Synthesis and characterization of fungal based bio-composite sheet (FBBS) using mushroom and lignocellulosic biomass

Thesis submitted in fulfillment of the requirement for the degree of

Master of Technology

In

Biotechnology

By

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PATENT FILED

The detail of the patent application is as follows:

TITLE OF INVENTION: BIODEGRADABLE FUNGAL BASED BIO-COMPOSITE SHEET (FBBS) FOR SUSTAINABLE MATERIALS MANUFACTURING

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CANDIDATE'S DECLARATION

I hereby declare that work presented in this report entitled **"Synthesis and characterization of fungal based bio-composite sheet using mushroom (FBBS) and lignocellulosic biomass''** in partial fulfillment of the requirements for the award of the degree of **Master of Technology in Biotechnology** submitted to the Department of Biotechnology & Bio-informatics, Jaypee University of Information Technology, Waknaghat is an authentic record of my own work carried out over a period from July 2023 to May 2024 under the supervision of **Dr. Ashok Kumar Nadda, Assistant Professor & co-supervisor Dr. Jata Shankar, Professor Department of Biotechnology and Bio- informatics** Jaypee University of Information Technology, Waknaghat Solan India**.**

I also authentic that I have carried out the above mentioned project work under the proficiency stream biotechnology.

The matter embodied in the report has not been submitted for the award of any other degree or diploma.

Manisha Sharma , 225011003

This is to certify that the above statement made by candidate is true to the best of my knowledge.

(Supervisor)	**(Co - supervisor)**
Dr. Ashok Kumar Nadda,	Dr. Jata Shankar,
Assistant Professor,	Professor,
Department of BT/BI (JUIT)	Department of BT/BI
Dated:	Dated:

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Manisha Sharma

(225011003)

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LIST OF ABBREVIATIONS

%	Percentage
^{o}C	Degree Celsius
Fig.	Figure
Concen.	Concentration
FBBC	Fungal based bio-composite
MBC	Mycelium based bio-composite
Agro	Agricultural

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ABSTRACT

This study is focused on the synthesis of green, sustainable, and cost-effective fungal based bio-composite (FBBC) using mushroom and lignocellulose biomass. Utilization of agricultural waste in the formation of an alternative synthetic bio-composite can minimize the environment pollution by replacing non-biodegradable materials. The used biocomposite materials can further be used as an bio-compost in the garden or farms. Here, *mushroom* is cultured with different lignocellulose biomass as substrate because of mushrooms adhesive network helps in the formation of efficient and cost-effective fungal bio-based composite. The fungus and agriculture biomass was incubated for 24-28 days under humid conditions (60-70%) in dark environment. The cultivated mycelium result in the formation of a highly condensed network of biomass entangled with mycelial network. The synthesized composites was dried at 100^{o}C for 24 h and then compressed with hot press machine at 150^{o}C for 20 minutes under pressure 6 MPa pressure.The synthesized fungal bio-based composite (FBBC) was found to be quite stable, durable and hard that can be used in the wood panel or plywood industry. The FBBC was tested for boiling water stability, water absorption and biodegradability *etc.* Mycelium based products are high in demand due to its light weight & eco-friendly nature. It has a vast number of applications in wooden panels, fiber board, building insulating material & electronic packaging material.

Keywords: *Fungal based Bio-composite (FBBC), mushroom ,Lignocellulose biomass, packaging material*

Chapter 1 Introduction

As the growing population, fast development & globalization these all are contributing factors from which the waste outputs are increasing. These days excessive use of synthetic products like synthetic clothes, plastics have great impact on environment. Nowadays , plastic have become easily accessible, widely utilized & to make items like boxes for food, household items.There is concern to protect the environment so lots of creative approaches are in progress to decrease the impact of filth on the environment and human society. Out of 280 million plastics tons produced annually, just ten percent are recycled , leading to serious environmental issues and concerns. So, to tackle these problems green and naturally derived products are needed like bio based composites which are eco-friendly , sustainable can be an alternative of synthetic based composites. Over the years consumption of plastics has increased globally because they are of long lasting, low priced, light weight. Annually 300 million tons of plastic is generated by plastic industry which is discarded after single use. Every year 14 billion pounds of garbage was dumped every year in the oceans According to the report of National Academy of Sciences in 1975 these wastes take thousands of years to decompose. In oceans alone, more than 10 million tons of plastic waste is dumped. Non-biodegradable plastics and synthetic biopolymers harms the environment . Lipids, Proteins, chitin and carbohydrates comprises the mycelium and these components comprise the mycelium.Via biological processes, bio plastics that degrade quickly can break into natural components & mix quietly with the soil through microbial processes. Bio-plastics can be used with crops waste items (such as maize & grain pees) to create composite substances for applications in industry. To tackle the sustainability issues related with traditional petroleum derived materials wide range of bio-plastics have been produced. Mycelium based biopolymers like PHB, PCL, PLA.

Rising worries about our planet have attracted increased awareness of bio based-composites. Earlier the non-renewable fibers were used causes many environmental effects & also are non-biodegradable. If we used resources from natural sources like environmental waste, municipal waste or agricultural wastes it would be more accessible and more affordable to the average person.

Mushroom species are widely produced and are crucial to the global economy. Roughly 25 % of all grown mushrooms worldwide are produced only from edible mushrooms. Mushrooms are rich in nutrients, carbohydrates, proteins, dietary fibers, essential amino acids, vitamins, & medicinal values. These were grown on wet logs, fallen branches in tropical forests. Due to presence of enzymes like laccase and peroxidases, it causes wood decay. Mushrooms have many therapeutic and medicinal & nutritional properties. They have variety of species like Grey, pink,white ,yellow etc. Roughly, 40 species are those which found in both temperature and tropical climates & 20 species genus which are edible. These Species are highly recommended because its growth time is fast and easy to cultivate. As estimated 998 million tons of agricultural trash , which includes wheat, rice straws, cereals are grown each worldwide. Its production contributes to reuse of agricultural residues with *Pleurotus spp.* mushrooms. Furthermore, also agricultural substrates are utilized to produce biogas, fertilizers and feed for cattle **[1].**

The processing and cultivating of mushroom is a highly sustainable and environmentally friendly way to turning variety of agricultural wastes into nutrition for humans**[2].** Africa is the world's largest producer of mushrooms, total production is roughly 27% **[3].** In India , cultivation of mushroom cultivation is gaining much popularity because of its substrates easily availability & low cost technology **[4].** Solid state fermentation of mushrooms and recycling of lignocellulose biomass. Different agricultural wastes were used to cultivate mushrooms.In Asia, widely used substrate is used rice straw and cotton waste for cultivation.

Mycelia of the fungi spread in the selected solid substrate during cultivation. A constant temperature was kept at 25°C to 30°C is generally required in the spawn room, Growth time of spawn is approximately, 24-30days. There is no requirement of light in the spawn chamber **[5].**

Every year large amount of agricultural waste produced in India. Annually, 682.6 million tons produced every year from agricultural fields. Crops leftovers are wastes are 500-683 million tons of agricultural waste produced annually in millions tons of agricultural waste produced annually in nation. Different kind of wastes generated includes rice husk, straws, crop waste, bagasse from sugarcane. 92 million tons of agricultural waste burned annually which may cause environmental & air pollution. Combustion of residues has developed into an environmental issue that will be contributing to global warming & harm public health. Preservation of crop residues combustion can be achieved by implementing sustainable crop management techniques with the governments support and system **[6].**

In recent decades, sustainable resources are in greater needs because disposal of plastic has generated major ecological issues. Alternative options are used to minimize these ecological issues like to use resources that are renewable**[7].**

Nowadays, (FBBC) are in demand to minimize the wastes and promote circular economy. FBBC are manufactured using renewable resources which are derived from natural resources . These are derived using agricultural waste because these show rapid growth in mushrooms growth. These are bonded together using natural binder which is mycelium. Mycelium binds the agriculture waste together with itself in dark conditions. FBBC was found to be stable, hard. Nowadays, demand of mushroom is high in demand because these have medicinal values, fast growth rate & cultivation is so easy. About 20 species of oyster mushrooms are edible. These fungal based bio-composites are found to be a good substitute for synthetic composites **[8].**

Synthetic composites are utilized in various industries because they have high durability , lightweight characterization.These synthetic composites are made up of Styrofoam which takes more time to recycle and cannot be broken down by nature fully. Moreover, recycling process is so costly.So, alternative of synthetic composites is replaced by sustainable fungal bio-based composites. The synthesized fungal bio-based composite

(FBBC) was found to b quite stable, durable and hard that can be used in the wood panel or plywood industry **[9].**

Synthetic composites are not preferred because

Highly expensive to produce : Composites can be of high price to generate because of the the excessive price of raw ingredients and its complicated methods of manufacturing.

Difficulty to repair : Breakages in synthetic composites is hidden & making it hard to find which makes it repair difficult.

Non - prone to ultraviolet light : Long term being exposed to ultraviolet rays may reducing their shelf life and strength & destroy composite materials.

Less recycling options : Makes it less sustainable.

Bio-based composites are preferred because of following reasons

Recyclable : They are more sustainable as compared to synthetic composites.

Minimal weight : Because of bio-composites light weight it is perfect for applications which require minimal weight.

Biodegradable : Biocomposite materials decompose organically with time via living microorganism.

Renewable : Bio-based composites are produced from natural resources so they are sustainable to the environment..

Cheaper : Less costly than synthetic composites**[9].**

Purpose of using agricultural waste

Contribution to sustainable practices: Using agricultural waste can promote sustainable practices and reduce environmental pollution.

Green house gases: these gases reduced by recycling agricultural waste & turning into useful bio-products.

Rationale of the study

1. To replace synthetic composites by using fungal bio-based composites
2. Currently, PVC(Polyvinyl chloride) based panels were used for temporary partitions, ceiling panels etc. When PVC is burned, it releases toxic substances which show harmful effects on environment.
3. Aims to replace PVC panels by using eco-friendly fungal bio-based panels.
4. Bio based composite is used as an alternative to synthetic composite.
5. Synthetic bio-composites are of high cost, low thermal properties, toxic, biodegradable so to making them sustainable and cost effective as an alternative we synthesized bio-composite.

Objectives of the study

1) Pre- conditioning and grinding of ligno-cellulosic biomass.
2) Culturing of mushroom under optimized conditions from spawn with biomass.
3) Co-culturing of agriculture biomass and mushroom under optimum conditions.
4) Synthesis of Fungal Bio-composite panel using hotpressing and characterization.
5) Re-usability of bio-composite as a bio-fertilizer after application.

Chapter 2 Review of literature

It is expected that due to the rising population and fast development yearly trash will be rise by 3.40 billion tons from 2025 to 2050 respectively. Economic areas like farms, homes, manufacturers are the main contributes of such kind of wastes. Pollution of water, air, soils has been caused by insufficient disposal of the garbage resulting from these areas. To reduce the impact of such trash/ garbage reprocessing technologies would be the best approach **[10].** This study aims to explore the use of ligno-cellulose biomass with mushroom to make a fungal based bio-composite material. Ligno-cellulose wastes used because they have polymers like lignin, hemicellulose, cellulose which helps in fast growth of mushrooms & used because it is easy to cultivate , no toxicity level, fast growth rate and in this study it is used as an adhesive binder to make a sustainable , cost-effective & hard bio composites which have vast applications in plywood industry & electronic packaging material. In order to maintain sustainable environment, green methods should be implemented such as a) cost-effective goods b) recyclable goods c) environmentally friendly substances d) locally available resources to save fuel as well as transportation costs. In 2007, Eben Bayer and Gavin McIntyre were the owners of Evocative company, they came up with the idea of using mycelium as the substance for making packaging materials & this company makes fully recyclable, harmless and good quality packaging material using mycelium as a substance **[11].**

In 2013, A startup named Evocative Design began using mycelium based material as a substitution to Styrofoam and plastic packaging. Fungal based composites can take place of traditional based composites. Because Fungal based composites can be turned into different shapes and are of light weight.

Hyphae of mycelium make a dense structure of tiny threads that spread & combine altogether to form a rigid substance, forms mycelium. Different species degrade lignin in different ways. Mycelium grows quickly on organic substrates.. Mycelium generates self assembled linkages & fibers which slowly consumed organic substrates & break down its organic content & bind the organic substrates with itself to make robust & three dimensional structure. Each species usually target hemi-cellulose**[12].** Fungi (mycelium) may break down both cellulose as well as lignin. During colonization , mycelium releasing an enzymes such as lignin peroxidase , laccase & manganese peroxidase.

Mycelium of mushroom have good growth rate, cultivation is easy, mushroom spread quickly on lignocellulose biomass which is easily available**[13].** Many researchers have chosen this phylum for the manufacture of biomaterials because of the phylum's natural binding capacity in mycelium. This phylum has 2 key characteristics are a) septa and b) anastomosis. Anastomosis has a unique property that causes two different hyphae to combine together **[14].** Mycelium can develop when two or more hyphae merge together in order to form complex structures that promotes the transfer of nutrients between the substrate and the cells. Mycelium is greatly impacted by the substrates type, species type & processing methods**[15].** Fungi type has more impact on bio-materials performance than other variables. While choosing a substrate we should follow these factors :- a) Biodegradability b) Abundant c) Adaptability. Materials with high cellulose materials can help fungi to develop quickly as compared to low cellulose materials. High cellulose materials have higher chitin level which helps in faster growth. Substrates which are used widely for the production of mycelium are straws, wood shavings, sawdust, coconut flour, sugarcane bagasse & garden waste (leaves)**[16].** Glucan is present in sawdust which has complicated structure as compared to sugarcane bagasse. On straw substrate the growth of mycelium is faster as compared to sawdust. This is because straws and sugarcane bagasse have smoother particles, which makes fungus so much easy to absorb nutrients from the surfaces. Fungus absorbs nutrients easily from the soft surfaces rather than hard surfaces. Different substrates were added to improve the nutritional value like rice husk, wheat straws and wheat straws **[17].**

Nowadays, the market demand of bio-based products like bio-composite, bio-plastics, are increasing due to environmental concerns to use product which is sustainable and environmentally friendly in nature. Due to this, scientists and researchers are finding and developing new methods to develop green products which are cheap , sustainable and eco-friendly. The production of different types of fungal based bio-composite using different ligno-cellulose biomass such as sugarcane bagasse, rice husk, wheat straw, sawdust etc are used for the development of a new material which is an alternative to replace petroleum based fiber boards , plastics etc. Animal fibers such as feathers, wool, silk and horns, which are made of proteins, can also be used as alternative sources of reinforcements and fillers for bio-composite are reported in literature , some of them are

discussed in this chapter of this report **[18].**

2.1 Biocomposite / Green Composites

Bio-composites are known as natural fiber composites with the reinforcement of natural fibers like sugarcane bagasse, sawdust, wheat straws etc. Agricultural waste can be used to make bio-composite that are eco-friendly, cheap & light weight. Bio-composite can be used as the future materials in many industries like construction, packaging etc & infrastructure like wood panels. Benefits of using bio based composites like low cost, availability & low energy consumption **[11].** Main aim is to replace synthetic petroleum based fibers with natural fibers. Synthetic petroleum fibers are expensive to produce & dangerous. Natural fibers are best alternatives for synthetic fibers like carbon fiber, glass etc . Because of its sustainable nature, recyclable, compostable etc **[l2].** Green composites mean composite material which is made up of natural resources. These composites have great applications in partition boards, insulating material in buildings, wood panels etc**.**

2.1.1 Agricultural wastes

Agricultural waste is the leftover residues from agricultural fields after processing. Agricultural wastes are also known as lignocellulose biomass which contains lignin, cellulose, hemicelluloses. Crop residues like,corn cob, pine needles,woodshaving,barley, wheat straws, rice straw,rice husk, corn husk etc are used because these have better thermal stability. Cellulose is extracted which can be used in making of bio plastics. These lignocellulose biomass are, non-toxic nature, easily available, light weight, low - density & abundant so it is considered best source for the replacement of petroleum based materials**[14]**

2.1.2 Classification (Agricultural biomass have different categories)

- **Plant and Crop based residues have two types:** Primary and Secondary residues
- **Primary residues -:** After harvesting, basically these residues are field based residues like - stalks, cereal straws, dry leaves *etc.*
- **Secondary residues -:** during industrial crop processing these residues are collected like husks, cobs, bagasse *etc.*

2.1.3 Crop residue

Crop residues are crops leftover in the landscape after harvest, or the leftover parts of plant like stalks, straws etc after harvesting are known as crop residues. Considering their use in substitute goods like food for cattle, animal bedding, fire wood and natural compost etc **[15].**

2.1.4 Plant fibers (Lignocellulosic fibers)

Plant fibers are composed of cellulose and mainly used as reinforcement agents. Recently, researchers are paying more attention to these lignocellulose fibers in industry because of their sustainable nature. These fibers utilized three components such as lignin ,cellulose, hemi-cellulose. These fibers could have more other components like protein, starch, pectin, waxes *etc.*

Cellulose: It is made up of D-glucopyranose atoms which is connected by 1,4-b-D-glycosidic linkages (C_1 and C_4) **.** Depending on what kind of fiber, cellulose can have a polymerization degree of approximately 10,000 and is hydrophilic.

Hemi-cellulose: It is the 2^{nd} main structural element of the fibers is hemicellulose. The structure of hemicellulose is more complex than cellulose. Hemicellulose serves as the matrix for cellulose microfibriils and are made up of various sugar units. They are also very hydrophilic and non crystalline.

Lignin: It is a polymer which is opaque, hydrophobic & aromatic in nature. It is mainly functions as fillers between pectin, hemicellulose, cellulose structures. It gives stiffness to the structures of hemicellulose and cellulose structures**[16].**

Bagasse: It is the also called sugarcane bagasse. It is the waste material after sugar production. These bagasses can be used to make biodegradable composite materials, papers etc **[17].**

2.2 Animal waste Chicken feathers

Poultry farms generated huge amount of wastes & are thrown away or disposed in landfills. So, to utilize these wastes (chicken feathers) or having good thermal stability these can be used bio-composites as fillers (reinforcement material). In chicken feathers , 90% of keratin is present. These are abundant, cheap, bio-degradable **[18].**

2.2.1Mycelium

Mycelium is the vegetative part of fungus, branches & with filamentous thread like cells called hyphae.Mycelium can grown on lignocellulose substrates from a spore under ambient conditions. Hyphae colonies forms 3 D filamentous structures and work together to form a filamentous network structures which act as a kind of natural self assembled glue to bind the substrate and mycelium together & form a composite material. Mycelium splits down different substrates into simple elements. Fungi can expand their growth by utilizing the substrates**[19]**

2.2.2 Mushroom

Mushroom is also known as mycelium/fungus. Mushroom species has proved useful in treating many kinds of human problems like cholesterol, sugar,high BP problems. Mushrooms are high in proteins & provides extremely low amount of carbohydrate, peptide *etc*. Generally mushroom is cultivated at 25ºC mushroom was cultivated.The lignocellulosic biomass can be easily broken down by these mushroom species. Many mushroom species have the ability to produce enzymes like glucosidases, endoglucanases, cellobiohydrolases ,these enzymes were involved in breaking down the cellulose . Particle board is formed using mushroom . It is an environmental friendly alternative to conventional materials using resins which have carcinogenic formaldehyde. Lignocellulose is considered to be the best substrates to form the wood particles which can be use as composites. Fungi degrades the lignin and left a white residues of cellulose. Biocomposite which are made from agricultural waste have been widely used because of its biodegradable nature. Fungal based bio- composites have been used in packaging materials, construction, household applications. Bio based material used for door panels, electronic packaging material **[20].**

2.2.3 Fungal mycelium based biocomposite

Edible mushroom is cultivated on different lignocellulose biomass. After days of incubation FBBC were produced. A fungus's mycelium is the vegetative part of the mycelium and is made up of hyphae which is the mass of branches & have spherical body that forms its adhesive matrix which expands rapidly & it is harmless. Its main function is to bind the organic material / agricultural waste & attaching it to surrounding to form a extremely complex matrix of fibers. As compared to synthetic materials, bio based materials are derived from fungi and have a number important advantages like eco-friendliness, lower cost, low density, less energy use**[21].**

2.2.4 Production synthesis of fungal based biocomposite

Firstly, agricultural waste (supplementary waste) is grinded finely into powdered form. Wheat straws were pasteurized .These wastes were cultivated with the mushroom spawns for the production of biocomposite material trays in a packed tray. Tray was incubated at 25^{o} C . Distilled water was used consistently throughout whole experiment. After 30 days , trays were dried fully in hot air oven to deactivate the growth. And then hot-pressing was done at 150^{o} C . After hot pressing, fungal based biocomposite is produced using mushroom (white oyster mushroom) with lignocellulose biomass**[22].**

2.2.5 Medicinal properties of mushroom

Anti-microbial
Anti-tumour
Anti-oxidant
Anti-inflammatory
Anti-ageing **[23]**

2.2.6 Advantages of using Bio-based biocomposite

Easily available
Less expensive
Light weight.

Renewable

Sustainable

Biodegradable **[24]**

2.2.7 Bio-based bio-composites preferred over synthetic ones

1. Renewable - these are more sustainable than synthetic ones because they are derived from renewable resources

2. Economical - production of natural bio composites are cheaper than artificial ones.

3. Minimal weight - they are perfect for applications which requiring minimal weight .

4.Recyclable - they are usable because of green product .

5. Biodegradable - living microorganism degrade it fully **[25].**

2.2.8 Applications of bio based composites

Fungal based bio-composite material are low cost material these type of bio-based material are used in construction industries for manufacturing of partition boards, panels packaging, storage device.

Bio-based composites are used as packaging materials for electronic devices & plywood industry **[26].**

2.2.9 Biofertiliser

After harvesting of mushrooms, remaining wasted material could be utilized as a fertilizer for plant development & for animal feed. An novel economic framework was put forward during 2015 edition of the "Closing the loop'' initiative which is launched by European Union. By using wastage its initial resources to create an additional value goods. By products and waste which are come from farms, food manufacturing & livestock dung areas which is needed to be used as bio fertilizers. To increase the fertility of soil animal waste (from poultry, cattle, pigs) can be used as source of nitrogen. Biofertiliser is an effective method for increasing the growth of environmentally friendly organic farming that doesn't harm environment materials which are hazardous are released into water, soil & atmosphere by agricultural systems. Agriculture is responsible for 14% of global emissions with a very large amount of carbon footprint. On agriculture

climate change has a significant impact which can results in flooding & extremely undesirable environmental conditions. Overuse of pesticides, fertilizers & pollutants such as heavy metals could escape from farming systems which travels to the organisms that are not targeted and scattered across an ecosystem. Rising faster need for fertilizers than the productivity of crops, indicating how important these chemicals are to agriculture **[27].**

2.2.9.1 Medicinal properties of mushroom

Mushroom has medicinal properties to deal with high cholesterol , cancer, high blood pressure etc. They have about 40 species. Mushroom species grows on lignocellulosic biomass which is considered to be the agricultural waste like pine needles,corncob, wood shavings,wheat straws, sugarcane bagasse, sawdust, corn husk, rice husk, etc**[28].** These were considered to be the best source for the growth of mushrooms and produce mushrooms which were considered to be rich in minerals, vitamins, proteins, less carbohydrates, less sugar and no cholesterol**[29].** The king of medicine is Ganoderma mushroom is known for its convenient treatments of HIV, lung cancer etc **[30]**. Mushrooms are rich source in proteins,vitamins. Mycelia and fruiting body of mushroom species acquires many therapeutic properties such as anticancer activity, anti inflammatory, anti-oxidants, hyperglycemia, hypocholestrolemic & anti-ageing **[31]**.

Species of mushrooms have many compounds like lectins, terpenoids, alkaloids, steroids. Mushroom have about 40 species which are edible. These mushrooms were cultivated artificially. Cell wall of mushroom are rich in Gallic acid, ascorbic acid, beta-carotene,beta-glucan, non-starch polysaccharides, lipids, vitamins like b1, b2, vitamin c, minerals, enzymes like laccase etc. Mushroom of species contains moisture upto 85-90%. Toxins produce by mushrooms are because of the presence of secondary metabolites. Mushrooms have many therapeutic properties to lower downs the high cholesterol, blood pressure. And have low glycemic index and less carbohydrate , less sugar which makes it suitable for people which have diabetes, heart diseases, high cholesterol level **[32].**

Mycelium based bio-composites gaining attention because of their biodegradable nature, light weight, low manufacturing cost, cheap, made of waste

agricultural material, renewable, sustainable etc **[33].** On agricultural waste or lignocellulosic biomass mycelium grows so easily by degrading its lignin, hemicellulose, cellulose, chitin, glucan and make a filamentous structure with agro. After days good growth was observed by fungus. Fungus fully degrade the lignocellulosic biomass. MBC were easily grown and have very low manufacturing cost/ process as compared to synthetic composite with very high manufacturing cost and process[34]. We can produce MBC by using different species of fungus. And fungus can adopt the shape of the mould in which it can be cultivated by digest the chitin, lignin, cellulose, hemicellulose of the fibers and straws (lignocellulosic biomass) and colonize over them and make a network structure. After the growth of mycelium with agro waste, to deactivate the growth of fungus drying method was applied. Fungal based product was dried to stop the growth of fungus. After, proper drying heating was done to make it a bio-composite. These fungal based bio-composite are sustainable, environment friendly, recyclable, cheap, light weight etc**[35].** Global and local organizations have been under pressure to boost the number of environmentally friendly items. Current generations are very much conscious to replace the synthetic items using sustainable materials. Bio-based biocomposite material are good way to replace synthetic composites **[36].** Use of MBC in various sectors has given business sectors new opportunities and is step towards for the production of renewable bio-based material for MBC uses in future generations meeting need of sustainable green technologies **[37].**

Chapter-3 Material and methods

3.1 Pre-treatment of waste chicken feathers biomass

Chemical required: Petroleum ether /hydrogen peroxide (to remove blood stains) , detergent, water.

Procedure

1) Chicken feathers were collected from slaughter house. Remaining skin residue have been removed from feathers and washed with normal tap water 2-3 times.

2) Feathers were soaked in detergent (labdet) with water for at least for 2 h.

3) Boiled at 90 ^{o}C for 1 h. After that soaked in petroleum ether. Washed two times with normal tap water. Dried for 2-3 h in direct sunlight or in incubator at 40degree Celsius. After drying, properly packed in plastic seal bags and stored them for further analysis.

3.2 Sub- culturing / Inoculation of fungus *(Aspergillus Niger)*

Equipments used: Loop , burner, match stick , ethanol, plates, gloves

Procedure

1) Sub-culturing/ inoculation of fungus was done in plates in laminar air flow in agar plates under sterile conditions.

2) Plate was incubated at 37^{o}C for 7-10 days as shown in Fig 3.1

Fig 3.1 Growth of *Aspergillus niger* after 10 days

3.3 Spore harvesting

1) *Aspergillus niger* culture was grown on PDA plates . Spores were harvested using a solution of PBST(Phosphate Buffer Saline and 0.05 (v/v) Tween 20) . Spores were purified by centrifugation and washing with PBS.

2) Haemocytometer was used for calculating CFUs (colony forming units) 1.35x 106 cells/ml was used as a working culture in studies.

3) The spores were then re-suspended in fresh PDA plate and grown for specific time point at 25^{o}C.

3.4 Spore counting

1) To count the quantity of cells or spores in a sample solution, a haemocytometer is utilized. It is a tool that makes counting the number of spores in a solution easier. Under a microscope, the cells per chamber could be counted.

Formula for haemocytometer cell counting

a) Determine the number of spores in each sizable chamber in the corner.

b) It is calculated how many spores there are overall.

Spores/ml = Average spores counted *10^4

Procedure

* With 70% ethanol, haemocytometer was cleaned.
* Haemocytometer was covered with cover-slip.
* From the edge of the haemocytometer, cell suspension was dispense.
* Each square number of spores were counted as shown in Fig3.2

Fig. 3.2 Spore harvesting

3.3.1 A) Growth optimization of *Aspergillus niger in media*

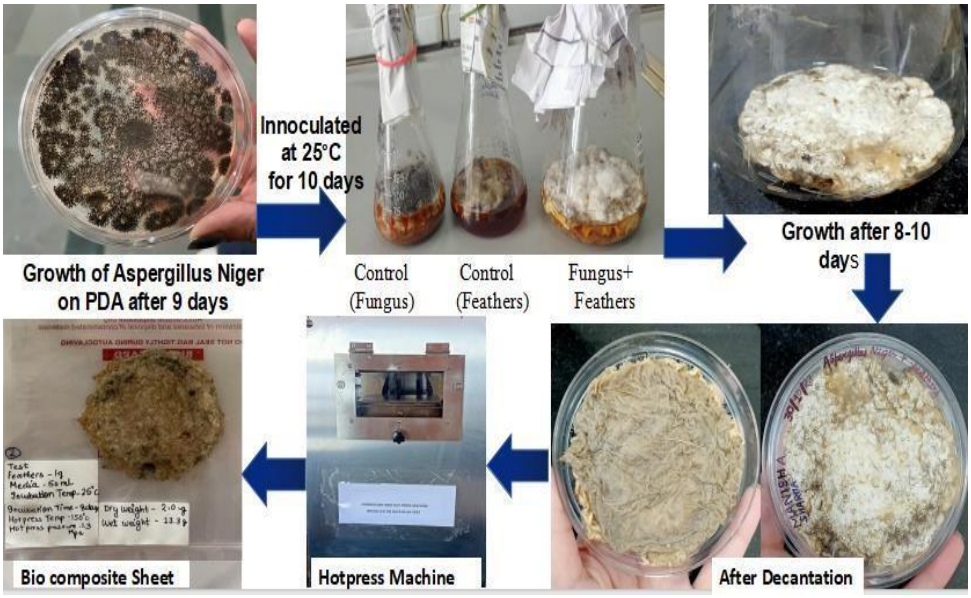

Fig 3.3 Growth optimization of *Aspergillus niger in media*

Procedure

50ml media was poured in different flasks two control, one test with 1g feathers, *Aspergillus Niger's* spores were inoculated in 250 ml beaker. Flasks were inoculated at 25°C for 10 days. Growth was observed after 10 days as shown in fig3.3.

3.3.2 B) Growth optimization of *Aspergillus flavus* (BT01,03,05) in batch culture

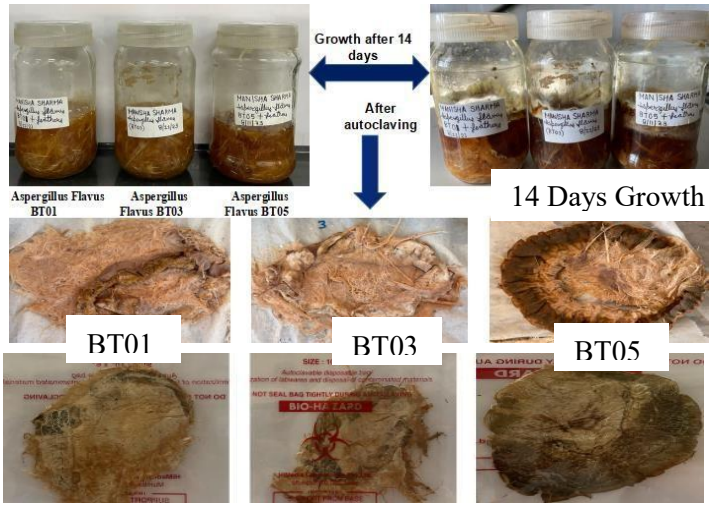

Fig 3.4 Growth optimization of *Aspergillus flavus* (BT01,03,05)

Procedure

A total 2g of Feathers, different fungus strain (*Aspergillus flavus* BT01, BT03, BT05) spores were together inoculated in media (100ml) in different jars.Jars were incubated at 25^{o}C for 14 days. After 14 days, growth was observed. Jars were autoclaved at 121 ^{o}C. Drying under sunlight for few hours. Then hot press the material as shown in fig 3.4.

3.3.3 Growth optimization of mushroom with different lignocellulosic biomass

Fig 1. a) wheat straw b)sugarcane bagasse c) rice husk d) Pine needles e)sawdust f) corn husk

Fig. 3.5 Growth optimization of mushroom with different lignocellulosic biomass

Procedure

1) Agricultural (lignocellulosic biomass) was grinded into fine particles/powder.

2) Seven flasks were taken, washed & label properly.

3) In each flask 100 ml of PDB was added with 5 g of different substrates and 2g of spawns of (mushroom) and covered it with cotton plug.

3.3.4 Cultivation of A) *Aspergillus niger,* B*) Aspergillus flavus* (spores) *&* C*)* mushrooms (spawns) with agricultural biomass

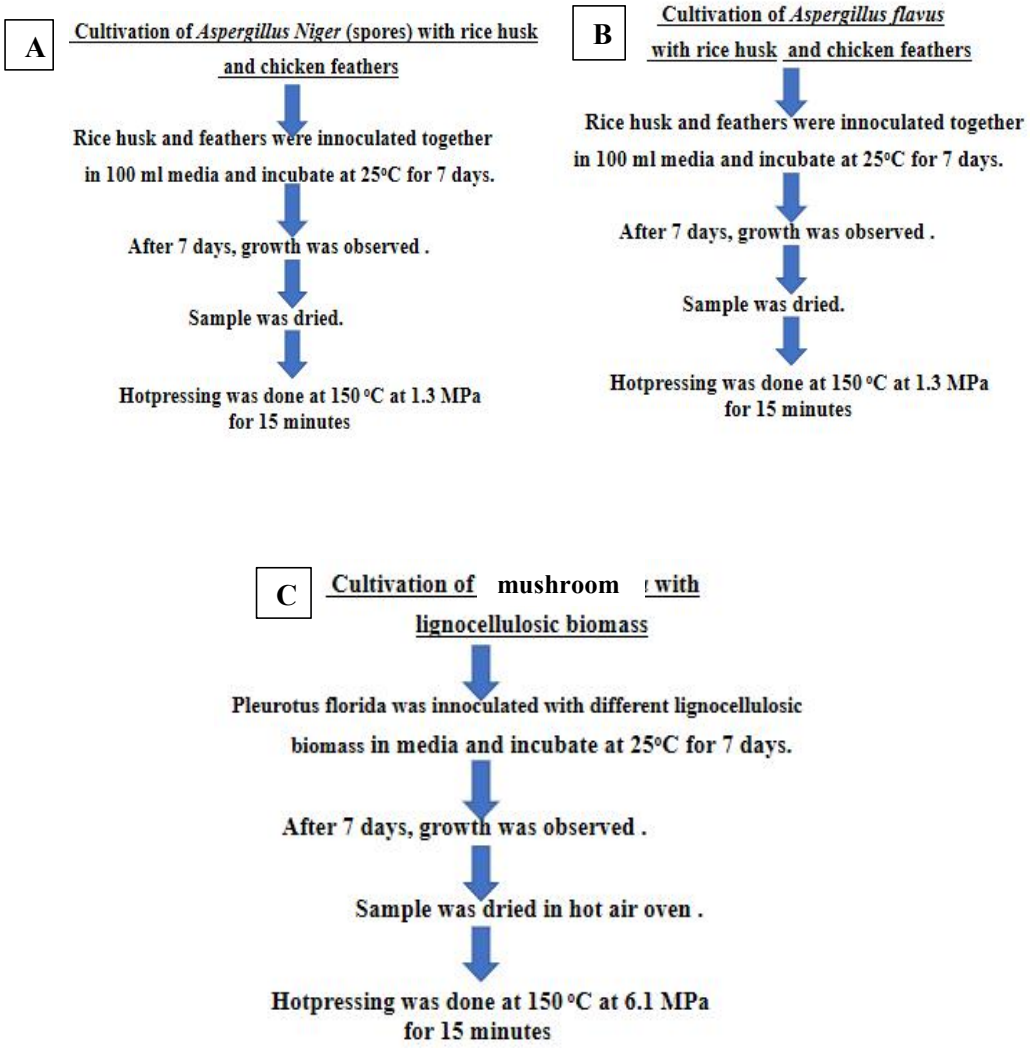

Fig 3.6 Cultivation of A) *Aspergillus niger,* B*) Aspergillus flavus* (spores) *&* C*)* mushrooms (spawns) with agricultural biomass

3.4 A) Inoculation of *Aspergillus niger* (spores) with different substrates for 14 days in cultural bags with knob

Procedure

1) Cultural bags were inoculated at 25 °C for 14 days with knobs which passes aeration.

2) After 14 days good growth of fungus was observed in each cultural bag.

3) Growth was taken out from the bags and remove the excess water from it.

4) Then let it dry under sunlight for few hours.Then hot press the material at 150 ºC and 1.3 MPa (Mega pascal) for few minutes as shown in fig 3.6.

Table 3.4 Inoculation of *Aspergillus niger* (spores) with different substrates

S No	Fungal species	Substrate	Media	Feathers	Rice straw	Rice husk	Spores
1	***A. niger***	Feathers+ Rice straw+ spores	500ml	2.5g	2.5g	**---**	5ml
2	***A.niger***	Feathers+ spores	500ml	5g	---	**---**	5ml
3	***A.niger***	Feathers+ rice husk+ spores	500ml	5g	---	5g	5ml
4	***A.niger***	Rice straw+ spores	500ml	---	5g	---	5ml

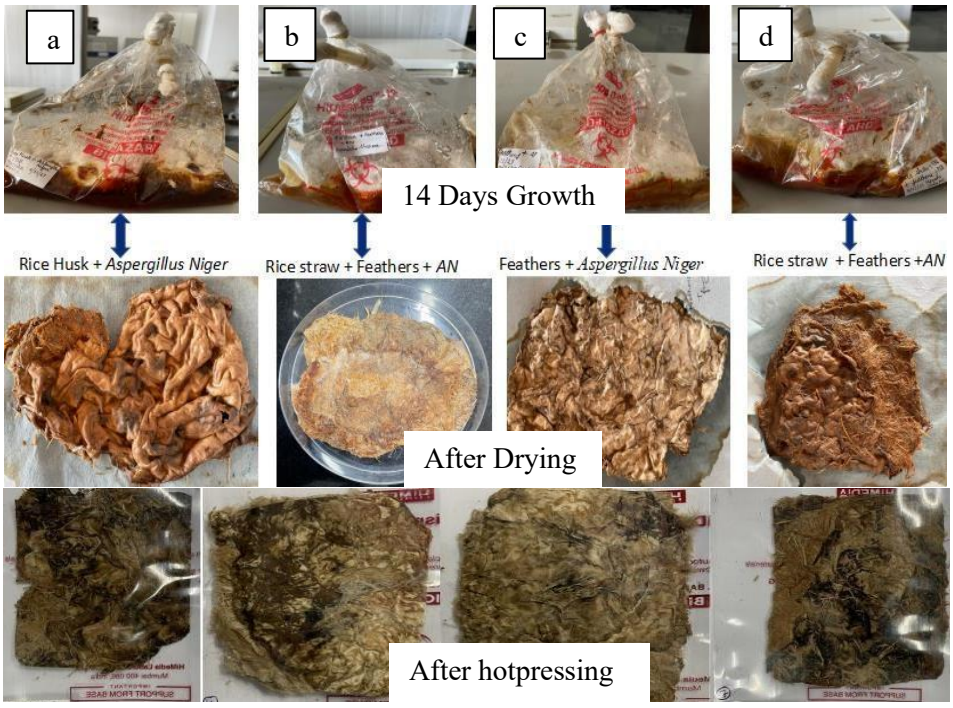

Fig 3.6 14 days growth of *Aspergillus niger* in cultural bags

Materials: Agricultural materials from a local source were employed as substrates. Grain spawns of mushroom were purchased from DMR Solan. Distilled water was used consistently throughout the whole experimental process.

Preparation of substrates: Agricultural waste used as substrates for growing mushrooms were finely grinded into powdered form. Pasteurization of wheat straws was done for 3-4 hours.

4.1 Preparation of plates

Chemicals required and equipment required:

* Agar
* PDB(Potato Dextrose Broth)
*Distilled water
*Flasks
*Cotton
*Petriplates
*Weighing balance
*Spatula
*Ethanol

Procedure

Total 3.6 g of PDB and 3g Agar was weighed and dissolved in 150 ml distilled water. Media was autoclaved. Then pouring was done under LAF (Laminar Air Flow) and after pouring let it set .After that, plates were labeled properly.

4.2 Spawns of *mushroom* were brought from Directorate of mushroom research (DMR) Solan

4.2.1 Sub culturing / Inoculation of *(*mushroom spawns*)* in agar plate

Equipments used

* Forecep
* Ethanol
* Cotton
* Spawns (mushroom)

* Gloves

* Burner

* Matchstick

* Plates

Procedure

1) Sub-culturing / inoculation of spawns were done in plates in laminar air flow in agar plates under sterile conditions.

2) Plate was inoculated at 25 degree Celsius for 7-10 days.

3) Growth was observed after 10 days as shown in fig 3.7.

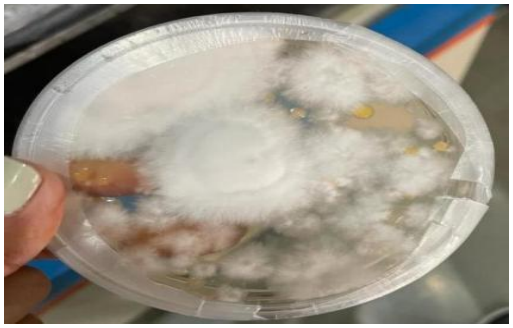

Fig 3.7 Growth of *Pleurotus florida* mushroom after 10 days

4.2.2 Pre- conditioning and grinding of Lignocellulose biomass

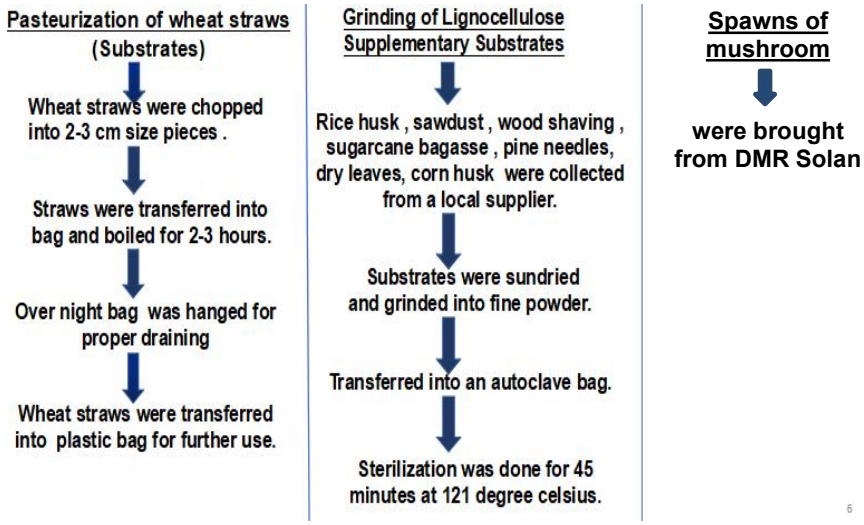

Fig 3.8 Pre-conditioning and grinding of lignocellulose biomass

STEPS

A) Cleaning of trays and autoclaving of agricultural biomass waste.

B) Pasteurization of wheat straws: The wheat straws were finely chopped for the substrate use. After chopping, substrate (wheat straws) was transferred in a jute bag or another bag i.e porous in nature. Water was boiled in large container & straws were kept in it for 1-2 hours.After, 2 hours kept hanging the bag overnight for proper draining. Next day transferred the wheat straws into an plastic bag for further use.

C) Grinding of supplementary substrates: Like wooden chips, wooden shaving ,sugarcane bagasse, pine needles etc. Supplementary substrates were grinded until become powdered like substrate as shown in fig 3.8.

4.3.3 METHODOLOGY:

Fungal based Bio-Composite (FBBC) using *Pleurotus florida* mushroom and Lignocellulosic biomass

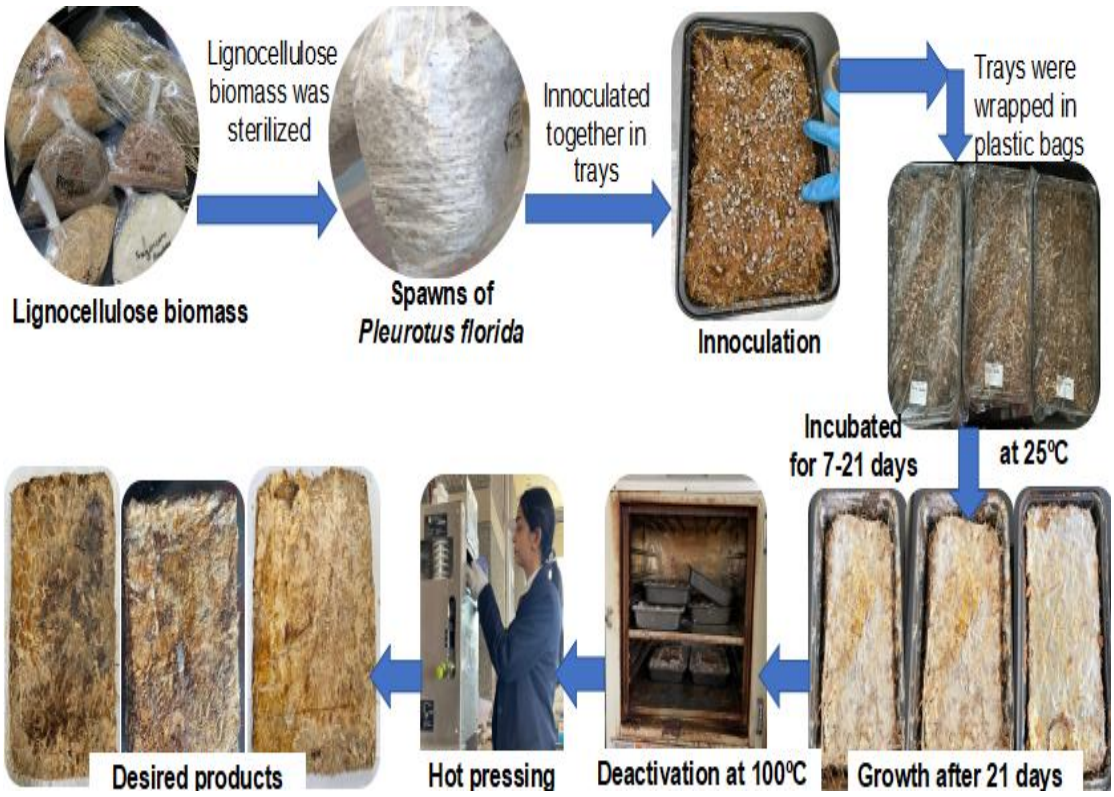

Fig 3.9 Fungal based Bio-Composite using mushroom & lignocellulosic biomass

4.3.4 For growth optimization of *mushroom* in PDB (Potato dextrose Broth) with different substrates

Material used- Wheat straw, pine needles, sugarcane bagasse, wooden sawdust, dry leaves, corn husk, 7 flasks, PDB, spawns, cotton.

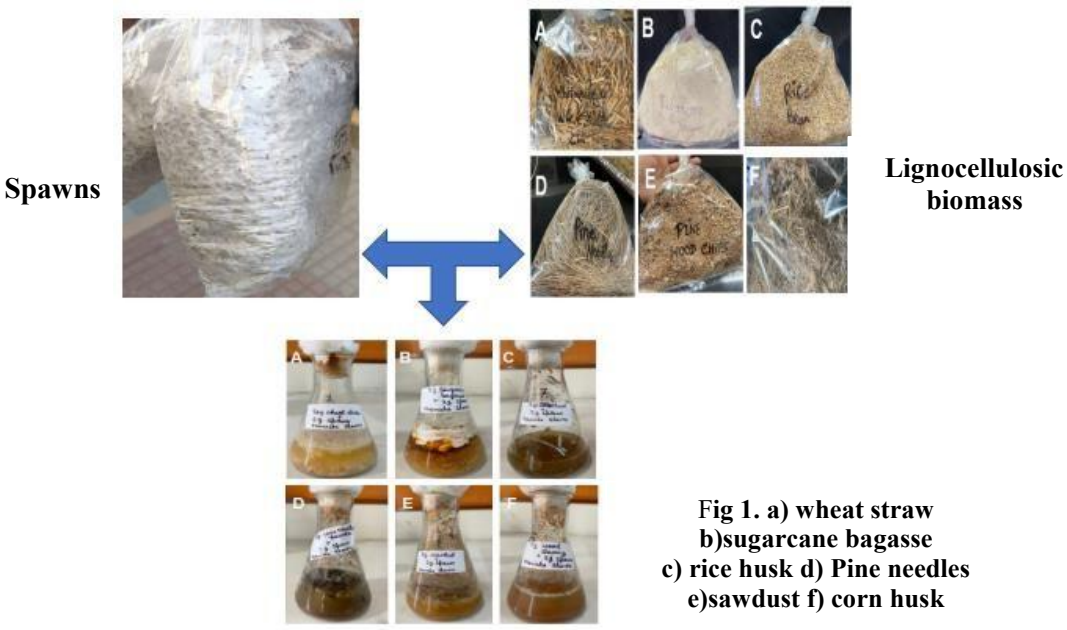

Fig 1. a) wheat straw b)sugarcane bagasse c) rice husk d) Pine needles e)sawdust f) corn husk

Fig 4. For growth optimization of *mushroom* in PDB (Potato dextrose Broth) with different substrates in different flasks.

4.3.5 Culturing of mushroom spawn in PDB with different agricultural wastes in different flasks

Procedure

1) Agricultural waste was grinded into fine particles.

2) Seven flasks were taken ,washed & label properly.

3) In each flask 100 ml of PDB was added with 5 g of different substrates(wheat straw, pine needle, sugarcane bagasse, wooden sawdust, dry leaves, corn husk) and 2g of spawn in it and covered it with cotton plug.

4) Flasks were incubated at 25 ^{o}C for 10 days for proper growth. As shown in fig 4.1.

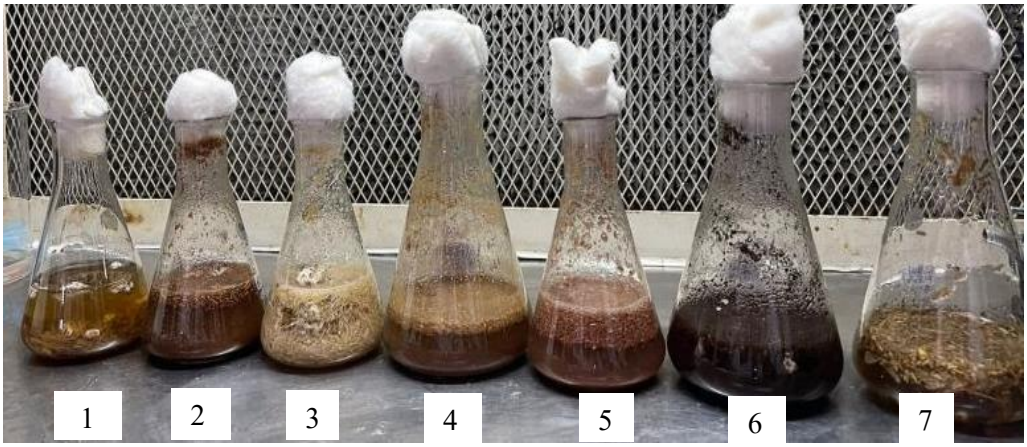

Fig. 4.1 growth optimization of *mushroom* in PDB

Table 4.3.5 Inoculation of Spawn with different agricultural substrate

S. No	Substrates	Spawns	Media
1	Wheat straw(5g)	2g	100ml
2	Pine needle (5g)	2g	100 ml
3	Sugarcane bagasse(5g)	2g	100 ml
4	Rice husk (5g)	2g	100 ml
5	Wooden sawdust(5g)	2g	100 ml
6	Dry leaves (5g)	2g	100 ml
7	Corn husk (5g)	2g	100 ml

4.3.6 METHODOLOGY

A flow chart of methods used is as given below

Wheat straws were pasteurized & Agricultural biomass was grinded and sterilized.

↓

Spawns of white oyster mushroom (obtained from DMR Solan)were inoculated with agricultural biomass in autoclaved tray.

↓

Small holes were made for aeration purpose & water was sprayed daily to maintain

humidity

After regular interval of time the growth was observed, and trays were removed and dried in oven at 100ºC for 4-5 hours.

After drying , product was hot-pressed for 20 minutes at 150ºC temperature and 6.1 MPa pressure.

4.3.7 (SET1) Culture conditions optimization: For determination of optimum temperature 25ºC -30ºC was selected .about 55-70% humidity was maintained . Distilled water was used consistently to maintain humidity through out. Small holes were punched for ventilation purpose. Incubation period was about 25-28 days for proper binding & strength. Mycelium growth using agricultural waste / lignocellulose biomass.

Co-culturing of *mushroom* (oyster mushroom's spawn) with different agricultural wastes in trays for 12 days.

Table: 4.3.7 Inoculation of mushroom with different agricultural waste for 12 days

S.No	Spawns	Wheat straws	Wood chips	Rice bran
(A) TRAY 1 Control	100g	200g	----	----
(B) TRAY 2	100g	200g	----	40g
(C)TRAY 3	100g	200g	40g	----

A) Culture condition optimization (SET 1)

Composition 200g = Wheat straws , 100 g = grain spawn.

Procedure

1)Tray was cleaned properly using soap and water.

2) 200g wheat straws, were properly mixed in plastic bag with 100g grain spawns

3) Residue mixture was transferred into tray.

4) Tray was packed in autoclave bag & sealed properly using cello tapes.

5) Small holes were made for the aeration purpose.

6)Tray was placed in incubator at 25 ^{o}C for 12 days.

7)Sterilized spray water was used during the time of inoculation in order to maintain moisture in trays.

8)After 12 days, tray was removed from incubator and dried in hot air oven at 100^{o}C for 4-5 hours.

B) Culture condition optimization (SET1)

Composition - 40g = Rice bran/husk

200g = wheat straw 100g = grain spawns

Procedure

1)Tray was cleaned properly using soap and water.

2)40g rice husk , 200g wheat straws, were properly mixed in plastic bag with 100 g grain spawns .

3)Residue mixture was transferred into tray.

4)Tray was packed in autoclaved bag & sealed properly using cello tapes.

5)Small holes were made for the aeration purpose.

6)Tray was placed in incubator at 25 ^{o}C for 12 days.

7)Sterilized spray water was used during the time of inoculation in order to maintain moisture in trays.

8)After 12 days, tray was removed from incubator and dried in hot air oven at 100 ^{o}C for 4-5 hours.

C) Culture condition optimization (SET1)

Composition :- 40g = Wood-chips

200g = wheat straws 100g= grain spawns

Procedure 1) Tray was cleaned properly using soap and water.

2) 40g wood chips , 200g wheat straws, were properly mixed in plastic bag with 100 g grain spawns .

3)Residue mixture was transferred into tray.

4)Tray was packed in autoclaved bag & sealed properly using cello tapes.

5)Small holes were made for the aeration purpose.

6)Tray was placed in incubator at 25^{o}C for 12 days.

7)Sterilized spray water was used during the time of inoculation in order to maintain moisture in trays.

8) After 12 days, tray was removed from incubator and dried in hot air oven at 100^{o}C for 4-5 hours as shown in table 4.3.7.

S.No	Spawn	Substrate (wheat straw)	Sugarcane bagasse	Sawdust (powder)/ wood shaving	Pine needle	Rice Husk (powder)
(A)TRAY4	150g	100g	---	50g,50g	---	---
(B)TRAY5	150g	100g	50g	---	---	20g
(C)TRAY6	150g	100g	---	---	50g	30g

4.3.8 Table Co-culturing of (oyster mushroom's spawn) with different agro waste for 7 days.

4.3.8 (SET 2) Agricultural biomass optimization

For determination of agricultural biomass.In SET1 the agricultural biomass with mushroom grain spawn was used in the ratio of 2:1(w/w). According to the, space available in the tray used for whole experimentation & similarly in Set 2 . [in each set total trays were 3]. In SET 1 & 2 , different types of agricultural biomass was used to

optimized the substrate and mycelium growth. In both sets agricultural biomass were used are wood-chips, rice husk, wheat straw, sugarcane bagasse, wood shavings, pine needles.

(A) Agricultural biomass optimization (SET 2)

Composition -: 50g = Sawdust

50g = wood shaving 150g = wheat straw 150g = spawn (grain)

Procedure

1) Tray was cleaned properly using soap and water.

2) 50g sawdust , 50g wood shaving, 100g wheat straws, were properly mixed in plastic bag with 150 g grain spawns .

3) Residue mixture was transferred into tray.

4) Tray was packed in autoclaved bag & sealed properly using cello tapes.

5) Small holes were made made for the aeration purpose.

6) Tray was placed in incubator at 25 degree Celsius for 7 days.

7) Sterilized spray water was used during the time of inoculation in order to maintain moisture in trays.

8) After 7 days, tray was removed from incubator and dried in hot air oven at 100 degree Celsius for 4-5 hour.

(B) Agriculture biomass optimization (SET 2)

Composition -: 50g = sugarcane bagasse

20g = rice husk powder 100g = wheat straw 150g = grain spawn

Procedure

1) Tray was cleaned properly using soap and water.

2) 50g sugarcane bagasse , 20g rice husk powder, 100g wheat straws, were properly mixed in plastic bag with 150 g grain spawns .

3) Residue mixture was transferred into tray.

4) Tray was packed in autoclaved bag & sealed properly using cello tapes.

5) Small holes were made made for the aeration purpose.

6) Tray was placed in incubator at 25 degree Celsius for 7 days.

7)Sterilized spray water was used during the time of inoculation in order to maintain moisture in trays.

8) After 7 days, tray was removed from incubator and dried in hot air oven at 100^{o} Celsius for 4-5 hours.

C) Agriculture biomass optimization (SET 2)

Composition: 50g = pine needle

30g = rice bran 100g = wheat straw

150g = grain spawn

Procedure

1) Tray was cleaned properly using soap and water.

2) 50g pine needle , 20g rice bran , 100g wheat straws, were properly mixed in plastic bag with 150 g grain spawns .

3) Residue mixture was transferred into tray.

4) Tray was packed in autoclaved bag & sealed properly using cello tapes.

5) Small holes were made for the aeration purpose.

6) Tray was placed in incubator at 25 ^{o}C for 7 days.

7) Sterilized spray water was used during the time of inoculation in order to maintain moisture in trays.

8**)** After 7 days, tray was removed from incubator and dried in hot air oven at 100^{o} C for 4-5 hour as shown in table 4.3.8.

4.3.9 (SET 3) Co-culturing of biomass and *mushroom* under optimum conditions

Table 4.3.9 Co-culturing with different agricultural wastes in trays for 21 days.

S.No	Wheat straw	Sugar cane pith	Corn husk	Grain Spawn	Sawdust powder	Mix of tray 3	Mix of tray 6	Mix of tray 1
(A) TRAY 7	100g	20g	---	150g	30g	30g	10g	---
(B) TRAY 8	100g	20g	---	150g	10g	---	30g	---

(C) TRAY 9	50	20g	50g	150g	---	20g	20g	20g

(A) Co-culturing of biomass and *Pleurotus florida* under optimum conditions SET 3

Composition: 100g = wheat straw

20g = sugarcane bagasse 30g = sawdust powder

30g = powdered mixture of tray 3,

10g = powdered mixture of tray 6

150g = grain spawn

Procedure

1) Tray was cleaned properly using soap and water.

2) 20g sugarcane pith , 30g sawdust, 100g wheat straws, 30g mixture of tray 3, 10g mixture of tray 6, were properly mixed in plastic bag with 150 g grain spawns .

3) Residue mixture was transferred into tray.

4) Tray was packed in autoclaved bag & sealed properly using cello tapes.

5) Small holes were made for the aeration purpose.

6) Tray was placed in incubator at 25 ^{o}C for 21 days.

7) Sterilized spray water was used during the time of inoculation in order to maintain moisture in trays.

8) After 21 days, tray was removed from incubator and dried in hot air oven at 100 ^{o}C for 4-5 hours.

B) Co-culturing of biomass and *Pleurotus florida* under optimum conditions (SET 3)

Composition: 100g = wheat straw

20g = sugarcane bagasse, 10g = sawdust powder

Procedure

1) Tray was cleaned properly using soap and water.

2) 20g sugarcane pith, 10g sawdust, 100g wheat straws, 30g mixture of tray 6, were properly mixed in plastic bag with 150 g grain spawns.

3) Residue mixture was transferred into tray.

4) Tray was packed in autoclaved bag & sealed properly using cello tapes.

5) Small holes were made for the aeration purpose.

6) Tray was placed in incubator at 25 ^{o}C for 21days.

7) Sterilized spray water was used during the time of inoculation in order to maintain moisture in trays.

9) After 21 days, tray was removed from incubator and dried in hot air oven at 100^{o}C for 4-5 hours.

C) Co-culturing of biomass and *Pleurotus florida* under optimum conditions (SET 3)

Composition: 50g = wheat straw

20g = sugarcane bagasse, 10g = sawdust powder

20g = powdered mixture of tray 6 150g = grain spawn

50g = corn husk , 20g = mixture of tray 3 , 20g = mixture of tray 1

Procedure

1) Tray was cleaned properly using soap and water.

2) 20g sugarcane pith , 10g sawdust, 50g wheat straws, 20g mixture of tray 6,50g corn husk, 20g mixture of tray3 , 20g mixture of tray1, were properly mixed in plastic bag with 150 g grain spawns .

3) Residue mixture was transferred into tray.

4) Tray was packed in autoclaved bag & sealed properly using cello tapes.

5) Small holes were made for the aeration purpose.

6) Tray was placed in incubator at 25 ^{o}C for 21 days.

7) Sterilized spray water was used during the time of inoculation in order to maintain moisture in trays.

8) After 21 days, tray was removed from incubator and dried in hot air oven at 100 ^{o}C for 4-5 hours as shown in fig 4.3.9.

4.4 (SET 4) Optimization of bio-composite with different concentration of sawdust

Table 4.4 Co-culturing of (oyster mushroom's spawn) with different agricultural wastes in trays for 28 days

S.No	Sugar cane pith	Saw dust Powder	Wheat straw	Grain Spawn	Mixture of tray 1	Mixture of tray 6	Starch Binder (10%)
(A) TRAY10	200g	150g	100g	200g	---	50g	600ml
(B) TRAY11	200g	100g	100g	200g	---	---	600ml
(C) TRAY12	200g	80g	50g	200g	50g	---	---

(A) Optimization of bio-composite with different concentration of sawdust (SET 4)

Composition:-100g = wheat straw

200g = sugarcane bagasse, 150g = sawdust powder

50g = powdered mixture of tray 6, 200g = grain spawn

To obtain starch based binder: After adding 10% starch (100g) to 1 litre water, the mixture was continuously mixed and heated to between 55 and 69^{o}C. After that, the remaining water was added. The gel was heated while being stirred continuously until it started to clear.To create a homogeneous mixture, the heating was stopped. The gelatinized starch was stirred for five minutes & prepared mixture was distributed throughout the whole form in tray.

PROCEDURE

1) Tray was cleaned properly using soap and water.

2) 200g sugarcane pith, 150g sawdust, 100g wheat straws, 50g mixture of tray 6, were properly mixed in plastic bag with 200 g grain spawns.

3) Residue mixture was transferred into tray with 600 ml starch binder slurry was added with aggregates & mixed for 5 minutes to obtain a homogeneous mixture. Side & bottom

parts of the tray was lubricated with oil.

4) Tray was packed in autoclaved bag & sealed properly using cello tapes.

5) Small holes were made for the aeration purpose.

6) Tray was placed in incubator at 25^{o}C for 10-13 days.

7) Sterilized spray water was used during the time of inoculation in order to maintain moisture in trays.

8) After 13 days, tray was removed from incubator and dried in hot air oven at 100^{o}C for 4-5 hours.

B) Optimization of bio-composite with different concentration of sawdust (SET4).

Composition:- 200g = sugarcane bagasse,

100g = sawdust powder 100g = wheat straw

200g = grain spawn.

PROCEDURE

1) Tray was cleaned properly using soap and water.

2) 200g sugarcane pith, 100g sawdust powder, 100g wheat straws, was properly mixed in plastic bag with 200 g grain spawns.

3) Residue mixture was transferred into tray with 600 ml starch binder slurry was added with aggregates & mixed for 5 minutes to obtain a homogeneous mixture. A side & bottom part of tray was lubricated with oil.

4) Tray was packed in autoclaved bag & sealed properly using cello tapes.

5) Small holes were made for the aeration purpose using cutter.

6) Tray was placed in incubator at 25 ^{o}C for 10-13 days.

7) Sterilized spray water was used during the time of inoculation in order to maintain moisture in trays.

8) After 13 days, proper binding was not observed .In this tray, yellowish fluid on top layer was observed.

C) Optimization of bio-composite with different concentration of sawdust (SET4)

Composition: 200g = sugarcane bagasse,

80g = sawdust powder 50g = wheat straw

50g = mixture of tray 1

PROCEDURE

1) Tray was cleaned properly using soap and water.

2) 200g sugarcane pith , 80g sawdust, 50g wheat straws, 50g mixture of tray 1, were properly mixed in plastic bag with 200 g grain spawns .

3) Residue mixture was transferred into tray with 600 ml starch binder slurry was added with aggregates & mixed for 5 minutes to obtain a homogeneous mixture.Side & bottom parts of the tray was lubricated with oil.

4) Tray was packed in autoclaved bag & sealed properly using cello tapes.
Small holes were made for the aeration purpose.

5) Tray was placed in incubator at 25 ^{o}C for 10-13 days.

6) Sterilized spray water was used during the time of inoculation in order to maintain moisture in trays.

7) After 13 days, in this no yellow fluid was observed .

8) Tray was removed from incubator after 26 days and dried in hot air oven at 100^{o} Celsius for 4-5 hours as shown in table 4.4.

4.4.1 (SET5) Co-culturing of *Pleurotus florida* with different agricultural wastes for 30 days

Table 4.4.1 Co-culturing of mushroom with different concentration of starch binder in trays for 30 days.

S No.	Sugarcane bagasse	Sawdust powder	Wheat straws	Wheat straw powder	Starch binder
(A) TRAY 13	150 g	50g	20g	30g	50g
(B) TRAY 14	150 g	50g	---	50g	100g
(C) TRAY 15	150 g	50g	30g	20g	150g

(A) Co-culturing of mushroom with different concentration of starch binder in trays for 30 days (SET 5)

Composition 150g = Sugarcane bagasse

250 = grain spawn 50g = sawdust powder

50 g = wheat straw (20 g wheat straw,

30g powdered wheat straw)

50g = starch binder

PROCEDURE

1) Tray was cleaned properly using soap and water.

2)150g sugarcane bagasse, 50g sawdust, 50g wheat straws, were properly mixed in plastic bag with 250 g grain spawns .

3)Residue mixture was transferred into tray & 50g of starch binder was added and mixed well. Distilled water was sprayed consistently throughout.

4)Tray was packed in autoclaved bag & sealed properly using cello tapes.

5)Small holes were made for the aeration purpose.

6) Tray was placed in incubator at 25 ^{o}C for 30 days.

7) Sterilized spray water was used during the time of inoculation in order to maintain moisture in trays.

8) Trays were observed days.

B) Co-culturing of mushroom with different concentration of starch binder in trays for 30 day (SET 5)

Composition 150g = Sugarcane bagasse

250 = grain spawn 50g = sawdust powder

50 g = powdered wheat straw 100g = starch binder

PROCEDURE

1) Tray was cleaned properly using soap and water.

2) 150g sugarcane pith , 50g sawdust, 50g powdered wheat straws, were properly mixed in plastic bag with 250 g grain spawns .

3) Residue mixture was transferred into tray & 100g of starch binder was added and mixed well. Distilled water was sprayed consistently throughout.

4) Tray was packed in autoclaved bag & sealed properly using cello tapes.

5) Small holes were made for the aeration purpose.

6) Tray was placed in incubator at 25 ^{o}C for 30 days.

7) Sterilized spray water was used during the time of inoculation in order to maintain moisture in trays.

8) Trays were observed daily.

C) Co-culturing of mushroom with different concentration of starch binder in trays for 30 day (SET 5)

Composition 150g = Sugarcane bagasse

250g = grain spawn 50g = sawdust powder50 g = wheat straw (20 g powdered wheat straw, 30g wheat straw, 30g powdered wheat straw) 100g = starch binder as shown in table 4.4.1

PROCEDURE

1) Tray was cleaned properly using soap and water.

2) 150g sugarcane pith , 50g sawdust, 50g wheat straws, were properly mixed in plastic bag with 250 g grain spawns .

3) Residue mixture was transferred into tray & 100g of starch binder was added and mixed well. Distilled water was sprayed consistently throughout.

4) Tray was packed in autoclaved bag & sealed properly using cello tapes.

5) Small holes were made for the aeration purpose.

6) Tray was placed in incubator at 25 ^{o}C for 30 days.

7) Sterilized spray water was used during the time of inoculation in order to maintain moisture in trays.

8) Trays were observed daily. After colonization samples were dried in hot air oven and after drying hot-pressing the FBBC and sheet was prepared.

4.2 Growth optimization of *Aspergillus niger* in cultural bags with agricultural waste

In cultural bag, spores of *Aspergillus* were inoculated with feathers, rice husk, rice straw, and incubate it for 2 weeks. Knob was attached with cotton plug in cultural bag for aeration purpose . After 2 weeks growth was observed and dried in oven. After drying, sample was hotpressed .But it is not fulfilling the criteria to be used in application of packaging material .All samples were brittle as shown in fig 4.2

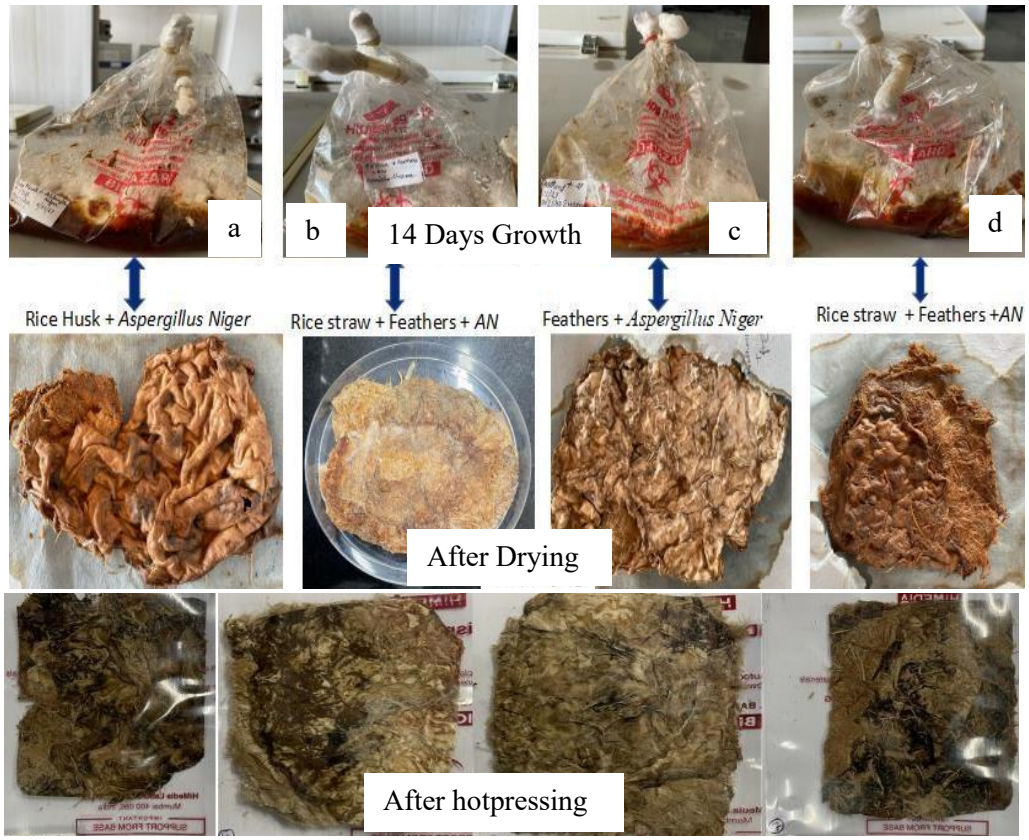

Fig 4.2 After 14 days growth in cultural bag a) Rice husk +A.N b) Rice straw + Feathers + AN c) Feathers + A.N d) Rice straw + Feathers +AN

4.3 Growth optimization using *Aspergillus flavus*

2g of Feathers, different fungus strain (*Aspergillus flavus* BT01,BT03,BT05) spores were together inoculated in media (100ml) in flasks. Flasks were incubated at 25^{o}C for 14 days. After 14 days, growth was observed. Flasks were autoclaved at 121 ^{o}C. Drying under sunlight for few hours.Then hot press the material .But is not considerable for application purpose because of its brittle nature as shown in 4.3

Fig 4.3 Growth optimization of *Aspergillus flavus* (BT01,03,05) in batch culture

4.4 Growth Optimization of *Pleurotus florida* in PDB media for 14 days

A)Flask 1st=100 ml of PDB, Sugarcane bagasse (5g) , *mushroom* spawn (2g)

B)Flask 3rd =100 ml of PDB , Saw dust (5g) , Spawn (2g)

C)Flask 4th =100 ml of PDB , Wheat straw (5g), Spawn (2g)

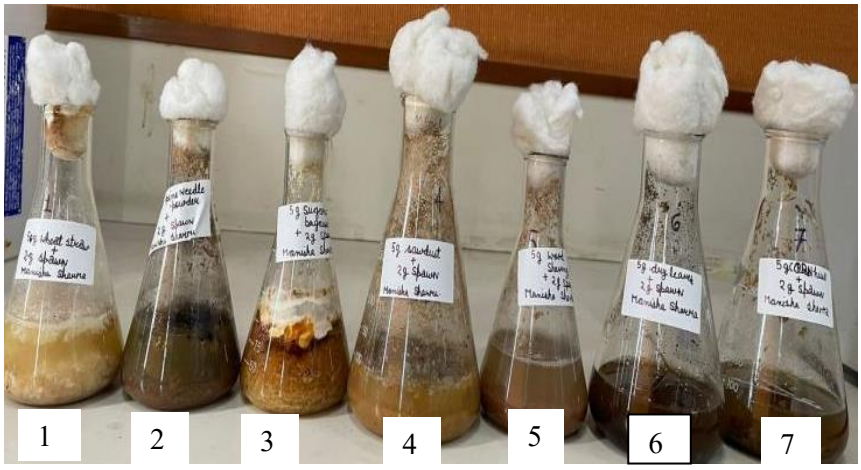

Fig 4.4 Growth after 2 weeks

Good growth was observed only in sugarcane bagasse, sawdust, wheat straw biomass, so further will use these agricultural biomass in different experiments to make biodegradable green product using oyster mushroom.

4.5 Culture conditions optimization

SET 1

Grew a thinner fungal layer additionally it displayed an uneven mycelial development with exposed areas. By day 7, over two thirds of the upper surfaces were coated in a mycelial layer. It took 11 - 12 days to complete full coverage. About two thirds of the top surfaces were coated with a mycelial layer on day seven. On day 9, the entire top surfaces were coated in an almost uniform fungal layer. The mycelium looked weaker & had cottony growth properties. A dense fungal skin formed after 9-12 days. It had a slower rate of colonization and a thinner layer of fungal growth. Additionally, until 12 days, it had in-homogeneous growth with exposed portions.

SET 1

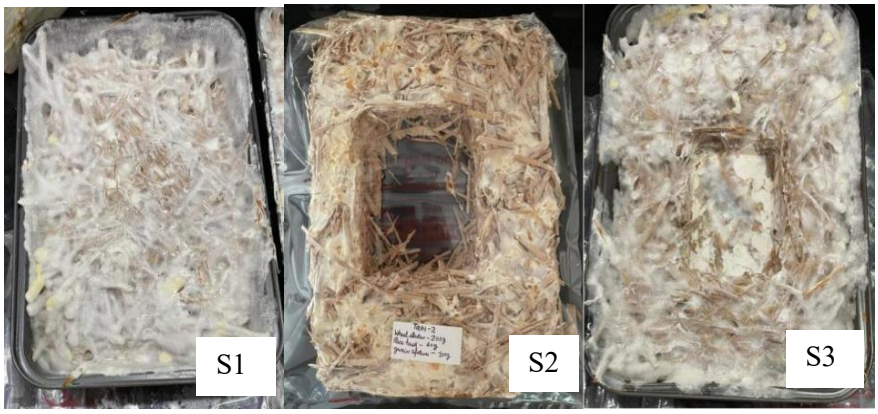

Fig 4.5 : S1**,**S2,S3 are the samples of tray 1,2,3 after 12 days growth**.**

Growth period of trays SET 1 were 12 days. All trays were brittle because wheat straws were not grinded finely & its size is (2-3cm). And proper binding of mycelium and biomass was not observed within 12 days.

4.6 Agricultural biomass optimization

(SET 2)

It has lower rate of colonization & a thinner layer of fungal growth. Additionally, until 12 days it had homogeneous growth with exposed portions. Cottony growth was observed which looked weaker (brittle). In tray 5, growth was on rice husk powder and sugarcane bagasse, mycelium grew a little bit more quickly. On day 5: the entire top surface was coated in an almost uniform fungus layer. Compact fungal layer was developed on day 7. It took 12 days to cover the entire surface. Although a soft , cottony structure was noticed, it has a fragile quality. In tray 6, there was a slow growth rate. On day twelve, the entire surface was not covered with mycelium, cottony growth was observed, indicating brittle nature.

SET 2

Fig.4.6 S4**,** S5, S6 are the samples of tray 4,5,6 after 7 days of growth.

As compared to samples 1,2,3 wheat straws were grinded more finely for samples 4,5,6 . more amount of spawns were added in each tray. Supplementary substrates (sugarcane bagasse, pine needle) were grinded more finely for these trays. But results were not as satisfactory as expected. All trays were so fragile. Sample 1,3,4 were grinded into fine powder & further will used in sample 7,8,9 as supplements. Because these trays were so brittle and have no strength at all.

4.7 Co-culturing of biomass and *mushroom* under optimum conditions

SET 3

In sample 7 late growth was observed. It has thinner layer of fungal growth till 12 days. After 21 days , soft cottony structure was noticed which shows its brittleness. In sample 8, fast growth was observed on sugarcane pith (inner portion of sugarcane) , sawdust powder & mixture of tray 6. It took 7-12 days to cover the entire surface. Upper growth was observed till 12 days. It takes 21 days to fully cover the entire surface & for proper binding of mycelium & agriculture waste. In this fungal mycelium acts as binding agent. Proper binding was observed after hot pressing for 20 minutes at 150^{o}C temperature & 6.1MPa. After hot pressing, it shows hardness & strong binding of mycelium and agricultural biomass which looks like a biocomposite.In sample 9 tray, again fast growth was observed on sugarcane pith, corn husk & supplementary mixtures. It took 21 days to fully cover the entire surface & for proper binding of mycelium and agriculture biomass.

SET 3

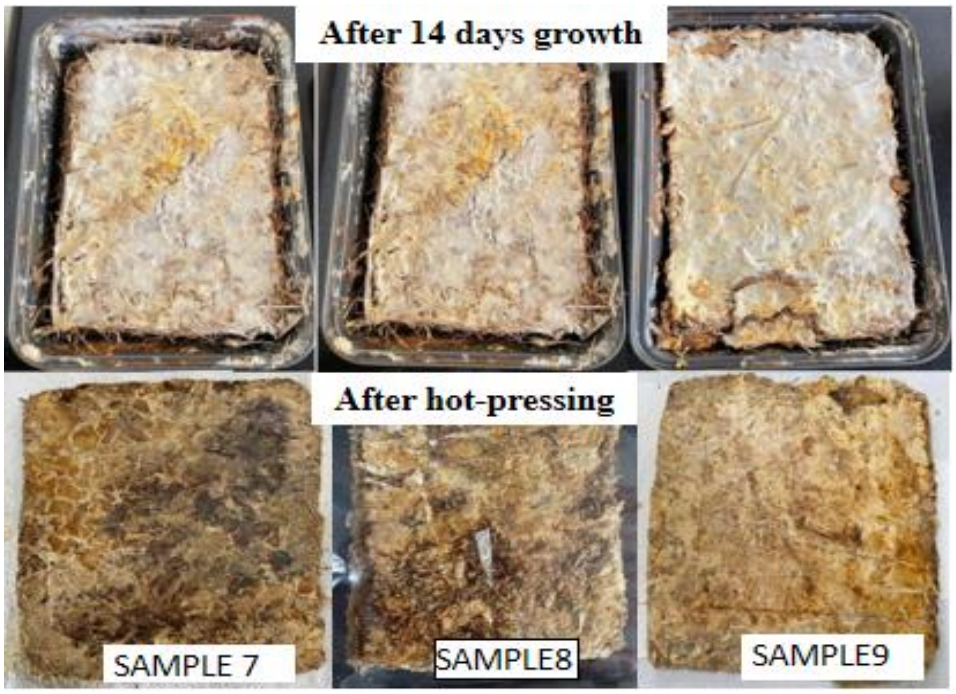

Fig 4.7 S7,S8,S9 are the samples of tray 7,8,9 after 26 days of growth

After dividing trays into three sections and creating a sandwich like structure, hot pressing was carried out at 6.1 MPa and 150 ^{o}C. In these cases, biomass is bound together by the fungal mycelium as shown in fig 4.7

4.8 Optimization of bio-composite with different concentration of sawdust

SET 4

After 13 days of growth, yellowish fluid was observed on the top layer in both trays (10,11) .Starch binder was added in the form of slurry , Growth was observed only on the top most layer , proper binding was observed after 26 days. Then trays were dried in hot air oven for proper drying & deactivation of mycelium growth.

After that, hot-pressing was done at 150 ^{o}C temperature and 6.1MPa. Good result was observed in tray11. Material was hard and binding of mycelia and substrate was good.

In tray 12, after 13 days of growth , no yellowish fluid was observed , in this no starch binder was added in the form of slurry. Growth was observed only on the top most layer , proper binding was observed after 26 days.

Then trays were dried in hot air oven for proper drying & deactivation of mycelium growth . While hot-pressing, the material at 150^{o}C temperature and 6.1MPa material was disintegrated & results were not as expected .

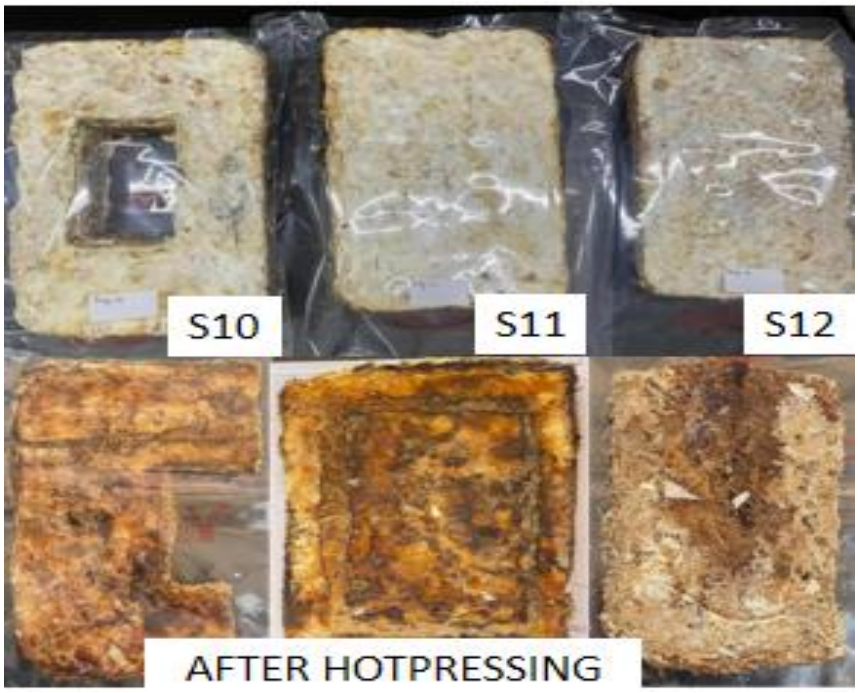

Fig 4.8 S10,S11,S12 were tray sample of 10,11,12 after hot pressing

4.9 Co-culturing of *Pleurotus florida* with different agricultural wastes for 30 days.

After 30 days, Cottony growth was observed. Less percentage of binder shows the weaker binding of mycelium and lignocellulosic biomass. While hotpressing the product was disintegrated. After 30 days best growth was observed in tray 14, which shows good binding of mycelium and lignocellulosic biomass and makes a network Structure. Stiffness and harness was also observed. Starch was added as binder and it shows the best results. After hotpressing, at 150^{o}C temperature and 6.0 MPa pressure it shows the best results it gives the product more stiffness and hardness.In tray 15, Best growth was observed after 30 days of growth. Which shows good binding of mycelium lignocellulosic biomass and makes a network Structure. Stiffness and harness was also observed. More percentage of binder was added more will be the good binding between both. After hotpressing, at 150^{o}C temperature and 6.0 MPa pressure it shows the best results it gives the product more stiffness and hardness.

SET 5

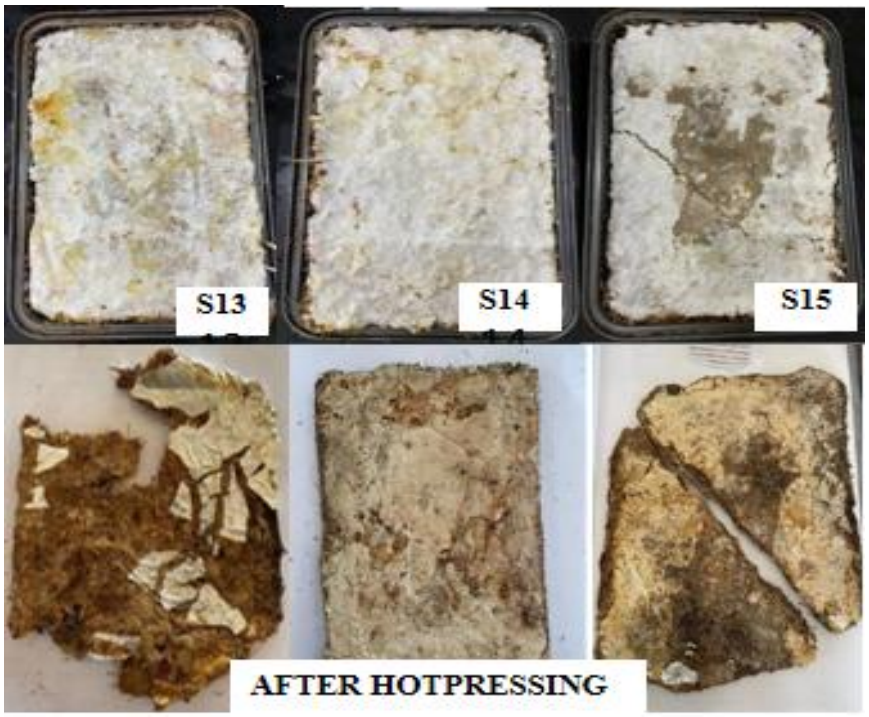

Fig 4.9 S13,S14,S15 shows sample of tray 13,14,15 growth after 30 days

As compared to tray 1,2,3 wheat straws were grinded more finely for tray 4,5,6 . More amounts of spawns were added in each tray. Supplementary substrates (sugarcane bagasse, pine needle) were grinded more finely for these trays. But results were not as satisfactory as expected. All trays were so fragile. Tray 1,3,4 were grinded into fine powder & further will used in tray 7,8,9 as supplements. Because these trays were so brittle and have no strength at all.

5. Characterization of synthesized Fungal based Bio-composites using lignocellulosic biomass & mushroom)

5.1 Biodegradability Test

In order to prove the biodegradable nature of the synthesized fungal based bio-composite Biodegradability test has been performed by exposing the biocomposite material in set of several environmental condition (in-vitro). Different environmental conditions can be seen in figure i,e control, wet soil, dry soil . All the observation of this experiment was recorded and reported in table .

Control - In control condition, biocomposite was leaved as it is in beaker to check the moisture absorbance from the air & to check the shelf life of the material at room temperature . Initial dry weight (0.5g)

Dry Soil - In this condition, bio-composite material was put in a petri plate having dry soil sample in order to check the biodegradability time.

Wet Soil - In this condition, bio-composite material was put in a petri plate having dry soil sample in order to check the biodegradability time.

5.1 Optimization of synthesized bio-composite material

Control Dry Soil Wet Soil

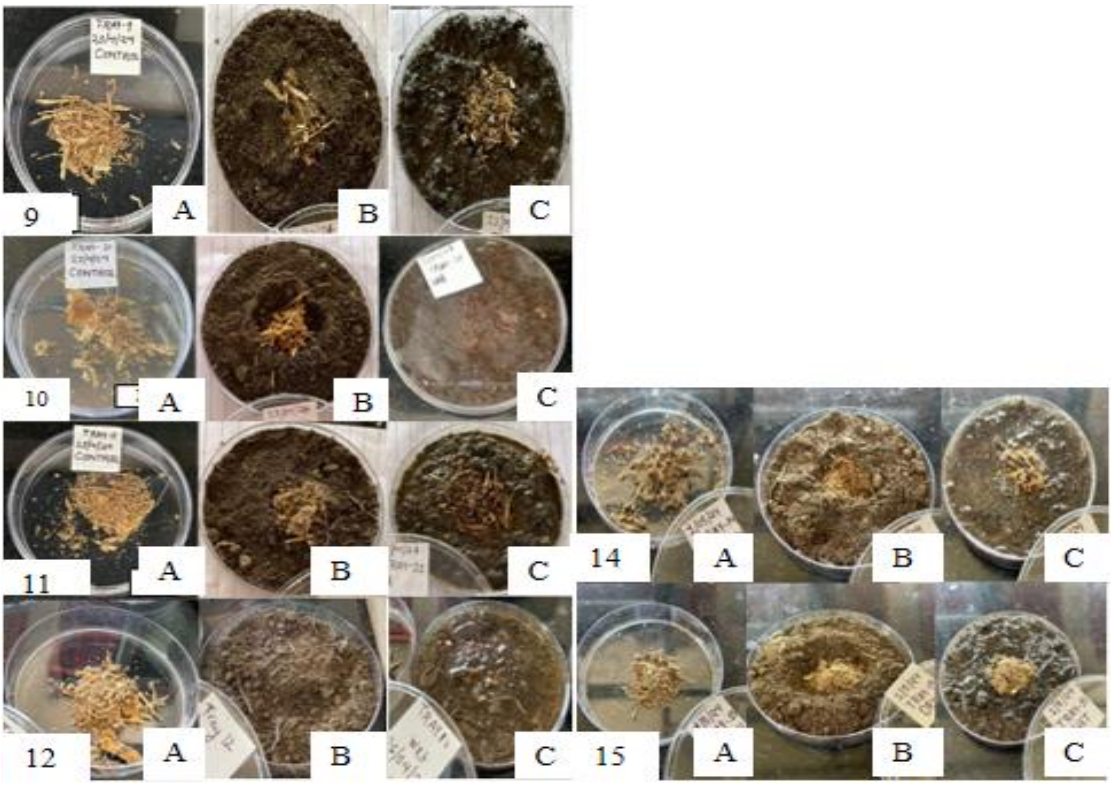

Fig.5.1 Biodegradability test (DAY1): A - Control, B- Dry soil, C-Wet soil

Table5.1:Biodegradability test of bio-composite under different condition.

S.No	Condition	Weight of the Biocomposite (g)	Observation and Result
1	**Control**	0.5	After 21 days no change was observed
2	**Dry soil**	0.5	No change was observed after 21 days.
3	**Wet soil**	0.5	After 21 days, biocomposite was not degraded.

CONTROL DRY SOIL WET SOIL CONTROL DRY SOIL WET SOIL

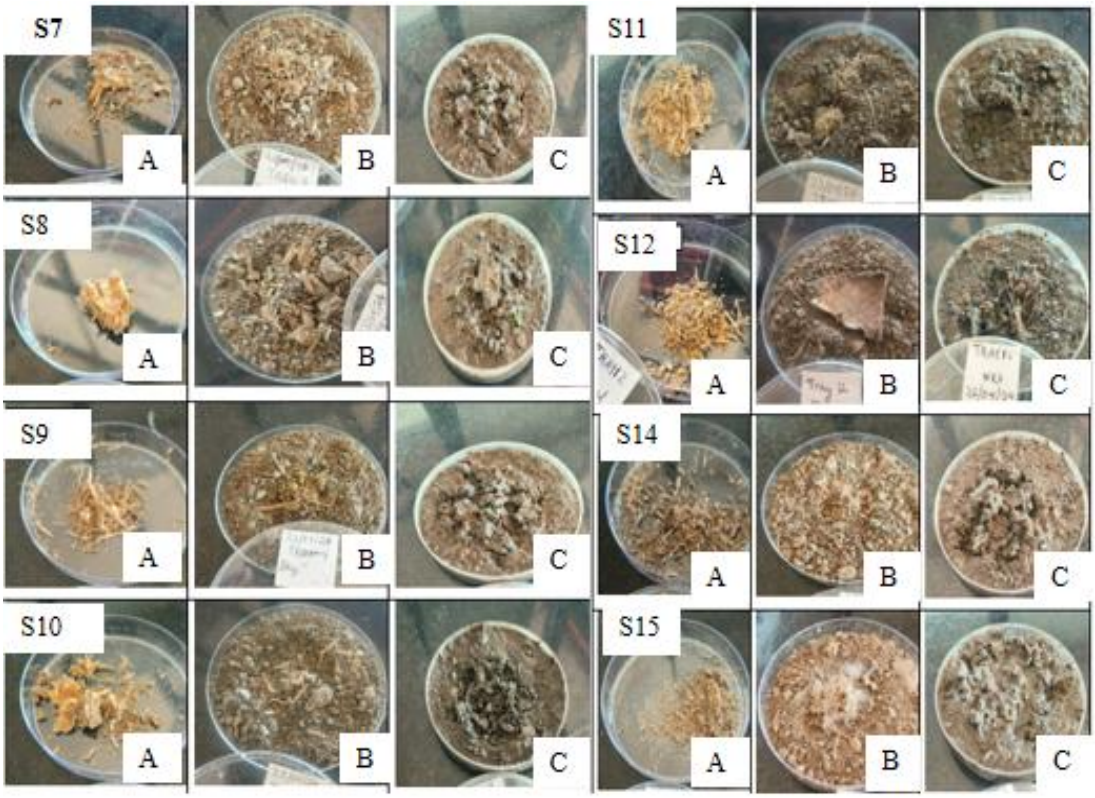

Fig 5.1 BIODEGRADABILITY TEST (AFTER 21 DAYS)

S7 represents samples of tray 7 represents A) Control B) Dry Soil C) Wet Soil **; S8** represents sample 8 A) Control B0 Dry Soil C) Wet Soil **; S9** represents sample 9 A) Control B) Dry Soil C) Wet Soil **; S10** represents sample 8 A) Control B) Dry Soil C) Wet Soil **; S11** represents sample 11 A) Control B) Dry Soil C) Wet Soil **; S12** represents sample 12 A) Control B) Dry Soil C) Wet Soil **; S13** represents sample 13 A) Control B) Dry Soil C) Wet Soil ; **S14** represents sample 14 A) Control B) Dry Soil C) Wet Soil ; **S15** represents sample 15 A) Control B) Dry Soil C) Wet Soil

Fig. 5.1 Sets of environmental condition : A is control, B is in biocomposite in dry soil, C is wet soil sample

- **Control :** to check the moisture absorbance from the air and to check the shelf life of the biocomposite material at room temperature and it is found that bio-composite didn't retain moisture and it does not change its morphology and initial weight (0.5g) around 21 days which shows that bio-composite have good shelf life.
- **Wet Soil :** to check sample in a petri plate in order to check the biodegradability time. It was found that in 21 days biocomposite is not degraded which shows its good shelf life. It takes longer degradation time to degrade it fully.
- **Dry Soil :** In this condition, bio based composite was put in dry soil sample in a petri plate in order to check the biodegradability time. It was found that, there is lack of moisture and humidity so that it will take more time to degrade.

5.2 Optimization of FBBC using water absorption test

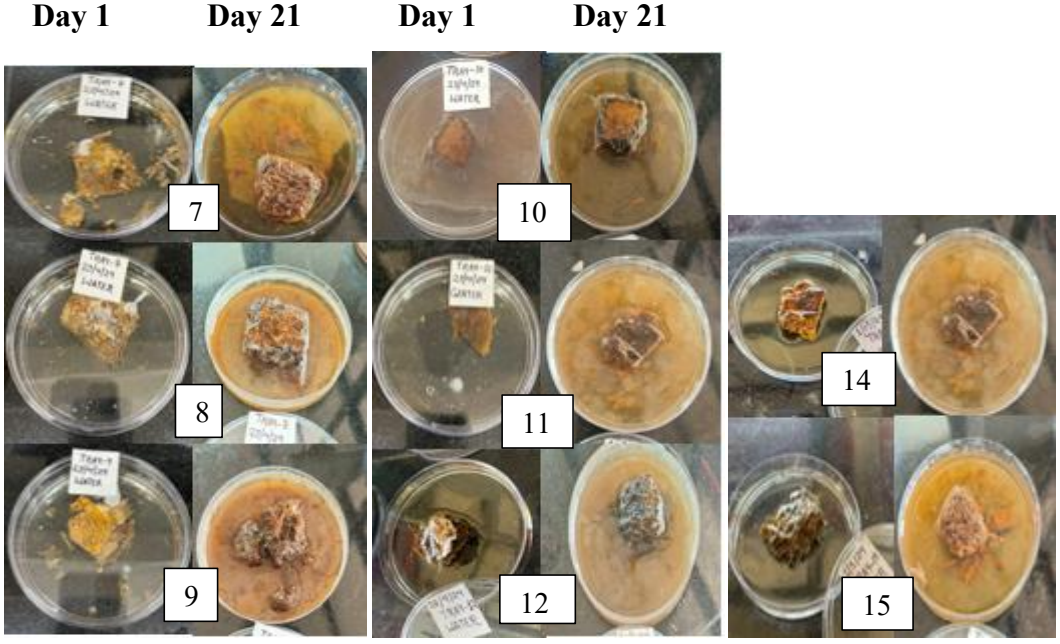

Fig: 5.2 Optimization of water absorption test A) Tray 7, B) Tray 8, C) Tray 9, D) Tray 10, E) Tray 11, F) Tray 12, G) Tray 14, H) Tray 15.

Table: 5.2 Optimization of water absorption test

TRAYS	Initial weight	1 day	7 days	14 days
TRAY 7	0.760g	3.76g	5.21g	5.79g
TRAY 8	3.16g	10.1g	11.6g	13.5g
TRAY 9	1.4g	7.08g	7.49g	7.9g
TRAY 10	1.01g	5.87g	6.65g	7.16g
TRAY 11	0.8g	3.49g	4.179	4.59g
TRAY 12	2.18g	6.83g	7.08g	8.01g
TRAY 14	1.36g	4.23g	4.62g	4.7g
TRAY 15	1.53g	5.53g	5.30g	6.33g

Conclusion -: Results obtained in water absorption test proves that all synthesized samples of bio-composite material absorbs water , it indicates its nature is hydrophilic.

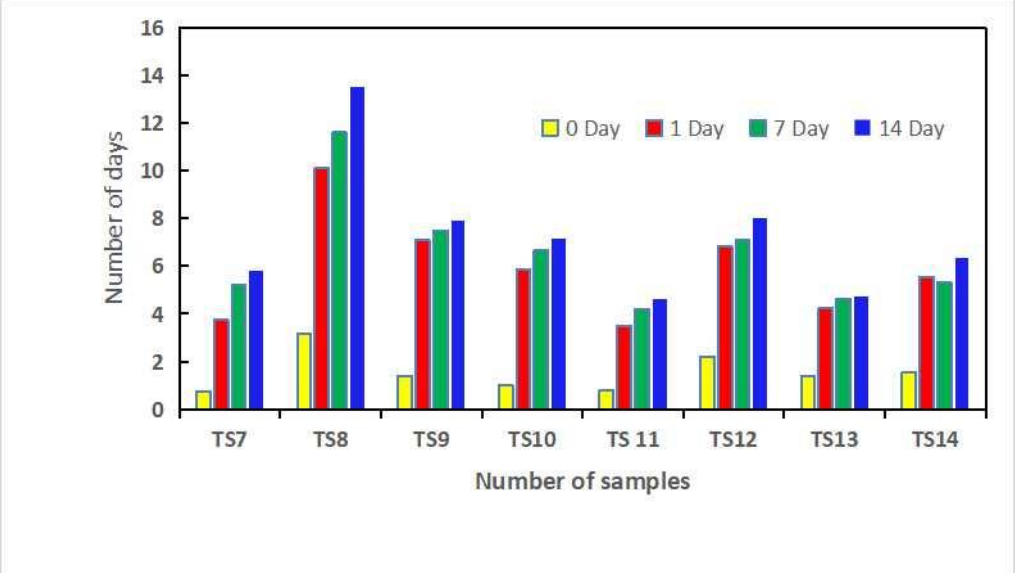

Fig 5.2 Graph of water absorption test

Yellow line represents day 0, Red line represents day 4;

Green line represents day7; Blue line represents day 14.

5.3 Characterization of FBBC using mechanical method (strength) test

All the fungal based bio-composite material shows good strength , tray 7,8,9,10,12 stable at 7 Kgs and tray 11 only tolerate weight upto 4 kgs and trays 12,14,&15 tolerate weight up too 7 kgs which shows its hardness, stiffness, compactness & binding between the mycelium and lignocellulosic biomass. Which is further used in many industries.

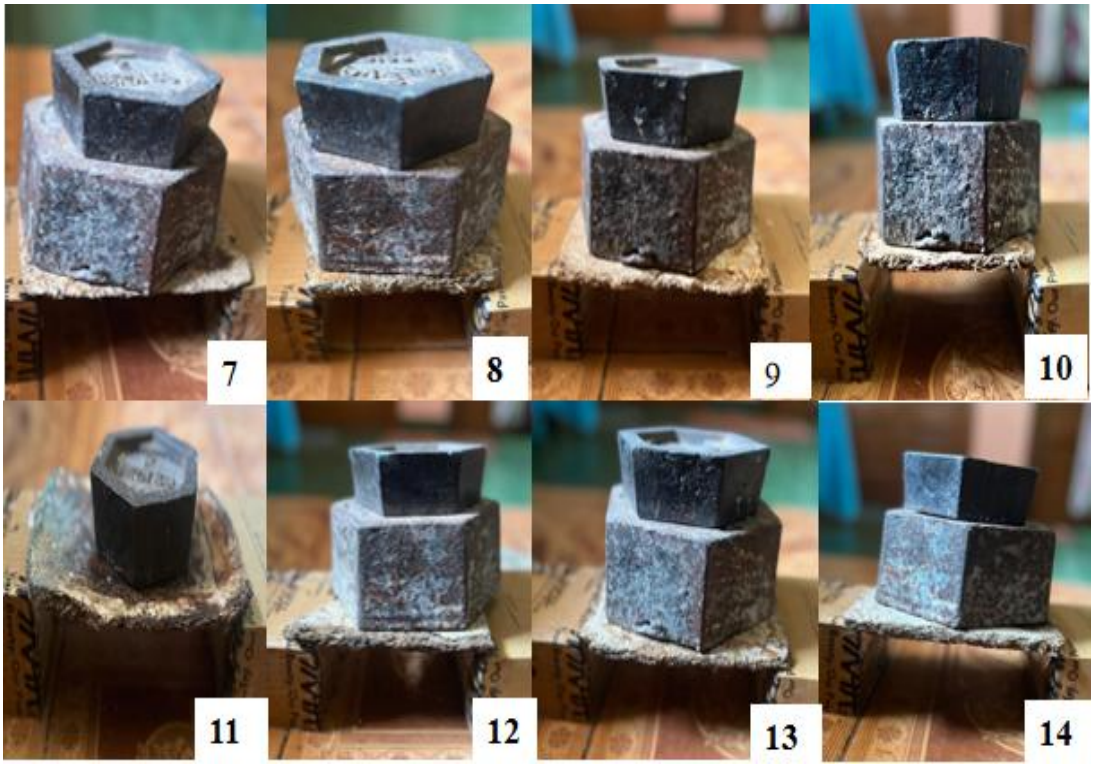

Fig 5.3 Mechanical method strength test which represents different ; 7 represents the sample of tray 7 ; 7 represents the sample of tray 8 ; 9 represents the sample of tray 9; 10 represents the sample of tray 10 ; 11 represents the sample of tray 11 ; 12 represents the sample of tray 12 ; 13 & 14 represents the sample of tray 13,14 as shown in fig 5.3

5.4 Physical Method (Boiling water test)

5.5 Optimization of FBBC at 100ºC Boiling water test

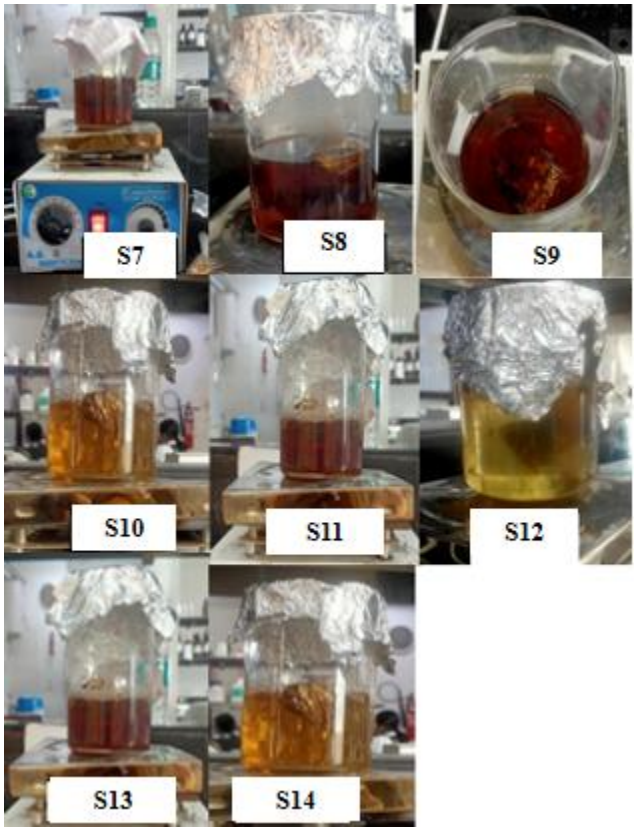

Fig 5.5 Optimization of FBBC at 100ºC

50 ml of distilled water was added into beaker and boiled at 100 ºC on hot plate. Pieces of biocomposite material were cut into square shapes and put it into beaker. Stability of the fungal based biocomposite have been recorded. All the products are stable at 100ºC . This shows that binding of mycelium of mushroom and lignocellulosic biomass biocomposite material shows better results. It will resist heat and will be use in construction as insulating sheets for heat resistant. And this fungal based bio-composite was stable up to some extent as shown in fig 5.5.

5.6 FUTURE PROSPECTIVE

Further, will try to make this fungal based bio composite (FBBC) , hydrophobic and fire resistant by using different method. FBBC has many applications in many sectors like packaging material, plywood industries and as biofertiliser.

5.6 APPLICATIONS

- These fungal based bio-composite material have applications in many industries like in **plywood industry** it is used as wood panels , ply.
- **In Construction industry -:** it is used as insulating sheet walls and wooden partition boards.
- **In Packaging industry-:** it is used for electronic devices while shipping as shown in fig 5.6.
- These fungal based bio-composite are made up of agricultural waste.
- **Biofertiliser** -: Shredded particles of fungal based bio-composite is used as bio-fertilisers for plants to increase the fertility of soil.

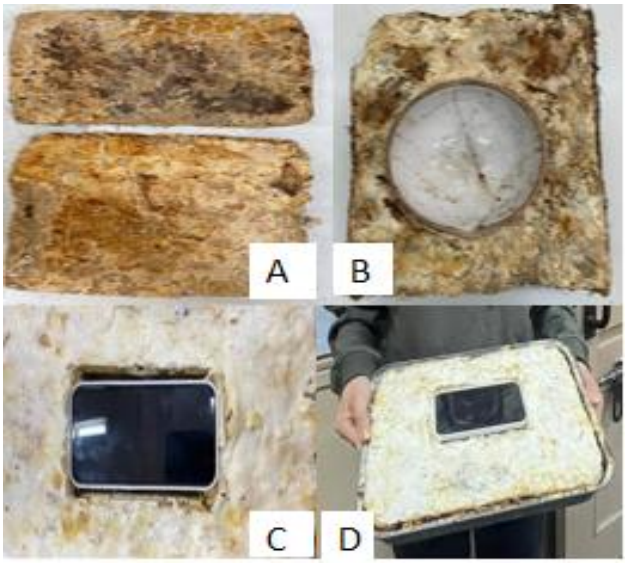

Fig 5.6 Applications of fungal based bio-composite

A) Wood panels, **B)** Packaging material,

C&D) Packaging material for electronic devices.

DISCUSSION

- Biocomposite sheets were made using fungus *Aspergillus niger* and *Aspergillus flavus* with different strains BT01, BT03, BT05 which is cultivated in PDB media with chicken feathers for about one week but the growth is not appropriate. Binding of feathers and fungus is not good enough to make a bonding between them. Product which is made after hotpressing is brittle in nature.
- Biocomposite is made using fungus *Aspergillus flavus* with three different strains with chicken feathers, they also lacks strength and proper binding of fungus and feathers.
- Further, spawns of mushroom is used with different agricultural biomass to make FBBC. By using *Pleurotus florida (*oyster mushroom spawns) with lignocellulosic biomass, it shows great results with proper binding of biomass and fungi. In this, Fungus is used as an adhesive binder which binds the biomass with fungus. After hotpressing at 150 ^{o}C and 6MPa pressure it shows the best results. Mushroom which is used in the production of FBBC is an edible mushroom which shows no toxic effect and is filamentous fungi. And mushroom is artificially cultivated on lignocellulosic biomass which is abundantly found in nature and considered as waste. This is a novel approach to make a fungal based biocomposite sheet using agricultural waste.

CONCLUSION

Fungal based bio-composite materials (FBBC) which was made using the mushroom with lignocellulosic biomass. This fungal species is chosen because of its natural adhesive nature it acts like glue with agricultural biomass. These substrates were chosen because these substrates have good lignin, cellulose, hemicellulose, glucan which is very good for growth of fungus. And these agricultural wastes are found in nature abundantly after harvesting mushrooms so to minimize the rate of agricultural waste, I utilised it with spawns of *mushroom* to make a fungal based bio-composite which is of very low cost, light weight, biodegradable, low manufacturing process, Heat resistant, and made using waste material which decreases the agricultural biomass, so that pollution will decrease. Fungal based bio-composite (FBBC) was cultivated in steel mould of dimensions 20*29.5*4.5 by layer by layer method using spawns of mushroom and lignocellulose biomass in the ratio of 1:1 (w/w). After inoculating together, distilled water was sprayed to maintain humidity. Then, it covered with autoclavable disposable bag for sterilization of laboratory and disposal of contaminated materials of dimensions 14’’x 20’’ and cover it using cellotape. 8-10 small holes were punched to maintain aerobic environment for fungus. Incubate it in incubator at 25^{o}C for 28-30 days for proper growth of fungus to make fungal network structure with lignocellulosic biomass. After days, good growth was observed. To deactivated the growth of fungus it was dried in hot air oven for 24-30 hours at 100^{o}C. After drying it was sandwiched and cover it using aluminium foil and hotpressed at 150^{o}C to make a hard, stiffed and compact fungal based bio-composite (FBBC). Applications of these bio based composites are in packaging materials of electronic devices, in construction wall insulating material, wooden partion boards etc. Heat boiling water test, Water absorption test, biodegradability test, Strength test were carried out. Its one limitation is it is hydrophilic in nature but its upper layer mycelium layer is hydrophobic In Heat boiling water test it was observed that fungal based bio-composite materials is stable at 100^{o}C for some extent & also it is heat resistant.Producing fungal based biocomposite by lignocellulosic biomass can be a game changer sustainable alternative.

References

[1] N.Majib, N.D.Yaacob, N.M. Rohaizad, W.L.Tan, "Characterization of Fungal Foams from Edible Mushrooms Using Different Agricultural Wastes as Substrates for Packaging Material''.*Polymers,*vol.15,pg no.873, 2023,doi: https:// doi.org/10.3390/polym15040873

[2] E.O.Ugumomore, N.Maduka, I.Ahaotu,"The effect of clarification methods on quality attributes of sugarcane - watermelon wine fermented by palm wine yeast *(Saccharomyces cerevisiae)". African Journal of Biotechnology ,vol.*23,no(2), pp.1358-1365 , 2012 doi: https:// doi :10.5897/AJB11.25

[3] D.Alemu, M.Tafesse, and A.K,Mondal "Mycelium - Based Composite : The future Sustainable Biomaterials," *International Journal of Biomaterials*,vol22,article ID 8401528, Mar. 2022

[4] M.Z.Islam, M.E.Sarker, M. M. Rahman, M.R.Islam, A. F. Ahmed, M.S. Mahumud, and M.Syduzzaman," Green composites from natural fibers and biopolymers: A review on processing , properties and applications," *Journal of Reinforced Plastics and Composites*,vol.41,no.14,pp.526557,Jul.2022,doi:https://doi.org/10.1177/0731684421105 8708

[5] O.L. Shanmugasundaram, " Green composites: manufacturing technology and application," *Indian Textile Journal (ITJ)*, vol.63,no.13-14,pp.63-69, Oct.2009

[6] R.Saberi-Riseh , M.G.Vazvani, M. Hassanisaadi, and V.K.Thakur," Agricultural wastes: A practical and potential source for the isolation and preparation of cellulose and application in agriculture and different industries," *Industrial Crops and Products,*vol.208, Article 117904, 2024

[7]H. Ahmadi, "Cellulose - Mycelia foam : novel bio-composite material,'' *Master`s Thesis, The University of British Columbia,* Vancouver,BC,Canada, 2016

[8]Y.Patel,R.Naraian, and V.K.Singh, "Medicinal properties of Pleurotus species (oyster mushroom) : a review," *World Journal of Fungal and Plant Biology*,vol.3,no.1,pp.1-12,2012.

[9] Y.Tsao, "Characterization of Mycelium Based composites as Foam - like wall Insulation Material". *Master`s Thesis, Eidhoven University of Technology*, Eindhoven, The Netherlands, pp.1-74, 2020

[10] A.Digafe, T.Mesfin and K. Ajoy " Mycelium Based Composites: The Future Sustainable Bio material" *International Journal of Biomaterials* , March 2022, doi: 10.1155 / 2022/ 8401528

[11] S. krivanek, "Fungal Mycelium ;the Key to a Sustainable Future" *University of Nebraska,*Lincoln,NE,USA,2020

[12] W.A.Elkhateeb and G.M.Daba, " The Amazing Potential of Fungi in Human Life," *ARC Journal of Pharmaceutical Sciences,*vol.5,no.3,pp. 12- 16, 2019

[13] O.P. Etinosa, Design and Testing of Mycelium Biocomposite, *African University of Science and Technology Research*, Abuja, Nigeria,PHD thesis, 2019

[14] F.V. Appels and H.A.Wosten, Mycelium Materials, *Encyclopedia of Mycology*,vol.2,pp.710-718, 2021

[15]R.Lelivelt, G.Lindner, P.Teuffel, and H.Lamers, "The production process and compressive strength of mycelium based materials,'' *in Proceedings of The First International conference on Bio-Based Building Materials*, pp.1-6, Clermont - Ferrand, France, June 2015

[16] F. Gauvin and I.J.Vette , " Characterization of Mycelium Based Composites as Foam - like wall insulation Material'', *Eindhoven University of Technology, Eindhoven, Netherlands*,2020

[17] K.Joshi, M.K.Mehar and K.M.Poluri, Fabrication and characterization of bio-blocks from agricultural waste using fungal mycelium for renewable and sustainable applications,'' *ACS applied Bio materials*, vol.3,no4,pp.1884-1892,2020

[18] D.Zou and L.Gao, " Preparation and properties of fungal mycelium based on garden waste," *Science Discovery*,vol.8, no.2,p.43,2020

[19] K. N. Bharath and S. Basavarajappa, " Applications of biocomposite material based on natural fibers from renewable resources : a review,'' *Science and Engineering of Composite Materials''* vol,23, no.2, pp 123- 133, 2016

[20] M.Akter, M H.Uddin, and I.S.Tania, " Bio-composites based on natural fibers and polymers : A review on properties and potential applications," *Science and Engineering of Composite Materials''*,vol.41,no.17-18,pp.123-133,2024.

[21]Thakur, Vijay Kumar, ed. *Green composites from natural resources*. CRC Press, 2013.

[22] R.Saberi-Riseh , M.G.Vazvani, M. Hassanisaadi, and V.K.Thakur,“ Agricultural wastes: A practical and potential source for the isolation and preparation of cellulose and application in agriculture and different industries,” *Industrial Crops and Products,*vol.208, Article 117904, 2024

[23] M. Rani et al., “Edible mushroom: occurrence, management and health benefits,” Food Materials Research, vol. 3, no. 1, p. 0, Jan. 2023, doi: 10.48130/fmr-2023-002

[24] YILDIZHAN, Şafak, et al. "Bio-composite materials: a short review of recent trends, mechanical and chemical properties, and applications." *European Mechanical Science* 2.3 , 83-91, (2018)

[25] B. Parameswaran, P.Nigam , A. Pandey, “Sugarcane Bagasse” B*iotechnology for Agro- Industrial Residues Utilization Springer, Dordrecht* 239-240, (2019).

[26] T. Tamrat, S. Bruce, R. Deresh, “ Valorisation of waste chicken feathers : Optimisation of decontamination and Pre- treatment with bleaching agents using response surface methodology”, *Sustainable Chemistry and Pharmacy*, Volume 8, Page 21-37, ISSN 2352-5541, 2018, https://doi.org/10.1016/j.scp.2018.02.003.

[27] W.Aiduang, A Chanthauck, J Kumla, K. Jatuwong, S.Srinuanpan , T.Waroonkun , R Oranratmanee, S. Lumyong , N. Suwannarach, *“Amazing fungi for Eco-Friendly Composite Materials : A Comprehensive Review : J. Fungi’’* vol 8, page 842 , 2022, https:// doi.org/10.3390/jof8080842

[28] Y. Patel, R.Naraian & V.K. Singh, *“ Medicinal Properties of Pleurotus Species (Oyster Mushroom) : A Review, World Journal of Fungal and Plant Biology*’’ 3(1), 01-12, 2012, DOI: 10.5829/I doi.wjfpb.2012.3.1.303

[29] W. Aiduang et al., “ Amazing fungi for Eco-friendly Composite Materials : *A Comprehensive Review* ,” *J Fungi,* vol. 8, no.8,p.842, Aug 2022, doi: 10.3390/jof8080842

[29] N.Majib, N.Yaacob , N.Rohaizad, W.Tan, “ Characterization of fungal Foams from Edible Mushrooms Using Different Agricultural Wastes as Substrates or Packaging Material ” , *Polymers,* 15, 873, 2023. https://doi.org/10.3390/polym15040873.

[30] Y.Patel, R.Naraian, V. Singh: “Medicinal Properties of *Pleurotus* Species (oyster mushroom) ”*: A review. World Journal of Fungal and Plant Biology*, 3(1), 01-12, 2012: Doi: 10.5829/idosi.wjfpb.2012.3.1.303

[31] R.D. Rai, B.L.Dhar, R.N.Verma " Medicinal Mushroom In: Advances in Mushroom Biology and Production , Eds" *Mushroom Society of India, National Research Centre for Mushroom, Solan-India,* pp: 355-368, 2007

[32] S.P.Wasser, A.L.Weis, "*Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: Current perspectives* Int.J.Med.Mush.,1:31-62,1999 ''

[33] S.P.Wasser,"Medicinal mushrooms as a source of anti tumour and immunomodulating polysaccharides (mini review). Appl. Microbial.Biotechnology,60: 258- 274, 2002.

[34] P.Yashvant, N.Ram,V.K.Singh , "*Pleurotus ostreatus* : an oyster mushroom with nutritional and medicinal properties" *World Journal of Fungal and Plant Biology 1-12,* doii: 10.5829/idosi.wjfpb.2012.3.1.303, 2012.

[35] D.Krishnamoorthy, M.Sankaran, "An oyster mushroom with nutritional medicinal properties'' *Biochemical Technology Society,113-198,* 24 July, 2014.

[36] L.Jiang, D.Walczyk, G.McIntyre, R.Bucinell, G.Tudryn, "Manufacturing of bio-composite sandwich structures using mycelium bound cores and preforms", *Journal of Manufacturing Processes,* 28,50-59, 2017.

[37] J. Shankar, P. Verma, M.P.Shah, " Microbial approaches for sustainable green technology'', *CRS Press ,* 2024, doi:10.1201/9781003407683