

**COMPARITIVE EFFECT OF POTASSIUM HUMATE ON EDIBLE MUSHROOM
(*Pleurotus ostreatus*) CULTIVATION IN TUBULAR SOLID STATE FERMENTATION
Vs CONVENTIONAL METHOD**

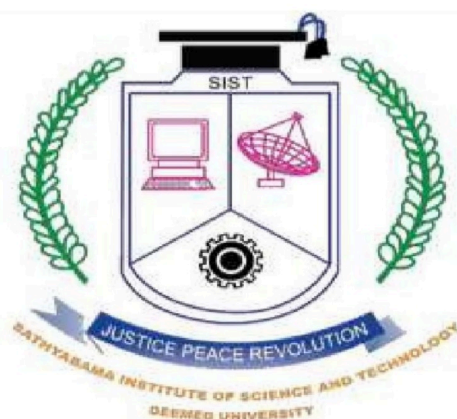
PROJECT WORK

Submitted in partial fulfilment of the requirements for the award of Bachelor of
Science degree in Microbiology

By

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**DEPARTMENT OF BIOTECHNOLOGY
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SATHYABAMA

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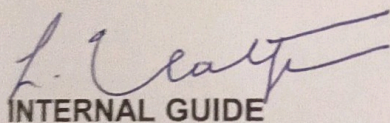
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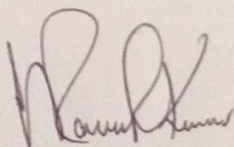
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **M. Imran nazir** (41300015) and **G. Baskar** (41300007) who carried out the project entitled "COMPARATIVE EFFECT OF POTASSIUM HUMATE ON EDIBLE MUSHROOM (*Pleurotus ostreatus*) CULTIVATION IN TUBULAR SOLIDSTATE FERMENTATION Vs CONVENTIONAL METHOD" under my supervision from January 2024 to April 2024.

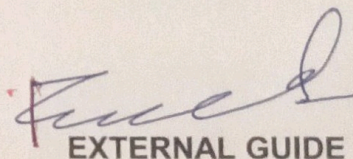

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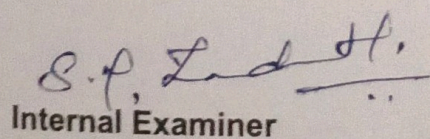
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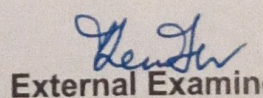
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DECLARATION

DECLARATION

We, M. Imran nazir (41300015) and G. Baskar (41300007) hereby declare that the Project Report entitled "CONVENTIONAL EFFECT OF POTASSIUM HUMATE ON *Pleurotus ostreatus* CULTIVATION IN TUBULAR SOLIDSTATE FERMENTATION Vs CONVENTIONAL METHOD" done by me under the guidance of Dr.L. Inbathamizh (Internal) and Dr. P. Prakash (External) at Sathyabama Institute of Science and Technology, Chennai is submitted in partial fulfilment of the requirements for the award of Bachelor of Science degree in Microbiology.

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ABSTRACT

Potassium humate is an organic molecule that is formed by the breakdown of coal, soil, peat and dystrophies lakes. The aim of the present investigation was to determine the effect of humic acid on growth rate and nutritional value of *Pleurotus ostreatus* was discussed. Humic acid was extracted from lignite by addition of alkaline solution and precipitated by acid. In the present study *Pleurotus ostreatus* was grown with different concentration of humic acid i.e. 1 to 10%. After incubation *Pleurotus ostreatus* at respective treatment is evaluated for total biomass, protein, Carbohydrates, fiber, and moisture contents. They were found to be increased in all the treated groups than the untreated control. The maximum yield and nutrient contents were recorded in 10% concentration of humic acid than untreated control. And oyster mushrooms are grown in two different methods. They are Tubular solid state fermentation method (TSF) and conventional method (CM). By comparing the yield and biochemical components. Therefore TSF method is more effective than CM.

Keywords: Potassium humate or humic acid, *P. ostreatus*, Tubular solid state fermentation (TSF), Conventional method (CM).

LIST OF CONTENTS

S.NO	TITLE	PAGE NO
	ABSTRACT	V
	LIST OF ABBREVIATION	VIII
	LIST OF FIGURES	IX
	LIST OF TABLES	X
1	INTRODUCTION	
	1.1 AN OVERVIEW ON PLEUROTUSOSTREATUS	1
	1.2 HEALTH BENEFITS OF OYSTER MUSHROOMS	2
	LITERATURE SURVEY	
	2.1 PROPERTIES OF OYSTER MUSHROOM	4
	2.2 NUTRITIONAL INFORMATION	
	2.3 INTRODUCTION ON HUMIC ACID	5
	2.4 FORMATION AND DESCRIPTION OF HUMIC ACID	5
	2.5 CHEMICAL CHARACTERISTICS OF HUMIC SUBSTANCES	7
	2.6 ECOLOGICAL EFFECTS OF HUMIC ACID SUBSTANCES	8
	2.8 MECHANISM OF HUMIC ACID	8
	2.9 APPLICATIONS OF HUMIC ACID	11

3	AIM & OBJECTIVES	
	3.1 AIM	12
	3.2 SCOPE	12
 4	 MATERIALS & METHODS	
	4.1 MATERIALS REQUIRED	13
	4.2 METHODOLOGY	14
	4.2.1 PREPARATION OF HUMIC ACID	14
	4.2.2 PEOCEDURE FOR MUSHROOM CULTIVATION	14
	4.3 NUTRIENT VALUE ANALYSIS	15
	4.3.1 ESTIMATION OF PROTEIN BY (LOWERY'S METHOD)	15
	4.3.2 ESTIMATION OF CARB (DNSA METHOD)	16
	4.3.3 DETERMINATION OF MOISTURE	17
	4.3.4 DETERMINATION OF CRUDE FIBER	18

5.1 EFFECT OF KHA OF <i>P.ostreatus</i>	19
5.1.1 GROWTH COMPARISON BASED ON METHODS AND CONCENTRATTION	21
5.2 EFFECT OF KHA OF <i>P.ostreatus</i> IN PROTEIN	23
5.3 EFFECT OF KHA OF <i>P.ostreatus</i> IN CARBOHYDRATES	26
5.4 EFFECT OF KHA OF <i>P.ostreatus</i> IN FIBER	29
5.5 BLUEPRINT AND DESIGN OF FERMENTOR	31

LIST OF ABBREVIATIONS

TSF	Tubular Solids State Fermentation
CM	Conventional Method
Kg	Kilogram
HA	Humic Acid
g	Gram
%	Percentage

LIST OF FIGURES

FIGURE NO NO	TITLE	PAGE
5.1.1	Growth comparison (Day - 1)	21
5.1.1	Growth comparison (Day - 5)	21
5.1.1	Growth comparison (Day - 10)	22
5.1.1	Growth comparison (Day - 12)	22
5.2	Graph of CM (lowry's method)	24
5.2	Graph of TSF (lowry's method)	24
5.3	Graph of CM (DNSA method)	27
5.3	Graph of CM (DNSA method)	27
5.5	Blue print of Fermentor	31

LIST OF TABLES

TABLE NO	TITLE	PAGE NO
4.3.3	Amount of moisture content	17
5.1	Effect of KHA of P.ostreatus (yield)	19
5.2	Effect of KHA of P.ostreatus in Protein	23
5.3	Effect of KHA of P.ostreatus in Carb	26
5.4	Effect of KHA of P.ostreatus in Fiber	29

CHAPTER 1

INTRODUCTION

1.1 AN OVERVIEW ON PLEURATUS OSTREATUS

Pleurotus is a genus of gilled mushrooms which includes one of the most widely eaten mushrooms, *P. ostreatus*. Species of *Pleurotus* may be called **oyster**, **abalone**, or **tree mushrooms**, and are some of the most commonly cultivated edible mushrooms in the world. *Pleurotus* fungi have been used in mycoremediation of pollutants such as petroleum and polycyclic aromatic hydrocarbons. Mycoremediation of pollutants such as petroleum and polycyclic aromatic hydrocarbons has been done using *Pleurotus* fungi.

Pleurotus fungi are found in both tropical and temperate climates throughout the world. Most species of *Pleurotus* are white-rot fungi on hardwood trees, although some also decay conifer wood. *P. eryngii* is unusual in its association with herbaceous plants, and *P. tuberregium* produces underground sclerotia. In addition to being saprotrophic, all species of *Pleurotus* are also nematophagous, catching nematodes by paralyzing them with a toxin.

The mushroom has a broad, fan or oyster-shaped cap spanning 5–25 cm; natural specimens range from white to gray or tan to dark-brown; the margin is enrolled when young, and is smooth and often somewhat lobed or wavy. The flesh is white, firm, and varies in thickness due to stipe arrangement. The gills of the mushroom are white to cream, and descend on the stalk if present. If so, the stipe is off-centre with a lateral attachment to wood. The mushroom's stipe is often absent. When present, it is short and thick.

The oyster mushroom is found worldwide in many temperate and subtropical forests, although it is not found in the Pacific Northwest of North America, where *P. pulmonarius* and *P. populinus* have taken its place. It is a saprotroph that serves as the main wood decomposer, breaking down wood primarily from deciduous trees,

especially beech trees. This fungus causes white rot and wood degradation. This fungus cause white rot and wood degradation.

The oyster mushroom is widespread in many temperate and subtropical forests throughout the world, although it is absent from the Pacific Northwest of North America, being replaced by *P. pulmonarius* and *P. populinus*. It is a saprotroph that acts as a primary decomposer of wood, especially deciduous trees, and beech trees in particular. It is a white-rot wood-decay fungus.

The oyster mushroom is one of the few known carnivorous mushrooms. Its mycelia can kill and digest nematodes, which is believed to be a way in which the mushroom obtains nitrogen.

The standard oyster mushroom can grow in many places, but some other related species, such as the branched oyster mushroom, grow only on trees. They may be found all year round in the UK.

While this mushroom is often seen growing on dying hardwood trees, it only appears to be acting saprophytic ally, rather than parasitically. As the tree dies of other causes, *P. ostreatus* grows on the rapidly increasing mass of dead and dying wood. They actually benefit the forest by decomposing the dead wood, returning vital elements and minerals to the ecosystem in a form usable to other plants and organisms. Despite this, the belief that *P. ostreatus* could damage New Zealand's forestry industry has led New Zealand to ban its importation. Despite this concern about potential damage to New Zealand's forestry industry have led to the ban of *P. ostreatus* importation.

1.2. HEALTH OF OYSTER MUSHROOMS

Cholesterol: Oyster mushrooms are also suitable additions to the diet of people with obesity, diabetes and high blood pressure. This is primarily because these mushrooms are low in sodium and have a low starch ratio. These mushrooms also contain statins which are cholesterol lowering drugs. It is a good idea to include oyster mushrooms in diet for high cholesterol. Despite this concern

about potential damage to New Zealand's forestry industry have led to the ban of *P. ostreatus* importation.

Cancer: Extract from the pink oyster has shown to stop the proliferation of cancerous cells. Extracts of king oyster mushroom has shown to stimulate the immune function. Use of mushroom in cancer treatment is an on-going research activity that has a lot of promise.

Hepatitis: Oyster mushrooms are also known to benefit people trying to quit tobacco products or even counter the Hepatitis C virus.

Other people who will benefit by including them in their daily diet are people suffering from anaemia, hyperacidity, constipation and of course, to boost general immunity.

This, however, does not mean you should discontinue drugs just because you have added mushrooms to your diet. All advice on drug courses should come from a certified doctor. Get to know detailed information on button mushroom

CHAPTER 2

LITERATURE SURVEY

2.1 Properties of Oyster Mushrooms

Oyster mushrooms offer a plethora of health benefits, including antioxidant and antibacterial properties. They are packed with essential nutrients, such as zinc, potassium, iron, calcium, phosphorus, and vitamins B1, B2, C, folic acid, and niacin. Incorporating oyster mushrooms into your diet regularly ensures a reliable intake of these vital nutrients, particularly essential vitamins.

Interestingly, the iron content in oyster mushrooms surpasses that found in meat, a common dietary source of iron. Additionally, the high potassium content in oyster mushrooms serves as a preventive measure against heart disease and hypertension. However, it's important to note that mushrooms have a tendency to absorb metals from the soil, including cadmium and mercury. Therefore, it's advisable to know the origin of the mushrooms to mitigate any potential health risks associated with metal absorption.

Vitamin Content

Mushrooms possess antimicrobial, antibacterial, and antioxidant qualities. These are parasites that release substances into the environment that enable them to live. When we eat mushrooms, this benefits us further. Ergothioneine is the chemical that gives mushrooms their antioxidant qualities, and it remains intact even after cooking. For instance, three ounces of mushrooms can include up to 13 milligrams of ergothioneine in them.

Ergothioneine is known to protect cells and build immunity. The anti-bacterial function in oyster mushrooms is because of the compound benzaldehyde. This compound reduces levels of bacteria in the body. Cooking these mushrooms provides a boost of riboflavin along with the earthy taste of mushrooms. Riboflavin is a form of

B12 vitamin and these mushrooms ensure you receive your daily intake of this vitamin. These mushrooms also contain up to 13 per cent of pantothenic acid which is also a form of vitamin B5. Along with B12 and B5, oyster mushrooms also contain thiamine which is a form of vitamin B1. The quantity of vitamin B3 is also about five times higher as compared to other vegetables. Mushrooms in general are very nutrient rich so you can mix up different mushrooms and add them to your sandwiches, salads and soups. It need not only be oyster mushrooms. You can mix it up with button, crimini, chanterelle, porcini, shitake and many other kinds of mushrooms.

Mushrooms are also known to provide vitamin D which helps strengthen bones.

2.3 INTRODUCTION ON HUMIC ACID

Humic acid is a principal component of humic substances, which are the major organic constituents of soil (humus), peat and coal. It is also a major organic constituent of many upland streams, dystrophic lakes, and ocean water. It is produced by bio degradation of dead organic matter. It is not a single acid; rather, it is a complex mixture of many different acids containing carboxyl and phenolate groups so that the mixture behaves functionally as a dibasic acid or, occasionally, as a tribasic acid. Humic acids can form complexes with ions that are commonly found in the environment creating humic colloids. Humic and **fulvic acids** (fulvic acids are humic acids of lower molecular weight and higher oxygen content than other humic acids) are commonly used as a soil supplement in agriculture, and less commonly as a human nutritional supplement. As a nutrition supplement, fulvic acid can be found in a liquid form as a component of mineral colloids. Fulvic acids are poly-electrolytes and are unique colloids that diffuse easily through membranes whereas all other colloids do not.

2.4 .FORMATION AND DESCRIPTION OF HUMIC ACID

Oyster mushrooms boast impressive nutritional content, with a 100-gram or 3.5-ounce serving containing only 33 calories and 0.4 grams of fat. Additionally, they provide 6.4 grams of carbohydrates and 33 grams of protein per serving. However,

the nutritional profile may alter depending on how they're prepared, such as being cooked with additional fats or served alongside bread or meat. Optimal cooking methods include quick stir-frying, sautéing, or incorporating them into larger dishes like stews, roasts, or salads.

Resembling oysters in appearance, oyster mushrooms derive their name from this likeness. They typically grow on fallen logs, at the base of dead trees, or even on organic matter like sawdust and straw. Oyster mushrooms exhibit various colours including grey, white, yellow, dark brown, and occasionally pink, with colour often influenced by growing conditions, which also impact taste. The tender stems and entire mushroom are edible, maintaining firmness even after cooking. Tender mushrooms tend to offer a more flavourful experience, while larger ones may be chewier yet still retain a pleasant sweetness.

Oyster mushrooms are commonly paired with chanterelles in culinary preparations. They earn their name not only from their appearance but also because they often exhibit a taste reminiscent of shellfish rather than the typical mushroom flavour. Known by various monikers such as elephant mushroom, oyster cap mushroom, tree mushroom, and shimeji, the oyster mushroom is scientifically classified as '*Pleurotus ostreatus*' and belongs to the fungus kingdom like other mushrooms.

Humic substances found in soils and sediments can be categorized into three main fractions: humic acids, fulvic acids, and humin. Humic and fulvic acids are extracted from solid phase sources into a strongly basic solution of sodium hydroxide or potassium hydroxide. Humic acids are then precipitated by adjusting the pH to 1 with hydrochloric acid, while fulvic acids remain in solution, marking the operational difference between the two. Humin, on the other hand, remains insoluble in dilute alkali. The alcohol-soluble portion of the humic fraction is commonly referred to as humic acid. Various types of humic acids, such as "gray humic acids" (GHA) and "brown humic acids" (BHA), exhibit solubility characteristics under specific alkaline conditions, while fulvic acids (FA) are soluble regardless of pH and ionic strength.

Methods like liquid chromatography and liquid-liquid extraction are employed to separate the constituents of humic substances. These substances include mono-, di-, and tri-hydroxyl acids, fatty acids, dicarboxylic acids, linear alcohols, phenolic acids, and terpenoids.

The use of organic matter soil amendments for enhancing plant growth has been practiced by farmers for ages. However, understanding the chemistry and function of organic matter has been a matter of debate since the 18th century.

2.5 CHEMICAL CHARACTERISTICS OF HUMIC ACID

A typical humic substance comprises a mixture of numerous molecules, many of which feature aromatic nuclei with phenolic and carboxylic substituents linked together, as depicted in the illustration. Phenolic and carboxylic groups are the primary functional groups contributing to the surface charge and reactivity of humic substances. Humic acids exhibit characteristics akin to mixtures of dibasic acids, with a pK_1 value of approximately 4 for the protonation of carboxyl groups and around 8 for the protonation of phenolate groups. Despite variations, there is a notable overall similarity among individual humic acids. Consequently, measured pK values for a particular sample represent average values pertaining to the constituent species. Another crucial aspect is the charge density. These molecules may assemble into a supramolecular structure maintained by non-covalent forces such as Van der Waals force, π - π , and CH- π bonds.

The presence of carboxylate and phenolate groups enables humic acids to form complexes with ions like Mg^{2+} , Ca^{2+} , Fe^{2+} , and Fe^{3+} . Many humic acids possess two or more of these groups arranged to facilitate the formation of chelate complexes. This formation of (chelate) complexes plays a significant role in the biological function of humic acids by regulating the bioavailability of metal ions.

2.6 MECHANISM OF HUMIC ACID SUBSTANCES

Humic acid improves plant growth in several ways:

Clay Dis aggregation

Water Penetration Enabled

Micro nutrient Transference

Water Sequestration.

These are mechanism involved on plants by humic acid substances for their nutrient uptake and production.

2.8 APPLICATION OF HUMIC ACID

Humic Acid (humus or humate) is extracted formed compressed, ancient plant matter in the form of leonardite (a soft brown coal) as one kind of high polymer heterogeneous aromatic materials contains hydroxyl, carboxyl, phenolic hydroxyl and other active groups with black brown appearance. While humic acid react with different alkaline and prepared by extraction of lignite, brown coal or leonardite, we can potassium humate, sodium humate, boron humate, magnesium humate etc. It is an ideal additive to boost bio-activity and improve the performance of compost or composted fertilizers. The composting process speeds the release of the humic and fulvic acids.

Function & Benefits: - Enhance nutrient uptake by combining nutrients and humic aid as well as keep a well-balanced nutrition. Improve the structure of soil, increase the buffering powder of soil, and optimize N. P. K absorption by plants. Neutralize both acid and alkaline soils, regulate the PH value of soils, with the prominent effect in alkaline and acidic soil. Reduce nitrate leaking into the groundwater and protect the underground water. Enhance the resilience of crops, such as cold, drought, pest, disease and toppling resistance Stabilize nitrogen and

improve nitrogen efficiency (as an additive with urea) Promote healthier, stronger plants and beautify appearance Buffering the effects of excessive elements (particularly sodium), toxic chemicals and heavy metals. Promoting seed germination in a shorter time.

There are three different types of mushrooms. They are,

Edible mushroom.

Medicinal mushroom.

Poisonous mushroom.

Mushrooms add something special to every meal and, when you are familiar with the different types of edible mushrooms, you can eat a different mushroom every night.

Syabryai *et al.* (1965) illustrated a doubling in yield for potatoes and cabbage when fertilizers containing NPK and humic acid were employed, also noting accelerated growth in barley crops with the use of humic acid.

Kononova *et al.* (1966) defined humus as a chemical amalgamation comprising resilient, hydrophilic, partially aromatic organic substances altered from original tissues or synthesized by soil organisms, varying in molecular weight.

Abolina and Tashkhadzhasv (1968) observed the impacts of coal-humic fertilizers on plant physiological processes and potato yield.

Freeman *et al.* (1970) emphasized notable increases in yield for potatoes, soybeans, and algal cultures through the application of humic acid.

Brams *et al.* (1973) explored the ability of humic acid to alleviate P fixation and solubilize insoluble P in acidic and alkaline soils, facilitating its uptake by plants.

Senn *et al.* (1974) highlighted the soil-enhancing properties of humic substances, including improved fertility, water retention, drought resistance, and seed germination stimulation.

Lee *et al.* (1976) observed a significant increase in corn yield by stimulating root and shoot development through the addition of humates to hydroponic solutions.

Noster *et al.* (1978) discussed the commercial significance of oyster mushrooms in Europe, particularly their cultivation practices.

Schnitzer *et al.* (1978) elucidated the intricate composition of soil humus, comprising humic acid, fulvic acid, and humin, and their role in soil dynamics.

Stevenson *et al.* (1982) clarified the insoluble nature of humic acid within humic substances at higher pH levels and outlined extraction methodologies.

Breene *et al.* (1990) acknowledged the nutritional and medicinal value of specific mushroom species, especially in Japan.

Brannon and Sommers (1985) demonstrated the effectiveness of humic acid in enhancing N and P contents, thereby augmenting crop yields.

Stevenson *et al.* (1986) described the negatively charged colloidal surface of humus, influenced by carboxylic or phenolic groups and pH levels.

Baysal *et al.* (2003) investigated the cultivation of oyster mushrooms on different substrates, with rice husk supplementation yielding optimal results.

Calzada *et al.* (1987) evaluated the growth of white rot fungi on various substrates, highlighting wheat straw as the preferred substrate for *Pleurotus* species.

Adadayo *et al.* (2011) analyzed the nutritional composition of edible mushrooms, affirming their safety and nutritional value.

Rajarithnam and Banoz (1987) discussed the characteristics and versatility of *Pleurotus* species in decomposing waste materials.

Hernandez *et al.* (2003) delineated a straightforward method for preparing substrates for cultivating *Pleurotus ostreatus*.

Hafez *et al.* (2004) demonstrated the positive effects of humic acid application on squash plant growth and fruit yield.

Shah *et al.* (2004) cultivated oyster mushrooms on various substrates, noting their nutritional and medicinal benefits, particularly with sawdust as a substrate.

Prakash *et al.* (2010) presented strategies for enhancing *Pleurotus ostreatus* production, highlighting the beneficial impact of humic acid addition.

Prakash *et al.* (2010) also reported on lignite solubilization into humic acid by *Trichoderma viridi*, showcasing its efficacy in promoting plant growth and biocontrol against phytopathogens.

CHAPTER 3

AIM AND OBJECTIVES

3.1 AIM

To compare the effect of potassium humate on edible mushroom cultivation in Tubular solid state fermentation Vs conventional method.

3.2 OBJECTIVES

To optimize the effect of humic acid on oyster mushroom

To estimate the physical and biochemical compounds such as moisture and growth yield, non-reducing sugar, protein, fiber.

To design solid state tubular fermentor and its effect on mushroom cultivation

CHAPTER 4

MATERIALS AND METHODS

4.1 MATERIALS REQUIRED

- Paddy straw
- Mushroom spawn
- Polythene bag
- 12 feet PVC pipe
- 14 feet PVC pipe
- Water
- Buckets
- Autoclave
- Rope
- Humic acid
- Alkaline reagent
- Folins reagent
- DNS solution
- Sodium hypochlorite
- Colorimeter
- Spatula
- Weighing machine
- Test tubes & beaker
- Micropipette
- Sieve

4.2 METHODOLOGY

4.2.1 Production of humic acid

- 50 grams of lignite were first sieved using sieves with 75 and 150 micron pore diameters.
- Response surface technique was employed to establish optimal circumstances. (RSM).
- The lignite and 150 milliliters of water were combined thoroughly in a beaker.
- Following that, 50 grams of water were used to dissolve 4 grams of potassium hydroxide. Potassium hydroxide was introduced drop by drop during sonication.
- After settling for a day, the mixture was further processed, and the supernatant was carefully removed.
- The supernatant was mixed with one milliliter of strong hydrochloric acid.
- The supernatant was collected again after being left undisturbed for an additional day.
- The residue that was left behind after the slurry was filtered through paper was gathered and dried for an entire night at 60°C in a hot air oven. From this procedure, crystals of humic acid were later produced.

4.2.2 Procedure for mushroom cultivation

- Heated rice straw has been consumed.
- 5 cm lengths of dry paddy straw were cut.
- After adding humic acid to the chopped paddy straw, it was left to soak in water for four to five hours.
- Boiling water (80°C) was used to soak the aforementioned for two hours. Paddy straw that had been treated was dried. Dried on a sanitized gunny rag in a well-ventilated space. A relative humidity of about 65% was maintained.
- We measured out 60 by 30 centimeter polythene bags and knotted the bottom of each with a rubber band or twine.

- Up to five centimeters of paddy straw were placed inside the bag, and around twenty-five grams of mushroom seeds were distributed throughout.
- For solid state fermentation, rice straw was similarly put into PVC pipes. using cellophane tape to shut and seal the pipe afterward.
- Once the layers were constructed, the rubber band or thread was used to secure the polythene bag's mouth.
- The polythene bag's surface was punctured with a needle to create holes (10–15). In 3–4 days, fruiting mushroom buds began to emerge from the mushroom bed's surface, following a 15-20 day spread of fungal mycelium.

4.3 NUTRIENT VALUE AND ANALYSIS

4.3.1 Estimation of protein by Lowry's method

PRINCIPLE

A blue color complex is produced when the protein sample's aromatic amino acids, tryptophan and tyrosine, react with folin's phenol reagent. Calorimetric measurement of the color intensity was done at 620 nm.

REAGENTS REQUIRED

Stock Solution: 100mg of BSA (Bovine Serum Albumin) is made up to 100ml with distilled water in SMF.

Working Standard Solution: 10ml of stock standard is made up to 100ml with distilled water in SMF.

Alkaline Copper Reagent :

Solution A: 2% Sodium Carbonate in 0.1N Sodium hydroxide

Solution B: 0.5% Copper sulphate in 1% Sodium potassium tartrate.

Mix 50ml of solution A with 1ml of Solution B prior to use.

Folin's phenol reagent: Dilute the reagent with water in 1:2 ratios.

PROCEDURE

The protein working standard solution is pipetted out into a clean, dry test tube designated S_1 to S_5 , accordingly, containing 0.5 to 2.5 ml. The extracted mushroom solution that is provided is T_1 to T_6 and control. Distilled water is added to get the total volume up to 1.0 ml. The blank is 3.0 milliliters of pure water. Alkaline copper reagent (4.5 ml) should be added. Once everything is well combined, let it sit at room temperature for fifteen minutes. Now, add 0.5 ml of Folin's reagent to each tube, thoroughly mix the contents, and let them sit at room temperature for half an hour. Calorimetric readings of the created color's intensity are made at 620 nm.

4.3.2 Estimation of carbohydrates by DNSA method

PRINCIPLE

Reducing sugar quantification using the 3, 5-dinitrosalicylic acid (DNS) technique in theory, the colorful reaction with DNS reagent is accompanied by the oxidation of aldehyde functional groups of reducing sugars to carboxylic acids. The intensity of color is proportional to the concentration of decreasing sugars.

REAGENTS REQUIRED

Standard dextrose: 200mg of dextrose is dissolved in distilled water and make up to 100ml in standard flask. Thus, prepared standard solution of maltose is used to plot the standard curve for maltose [concentration = 2mg/ml].

PROCEDURE

- The standard maltose solution (0.5 ml) was added to five test tubes labeled S_1 through S_5 , denoting the concentration ranges of 200 to 1000 cups. In each test tube, add 1 milliliter of dinitro salicylic acid to this solution.
- Test tubes are cooled to room temperature after being submerged in a boiling water bath for three minutes.
- Next, distilled water is added to each test tube to fill it to the brim with 10ml.

- Calculate the optical density (OD) at 540 nm and use the absorbance at 540 nm along the y-axis and the maltose concentration on the X-axis to plot a common curve.

4.3.3 Determination of moisture

- By weighing the fresh and dry samples of mushrooms, the moisture content of the mushrooms is ascertained following the drying process. Ascertained using an electronic balance.
- The moisture percentage are calculated by,

Moisture (%) =

Weight of original sample – weight of dried sample (g) *100

Weight of original sample (g)

Table 4.3.3 Amount of moisture content – TSF Vs CM

Concentration of HA (%)	Total moisture in (TSF) method (%)	Total moisture in (CM) method (%)
1	89	84
2	82	83
3	87	89
4	95	92
5	89	86
6	88	82
7	82	89
8	93	91
9	91	87
10	88	86
Control	90	88

4.3.4 Determination of fiber

PRINCIPLE

Fiber content in food samples is measured using the gravimetric method of fiber estimation. To extract proteins, lipids, and carbs from the food sample while preserving the fiber, the sample is subjected to a variety of reagent treatments, including acids and alkalis.

MATERIALS REQUIRED

- Alkali solution like sodium hydroxide, sulfuric acid and other enzymes etc.
- Mortar and pestle
- Distilled water
- Weighing machine.

PROCEDURE

- Weigh the mushroom sample accurately.
- Treat the sample with alkali reagents like sodium hydroxide to remove fats, proteins and carbohydrates, leaving behind the fiber
- Filter and wash the residue to remove any impurities.
- Dry the residue in an oven to remove any moisture.
- Weigh the dried residue to determine the fiber content.
- Calculate the percentage of fiber in the sample based on the weight of the residue.

CHAPTER 5

RESULT & DISCUSSION

In this study, we concentrated on exploring the impact of humic acid extracted from lignite on mushroom cultivation in humus-rich environments. Various concentrations of humic acids were applied, and the yields of mushrooms were meticulously recorded and tabulated.

5.1 EFFECT OF KHA OF (*P.ostreatus*)

TABLE: 5.1: Amount of yield produced

S.NO	KHA%	YIELD OF TSF METHOD	YIELD OF CONVENTIONAL METHOD	DIFFERENCE
1	1	415	259	156
2	2	109	262	-153
3	3	438	309	129
4	4	491	278	213
5	5	453	211	242
6	6	320	441	-121
7	7	94	338	-244
8	8	273	276	-3
9	9	554	302	252
010	10	617	139	478
CONTROL		472	222	250

YIELD OF TSF Vs CM



Fig: 5.4 Yield of TSF Vs CM

- The tubular fermentation method produces a higher yield compared to the conventional method.

Oyster mushrooms were cultivated through two distinct methods: the Tubular Fermentation Method (TSF) and the Conventional Method (CM). Among the two, TSF demonstrated greater effectiveness, resulting in a total yield of 7 kg. Within this yield, TSF accounted for 4.5 kg, while CM yielded 2.5 kg. This success of the TSF method can be attributed to its superior water retention capacity during fermentation, leading to higher mushroom yields. Additionally, during the process, various concentrations of humic acid ranging from 1 to 10 were utilized. It was observed that the 10th concentration notably outperformed others in terms of yield, and the rate of yield was notably faster compared to the control group.

5.1.1 Growth based on method

DAY: 1

No growth seen in day -1

CONVENTIONAL METHOD

TSF METHOD



Fig: 5.1.1 Day-1 Comparison

DAY: 5

Pin head is visible in TSF (day - 5)

No growth seen in Conventional method CM

CONVENTIONAL METHOD

TSF METHOD



Fig: 5.1.1 Day-5 Comparison

DAY: 10

Visible and large amount of growth seen in TSF method (day - 10)

Mycelium growth seen CM.

CONVENTIONAL METHOD

TSF METHOD



Fig: 5.1.1 Day-10 Comparison

DAY: 12

Full growth seen in TSF method

In conventional method .Pin head is projected out from mycelium

CONVENTIONAL METHOD

TSF METHOD



Fig: 5.1.1 Day-12 Comparison

5.2 EFFECT OF KHA OF (*P.ostreatus*) IN PROTEIN

Biochemical testing was conducted after cultivating oyster mushrooms to evaluate the protein content using Lowry's method. Folin's reagent was utilized as a derivatizing agent to quantify the levels of amines and amino acids. Upon reacting in an alkaline solution, this reagent produces a fluorescent material that can be detected by a calorimeter at 620nm.

Table: 5.2: AMOUNT OF PROTION IN TSF VS CM

S.NO	KHA%	PROTIEN TSF%	PROTEIN CM%	DIFFERENCE
1	1	8	2	6
2	2	5	3	2
3	3	7	4	3
4	4	10	4	6
5	5	6	3	3
6	6	5	3	2
7	7	8	4	4
8	8	7	5	2
9	9	9	5	4
10	10	8	6	2
11	Control	9	5	4

GRAPH

Conventional method

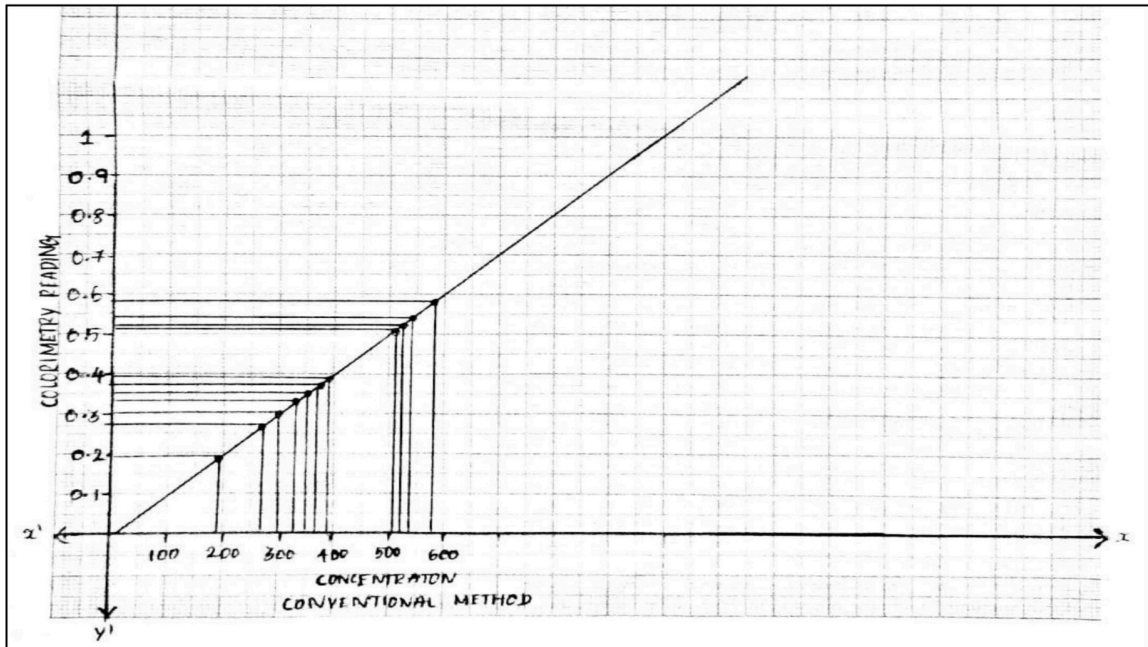


Fig: 5.2 Graph of CM (Lowry's method)

TSF method

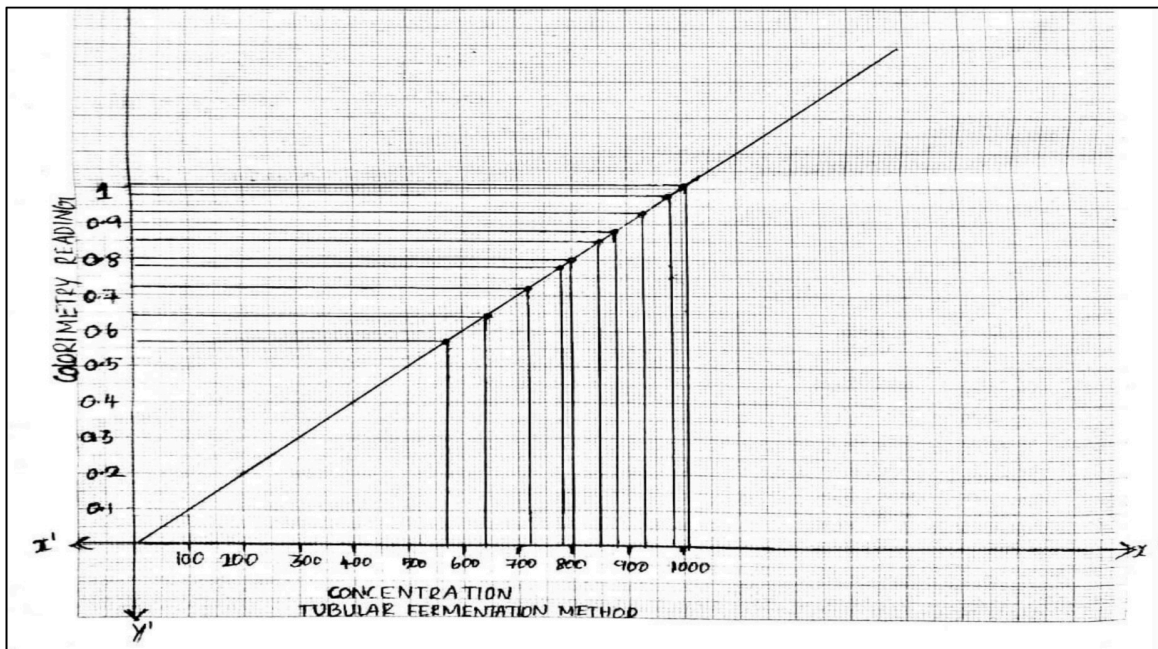


Fig: 5.2 Graph of TSF (Lowry's method)

PROTEIN CONTENT (%) – TSF Vs CM

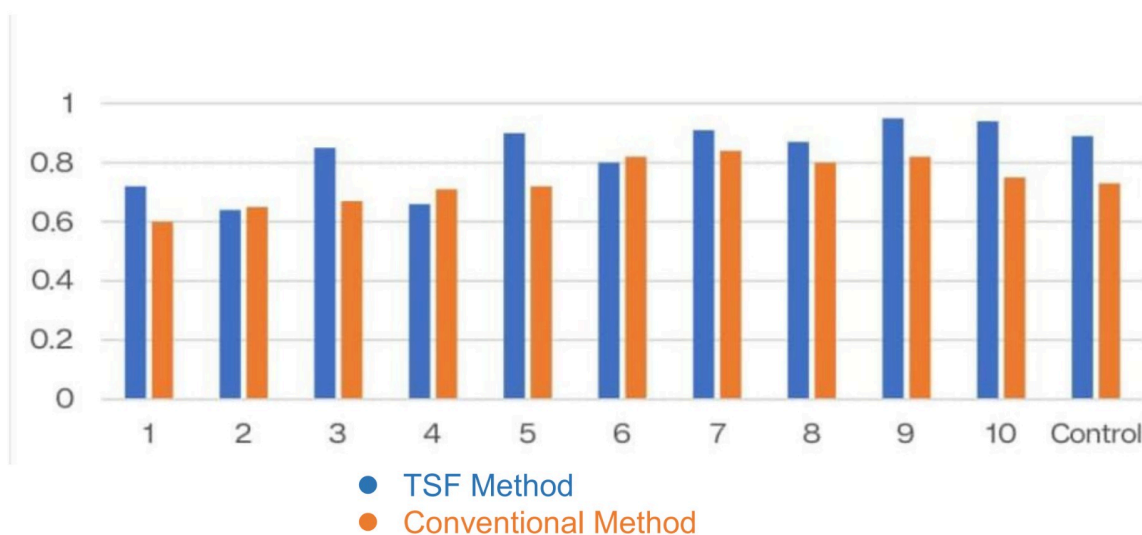


Fig: 5.2 Protein content TSF Vs CM

Following the cultivation of oyster mushrooms, a biochemical test was conducted to assess the protein content using Lowry's method. Folin's reagent was employed as a derivatizing agent to measure the levels of amines and amino acids. Upon reaction in an alkaline solution, this reagent produced a fluorescent material detectable by a calorimeter at 620nm.

Two methods were utilized in this study: TSF and CM. Of these, the TSF method demonstrated a higher protein content compared to CM. Furthermore, it was noted that the 10th concentration of humic acid exhibited a slightly increased protein content compared to both the control and other concentrations.

5.3 EFFECT OF KHA OF (*P.ostreatus*) IN CARBOHYDRATES

Biochemical analysis was conducted after oyster mushrooms were grown to determine the carbohydrate levels using the DNSA method. In biochemistry, this method commonly utilizes 3,5-Dinitrosalicylic acid (DNSA) to measure reducing sugars. It operates by detecting the presence of free carbonyl groups (C=O) within these sugars, which entails oxidizing both the aldehyde and ketone functional groups.

Table: 5.3: AMOUNT OF CARBOHYDRATES IN TSF VS CM

S.NO	KHA%	CARB IN TSF%	CARB IN CM%	DIFFERENCE
1	1	2	2	0
2	2	3	2	1
3	3	3	2	1
4	4	3	3	0
5	5	3	2	1
6	6	2	3	-1
7	7	2	2	0
8	8	2	3	-1
9	9	3	2	1
10	10	3	2	1
11	Control	3	2	1

GRAPH

Conventional method

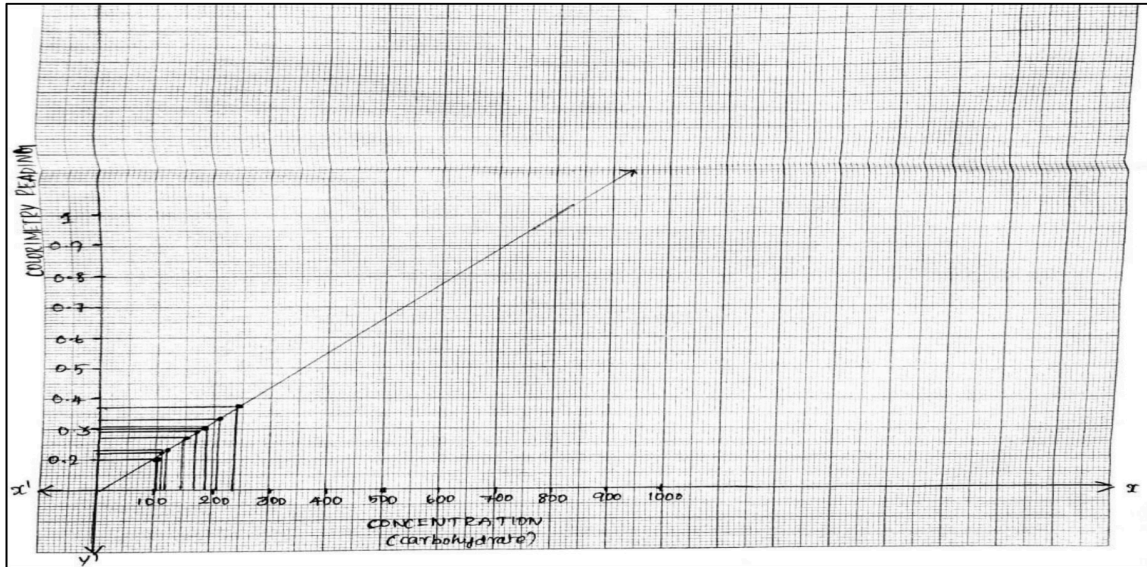


Fig: 5.3 Graph of CM (DNSA method)

TSF method

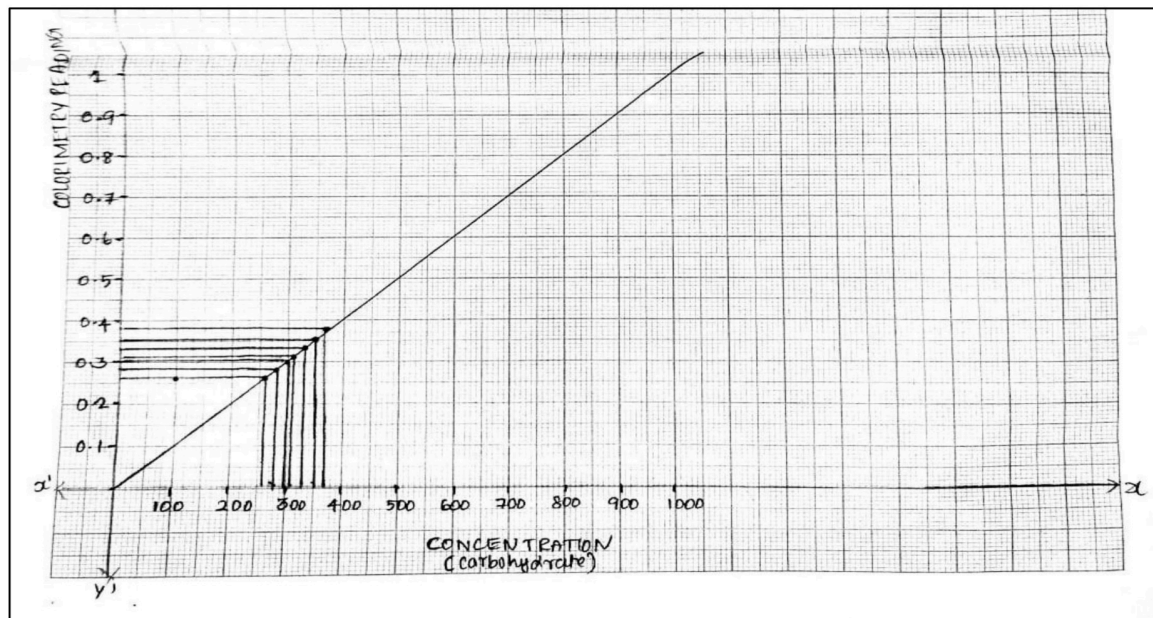


Fig: 5.3 Graph of TSF (DNSA method)

CARBOHYDRATES CONTENT (%) – TSF Vs CM

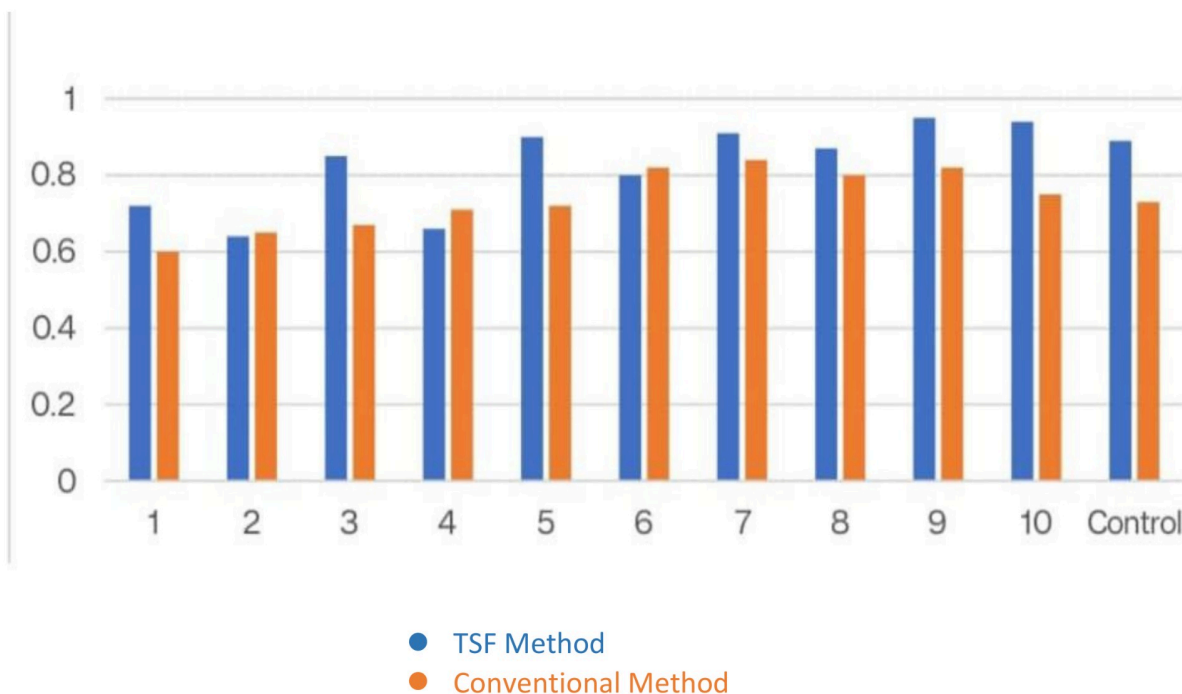


Fig: 5.3 Carbohydrates Content TSF Vs CM

After cultivating oyster mushrooms, a biochemical assessment was conducted to analyze the carbohydrate content using the DNSA method. 3,5-Dinitrosalicylic acid (DNSA) is commonly utilized in biochemistry to estimate reducing sugars by detecting the presence of free carbonyl groups (C=O) within them. This process involves the oxidation of both the aldehyde and ketone functional groups. The results were measured using a calorimeter at 540nm.

Two methods were employed in this study: TSF and CM. Among these, the TSF method exhibited a higher carbohydrate content compared to CM. Additionally, it was observed that the 10th concentration of humic acid showed slightly elevated protein content compared to the control and other concentrations.

5.4 EFFECT OF KHA OF (*P.ostreatus*) IN FIBER

Biochemical testing is conducted after growing oyster mushrooms to determine the fiber content using the gravimetric method. A strong alkali reagent such as sodium hypochlorite is employed to eliminate other biochemical components like protein and carbohydrates, leaving behind the fiber. The sample is then crushed with a mortar and pestle, and the residue is collected and dried. Subsequently, it is measured using a weighing balance to ascertain the fiber content.

Table: 5.4: Amount of fiber content based on methods

S.NO	KHA%	Fiber in TSF(g)	Fiber in CM(g)	Difference
1	1	0.72	0.6	0.12
2	2	0.64	0.65	-0.01
3	3	0.85	0.67	0.18
4	4	0.66	0.71	-0.05
5	5	0.9	0.72	0.18
6	6	0.8	0.82	-0.02
7	7	0.91	0.84	0.07
8	8	0.87	0.8	0.07
9	9	0.95	0.82	0.13
10	10	0.94	0.75	0.19
11	Control	0.89	0.73	0.16

FIBER CONTENT (%) – TSF Vs CM

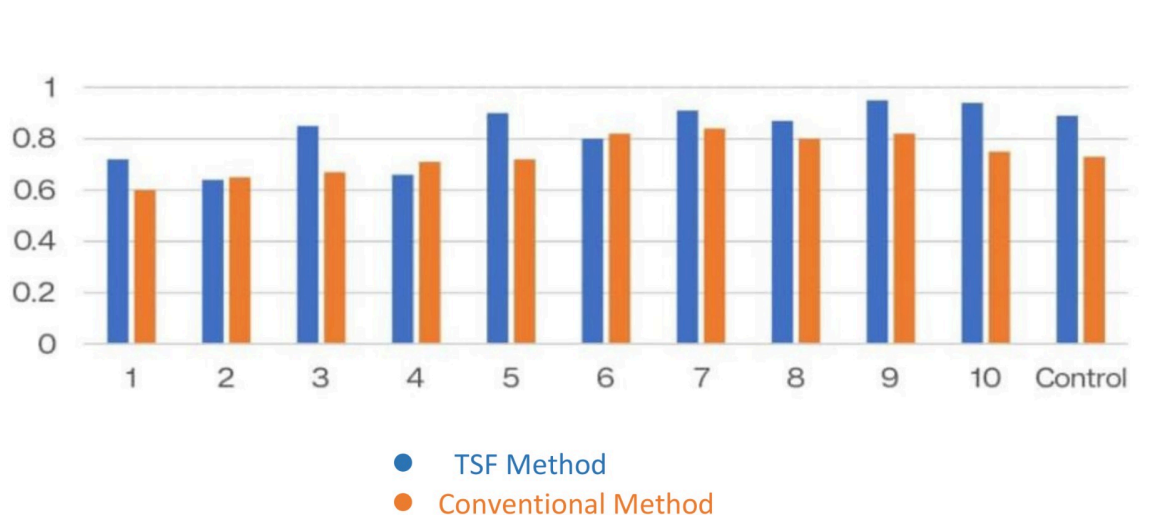


Fig: 5.4 Fiber content TSF Vs CM

After cultivating oyster mushrooms, a biochemical test is conducted to determine the fiber content using the gravimetric method. A strong alkali reagent like sodium hypochlorite is utilized to remove other biochemical components such as protein and carbohydrates, leaving behind the fiber. The sample is then crushed with a mortar and pestle, and the residue is collected and dried. Subsequently, it is measured on a weighing balance to ascertain the fiber content.

Two methods were employed in this study: TSF and CM. Among these, the TSF method exhibited a higher fiber content compared to CM. Additionally, it was observed that the 10th concentration of humic acid showed slightly elevated fiber content compared to the control and other concentrations.

5.5 BLUE PRINT AND DESIGN OF FERMENTOR

Tubular solid-state fermentation yields a higher output compared to the conventional method. Additionally, its biochemical components exhibit a slight increase, with no occurrences of contamination in TSF.

Consequently, we have designed a fermentor for the automatic and easy cultivation of oyster mushrooms, facilitating large-scale production.

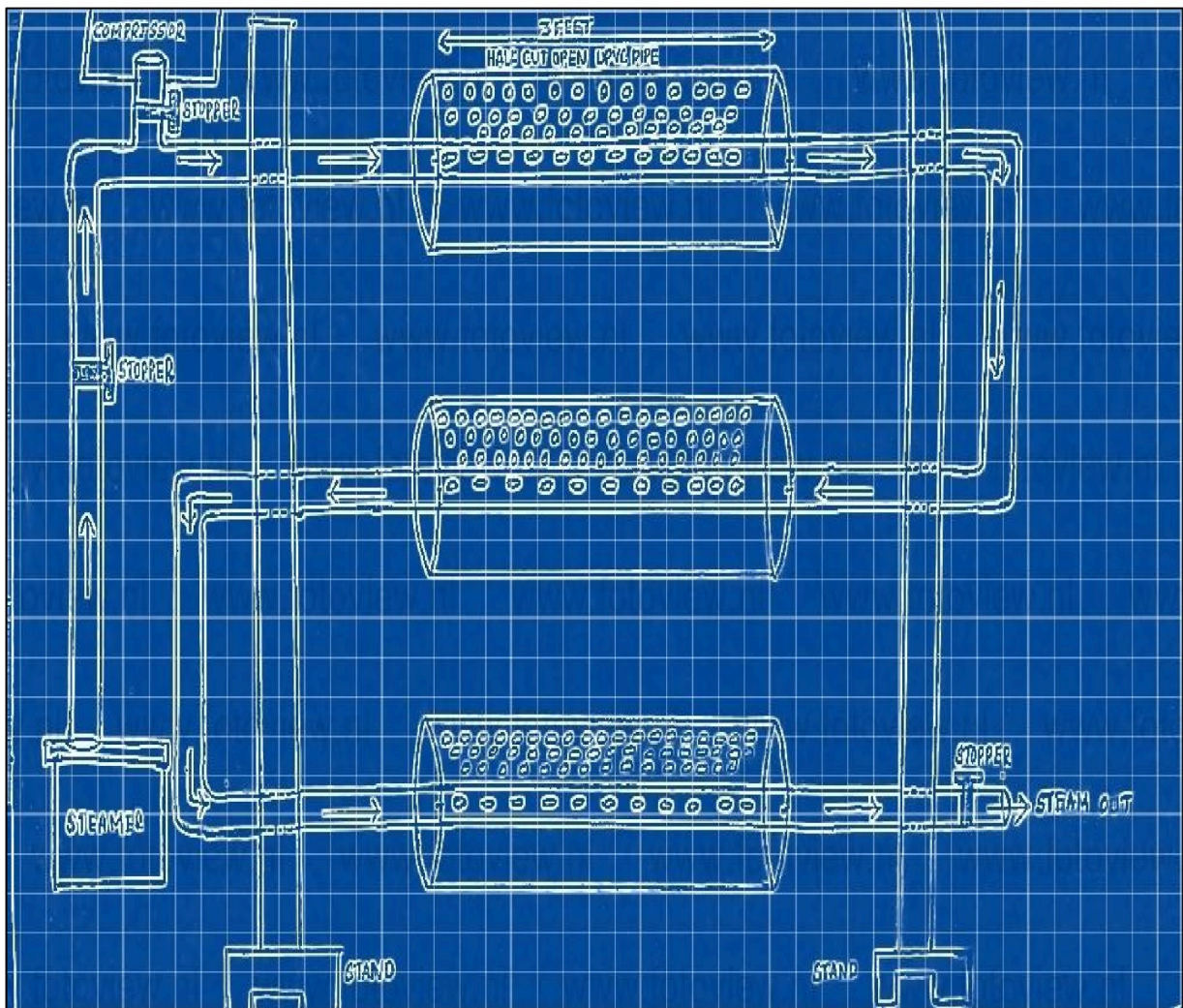


Fig: 5.5 Blue print of fermentor

CHAPTER 6

SUMMARY AND CONCLUSION

The investigation aimed to assess the impact of humic acid on growth rate and nutritional quality. Humic acid, derived from lignite through alkaline solution and acid precipitation, was applied at concentrations ranging from 1% to 10% during *Pleurotus ostreatus* cultivation. Subsequent analysis revealed increased total biomass, protein, fiber, ash, and moisture content across all treated groups compared to the control. The highest biomass and nutrient levels were observed at the 10% humic acid concentration. Substrate optimization indicated that 10% humic acid led to superior yield and nutrient content compared to other concentrations. Thus, the 10% humic acid concentration emerged as the optimal level. Notably, cultivating *Pleurotus ostreatus* mushrooms on paddy straw treated with 10% humic acid exhibited promising results, yielding maximum output according to biochemical analysis.

Based on our findings, it is evident that a 10% concentration of humic acid yields greater output compared to other concentrations. Additionally, we employed two cultivation methods for oyster mushrooms: the conventional method (CM) and the tubular solid-state fermentation method (TSF). Results indicate that TSF yielded 619g, whereas CM yielded 139g. Notably, TSF not only produced higher quantities but also exhibited superior quality and biochemical composition compared to CM. ,

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