precision. Ultimately, the genome determines the pattern. The genome is normally identical in every cell; the cells differ not because they contain different genetic information, but because they express different sets of genes. This selective gene expression controls the four essential processes by which the embryo is constructed: (1) Cell proliferation, producing many cells from one, (2) Cell specialization, creating cells with different characteristics at different positions, (3) Cell interactions, coordinating the behavior of one cell with that of its neighbors, and (4) Cell movement, rearranging the cells to form structured tissues and organs. In a developing embryo, all these processes are happening at once, in a kaleidoscopic variety of different ways in different parts of the organism. Regulated activation and inactivation of genes leading to time- specific protein synthesis (temporal gene expression) is largely responsible for the intricate developmental processes. The high concentration of proteins observed in the early growth stages of *Pleurotus florida* reflects the fact that a large number of proteins are required during development at different times. These include a large number of developmental proteins, many metabolic enzymes as well as source of nitrogen for nucleotide synthesis. After differentiation event accomplishes, many proteins cease to occur as these are no more needed and also intensity of production of many proteins reduces that were profusely present during development. This accounts for the reduction in total protein contents of the mature fruiting bodies of *Pleurotus florida*. From nutritional viewpoint also, the pin- head stage of *Pleurotus florida* is rich in proteins and can be prescribed for consumption to the patients suffering from overall protein deficiency or PEM, or strict vegetarians. From commercial viewpoint, harvesting at pin- head stage will produce very little mushroom and also due to toughness pin- heads are difficult to cook and not of very good flavour. Hence, this will lead to increase in production cost and the decrease in consumption as well. So, this will be a costly business. So, from commercial point of view, fruiting bodies of higher stages are harvested for sell. Pin- heads are richest but higher stages are also not poor in proteins. In fact,

the stage at which *Pleurotus florida* is commercially harvested is good both in terms of flavour as well as protein contents for consumption. Although the present work is the result of great efforts and intensive labour, both physical and mental, it still may have many flaws or lacunae due to lack of time and technical hurdles. The present work focuses on temporal changes in the overall protein contents. It would not be an exaggeration to say that if the present work would be extended with some more sophisticated techniques as mentioned above, a more significant result could be expected.

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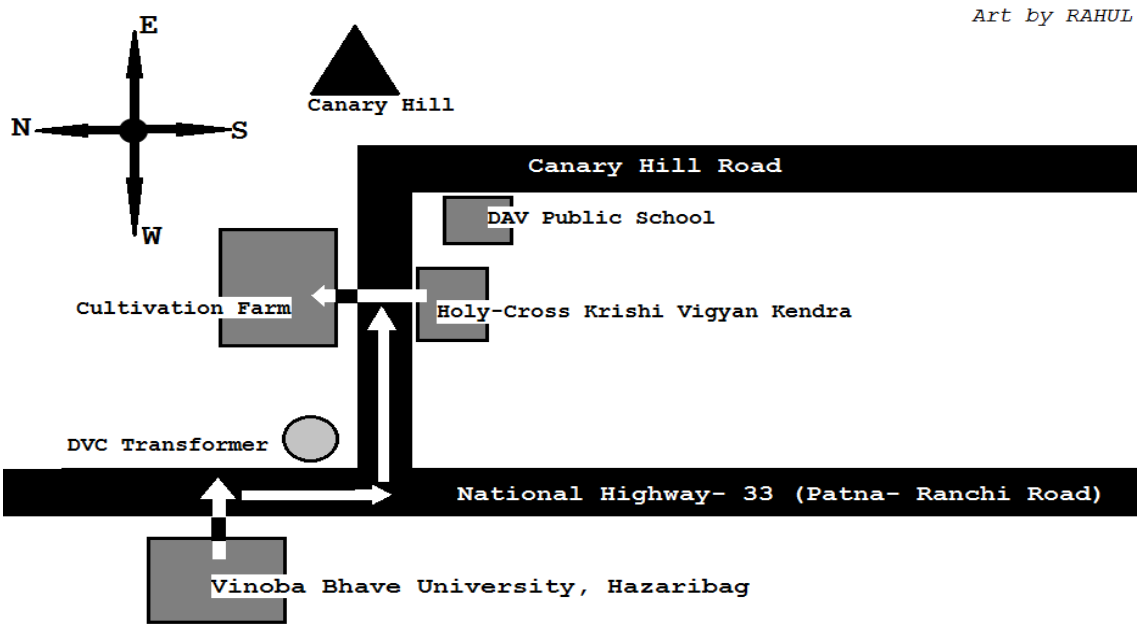


FIGURE: Road-map to Holy-Cros Krishi Vigyan Kendra, Hazaribag

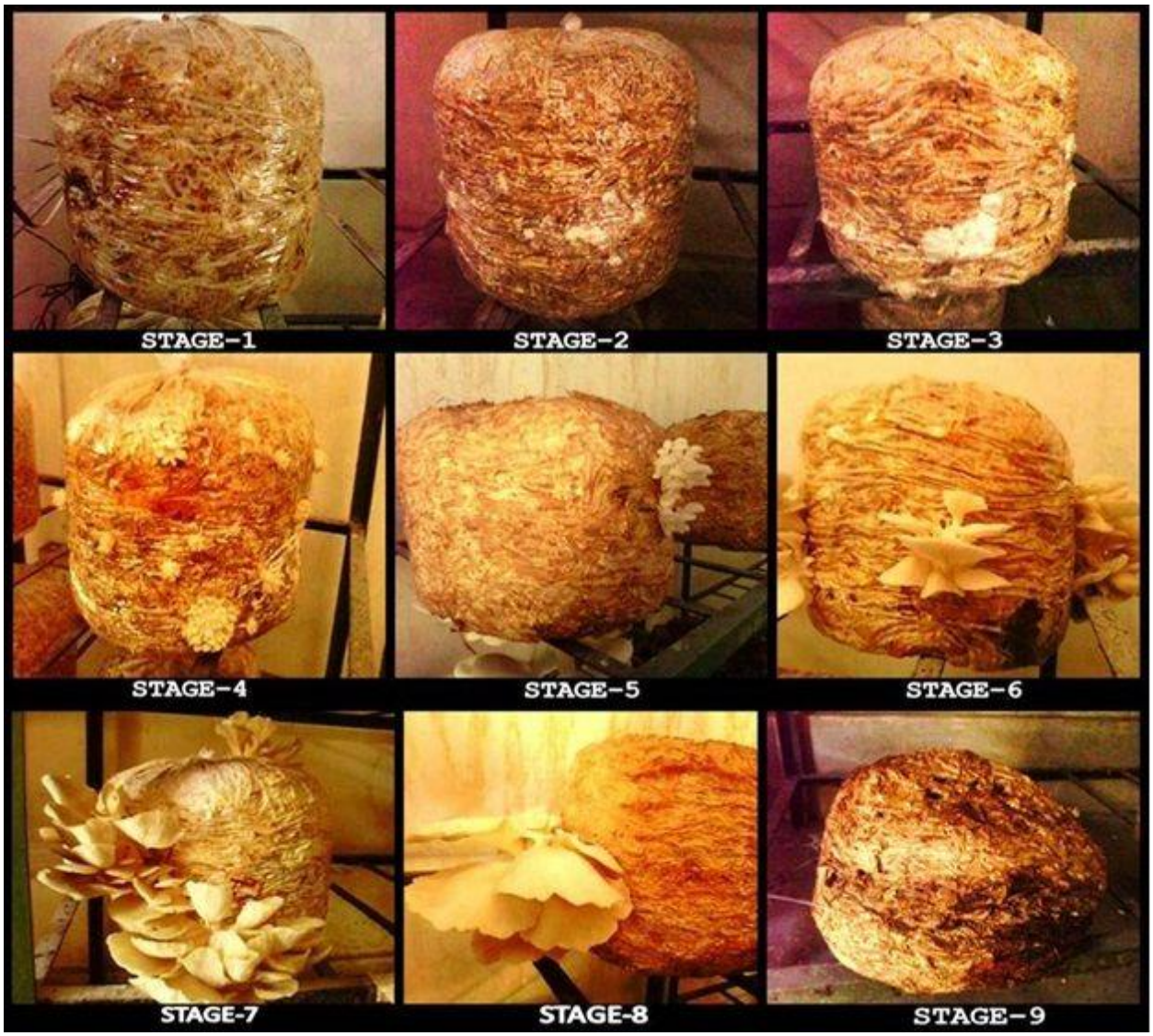
DESCRIPTION OF PHOTOGRAPHS:

Photograph-1: Mother inoculum containing pure culture of *Pleurotus florida* on wheat grains
Photograph-2: Inoculation on sterile wheat grains to prepare spawns of *Pleurotus florida* in the laminar flow chamber
Photograph-3: Freshly inoculated spawns
Photograph-4: Shelf containing large number of spawns at Mushroom Spawn Unit, Holy-Cross Krishi Vigyan Kendra, Hazaribag
Photograph-5: A worker at Mushroom Spawn Unit, Holy-Cross Krishi Vigyan Kendra, Hazaribaag; showing freshly inoculated spawn (right) and 25 days old spawn (left) for comparison
Photograph-6: Collection of paddy straws for bundle preparation for cultivation of *Pleurotus florida*

DESCRIPTION OF PHOTOGRAPHS

PHOTOGRAPH-1: Straw bundles showing different stages of active fungal growth

PHOTOGRAPH-2: Old nutrient-exhausted straw bundles showing minimum fungal growth



DESCRIPTION OF PHOTOGRAPH

Different Growth Stages of *Pleurotus florida:*

STAGE-1: Mycelial stage;

STAGE-2, 3: Mycelia emerging out of straw bundle to form fruiting bodies;

STAGE-4: Early aerial fruiting bodies showing clusters of pin-heads;

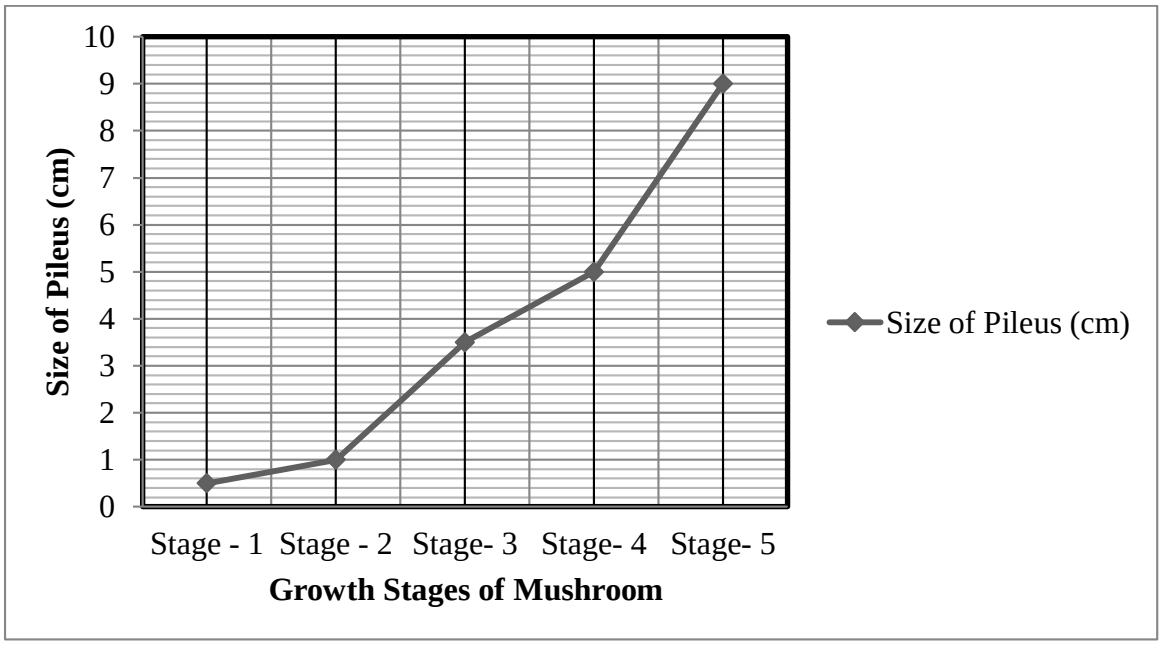
STAGE-5, 6, 7: Fan like fruiting bodies of different sizes (5<6<7);

STAGE-8: Fully grown fruiting bodies ready for commercial harvesting;

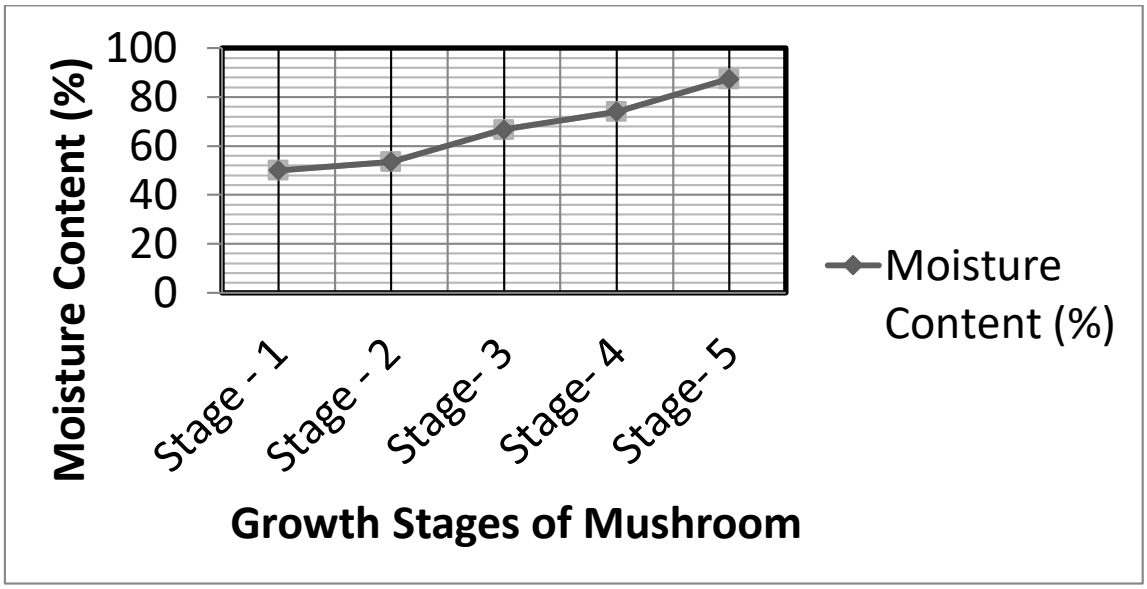
STAGE-9: Completely used-up straw bundle (exhausted of nutrients) showing no further growth

TABLE-1: Observation Table Showing Size and Moisture Contents of *Pleurotus florida* Different Growth Stages

Sr. No	Growth Stage	Size of Pileus (cm)	Weight (g)		$W_1 - W_2$	Moisture Content (%)
			Fresh Pileus (W_1)	Dry Pileus (W_2)		
1.	Stage- 1 (Pin- Head)	**0.5**	0.12	0.06	0.06	**50.00**
2.	Stage- 2 (Fan Shaped)	**1.0**	0.28	0.13	0.15	**53.57**
3.	Stage- 3 (Fan Shaped)	**3.5**	3.52	1.17	2.35	**66.76**
4.	Stage- 4 (Fan Shaped)	**5.0**	6.08	1.58	4.50	**74.01**
5.	Stage- 5 (Fan Shaped)	**9.0**	15.32	1.92	13.40	**87.48**



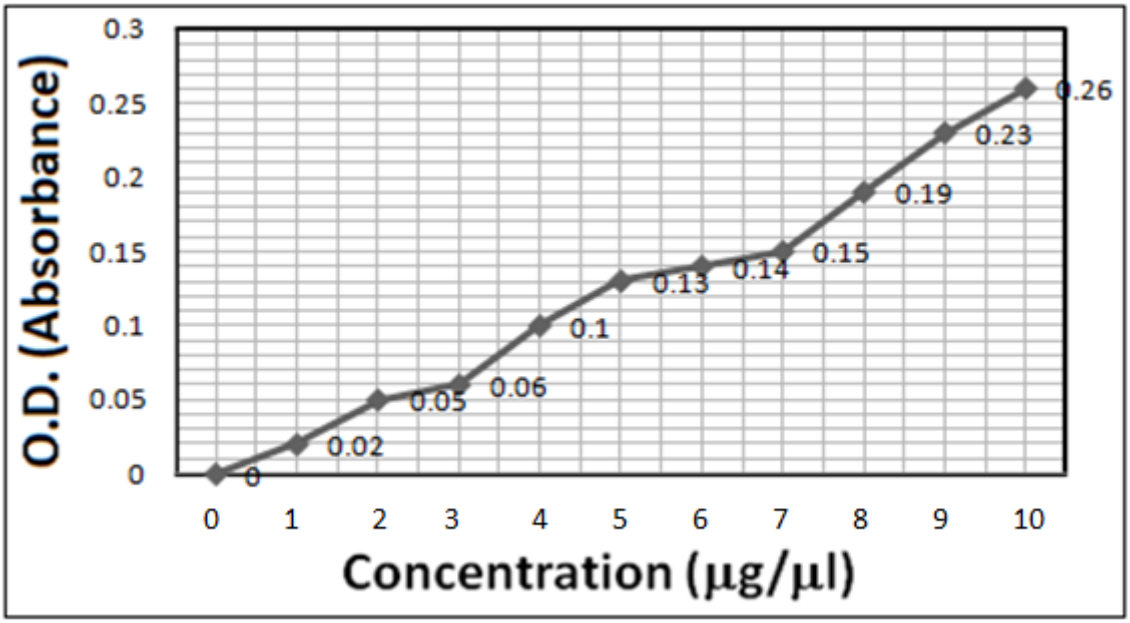
GRAPH-1: Pattern of Changes in Diameter of Fruiting Body Relative to Growth of *Pleurotuis florida*



GRAPH-2: Pattern of Changes in Moisture Content Relative to Growth of *Pleurotus florida*

TABLE-2: Observed Values of Optical Densities (Absorbance at 540nm- A540) of Different Grades of BSA for Protein Estimation by Biuret Method

Serial No.	Standard BSA/Blank	Volume of Standard BSA/Blank (ml)	Volume of distilled water (ml)	O.D. Reading (A_{540})	Concentration of Protein (µg/µl)
1.	Blank	0	1	0.00 (Set Value)	0
2.	BSA	0.1	0.9	0.02	1
3.	BSA	0.2	0.8	0.05	2
4.	BSA	0.3	0.7	0.06	3
5.	BSA	0.4	0.6	0.10	4
6.	BSA	0.5	0.5	0.13	5
7.	BSA	0.6	0.4	0.14	6
8.	BSA	0.7	0.3	0.15	7
9.	BSA	0.8	0.2	0.19	8
10.	BSA	0.9	0.1	0.23	9



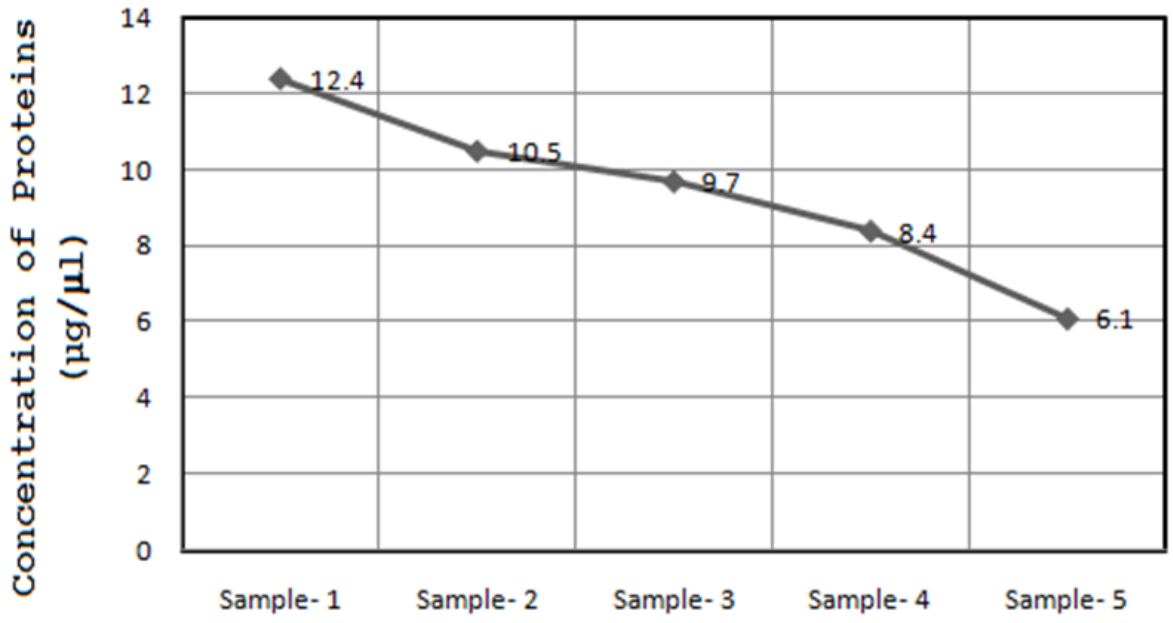
GRAPH-3: A Concentration- Absorbance Plot for Table-2

TABLE-3: Observed Values of Optical Densities (Absorbance at 540nm- A_{540}) of Mushroom Extract Samples

Growth Stage	Mushroom Extract	Volume (ml)	O.D. Reading (A_{540})
1	Sample- 1	1	**0.31**
2	Sample- 2	1`	**0.27**
3	Sample- 3	1	**0.24**
4	Sample- 4	1	**0.22**
5	Sample- 5	1	**0.15**

TABLE-4: Concentration of Proteins in Respective Mushroom Extract Samples as Calculated using Graph 3 and Table 3

Growth Stage	Mushroom Extract	Concentration of Proteins (µg/µl)
1	Sample- 1	**12.4**
2	Sample- 2	**10.5**
3	Sample- 3	**9.7**
4	Sample- 4	**8.4**
5	Sample- 5	**6.1**



GRAPH-4: Graph Plotted for Data of Table-4

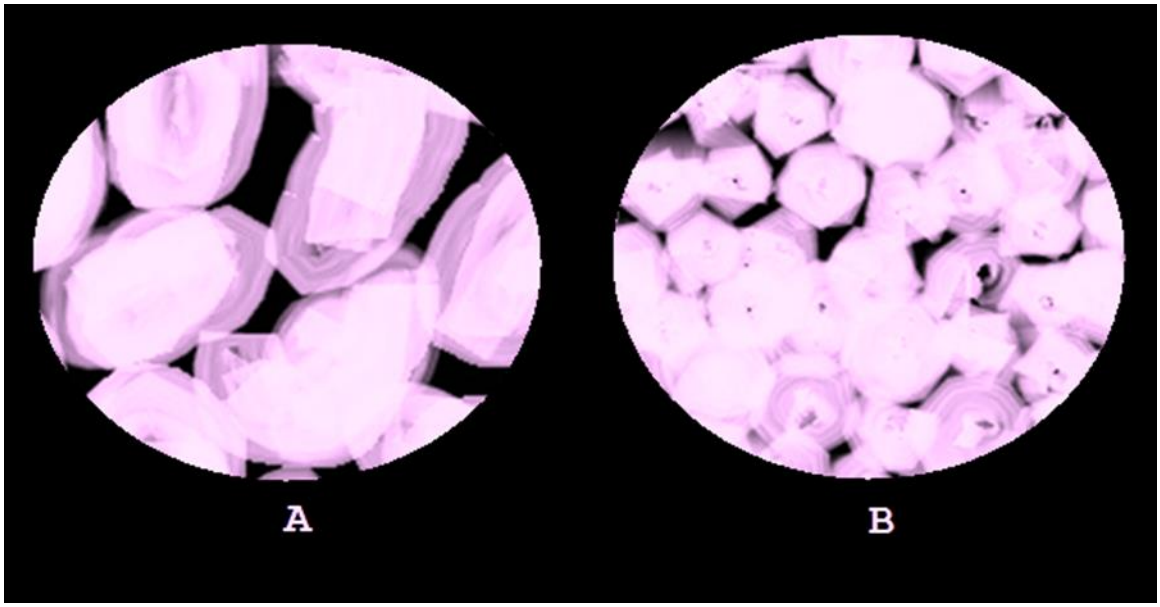


IMAGE: Schematic Drawings of Transverse Sections of fruiting body of *Pleurotus florida* as observed under Dark Field Illumination of Compound Microscope (at 40X):

A. Transverse section of disc of fully mature fruiting body (pre-harvesting stage) showing conspicuous intercellular spaces

B. Transverse section of disc of fruiting body at earliest growth stage (pin-head stage) showing compact cellular structure

Note that the number of cells per unit area (or volume) in B is greater than that in A.