STUDIES ON UTILIZATION OF PADDY STRAW FOR COMPOST PREPARATION OF BUTTON MUSHROOM (*Agaricus bisporus*)

Thesis

by

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CERTIFICATE-I

This is to certify that the thesis titled, **“Studies on utilization of paddy straw for compost preparation of button mushroom (*Agaricus bisporus*)”** submitted in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy Plant Pathology** in the discipline of **Plant Protection** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, (Nauni) Solan (HP)- 173 230 is a bonafide research work carried out by **Ms. Rajneesh Thakur (H-2019-32-D)** daughter of Shri Ramesh Chand under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation has been fully acknowledged.

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Dated:

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CERTIFICATE-II

This is to certify that the thesis titled, **"Studies on utilization of paddy straw for compost preparation of button mushroom (*Agaricus bisporus*)"** submitted by **Ms. Rajneesh Thakur (H-2019-32-D)** daughter of Shri Ramesh Chand to the Dr. Yashwant Singh Parmar University of Horticulture and Forestry, (Nauni) Solan (HP)- 173 230 India in partial fulfilment of the requirements for the degree of **Doctor of Philosophy Plant Pathology** in the discipline of **Plant Protection** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

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Needless to say, errors and omissions are mine.

Place: Nauni, Solan
Date: **(Rajneesh Thakur)**

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ABBREVIATIONS

°C	:	degree Celsius
µl	:	Miroliter
e.g.	:	For example
cm	:	Centimeter
mg	:	Milligram
g	:	Gram
mm	:	Millimeter
kg	:	Kilogram
µM/min/ml	:	Micro mole per minute per milliliter
ml	:	Milliliter
@	:	at the rate
Fig.	:	Figure
i.e.	:	that is
et al	:	co-worker
%	:	per cent
/	:	Per
BOD	:	Biological Oxygen Demand
PDA	:	Potato Dextrose Agar
MEA	:	Malt Extract Agar
CD	:	Critical Difference
etc.	:	Et cetera
UHF	:	University of Horticulture and Forestry
Cfu	:	colony forming unit
viz.	:	vide licet (namely)
ppm	:	Parts per million

pH	:	Potential of Hydrogen
EC	:	Electric Conductivity
CRD	:	Completely Randomized Design
ITS	:	Internal transcribed spacer
deci S m-^{1}	:	Deci simens per minute
BLAST	:	Basic Local Alignment Search Tool
OD	:	Optical Density
NCBI	:	National Centre for Biotechnology Information
YpSs Agar	:	Yeast Phosphate Soluble Starch Agar

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Chapter-1

INTRODUCTION

Mushroom is a macrofungus with distinctive fruiting body that can be either epigeous (above ground) or hypogeous (underground) and large enough to be seen with the naked eye and to be picked by hand (Chang and Hudson 2001). Cultivation of edible mushrooms carries great relevance in today's world in the context of a burgeoning population growth and extreme pressure on the environment. Mushrooms have been recognized by Food and Agriculture Organization (FAO) as food item contributing to the protein nutrient to the diet of developing countries like India, where there is heavy dependence on cereal diets. Mushrooms are frequently mentioned as alternative source for food.

The major constituents of mushrooms are water, proteins, carbohydrates, fiber, fat and ash along with minerals and essential amino acids (Heleno et al. 2010 and Alispahic et al. 2015). Mushrooms are highly nutritious and environment friendly crop that carry numerous medicinal benefits (Keles et al. 2011; Verma et al. 2013 and Muszynska et al. 2017). Mushrooms are a low-cost food with essential fatty acids, vitamins and are important source of protein to fight against malnutrition (Murugkar and Subbulakshimi 2005; Kayode et al. 2015 and Han et al. 2016). They contain low fat content, high fibre, all essential amino acids and important minerals (Sadler 2003). Mushrooms have numerous bioactive compounds including polysaccharides, tri-terpenoids, glycoproteins and immunomodulating compounds (Andrade et al. 2014). Mushrooms are well known for their high medicinal properties such as anti-ageing, antiviral, anti-hypertensive, antimicrobial, antibacterial, anticancer, anti-tumor, anti-inflammatory, anti-hypertensive, immuno regulator, health booster, help balancing blood sugar and support the body's detoxification mechanisms (Patel et al. 2012). Mushrooms are rich in antioxidants, including phenolics, carotenoids, ascorbic acid, tocopherols and ergosterol (Kumari et al. 2011 and Sanchez 2017).

Mushroom helps in smooth functioning of digestive system, brain function, cell formation in the body, heart, respiratory system, nervous system, skin, liver, kidney and slows down cell maturation by eliminating free radicals from the body (Kumari and Jaiswal 2023). They are also considered to reduce cholesterol, relieve stress and help to fight against coronary heart diseases, diabetes mellitus, bacterial or fungal infections, immune system disorders and cancer (Dhamodharan and Mirunalini 2010). Mushrooms are considered to be a complete, healthy food suitable for all age groups from children to adults.

Mushroom production occupies an important position in the agricultural businesses of the world. Mushrooms have been consumed in the world for thousands of years since ancient times. Major mushroom producing countries are China, Japan, United States, Netherlands, India, Poland, Spain, Canada, United Kingdom, Iran, Russia, France, Germany, Ireland, Italy, Turkey and Australia. The first specialized commission for the development of mushroom cultivation was founded in Pennsylvania (U.S.), since referred as the "mushroom capital of the world" (Beyer 2003). Commercial cultivation of mushroom started in India in 1960, under the scheme entitled "Development of Mushroom Cultivation in Himachal Pradesh" started at Solan by H.P. Government in collaboration with ICAR, New Delhi. Thus, the city of Solan is known as "Mushroom City of India".

The global market production of mushrooms was 44.00 million tonnes in 2020 FAOSTAT 2022). China is leading in global mushroom production both in cultivation of edible and medicinal types. China produces approximately 87 per cent of world mushroom production (Singh et al. 2020). In India, the best inclination of farmers towards mushroom cultivation and business has been seen. The total production of mushroom in India was 2,42,000 tonnes in 2021-2022 (Kumari and Jaiswal 2023). The main mushroom cultivating states in India are Himachal Pradesh, Punjab, Haryana, Karnataka, Andhra Pradesh, Utter Pradesh, Tamil Nadu, Maharashtra and Bihar. According to Upadhyay et al. (2008), Himachal Pradesh is a hotspot for biodiversity and has a climate that is favorable for growing mushrooms all year around. It also has a variety of rich, wild edible mushrooms. Mushrooms appear in both toxic (toadstools) and edible (medicinal) forms.

Commonly cultivated species of mushrooms are the button mushroom (*Agaricus bisporus*), shiitake mushroom (*Lentinus edodes*), oyster mushroom (*Pleurotus ostreatus*), oriental enoki or velvet stem mushroom (*Flammulina velutipes*), paddy straw mushroom (*Volvariella volvacea*) and black ear mushroom (*Auricularia auricula*). Other cultivated mushrooms are the reishi mushroom (*Ganoderma lucidum*), nameko (*Pholiota nameko*), white jelly fungi (*Tremelia fuciformis*) and truffle (*Tuber aestivum*) (Chakravarty 2011). In India, white button mushroom share is 73 per cent followed by oyster mushroom 16 per cent, paddy straw mushroom 7 per cent and milky mushroom 3 per cent (Sharma et al. 2017).

Agaricus bisporus mushroom is a useful bio-factor for agrowaste recycling. It is environmental friendly, capable of converting the lignocellulosic waste materials into food, feed and fertilizers (Jaradat 2010). The button mushroom is grown on composted lignocellulosic substrates and a variety of raw materials have been used in composting all over the world. The raw materials can be wheat straw, paddy straw, reed plants, waste paper, oat straw, waste tea leaves and some water plants. Cellulose, hemicellulose and lignin are the major components of lignocellulosic materials. The bioconversion of lignocellulosic biomass by the mushroom industry to food and useful products is already a significant contribution to the management of agricultural and industrial wastes at regional and national levels.

World production of agricultural wastes is about 4664.63 million tonnes, of which about 600 million tonnes is produced in India. 5 per cent of the global biomass is burnt in India alone, contributing to ozone depletion (Tewari and Ahlawat 2007). It is estimated that about 15 million tonnes of paddy straw is burnt in Punjab itself. Consequently, the growing of mushrooms on paddy straw is one such avocation, which if put into practice, can not only lead in improvement of dietary and economic standards of the masses but also in combating environmental pollution due to burning of agro-waste. Moreover, paddy straw is cheaper and therefore can be profitably utilized for the mushroom production to combat the day by day increasing cost of mushroom production. Paddy straw though does not provide good physical structure to compost but gives good results when mixed with wheat straw in different ratio (Rana 1998).

Paddy straw burning increased dramatically over the last decade, despite being banned in most rice- growing countries because of pollution and the associated health issues. So, it is important to look for sustainable solutions that can reduce the environmental footprint and upgrading the value chain of rice straw byproducts by using it as compost for growing button mushroom. Thus, sustainable straw-management practices by mushroom cultivation influence farmers not to do open-field burning of paddy straw to avoid negative environmental and health consequences and also to generate income.

Keeping above in view, the use of paddy straw with wheat straw in different combinations and singly for compost preparation of button mushroom (*Agaricus bisporus*) cultivation was tried with the following objectives:

Objectives:

1. Evaluation of different paddy straw formulations for button mushroom production.
2. Evaluation of physicochemical changes in compost, their effect on productivity and quality of button mushroom.

3. Isolation and characterization of thermophillic fungi from the compost.
4. Estimation of different enzyme activity and biochemical changes during button mushroom production in different compost formulations.

Chapter-2

REVIEW OF LITERATURE

Mushroom is the fruitbody of a fungus, which is neither a plant nor an animal, but having a separate kingdom of its own. Fungi have some characteristics of plants, some of animals and some of its own when we look at its biology, physiology and phylogeny. Fungi as a broad group either live parasitically on plants and animals or live saprophytically on dead organic matter. They cause numerous diseases of plants and animals and have been reported to cause considerable crop losses with tremendous suffering to mankind from time immemorial. The role of fungi as a friend of a human being is of recent origin, with the generation of information on existence of microorganisms and their importance to man on this earth.

Mushrooms are rich in minerals as well as vitamins and nutrients (Mattila et al. 2001). Mushrooms contain a variety of bioactive compounds (Sanchez 2004) and the most well-known polysaccharides with anti-tumor and immune-modulating activities (Wasser 2002). Mushrooms are thought to be a potential replacement for muscle protein due to their high digestibility (Pavel 2009). Mushrooms are a good source of protein, vitamin D and minerals (potassium, iron, copper, zinc and manganese) as well as being low in calories, fat, cholesterol, gluten and sodium (Heleno et al. 2010 and Sharma et al. 2017).

Mushrooms are a combination food with medicinal and nutraceutical properties. Mushroom have a variety of phenolic compounds, polyketides, terpenes, steroids and many other compounds with immunomodulatory, cardiovascular, liver protective, anti-fibrotic, anti-inflammatory, anti-diabetic, anti-viral, anti-microbial activities and anti-tumor properties (Kalac 2013). The food value of mushroom is being increased because of its low calories and high nutritive value. Mushroom can serve as a protein rich food in developing countries, where the population mainly depends on cereal based foods. Further, mushrooms are great recyclers, decomposers and therefore play a significant role in the ecosystem.

Among cultivated edible mushrooms, the button mushroom *Agaricus bisporus* (Lange) Imbach is one of the most widely cultivated species and consumed worldwide (Carrasco et al. 2018a and b). Their consumption is gradually increased due to their appreciated flavor, the interesting nutritional value such as protein content similar to meat or eggs (Liu et al. 2014) and low calories content comparable to vegetables (Wani et al. 2010).

2.1 COMPOST AS SELECTIVE SUBSTRATE FOR BUTTON MUSHROOM CULTIVATION

Button mushroom (*Agaricus bisporus*) is secondary decomposer requiring selective composts for their growth. Compost is a selectively decomposed lignocellulosic substrate as results of microbial succession initiated by mesophiles, over taken by thermotolerant and finally picked by thermophilic microflora. Paddy straw, wheat straw, maize straw, cotton waste and sugarcane waste are some of the most widely used substrates for compost production. Some others are reed plants, waste paper, oat straw and waste tea leaves etc (Kumla et al. 2020). Selectivity of these composts is influenced by both biological and changes in physico-chemical characteristics during composting process.

Composting is a process of transforming organic materials of either plant or animal origin into simpler compounds (Scialabba et al. 2017). It is an aerobic process, during which organic material is biologically decomposed by microorganisms (Jurak et al. 2015). Microorganisms such as bacteria, actinomycetes and fungi produce enzymes that breakdown complex organic compounds into simpler forms and release nutrients. Composting is one of the promising technologies to treat waste in organic material into a nutrient-rich substrate in an economical manner.

2.2 THE COMPOSITION OF AGRO-INDUSTRIAL WASTES

Agro-industrial waste mainly consists of cellulose, hemicellulose and lignin, all of which are collectively defined as lignocellulosic materials that are hard to degrade (Kumla et al. 2020). Cellulose is the most abundant component, followed by hemicellulose and lignin. Cellulose and hemicellulose are sugar derivative macromolecules; whereas lignin is the aromatic polymers made from the phenylpropanoid precursors. Cellulose is (35-50%) followed by hemicellulose (20-35%) and lignin (10-25%) (Rangabhashiyam and Balasubramanian 2019). Straws and stalks are the most abundant lignocellulosic residues/by-products of lignocellulosic crops. Majority of the consumable fungi has enzymatic frameworks that can break these complex substances.

Mushrooms degrade lignocellulosic substrates through lignocellulosic enzyme production and utilize the degraded products to produce their fruiting bodies. Therefore, mushroom cultivation can be considered a biotechnological process for the reduction and valorization of agro-industrial waste. Such waste is generated as a result of the eco-friendly conversion of low-value agri by-products into new resources that can be used to produce value-added products. It is apparent from the nature of the raw materials and their heterogeneity that moisture and nitrogen contents can vary considerably. However, known or estimated values of nitrogen and water contents of the raw materials can be used by an experienced operator to adjust compost formulae to avoid gross deficiencies or excesses, especially in nitrogen content. The composting process itself, which includes physical mixing and biochemical conversions, reduces the stacked mixture to a compost of uniform quality in which the carbon: nitrogen forms the bulk of the raw materials, but alternatives, including hay, corn cobs, sugar-cane bagasse, cotton and coconut wastes are used according to local supply. Whatever the materials, the same principles apply in formulating mixtures for composting. Consideration has to be given to the dry matter, water and air content of the mixture. These factors contribute not only to the chemical requirements to initiate aerobic fermentation but also to the physical properties of the final product mushroom compost.

Straw quality affects the physical structure of a compost stack. Cultivars of wheat with thin-walled straw and a hollow centre are preferred to those with thick-walled straw the centres of which are almost entirely filled with soft tissue. These straws remain rigid and can become compacted and waterlogged, restricting ventilation and consequently reducing aerobic decomposition (Flegg and Loughton 1961). It is important to consider the cultivar of cereal straw to be composted; straw from different cultivars of winter wheat, for example, varies significantly in its biodegradability. The properties of the straw also depend on conditions at harvest; late harvesting not only reduces the yield of straw but its fibre content is increased at the expense of crude protein. Also, if baled wet, or stored in damp conditions, the straw decomposes in the bale more rapidly than normal. In practice most straw can be used for mushroom compost providing that its quality, whether used from the bale or as horse manure, is recognized and the composting procedure is adjusted accordingly. For example, straw is more easily wetted and becomes mechanically weaker as it ages.

Deep-litter chicken manure is a useful source of nitrogen but it is a bulky additive which consists of wood-shavings, faeces and feathers. The moisture content is usually not more than 30 per cent and there is about 3 per cent nitrogen in the dry matter. Proprietary compost activators, with high nitrogen content, are processed organic wastes such as sewage sludge’s, brewer’s grains, dried chicken manure and dried blood. Compounded activators may also contain soluble forms of nitrogen such as urea or minerals such as ammonium sulphate which having a high nitrogen content can raise the specification to 8 per cent of the dry matter. Also, following the work of Hayes and Randle (1968), soluble carbohydrate sources such as sugar-beet pulp or molasses are included in some proprietary activators. Other products such as cotton-seed and soya-bean meal, which are mainly used by the food industry and for animal feedstuffs, are sometimes used as compost activators. Gypsum is usually added at stacking or during the first week of composting. The original purpose of using gypsum (Pizer 1937) is to improve the physical structure of compost by flocculating colloidal particles which caused the greasiness observed in 'heavy' horse manure.

At this time the manure was composted for a longer time, without other additives, in wider stacks and turned (manually) less frequently than in modern practice. Gerrits (1977) re-examined the function of gypsum when composting mixtures of horse and chicken manure. He found that gypsum had a stabilizing effect on the ammonium content. An increased ammonium concentration was obtained in the presence of gypsum and the higher ammonium content was associated with a higher yield. Gerrits also demonstrated an interaction between ammonium, gypsum and supplementation with soya-bean meal at casing - mushroom yield being improved in the presence of gypsum. Gerrits standard rate of application was 25 kg of gypsum per tonne of horse manure but Edwards (1974) showed that even 10 kg per tonne exceeded the amount required to saturate the water present in the manure. However, the excess is apparently needed to compensate for deficiencies in mixing.

The moisture content of mushroom compost at filling should be about 70 to 75 per cent. Horse manure containing 50 per cent water at stacking, will require about 1000 litres of water per tonne of manure (Gerrits 1972). ‘Synthetic compost, based on wheat straw requires 3000 to 4000 litres of water per tonne of straw’ whether it is pre-wetted (Gerrits 1974) or added within two or three days after stacking (Randle 1974). When Phase I of composting is complete, water should not run out of the compost when it is squeezed by hand. If excess free water is present, air space is reduced and consequently air movement through the compost is impeded. The water holding capacity of the compost stack increases as composting proceeds. For example, the biomass, which is made up of microbial cells containing up to 90 per cent water accumulates and forms an increasing reservoir of bound water thus raising the water-holding capacity of the compost. Therefore, water squeezed out of compost at a given pressure is an indication of its water holding capacity rather than the total water content.

2.3 COMPOSTING PROCESS

The purpose of composting is to prepare a substrate of such characteristics that the growth of mushroom mycelium is promoted to the practical exclusion of other microorganisms (Till 1962). During composting certain physical qualities and chemical properties are developed, all of which are important and interdependent. The physical qualities of the substrate are that it must be freely permeable to air, hold water without becoming waterlogged and have the appropriate pH. The chemical state of the compost must be such that the materials required to provide the nutrient needs of the mushroom are accumulated and are readily available. The accumulation of substances such as ammonia, capable of inhibiting mushroom mycelial growth, must be avoided. Composting is achieved by manipulating the natural succession of microorganisms present in the raw materials. During the process the chemical composition and physical state of the substrate change and the species of micro-organisms involved also change.

Although mushrooms have been regarded as a delicacy for many centuries, cultivation was not attempted until the seventeenth century. Possibly the earliest known reference to compost preparation and mushroom cultivation can be attributed to Tournefort (1707) at the Royal Academy of Science, France. At that time, the knowledge of composting was extremely vague and emphasis was directed towards the design and preparation of horse-manure beds for growing the mushrooms rather than to the composting procedure. By the late eighteenth century, composting methods were beginning to resemble the methods of today. Abercrombie (1779) described a method of composting stable manure in stacks about 16 meter long, 1.5 meter wide and 1.5 meter high. He also emphasized the need to mix and wet the composting ingredients every other day in order to achieve a uniform substrate on which to cultivate the mushroom. Callow (1831) realizing that compost variability was related to weather conditions was possibly the first to suggest that fermentation of stable manure stacks should be pursued under covered barns or sheds especially during the winter. Very little advance in composting methods and techniques were made until the start of 20th century when a considerable amount of experimentation was done by growers and research workers in an attempt to achieve predictable mushroom crops. The mushroom industry was becoming well established in France, England and the United States, so the change from horses to motor vehicles in the 1920s presented a threat to mushroom growing and stimulated work by Waksman and Reneger (1934); Sinden (1938); Lambert (1941); Stoller (1943) and Edwards (1949) to look for alternative ingredients suitable for composting. Although research workers approached the problem in different ways and employed various ingredients, similar conclusions were reached. Mushrooms could be grown successfully on so-called synthetic composts prepared from mixtures containing wheat straw as the basic component with additional carbon sources such as corn cobs or brewers grain and nitrogen sources such as ammonium nitrate, urea or calcium cyanamide. It was shown that there was nothing unique in horse manure that could not be provided by other suitable raw materials. With this important advance came the realization that wheat straw was an important basic constituent as a carbon source and not, as many considered, necessary only for its physical effect on water-holding capacity and aeration. Around 1915, growers in the United States introduced a second stage or phase of composting which they called 'sweating-out'. Composts were allowed to complete thermogenesis in shelved compost beds within the growing house prior to spawning. As temperatures were attained around 60°C (140°F), insect pest and troublesome moulds were killed or reduced to a low level, and generally composts treated in this manner gave more consistent yields. Although the ‘sweating-out process’ became standard, its significance in producing homogeneity in composts were not fully understood until Lambert (1941) questioned the major physical and environmental variables associated with composting. Lambert identified the main regions in the compost stack where different conditions existed and showed that composts most suitable for mushroom culture came from parts where the temperature had been maintained between 50 and 60°C (122 and 140°F) and where there was adequate aeration. Compost from an aerobic zone, if isolated and subjected to further fermentation within this temperature range under aerobic conditions, became more productive. Lambert therefore suggested that ‘sweating-out’ should be regarded as an integral part of composting by “conditioning” the compost for mushroom growing, and not merely a means to eradicate insects and competing fungi. This part of composting was later to become known as the ‘pasteurization’ or ‘peak-heat’ period, also termed Phase II of composting.

Aware of the dwindling manure supplies in the early 1940s, Sinden and Hauser (1950) introduced the so-called ‘short method’ of composting. The early part of composting in the yard (termed Phase I) was reduced from 3 weeks or more to 7-14 days. Yield measured in weight of mushrooms harvested per unit area of bed reached its maximum after only 11 days of Phase I composting when little decomposition of the material had occurred. Preparing composts by the shortened method not only made efficient use of the then limited supply of horse manure but also saved time, space and labor. Sinden and Hauser (1953) emphasized that Phase I and Phase II composting were related parts of the process dependent on each other, but, at the same time, differing in nature and function, thus agreeing with Lambert. This short method of compost preparation has become the most popular method employed by mushroom growers throughout the world.

The early work on chemical changes in the farmyard heap helped to give a basis to the scientific understanding of the preparation of composts suitable for mushroom cultivation. Hebert (1892) claimed that during the aerobic decomposition of straw and stable manure at high temperatures, about 50 per cent of the total organic matter was lost. The large losses were attributed mainly to cellulose degradation; losses of lignin were small. The extensive synthesis of microbial protein from simple nitrogen compounds in manure was recognized by Deherain (1889) and confirmed by Hebert (1892). Both workers suggested that during composting a protein-lignin complex accumulated which was resistant to further microbial attack. Although composting of a nutrient substrate to produce a selective medium on which growing of mushroom had been practiced for more than two centuries (Tournefort 1707) the processes involved remained unclear.

2.3.1 Formulating mixtures for composting

To standardize composting time, initiate fermentation and minimize loss of dry matter during composting about 1.5 per cent nitrogen is required in the dry matter of the raw materials (Sinden and Hauser 1950; 1953). The main bulky ingredients, horse manure and/or straw have low nitrogen content, usually less than 1 per cent, therefore materials with a higher content of nitrogen have to be added to raise the nitrogen content of the mixture to a satisfactory level. The five formulations have been calculated to give mixtures with 1.5 per cent nitrogen in the dry matter, based on 1 tonne of horse manure and/or straw as the main bulky ingredient. Formulation 1 shows that horse manure with deep litter chicken manure can provide a satisfactory mixture for composting. Alternatively, as in formulation 2 and 3, activators with specified nitrogen contents may be used to adjust the amount of nitrogen in the mixture. Formulation 4 shows that fresh straw can be added to ‘heavy’ horse manure to improve its physical structure and to obtain the required nitrogen content and formulation 5 as a control. Also, other farmyard manures which are too ‘heavy’ for use alone can be used in this way.

Synthetic compost is the name given to compost prepared without horse manure in which organic, mineral fertilizers and even other manures are added to cereal straw. In earlier formulae (Demelon et al. 1937; Stoller 1943 and Edwards 1950) emphasis was placed on mineral additions. More recently (Overstijns 1964; Hayes and Randle 1969; Gerrits 1974 and Randle 1974) the quality of the organic materials has been emphasized. Generally, minerals are not limiting either for satisfactory composting or for subsequent mushroom nutrition.

Synthetic mixture which includes an activator with soluble carbohydrate to promote the development of thermophilic bacteria in the stack (Hayes and Randle 1969). The disadvantages of preparing synthetic compost from fresh straw are that (apart from cost) the material is difficult to wet and normally requires chopping or crushing to assist the pre-wetting process. However, synthetic mixtures are easier to formulate, and are advantageous when the supply and quality of horse manure are unreliable and also when relatively small quantities of compost are being made. The usual variation in ratio inevitably lies within certain limits as shown by Burrows (1951) and Gerrits et al. (1967). Consequently, there is less variation between successive composts at spawning than between the mixtures of raw materials from which they were derived.

Formulation affects the time taken to prepare the compost and the yield of compost from the raw materials. Composting experiments by Flegg and Randle (1980 and 1981) have shown that as the initial nitrogen content increases as does the time required to complete the composting process. The short method of Sinden and Hauser (1950; 1953) has become the normal method of composting and is the basis of most mushroom composting procedures today. Phase I takes 7 to 10 days followed by 5 to 7 days for Phase II. Because of losses during composting, generally 1.5 tonnes of prepared synthetic compost can be obtained from 1 tonne fresh weight of starting materials (Flegg and Randle 1980) and probably about the same for horse manure compost. As decomposition proceeds microbial biomass accumulates; this protein has been shown to be important in mushroom nutrition (Eddy and Jacobs 1976; Fermor and Wood 1981; Sparling et al. 1982).

Rapid composting methods (Laborde and Delmas 1969; Smith 1978 and 1990) can give substrates selective for mushroom growth. Smith (1978) composted mixtures in which the initial nitrogen content was less than 1.5 per cent and found that with reduced decomposition the yield of compost was about 2 tonnes of prepared substrate per tonne of fresh weight starting materials. However, mushroom yields tended to be lower than from normal compost and this may be due to reduced microbial protein in ‘rapid’ composts. When nitrogen exceeds 2 per cent in the initial mixture prolonged and uncertain composting times with high dry matter loss may follow. The high nitrogen content induces production of free ammonia which may be difficult to eliminate in the conditioning period following ‘peak-heat’. Flegg and Randle (1981) have developed a unifying concept based on the relative durations of Phases I and II, which can be used as an aid to remedying mistakes in compost preparation, particularly in adjusting the durations of Phases I and II in relation to the initial nitrogen content of the stack. All composting procedures can be classified on the basis of the duration of Phases I and II. Those composts requiring only a short period (Phase I and Phase II) to prepare result in greater conservation of raw materials than those requiring longer periods to make. Also, the optimum nitrogen content (as per cent of the dry matter) at the start of composting can be lower with shorter duration required to complete the composting process. Similarly, the final bulk density compost production is the most important and integral part of *Agaricus bisporus* (white button mushroom) cultivation. It is a product of fermentation brought about by the variety of organisms including bacteria, actinomycetes and fungi. These organisms convert and degrade the straw to form lignin humus complex and also convert soluble form of nitrogen into microbial cell substances (Waksman and Cordon 1939; Waksman et al. 1939a and Waksman et al. 1939b). This decomposed straw along with microbial biomass both become a source of organic and inorganic nutrition for the mushroom mycelium (Wood and Fermor 1981). The process of composting is governed by a carefully ordered changing population of organisms (Chang and Hudson 2001). Further, these mycoflora also play a key role towards selectivity and conditioning of the compost and make the growth of competitor microorganisms more difficult (Ross and Harris 1983). Compost if properly prepared, *A. bisporus* can only successfully grow in it at the practical exclusion of the competing organisms. Various kinds of flora encountered during whole process of composting have different role to play. Mesophilc flora present in the initial process of composting bring about the biodegradation of the straw and other ingredients which results in heat energy resulting in establishment of thermophilic flora in the compost which later on govern the whole process of composting.

In short method of composting, the process is conducted in two distinct phases, i.e., phase-I and phase-II (Sinden and Hauser 1950). In phase-I, after pre-wetting and mixing of raw material, a heap is made to initiate the quick fermentation process. The stacks are made in open or under open-sided compost sheds. Turning is given to the substrate to aerate the pile. The temperature of the central portion of stack reaches 65-70°C, enough to kill most of the pests and competitor/ parasitic moulds. However, the outer layers of pile do not attain such temperature. This phase lasts for 8-10 days after pre-wetting. On fourth turning, compost is filled in pasteurization chamber for phase II. Phase-II composting is referred as ‘peak-heating’ or ‘pasteurization’. The composting process is carried out under controlled environmental conditions and continued to make the suitable for the growth of mushroom mycelium. This phase normally lasts for 6-8 days. After about 12-15 hours of compost filling in chamber, the temperature started rising automatically and when it reached at 48-50ºC, it is maintained for 36-40 hours with the manipulation of ventilation system. This temperature range is achieved by self generation of heat by the compost mass and steam injection. Temperature is raised to 58-59ºC by injecting the live steam in the pasteurization chamber and it is maintained for 5-6 hours to ensure proper pasteurization. After that conditioning of compost takes place by introducing fresh air in chamber till the compost cooled down to 25ºC, a favorable temperature for spawning. After that compost ready for the spawning was done. Spawning is done in bags and placed the compost bags after spawning in cropping rooms for spawn runs at 20-25 °C in dark conditions. White button mushroom requires 22-25℃ for vegetative growth (spawn run) and 14-18℃ for reproductive growth. Besides that, it requires relative humidity of 85-90 per cent, CO_2 concentration (>10000 ppm) for spawn run while for fruit body initiation, it requires 80-85 per cent RH and <1000 ppm CO_2. Maintaining these conditions, pin heads start growing, which gradually develop into button stage.

2.4 UTILIZATION OF AGRO-RESIDUES IN COMPOST PREPARATION FOR BUTTON MUSHROOM CULTIVATION

Compost plays a comprehensive and important role in mushroom production like soil does in higher plants (Sharma 1991). Composted substrates exhibit extreme variability in the yield of button mushrooms, highlighting the importance of numerous physicochemical parameters and substrate compositional changes. These factors have a significant impact on the composting process and consequently on production. It has been estimated that around 50 per cent of the costs associated with growing of *Agaricus bisporus* are related to the production of the compost (Royse and Chalupa 2009; Royse 2010). In India, mainly four agricultural crops (maize, wheat, rice and sugarcane) are responsible for producing the maximum amount of lignocellulosic biomass in the agriculture sector. About 620 million tonnes of agricultural waste is produced in India every year, of which 50 per cent is produced form rice, wheat and oilseed (Kumari and Jaiswal 2023). Rice straw represents one of the most prominent lignocellulosic waste materials that include the leftover residue (stems, leaf sheaths, blades and panicle remains after threshing).

Farmers burn the agricultural residues, produced by the harvesting of paddy, directly in the field, due to which a huge amount of carbon dioxide, methane and carbon monoxide are released in the atmosphere. These green house gases cause severe air pollution and have a direct effect on global warming (Sharma et al. 2010 and Abdurrahman et al. 2020). One of the most promising approaches for treating garbage more cheaply is composting. Composting has been used to recycle organic materials by transforming the organic matter into a valuable product that is rich in nutrients (Sayara et al. 2020). Consequently, the growing of mushrooms on paddy straw is one such avocation, which if put into practice, can not only lead in improvement of dietary and economic standards of the masses, but also in combating environmental pollution due to burning of agro-waste (Kaur et al. 2019).

2.5 DIFFERENT COMBINATION USED IN COMPOST PREPARATION FOR BUTTON MUSHROOM

Agaricus bisporus can be cultivated on various lignocellulosic materials. Paddy straw, wheat straw, maize straw, cotton waste and sugarcane waste are the most widely used substrates (Martinez Carrera 1989 and Pabhabraom et al. 2007). These agricultural residues can either be used alone or in a combination of several residues to enrich the nutrients required for mushroom growth (Gowda and Manvi 2019). Shandilya (1989) studied paddy straw compost formulations for growing button mushrooms and also compared it with traditional wheat straw-based compost. Tewari and Sohi (1976) used paddy straw and maize stalks (1:1 w/w) as a substitute for wheat straw for the cultivation of *Agaricus bisporus*. They found that synthetic compost prepared out of maize stalks and paddy straw with other ingredients *viz*., ammonium sulphate, super sulphate, urea, chalk, gypsum and rice bran as a good medium for mushroom cultivation giving an average yield of 145.5 kg/tonne of compost. Mohan et al. (2014) made compost by using sugarcane trash, wheat straw and paddy straw substrate in different combinations i.e., wheat straw + sugarcane trash (3:1 w/w) and for paddy straw + sugarcane garbage (3:1 w/w). Singh (1991) also used sugarcane bagasse along with various amounts of wheat straw and paddy straw for making compost.

Two formulations of compost with wheat straw+ paddy straw (1:1 w/w) and wheat straw+ paddy straw (1:2 w/w) have been prescribed by Punjab Agricultural University for the growers of Punjab (Khanna and Kapoor 2016). Rana (1998) additionally demonstrated that paddy straw does not give great physical structure to compost but rather gave a decent outcome when blended with wheat straw in equal amounts. Kaur and Khanna (2001) assessed two synthetic compost preparations wheat straw+ paddy straw (1:1) and wheat straw+ paddy straw (1:2) producing a yield in the range of 17.9-23.7 kg/100kg compost. Garg (2014) evaluated compost made from wheat straw and maize stalks in different ratios (1:1 and 2:1) and compared it to conventional compost made from wheat straw for the growth of *Agaricus bisporus.* Khanna and Kapoor (2016) evaluated compost formulations containing wheat straw: paddy straw (1:1 w/w) and wheat straw: paddy straw (1:2 w/w). Kaur et al. (2019) evaluated composts made from paddy straw and maize stalks (1:1 w/w), paddy straw and maize stalk (2:1 w/w) and paddy straw alone. Uddin et al. (2012) evaluated wheat+ paddy straw compost (1:1) along with paddy straw-based compost for production of *Agaricus bisporus*.

2.6 PHYSICO-CHEMICAL PARAMETERS OF COMPOST FOR GROWTH AND FRUCTIFICATION OF BUTTON MUSHROOM

Composting is a natural process that converts organic material into a nutrient-rich substrate (Sunar et al. 2009 and Karthika et al. 2022). Composting is an environmentally acceptable method that turns agro-waste and organic residues of animal origin into suitable materials for re-utilization. It is an aerobic biological process which uses naturally occurring microorganisms to convert biodegradable organic matter into humus like substance (Van Elsas et al. 2017). The process destroys pathogens, converts N from unstable ammonia to stable forms, reduces the volume of waste and improves the nature of the waste (Imbeah 1998). Composting is the decomposition of organic wastes in the presence of oxygen; produce from this process includes CO_2, NH_3, water and heat. Effective composting requires right blend of ingredients and conditions (Gonawala and Jardosh 2017). Composting is influenced by a variety of parameters, including temperature, oxygen supply (i.e., aeration), moisture, organic matter, carbon content, pH and C: N ratio. Beneficial microorganisms have a definite impact on the composting process since they are crucial for maintaining ideal composting conditions (Jusoh et al. 2013).

2.6.1. Moisture content

In composting, moisture content is a crucial ecological factor because it provides a medium for the disintegrated nutrients needed for microorganisms ‘physiological and metabolic activities’ (McCartney and Tingley 1998). Moisture content affects microbial activity, as well as the physical structure, in the composting process, and thus has a central influence on the biodegradation of organic materials. Very low moisture content values would cause early dehydration of composting material during composting, which will arrest the biological process, thus giving physically stable but biologically unstable composts (De-Bertoldi et al. 1983). On the other hand, anaerobic conditions brought on by water logging may result from high dampness (moisture), which will halt and stop the current composting activity (Schulze 1962 and Tiquia et al. 1996). The ideal moisture content for natural compost is between 65 and 67 per cent, but it is between 68 and 72 per cent for synthetic compost. If the moisture content of the final compost is more than 72 per cent, the empty space will be taken up by water and effective air circulation might not be possible. Under these circumstances, the anaerobic state may prevail and destroy the *Agaricus bisporus* mycelium while the compost may favour moulds like white plaster (*Scopulariopsis fimicola*) and brown plaster mould (*Papulospora byssina*).

During composting, an extensive amount of water can evaporate in order to control the excessive temperature resulting in low water content. This reduces the rate of decomposition of compost raw material. At that point, rewetting ought to be required in order to maintain the optimum moisture content for the microbial movement (Bernal et al. 2009). Water supply to mushroom during the fruit body growth extraordinarily impacts the quality and quantity of harvest and amount of water extracted from the casing soil was corresponding to the weight of the fruit bodies (Kalberer 1990). The fruit bodies take 54- 83 per cent of the water from the substrate and 17-46 per cent from the casing soil, which causes decrease in moisture content of the substrate and the casing soil during the growth of solid flush (Kalberer 1991). Kaur and Khanna (2001) reported the moisture content of three compost formulations namely, wheat straw + paddy straw (1:1 w/w), wheat straw + paddy straw (1:2 w/w) and paddy straw alone to range between 59-72 per cent. According to Almomany and Masaed (2019) the moisture content of compost ranged between 50-72 per cent, and it decreased during the crop cycle as the mushroom mycelium consumed water from the compost and casing layer, with some fluctuations as a result of irrigation process. On the other hand, the dry matter decreased as a result of degradation and utilization of organic matter by the microorganisms.

2.6.2 Temperature

Temperature is an important indicator of the composting process (Liang et al. 2003) and plays a selective role in the succession of microbial communities (Zhou et al. 2019). Composting process can be basically divided into four phases, (a) Mesophilic or initial low active phase during which the readily available constituents are used up by mesophilic microorganisms (20-35°C) leading to rise in temperature, (b) Thermophilic phase in which the temperature is high encouraging thermophilic microorganisms that are medium-temperature loving (45-70°C) and degrade more complex constituents of organic matter (maximum decomposition takes place in this phase) (c) Cooling phase where the mesophilic phase with mesophilic microorganisms is restored (d) Maturity or stabilization phase with least microbial activity as most of the microbially degradable substances have already been utilized by the microorganisms. Microorganisms derive their energy and carbon requirement from the decomposition of organic residues (Maheshwari et al. 2000 and Zhang et al. 2022). When organic material is mixed for composting some of the energy released by the breakdown of the material causes a rise in temperature (Smith and Collins 2007; Sinha et al. 2020). With the ample availability of oxygen, composting material breaks down quickly and passes through a temperature peak. Kaur and Khanna (2001) tested four composts *viz*., wheat straw, wheat straw + paddy straw (1:1 w/w), wheat straw + paddy straw (1:2 w/w) and paddy straw alone and observed that all formulations reached a maximum temperature of 67- 73°C during the composting process. In all the composts, it was observed that the centre zone's temperature was greater than the top and bottom zones. In case of the compost made from wheat straw + paddy straw (1:2 w/w), the central zone recorded the highest temperature (73°C). In case of composted wheat straw highest temperature ever measured was 69°C. The maximum temperature in the other two composts was roughly 67°C.

Miller (1992) concluded that increase in temperature within the composting materials as a component of initial temperature, metabolic heat evolution and heat conservation contributes substantially to decomposition during composting. The achievement of minimum temperature levels is essential to an effective composting process (Finstein and Morris 1975). According to Mosher and Anderson (1977) temperature of composting material below 20ºC lead to significantly slow or even stop the composting process. Temperatures in excess of 60ºC have also been shown to reduce the activity of the microbial community and above this temperature, microbial activity declines as the thermophilic optimum of microorganisms are surpassed. If the temperatures reach 82ºC, the microbial community is severely impeded (Fermor et al. 1989). Mc Kinley et al. (1985) reported that small variations in temperature can affect microbial activity and biomass in composting much more dramatically than small changes in moisture, pH, organic matter or C/N ratio.

2.6.3 Electrical conductivity (EC)

EC reflects the total salt concentration of the compost (Gao et al. 2010) which affects the mushroom yield. Cation exchange capacity of a material may determine its suitability as a casing medium (Stoller 1952). EC Value of the compost lower in the mesophilic phase. This also implied that a large amount of NH_3 was released during the mesophilic phase. In the thermophilic phase, the EC value increased rapidly may be resulted from the release of mineral salts due to the degradation of organic matter. At the maturation phase, microorganisms use lignin core, fulvic acid and amino acids as raw materials to synthesize macromolecular humus, resulting in the decrease of EC value. At the end of composting the EC value was lower, indicating that the composting quality was better (Zhang et al. 2022).

Addition of soluble salts to the casing layer affects the mushroom fruiting and these effects are related to the electrical conductivity irrespective of the salt used. Also, the high levels of soluble salts in the casing are detrimental to fruit body formation. With the progression of crop ions/soluble salts accumulate in the casing layer which ultimately affects the electrical conductivity of the casing soil (Yeo and Hayes 1979; Shandilya and Hayes 1987; Shandilya 1989). However, according to Hayes (1981) a good casing should have low availability of soluble inorganic ions. Shandilya and Agarwal (1983) obtained higher yields of *Agaricus bisporus* with the ageing of casing and salt concentration was one of the important chemical factors responsible for increased mushroom yield. According to Shandilya (1989) calcium, potassium, sodium, chloride and sulphate ions showed increased levels in casing layer with the crop progression and are the main ions responsible for increase in the salinity of casing media. A delay in the initiation of fruit bodies and decrease in the yield has been reported by addition of cobalt chloride to casing (Kurtzman 1995).

According to Singh et al. (2000) electrical conductivity plays an important role in the production of *Agaricus bisporus*, but it is not the sole controlling factor. Shandilya and Hayes (1987) reported a decrease in the number of pin heads with an increase in the conductivity. De Ger (2000) also suggested that addition of salts led to increase in the electrical conductivity value of the casing soil making it more difficult for mushroom to extract water from the soil. He also stated that this resulted in a firm mushroom with a high dry matter content and whiter color. He reported that an electrical conductivity value of more than 7 to 9 mho had a negative influence on the quality and especially quantity of mushrooms. Jarial and Shandilya (2004) also reported a negative correlation between electrical conductivity and mushroom yield indicating a decrease of 0.02 units in mushroom yield with every unit increase in electrical conductivity.

2.6.4 pH

The early stages of the composting process involved the generation of organic acids, which caused the pH to become acidic. The medium becomes more alkaline during the thermophilic phase as a result of the conversion of ammonium into ammonium hydroxide and the pH eventually stabilizes at values that are close to neutral (Rich et al. 2018). Each group of microorganisms has an ideal pH range for development and multiplication, which affect their survival. Whereas, most fungal activity takes place between pH 5.5 and 8.0, most bacterial activity happens between pH 6.0 and 7.5. The best range is between 5.8 and 7.2 (Dalzell et al. 1987). Kaur and Khanna (2001) demonstrated the pH range of 6.9–8.3 for compost formulations, i.e., wheat straw, wheat straw + paddy straw (1:1 w/w), wheat straw + paddy straw (1:2 w/w) and paddy straw alone. The pH profiles of all four composts showed a set pattern during composting. It was low towards the beginning, increased steadily in the middle of the turning, and afterward settled towards the end of composting. The pH of wheat straw compost was most reduced (6.9) towards the end of composting, and the pH of paddy straw compost was most astounding (8.3). *Agaricus bisporus* mycelium grows most effectively in the pH range of 7.2 to 7.8. However, at low pH, white plaster mould (*Scopulariopsis fimicola* and *Scopulariopsis brevicaulis*) may attack the compost and *Agaricus bisporus* development will be moderate. The pH of casing media anyway diminishes with the progress of the crop because of accumulation of different salts (Shandilya and Hayes 1987).

2.6.5 Role of carbon in mushroom production

During the thermophilic phase, thermophilic microorganisms began to decompose lignocellulose and to multiply (Bhattacharya and Pletschke 2014) which produced a large amount of CO_2 and assimilated protein, resulting in the rapid decline of total carbon content and the rapid rise of total nitrogen. Therefore, the thermophilic phase is the main phase of lignocellulose degradation (Zhsang et al. 2022). During spawn run time of compost, lingo-protein complex made during composting are degraded by the extracellular enzymes such as lignin peroxidases, manganese peroxidase and versatile peroxidases (Anastasi et al. 2009 and Anasonye et al. 2015). During the mushroom farming process, carbon, mainly microbial carbon, is transferred from the growth substrate to casing soil through physical processes such as capillarity and rate of water evaporation from casing soil (Major et al. 2010). Carbon provides both an energy source and the basic building block making up about 50 percent of the mass of microbial cells. Metabolites of carbon sources travel through primary pathways: 1) conversion of mycelium biomass and formation of fruiting bodies; 2) carbon dioxide emissions through the respiration of mushroom mycelia and other microbes (Hu et al. 2021).

2.6.6 Role of nitrogen in mushroom production

Nitrogen is a major component of the proteins, nucleic acids, amino acids, enzymes and co-enzymes necessary for cell growth and function (Upadhyay et al. 2002). Since the mode of nutrition of mushroom is by assimilation (Ghosal et al. 2016), increased protein level of fruiting bodies can be achieved by using substrates rich in nitrogen and low in carbon contents. Organic sources of nitrogen can be easily used by fungi because the absorption of these molecules is more energetically efficient than synthesizing the molecules, which allow the fungi to obtain more energy for mycelia growth and mushroom formation (Nunes et al. 2012). Total nitrogen content lower in initial phase (day 0-2) of compost preparation due to the release of ammonia and heat from degradable small molecules decomposition (Yan et al. 2019) and increased rapidly in thermophilic phase (day 2 to 15) indicated continuous assimilation of nitrogen and decomposition of organic carbon. N level gradually decreased in the cooling phase (day 15-18) and then stabilized in the maturation phase (day 18-21) (Zhang et al. 2022).

2.6.7 C: N ratio

The process of composting is aimed to convert complex carbohydrate sources in to proteins in presence of nitrogen. Hence the C: N ratio plays a key role in composting process. The ideal C/N ratio for composting is generally considered to be around 30:1, or 30 parts of carbon for each part of nitrogen by weight at the initial phases of composting. Later, the C: N ratio is reduced to 18:1 due to emission of CO_2 through microbial respiration during the composting. However, combination of agriculture ingredients plays a major role in balancing the C: N ratio and proper degradation. Pace et al. (1995) reported that the organic material consisting of 20-30 parts of carbon and one part of nitrogen is good for composting. To guarantee a good composting process, the starting mixture has to be an adequate C: N ratio between 25-35:1 (Alsanius et al. 2016). Breakdown of organic residues serves as the source of microorganism’s energy and carbon needs. Nitrogen is needed to build up the protoplasm of the cell for every ten parts of carbon. Fungi are highly efficient in carbon assimilation as compared to bacteria and actinomycetes.

One of the key elements that can impact composting is the C/N ratio (Onwosi et al. 2017). A low C/N ratio can result in high NH_3 emissions, where as a high C/N ratio can make this process very slow due to an abundance of degradable organic material (Bernal et al. 2009). It is generally recognized that a C/N ratio between 15:1 and 30:1 particularly is ideal for composting (Guo et al. 2012). It is crucial to maintain a balanced ratio of carbon to nitrogen (C: N) in the mushroom mycelium (Gea et al. 2009). Demirer et al. (2005) opined that to improve the C: N and to accelerate composting process, all substrate formulae require the addition of nitrogen-rich supplements at the beginning of composting. Pandey et al. (2009) studied impact of bioaugmentation and nitrogen supplementation on composting of paddy straw and showed that the compost prepared from bioaugmented paddy straw with poultry manure accomplished alluring C: N of 21:1 in one month and proved to be perfect for mushroom cultivation.

According to Kaur and Khanna (2001) the final C: N ratio for compost made from wheat and paddy straw mixtures in the ratios of 1:1 (w/w), 1:2 (w/w) and paddy straw alone ranged from 16.4 to 20.3:1. Additionally, the carbon: nitrogen (C/N) ratio and these parameters are equally crucial to the development of the many microorganisms that live in compost, causing rapid degradation and favoring the growth of *Agaricus bisporus* in the compost (Levanon et al. 1983; Kaul and Khanna 2001). According to Andrade et al. (2014) optimal C: N ratio for *Agaricus bisporus* compost should range from 15 to 21:1 and not exceed 30:1. The abundance or absence of N content in the substrate might be a restricting element for fungus growth. According to Rajarathnam et al. (1989) when N is added in excess, substratum degradation is reduced. There is also a wide range of N content and C: N ratios in agricultural or agro-industrial by-product substrates. C: N ratio of 33:1 during fermentation, 18:1 during the formation of the mycelia, and 14:1 during fructification has been standardized to produce quality mushrooms with the appropriate yield (Randle and Flegg 1985; Zheng et al. 2018).

2.6.8 Organic matter

Organic matter quality is largely determined by the quality of humic acids, which constitute one of its major fractions in the compost (Canellas et al. 2010; Lanyi 2010 and Weber et al. 2018). Formation of the humic substances results from transformation and decomposition processes, which are collectively called ‘humification’. Organic matter content averaged 25.86 per cent (wet weight) or 60.97 per cent (dry weight). Organic matter in mushroom compost consists of decomposed plant, animal and fungal residues materials and is often recommended for use in land reclamation or soil remediation (Rupert 1995) as well as plant production (Davis et al. 2006).

Organic matter content in compost varied between 31.65 to 24.49 per cent and carbon 39.71 to 17.81per cent (wet weight basis). Organic matter and carbon are also reported to be essential for enhancement of agriculture production (Davis et al. 2006 and Sinha et al. 2020). The organic matter content of compost ranged between 50- 69 per cent (dry weight basis) and it decreased with the time as a result of degradation and carbon utilization by the mushroom mycelium during growth. On the other hand, ash content remained constant or increased during the mushroom growth and it affected yield and productivity indirectly, because when the organic matter increased, the porosity increased while the bulk density decreased (Almomany and Masaed 2019).

2.6.9 Changes in lignocellulosic fractions of growing substrates

Lignocellulosic substrate is comprised of cellulose, hemicellulose, and lignin. Mushrooms seem to be the most important players in recalcitrant lignin degradation by producing both hydrolytic and oxidative enzymes. This lignocellulose degrading ability of the fungi can be attributed to their highly well-organized enzymatic system. There are two types of extracellular enzyme system, one which produces hydrolases for the degradation of polysaccharides and another one a unique extracellular and oxidative liginolytic system, which cleaves open phenyl rings and thus degrades lignin (Sanchez 2009). Hydrolytic enzymes (cellulases and hemicellulases) are known to be responsible for polysaccharide degradation, while oxidative enzymes (ligninases) are responsible for lignin modification and degradation. Cellulose and hemicellulose are carbohydrates that act as carbon sources. Lignin provides carbon that is used by mycelium. Ultimately, lignin is converted into a nitrogen-rich lignin–humus complex (Wang et al. 2016).

2.6.10 Effect of bulk density and porosity of compost on yield and productivity of mushroom (*Agaricus bisporus*)

The bulk density of the compost ranged between 0.58 g/cm^3 at the beginning of the composting process and 0.78 g/cm^3 at the end of the crop cycle. It was noticed that the bulk density increased as the mushroom mycelium grown. In contrast, the porosity of the compost decreased with time and it ranged between 96 to 87 per cent, and it was noticed that when bulk density increased the porosity decreased, and vice versa. The bulk density means the weight per unit volume of compost, whereas the porosity means the percentage of pore space inside the compost particles and these two parameters affects yield and productivity significantly. Although it was noticed that when the porosity increased (less bulk density), the yield increased and when the porosity decreased (high bulk density) the yield decreased (Almomany and Masaed 2019).

2.6.11 Macronutrients and micronutrients in the button mushroom compost

The composting process depends upon the activity of microorganisms, which require a source of carbon to provide energy and material for new cells, together with a supply of nitrogen for cell proteins. C and N are the two main macronutrients required by fungi for structural and energy requirements; P, K and Mg are also considered macronutrients for mushrooms. In addition, trace elements such as Fe, Se, Zn, Mn, Cu and Mo appear to be needed for diverse functions (Chang and Miles 2004).

Desirable ratio of carbon to nitrogen in compost is in the range of 25 to 35/1. In general, composts have low nitrogen (N) content, typically in the 1 to 3 per cent range (Acquaah 2009). Average phosphorus (P) content was 0.29 per cent (wet weight) or 0.69 per cent (dry weight). Average potassium (K) content was 1.04 per cent (wet weight) or 2.44 per cent (dry weight). Micronutrients must be present for optimum growths and required in small amounts (Sinha et al. 2020). On an average, fresh mushroom compost contains the secondary macronutrients calcium (Ca) at 2.32 per cent (wet weight) or 5.38 per cent (dry weight), magnesium (Mg) at 0.36 per cent (wet weight) or 0.83 per cent (dry weight), and sulphur (S) at 0.86 per cent (wet weight) or 2.02 per cent (dry weight) (Wuest 1982 and Beyer 2003). The micronutrients such as iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), sodium (Na) and aluminum (Al) were detected in fresh mushroom compost at a very low average range of <0.01to 0.18 per cent (wet weight) or 0.02 to 0.43 per cent (dry weight) (Wuest and Fahy 1992).

2.6.12 Microflora of the compost

Compost is the end product of fermentation carried out by a range of organisms, including bacteria, actinomycetes and fungi. These organisms transform and decompose the straw to create lignin humus complex and also transform the nitrogen into microbial cell components (Waksman and Cordon 1939). Composting is managed by a carefully ordered, ever-changing population of micro-organisms (Chang and Hudson 2001). Further, this microflora additionally assumes a key role in the selectivity and conditioning of the compost and makes the growth of competitor microorganisms more difficult (Ross and Harris 1983). The presence of a microbial population in both compost and casing soil plays a vital role in *Agaricus bisporus* production. A diverse population of bacteria and fungi are involved throughout the production of Agaricus. A range of successional taxa convert the wheat straw into compost in the thermophilic composting process (Kertesz and Thai 2018). In mushroom crop production, bacterial community increases with every step of mushroom cultivation compare to fungal community in mushroom cropping process (Vieira and Pecchia 2018). These microorganisms initially break down readily accessible compounds and release ammonia, and then assimilate cellulose and hemicellulose into compost microbial biomass that forms the primary source of nutrition for the Agaricus mycelium.

Composting is performed by a microbial consortium initially by mesophilic microorganisms which digest easily degradable polysaccharide. Mostly found mesophilic bacterial genera are *viz*., Solibacillus, Comamonas, Acinetobacter, Pseudomonas, Sphingomonas (Kertesz and Thai 2018) and maximum found fungal genera are Lewia, Rhizomucor, Aspergillus (Kertesz et al. 2016). Later on, raise the temperature and cause the shifting of mesophilic microorganisms to thermophilic microbial community (Smith et al. 1995). Mostly found thermophilic bacterial species are *Bacillus* spp., *Paenibacillus* spp., *Actinobacteria* spp., *Corynebacterium* spp. and *Streptomyces* spp. (Zhang et al. 2015 and Vieira and Pecchia 2018) and dominat thermophilic fungi species present in compost are *Mycothermus thermophilus (Scytalidium thermophilum*), *Aspergillus* spp., *Rhizopus oryzae, Trichoderma viride, Chaetomium* spp., *Penicillium* spp., *Talaromyces* spp., *Thermomyces* spp., *Thermomyces ibadanensis*, *Thermomyces lanuginosus* (Vajna et al. 2012; Zhang et al. 2015; Kertesz et al. 2016 and McGee et al. 2017). The microbial biomass present in compost affects the mycelial distribution during the spawn run phase, whereas it causes the *Agaricus bisporus* life cycle's reproductive phase to be induced in casing soil (Ahlawat and Verma 2001). Certain microbial communities are promoting elongation of the Agaricus hyphae, and bacterial activity is required to induce production of the mushroom fruiting bodies during cropping (Kertesz and Thai 2018).

2.6.13 Good quality compost

A good quality compost contains a moisture level between 66 and 70 per cent and a C: N ratio of 23.18: 1 (Sarkar and Chanda 2016; Sofi et al. 2016 and Sinha et al. 2020). By monitoring variations in the compost's C/N ratio, it is feasible to monitor the decomposition and stabilization of organic materials (Kalamdhad et al. 2009). The C: N ratio of 30:1 at the initial phases of composting is crucial for creating high-quality compost and imparting selectivity. Composting is the process of decomposing organic wastes in the presence of oxygen and produces CO_2, NH_3, water and heat (Sofi et al. 2014, 2016 and Sinha et al. 2020). Effective composting requires the optimal settings and a perfect blending of nutrients.

According to Alsanius et al. (2016) a sufficient C:N ratio between 25 and 35:1 must be present in the initial mixture to ensure a successful composting process The final compost produced in this manner must have a pH of about 7.5 and a moisture level of 66-68 per cent (Shandilya 1989). Final temperature of compost must be at room temperature during spawning which gradually fall from 70-76℃ at the time of filling for phase-II of composting and C: N ratio to be 20:1 (Levanon et al. 1983). Zheng et al. (1995) observed a C: N ratio of 33:1 during fermentation, 18:1 during mycelia growth and 14:1 during fructification for *Agaricus bisporus* production.

2.7 ISOLATION AND CHARACTERIZATION OF THERMOPHILLIC FUNGI FROM THE COMPOST

To make compost successfully, attainment of high temperature is very important (Sinha et al. 2020). During the heating phase the temperature in the heap rises to 60-70 °C, due to activity of bacteria and fungi. The high temperature is caused by the energy generated by fungus and bacteria during breaking down the easily decomposable materials. A temperature increase in the composting pile is a good indication of high microbial activity (Diaz et al. 1993). Diseases, pests and weeds are all destroyed by this heat. Proper aeration is also needed for compost, which is provided by turning. If there is not enough air in the heap, fungal and bacterial growth will be hampered due to anaerobic conditions and the compost will begin to smell bad. Fungi and bacteria need an appropriate amount of oxygen to survive. In the composting process, moisture is also crucial as it allow fungi and bacteria to grow and function.

Composting is a microbial succession process starting with mesophiles, leading to increase in temperature. Thermotolerant organism take over from these point and temperature is further increased which is suitable for growth of thermophiles (Bahl et al. 1989). The presence of thermophilic organisms in compost or hay compounds plays a fundamental role in the breakdown of the substrate and the creation of stable products. Waksman and Cordon

(1939); Waksman et al. (1939 a) and Waksman et al. (1939 b) were the first to recognize the significance of microorganisms in the breakdown of plant wastes. At various stages of the composting process, both thermophilic and mesophilic bacteria and fungi are found. The population dynamics of thermophilic microorganisms increases than those of mesophilic microorganisms with the composting time.

Thermophilic fungi are those with a maximum growth temperature at or above 50°C and a minimum growth temperature at or above 20°C. According to Crisan (1959) thermophilic fungi are those whose ideal temperature for normal growth is at or above 40°C. Thermophilic fungi are able to adapt to high temperatures due to the phospholipids and a small number of neutral lipids found in the cell membrane of thermophilic moulds (Sumner et al. 1969). Distinct mesophilic genera of organisms included *Cellulomonas folia, Chondrococcus exiguous* and *Myxococcus virescens*, while more thermophilic genera of class Thermoactinomyces; Micropolyspora and Thermomonospora are predominant in the wetter and more humid roughages. Nine out of the thirty-four thermophilic fungi tested, including *Chaetomium thermophilum,* another *Chaetomium* species, *Malbranchea sulfurea, Myriococcum thermophilum, Symbiobacterium thermophilum, Stilbella thermophila, Thielavia terrestris* and two unidentified basidiomycetes, promoted the mycelial growth of *Agaricus bisporus* on sterilized compost (Gerben et al. 1994). Only *Symbiobacterium thermophilum* and *Myriococcum thermophilum* out of nine thermophilic fungus species grew successfully in test tubes on pasteurized compost. The successfully colonized organisms were re-isolated and counted after incubation, demonstrating the injected organisms' success. When compared to the pasteurized control, the yield of mushrooms on inoculated composts was almost two times higher (Gerben et al. 1994a).

Dhar and Munjal (1976); Hayes and Shandilya (1981) and reported thermophilic fungi, including *Humicola* spp. from the beginning of the composting. Fergus and Sinden (1969) isolated *Aspergillus fumigatus*, *Humicola grisea* var. *thermoidea*, *Humicola insolens* and a new species of *Stibella thermophila* from phase II of composting. Under aseptic conditions, a variety of thermophilic fungi can transform agricultural waste into a substrate that the *Agaricus bisporus* mycelium can use (Ross and Harris 1983). The use of thermophiles in making *Agaricus bisporus* compost was investigated by Pope et al. (1962). They said that *Penicillium*, *Anixia* and *Mucor* sp. can produce high-quality compost, whereas *Torula*, *Humicola*, *Monotospora* and *Chaetomium* sp. can produce acceptable compost. Straw and its components are biodegraded into compost by a variety of microorganisms (bacteria, actinomycetes and fungi), most of which prefer a warmer environment. It has been noted that decomposed straw is covered in microbial cell components and their matrix during the composting process (Eddy and Jacobs 1976; Atkey and Wood 1983). These organisms either die or become latent during composting because of the low temperature. This microbial mass acts as a concentrated source of nutrients for the development of *Agaricus bisporus.*

The temperature of the compost slowly drops until it consistently reaches room temperature as a result of nutrient shortage, which slows or stops the development rate of thermophilic fungus. These thermophilic fungi are still present in the compost after it has totally cooled, but they are in sporulated forms. Low temperatures promote the growth of mesophilic fungi, but at these temperatures, some of these fungi, particularly mycophagous ones such as *Trichoderma viride, Papulospora* sp., *Scopulariopsi*s sp. and *Doratomyces* sp. also become active (Bisht and Singh 1986). *Aspergillus terrus* and *Trichoderma viride* were identified by Eicker et al. (1991) at the spawn running stage, demonstrating that *Trichoderma viride* is a potent competitor of *Agaricus bisporus* compost (Thapa et al. 1977). The quality of compost is believed to be greatly enhanced by thermophilic fungi (Gerrits 1988a and 1988b). These fungi have been shown to affect the growth of mushroom mycelia and the production of mushrooms at three different levels (Wiegant et al. 1992).

i) They lessen the quantity of ammonia in the compost, which would otherwise inhibit the mushroom mycelium from growing.

ii) They are said to immobilize nutrients in a way that the mushroom mycelia can use them.

iii) They might promote the growth of mushroom mycelia, as has been demonstrated by *Scytalidium thermophilum* and several other thermophilic fungi.

Lacey (1973) observed that during composting, *Streptomyces* was dominant at mild heating stage (45-50°C) and more thermophilic genera of class like Thermoactinomyces; Micropolyspora and Thermomonospora prevailed in the wetter and hotter hays. Straatsma et al. (1994) recorded two-fold increases in the yield of mushrooms on inoculated compost as compared to the pasteurized control, demonstrating the efficacy of *Scytalidium thermophilum* in compost preparation for *Agaricus bisporus.*

2.8 ESTIMATION OF DIFFERENT ENZYME ACTIVITY AND BIOCHEMICAL CHANGES DURING BUTTON MUSHROOM PRODUCTION IN DIFFERENT COMPOST FORMULATIONS

2.8.1 Different enzyme activity during button mushroom production in different compost formulations

The compost becomes specialized for the growth of *Agaricus bisporus* due to the presence of various thermophilic fungi and the lignin humus complex that is produced by the breakdown of the compounding mixture. *Agaricus bisporus* consumes the resident microorganisms in the composted substrate as a source of nourishment. Mycelium (the vegetative or colonization phase) and the fruiting body are typically the two stages of a mushroom's life cycle (a reproductive phase that bears spores). Mycelia release enzymes that break down elements in the substrate, like cellulose and lignin, during the vegetative development phase (Gowda and Manvi 2019). Mushrooms produce a number of enzymes including lignin-degrading enzymes (laccases, lignin peroxidases, manganese peroxidases, arylalcohol oxidase and aryl-alcohol dehydrogenases or quinone reductases) hemicellulose and cellulose-degrading enzymes (xylanase and cellulases or cellobiose dehydrogenase) to facilitate the degradation of lignocellulosic substrates (Sanchez 2009 and Vos et al. 2017) and then utilize the end products to produce fruiting bodies. Therefore, mushroom cultivation is a crucial biotechnology technique for decreasing and valorising agro-industrial waste. Such compost is created as a result of the environmentally appropriate conversion of low-value by-products into new resources that could be used to build value-added products (Kumla et al. 2020).

In plant biomass, lignin and cellulose are closely related. Lignolytic bacteria and fungi are essential for this process because it allows bound cellulose to be transformed into free form (Tuomela et al. 2000). The enzyme endoglucanase converts crystalline cellulose into the soluble carbon compounds required for nutrition of fungal mycelium and can be used to evaluate the capacity to digest cellulose-complexes (Manning and Wood 1983). Sharma (1991) used derivative thermogravimetry to examine compost samples collected at various stages of composting to determine the degree of fibre breakdown and elemental analysis. He reported that the hemicellulose in the compost was significantly and thoroughly destroyed by the microorganisms present, resulting in just 5 per cent being found at the conclusion of phase II. It was also noted that there was an increase in ash content and the amounts of P, K, Na, Ca, Mg, S, Cu, Zn, Fe and Mn during composting. However, the primary component of straw, i.e., cellulose was less decomposed during phase I, and its significant degradation only occurred during phase II. The results of thermal analysis were consistent with information obtained using common gravimetric techniques to measure cellulose, hemicellulose, lignin and nitrogen (Kumla et al. 2020). Sharma (1991) reported that the hemicellulose in the compost was significantly and thoroughly destroyed by the microorganisms present, resulting in just 5 per cent being found at the conclusion of phase II. It was also noted that there was an increase in ash content and the amounts of P, K, Na, Ca, Mg, S, Cu, Zn, Fe and Mn during composting. However, the primary component of straw, i.e., cellulose was less decomposed during phase I, and its significant degradation only occurred during phase II.

According to Bonnen et al. (1994) *Agaricus bisporus* produces good amount of laccase and manganese peroxidise enzymes, which are necessary for the oxidation of polyphenols. Some bacteria and fungi produce enzymes during the composting process that break down the organic plant material's cell walls such as cellulose (Shina et al. 2020). The main ingredients of mushroom composts are lignin, carbohydrates and sources of organic and inorganic nitrogen (Bonnen et al. 1994). Mushrooms can completely break down lignin into the simpler molecule using LiP, MnP and laccase. They may also use the cellulose component as a source of carbon (Lara et al. 2003). Therefore, the amount of secreted laccase, LiP and MnP enzymes by mushrooms will have an effect on their growth cycles. The thermophilic fungi *Scytalidium thermophilum, Humicola insolens* and others secrete large amounts of cellulase and hemicellulase enzymes to break down diverse agricultural wastes (Fergus and Sinden 1969; Tansey 1971). At pH 5.5-9.0 and 47°C, *Humicola insolens* secretes endoglucanase, exoglucanase and β-glucosidase (Hayashida et al. 1980 and 1988). *Humicola insolens* achieved xylanolytic breakdown utilising wheat bran as substrate in submerged environment. Also, *Humicola insolens* generates xylanase on substrates made of rice straw, birch wood, wheat straw, barley straw and corn cobs (Dubeau et al. 1987; Blanco and Pastor 1993 and Filho et al. 1993) and starch-degrading enzymes (Flannigan and Sellars 1977; Ogundero 1979; Adams 1990 and Allison et al. 1992).

2.8.2 Effect of Thermophillic fungi on production and biochemical changes during button mushroom cultivation

The quantity of mushrooms produced by the *Malbranchea sulfurea + Torula thermophila* inoculated compost was significant (1990 g/5 kg of compost), about twice as much as the pasteurized control compost. The yield from control compost (1020 g/5 kg of compost) was plainly and significantly lower than that from inoculated compost. It seems essential in commercial practice to inoculate compost with certain isolates (Salar and Aneja 2007). A consortium of *Staphylococcus* sp. and *Bacillus megaterium* can be formed to achieve superior compost quality. A total of 09 thermophilic bacteria were isolated from white button mushroom (*Agaricus bisporus*) compost samples and examined for their tolerance to high temperatures, capacity to produce compost and extracellular lignocellulolytic enzyme activity (Ahlawat and Vijay 2010). *Staphylococcus* species had the highest levels of exoglucanase and endoglucanase activity, whereas *Bacillus brevis* and *Bacillus megaterium* had the highest levels of glucosidase and *Bacillus stearothermophilus* had the highest levels of xylanase and laccase. Compost treated with *Staphylococcus* sp. fruited 1.5-2.0 day earlier and gave noticeably greater mushroom output in two growing trials. *Staphylococcus* sp. has the capacity to convert agricultural waste into selective and rewarding compost for the growth of white button mushrooms (Ahlawat and Vijay 2001; Ahlawat and Vijay 2010).

Salar and Aneja (2007) isolated eighteen species of thermophilic and thermotolerant fungi from mushroom compost. They studied the growth of *Agaricus bisporus* on sterile compost pre-colonized with four thermophilic fungi *viz*., *Chaetomium thermophile, Malbranchea sulfurea, Thermomyces lanuginosus* and *Torula thermophila*. All the four fungi were inoculated singly and in different combinations on sterilized compost to evaluate their potential to promote growth and yield of *A. bisporus*. A mixed inoculum of *Malbranchea sulfurea* and *Torula thermophila* was found to be the best amongst the various treatments that promoted the growth of *Agaricus bisporus* to 7.7 mm/day in Petri plates and the yield of the mushroom was almost twice compared to the pasteurized control. Enzymatic studies conducted by Anandh and Prakasam in 2002 revealed that cellulose activity in the cased beds increased up to second harvest and was associated with the start of mushroom flushes. Hemicelluloses are hydrolyzed quickly because they are less resistant to catalyst activity than celluloses. Several extracellular enzymes activities vary during several growth phases of *Pleurotus citrinopileatus* growing on compost derived from fermented cotton seed hulls.

Endoglucanase, filter paperase and hemicellulase activity peaks emerged between the stages of primordial development and fruit body maturity (Klamis et al. 2008). The C: N ratio and lignocellulosic components are crucial for mushroom mycelial growth and primordial development (Narain et al. 2009). Sharma (1991) found that the substrate's cellulose and lignin content had an impact on the quantity of mushrooms produced. Ajonia and Tatah

(2012) found that the biological yield varies significantly depending on the substrate composition in several flushes. They opined that the right proportions of lignin and alpha cellulose were probably likely led to a faster rate of mycelium running. *Pleurotus* species create a variety of hydrolytic and oxidative catalysts that allow them to effectively colonize, break down and convert a range of lignocellulosic materials into sugars. Jain et al. (1989) have also reported 11-29 per cent of weight loss and 18-48 per cent of lignin loss in wheat straw, which was artificially inoculated by various pure cultures of thermotolerant and thermophilic fungi in 60 days. Satyanarayana (1978) showed 9-40 per cent weight loss of paddy straw due to decomposition by thermophilic fungi in 40 days at 45℃. Analysis of the plant residues after decomposition by pure thermophilic fungal culture have suggested that the biochemical changes are more or less similar to those observed during composting of materials by natural mixed flora.

Agaricus bisporus was axenically produced on composted straw. Examining this culture media during growth and fruiting revealed that the cellulose of the straw was degraded after the development of the fruit bodies, while the lignin part of the straw was degraded, notably during the vegetative growth phase. A novel method was developed in which the rate of mineralization to CO_2 during the entirety of *Agaricus bisporus* life cycle was continuously monitored without aggravating the growth. This method involved mixing ordinary or engineered radio-labeled lignin by hand with axenic compost (Singh et al. 2020). According to Gerben et al. (1995) fungus in compost is a positive correlate of the yield of mushrooms. *Scytalidium thermophilium* has been confirmed to enhance and promote the growth of *Agaricus bisporus*. It has been suggested that this fungal species provides a trigger for enhanced growth of button mushrooms.

Most agro-industrial waste is disposed of in landfills or burned, leading to various environmental problems and pose potential harm to the health of humans and wildlife (Anwar et al. 2014; da Silva 2016 and Hongzhang 2016). However, agro-industrial waste can potentially be converted into different high-value products, including biofuels, value-added fine chemicals and cheap energy sources for microbial fermentation and enzyme production (Sanchez 2009; da Silva 2016 and Hongzhang 2016). These waste products can represent a source of energy, as well as sources of carbon. Additionally, this form of waste is a source of the nutrients that are required for mushroom growth and lignocellulolytic enzyme production *via* solid state fermentation (Sanchez 2009; Knob et al. 2014; Grimm and Wosten 2018).

These agrowaste are used as a substrate for preparation of mushroom compost singly or with different combinations. Different substrate compositions used for compost preparation can directly affect the quality and quantity of mushroom production or yields. The efficiency of the mushrooms to utilize the various constituents of compost depend upon the substrate used in composting which further depends upon many physiochemical factors responsible during the composting process and mushroom growth (Kaur et al. 2019). Most substrates used for mushroom cultivation are complex structures that are mainly composed of cellulose, hemicellulose, and lignin known as lignocellulosic substrate. Mushrooms degrade lignocellulosic substrates by producing both hydrolytic and oxidative enzymes. Hydrolytic enzymes (cellulases and hemicellulases) are known to be responsible for polysaccharide degradation, while oxidative enzymes (ligninases) are responsible for lignin modification and degradation (Kumla et al. 2020).

Consequently, the microbial dynamics and communities of the composting substrates can significantly affect mushroom production. In microbial communities' thermophilic bacteria and fungi play important role because maximum degradation of substrate occurs by these microorganisms at thermophilic phase of compost. Thermophilic fungi are believed to contribute significantly to the quality of compost (Seal and Eggins 1976; Eicker 1977; Ross and Harris 1983; Gerrits 1988b). The effects of these fungi on the growth of mushroom mycelia and mushroom yield have been described at three distinct levels (Wiegant 1992). First, they decrease the concentration of ammonia in the compost, which otherwise would counteract the growth of the mushroom mycelium. Second, they immobilize nutrients in a form that apparently is available to the mushroom mycelia. Third, they may have a growth promoting effect on the mushroom mycelia (Salar and Aneja 2007). Therefore, changes in both substrate composition and microbial diversity during the cultivation process can impact the production of high-quality substrates and result in a high degree of biological efficiency of mushroom (Suwannarach et al. 2022). Increase mushroom productivity through the selection of suitable substrate compositions and their relation to the microbial community. The cultivation of edible mushrooms using agro-industrial waste represents the bioconversion of that waste into edible protein by using different thermophilic microorganism in compost (Kumla et al. 2020). Ultimately, utilization of agro-industrial waste in mushroom cultivation and the production of lignocellulolytic enzymes can facilitate the reduction of some global waste management problems.

Chapter-3

MATERIALS AND METHODS

The research work on "**Studies on utilization of paddy straw for compost preparation of button mushroom (*Agaricus bisporus*)**" was carried out in Mushroom Section of Department of Plant Pathology, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.). The details of experiment, materials used and methodologies adopted during the course of investigation are described in this chapter. The details of Materials and Methods for carrying out the present studies are given below under the following heads:

3.1 LAYOUT OF THE EXPERIMENT

3.2 PREPARATION OF SPAWN

3.3 PREPARATION OF DIFFERENT COMPOST FORMULATIONS

3.4 QUALITY ASSESSMENT OF COMPOST

3.5 YIELD DATA AND GROWTH ASSESSMENT OF MUSHROOM

3.6 ISOLATION OF THERMOPHILIC FUNGI FROM THE PREPARED COMPOST FORMULATIONS

3.7 IDENTIFICATION OF THERMOPHILIC FUNGAL ISOLATES

3.8 ESTIMATION OF ENZYMES ACTIVITY IN DIFFERENT COMPOST FORMULATIONS

3.9 STUDIES ON IMPROVEMENTS IN *A. BISPORUS* COMPOST USING THERMOPHILIC FUNGI

3.10 NUTRITIONAL PARAMETERS OF MUSHROOM FRUITING BODIES

3.1 LAYOUT OF THE EXPERIMENT

The experiments were laid out in the Mushroom Production Unit of the Department of Plant Pathology, which is situated at an elevation of 1269 m above mean sea level and falls at a latitude of 30°51' 55" North and longitude of 77° 10'14" East. The area falls under mid-hill zone that represent the transitional zone between sub tropical and sub temperate regions of Himachal Pradesh.

For cultivation of button mushroom, standard procedures were followed for composting and spawning. After spawn run, the top of compost bags were covered with a layer of pasteurized casing soil mixture, which was spread evenly to a height of 4 to 6 cm.

3.2 PREPARATION OF SPAWN

Spawn of U3 strain of *Agaricus bisporus* was prepared on wheat grains as per the standard procedure given by Sharma and Kumar (2011). Preparation of master culture or mother spawn was carried out under completely sterile conditions. Pure culture of the strain was inoculated in wheat grain to prepare grain spawn. Ten kg of wheat grains were boiled in 20 litres of water till the grain become soft but not ruptured. Water was then drained off and the grains were spread on a wire mesh tray for 8-10 hours to dry or remove excess of water. Grains were mixed with gypsum (calcium sulphate) and chalk powder (calcium carbonate) at the rate of 2 per cent and 0.5 per cent, respectively on dry weight basis. 10 kg of dry wheat grains required about 200g gypsum and 50g chalk powder. This helped to maintain the pH of the medium and also prevented sticking of grains with one another.

The grains were filled into half litre glucose bottles and PP bags, which were plugged with non absorbent cotton and sterilized at 22 psi pressure for 2 hours. Sterilized bottles were allowed to cool down overnight. Next day bottles were inoculated with the bits of pure culture of *A. bisporus*. Inoculated bottles were incubated at 25± 1ºC. Two weeks after inoculation, the bottles were ready as stock culture for further multiplication of spawn. One bottle of stock culture or master culture or mother spawn was sufficient to multiply 30-40 grain bottles or 15-18 PP bags of 1kg capacity**.**

3.2.1 Multiplication of spawn from stock/ master culture

Master spawn or master culture bottles/ bags were further used for inoculation of large number of other grain bags / bottles prepared by the same technique and resultant was the commercial spawn. Generally, few mycelium coated grains from one master culture bottle / bag were used to inoculate 30-40 grain bags aseptically under Laminar flow conditions, which were then incubated in a room at 25 ± 1 °C for 12-15 days. The commercial spawn thus prepared was used for inoculating the compost bags.

3.3 PREPARATION OF DIFFERENT COMPOST FORMULATIONS

Five different formulations of compost were prepared by using different substrates for growing button mushroom (*Agaricus bisporus)* using short method of composting (Sinden and Hause 1950). The ingredients used in different formulations are given below (Table 3.1):

Table 3.1 Different ingredients of button mushroom compost formulations

Treatment Substrate	T 1 (Kg)	T 2(Kg)	T 3(Kg)	T 4(Kg)	T 5(Kg)
Paddy straw	600	750	500	1000	-
Wheat straw	400	250	500	-	1000
Wheat bran	100	100	100	100	100
Chicken manure	600	600	600	600	600
Urea	15	15	15	15	15
Gypsum	30	30	30	30	30
Nitrogen (%)	1.68	1.69	1.66	1.72	1.60

Experiment size: - No. of treatments: 5, No. of replications: 4, No. of bags per treatment: 10, Bag size: 10Kg

Statistical design: CRD (Completely Randomized Design)

3.3.1 Compost preparation

Compost preparation was completed in two phases i.e., Phase I (outdoor composting) and Phase II (Indoor composting).

1) Phase I

The raw material (ingredients) were mixed together, watered and periodically turned according to following schedule:

i) -4 day: Paddy Straw, wheat straw, chicken manure were mixed by trampling to encourage uptake of moisture and aerobic fermentation.

ii) -2 day: Whole of mass was turned and made into slightly smaller stacks and more water was added.

iii) 0 day: On this day, the stack was again broken and the entire quantities of other raw material like wheat bran and urea were added. Water was also added according to the requirement. On this day a high aerobic stack of 5’×5’ (L× B) was made with the help of iron boards.

iv) +2 day: At this stage, first turning was given to compost heap.

v +4 day: Second turning was given to heap.

vi) +6 day: Third turning was given to heap and gypsum was added.

vii) +8 day: At this stage, the compost was filled in the pasteurization chamber for Phase 2 (pasteurization).

2) Phase II

Filling of compost in chamber:

On +8 day, the compost was filled in pasteurization chamber. As soon as the compost in chamber was completely filled, the door and fresh air ventilator were closed. The blower was put on for circulation of air @ 150-250 cubic meter/1000 kg of compost/hour.

The Phase II was completed in 3 stages as under:

a) Pre- peak heat stage:

After about 12-15 hours of filling of compost in the chamber, the temperature started rising automatically. The temperature was maintained at 48-50°C for 36-40 hours with the manipulation of ventilation system. This temperature range was achieved by self generation of heat by the compost mass.

b) Peak heat stage:

Temperature was raised to 58-59ºC by adjustment of fresh and recirculation air in the pasteurization chamber and it was maintained for 6-8 hours to ensure proper pasteurization.

c) Post- peak heat stage:

The temperature was gradually lowered down and maintained at 45-55ºC till no traces of ammonia were detected in the compost. It took 3-4 days for ammonia to cease off. When the compost was free from ammonia, fresh air was introduced by opening the damper to the maximum capacity till the compost cooled down to ambient temperature for spawning.

3.3.2 Spawning:

Spawn was mixed with compost in layers. In compost bags, about 3–4 layers of spawn and compost were layered and finally, one layer of spawn spread on the top of the compost. The spawning rate was kept at 0.5–0.7 per cent, i.e. 50-70 g/10 kg bag of compost. The bags were placed in cropping rooms for spawn run at 20–25 °C in dark conditions.

3.3.3 Preparation of casing soil

Casing material was steam pasteurized at 65 ± 1ºC for 6-7 hours. Hydrogen ion concentration (pH) of casing soil was adjusted to around 8.0 by adding calcium carbonate before pasteurization. The casing material was used to case the spawn run bags after a period of 2 weeks from the day of spawning. The moisture level of casing was adjusted to 60 per cent before placing it on the spawn run compost. After 10-12 days of casing, the bags were shifted to cropping room having 14-18ºC temperature with 80-90 per cent relative humidity, which was maintained till the crop was over. For maintaining the humidity, water was sprayed on the walls and floor of the growing chamber and cropping bags were also sprayed with water whenever required.

3.3.4 Fruiting

White button mushroom requires 22-25℃ temperature in cropping room for vegetative growth (spawn run) and 14-18℃ for reproductive growth. Besides, it requires relative humidity of 80-90 per cent and CO_2 concentration (0.08-0.15 %) for fruit body initials to form. During cropping, ventilation was provided to pinheads, which gradually developed into button stages.

3.3.5 Harvesting

Mushrooms were harvested in the button stage. Harvesting was done by holding the cap with forefingers and thumb by slightly pressing against the soil and twisting it off. The soil particles and mycelial threads clinging to the base of the stalk were chopped off.

The studies were conducted to select best compost formulation to study their quality and effect on growth and yield of white button mushroom in most economical manner.

3.4 QUALITY ASSESSMENT OF COMPOST

3.4.1 pH of compost

pH of compost was measured with the help of a pH meter as per the standard procedure given by Jackson (1973). For determination of pH of compost, samples were taken from each replication of different treatments. Collected samples were weighed and prepared for pH determination.

3.4.2 Electrical conductivity (EC)

EC of the compost was measured as per the standard procedure given by Jackson (1973). For determination of EC of compost before cropping, samples were taken from each replication of different treatments. Collected samples were weighed and prepared for EC determination.

3.4.3 Organic carbon (OC)

Organic Carbon of compost was measured according to "Loss of Ignition Method" following the procedure given by Kalra and Maynard (1991). For determination of OC of compost before cropping, samples were taken from each replication of different treatments. Collected samples were weighed, dried and prepared for OC determination. In this method, 5g of dried compost sample was transferred to silica crucible with known weight. Combustion process of the material was carried out in a muffle furnace at 550$\pm$50°C for 6 to 7 hours till constant weight of crucible was obtained.

$$\text{Approx Organic matter (\%)} = \frac{\text{Weight of oven dry sample (g)} - \text{Weight of sample after ignition(g)}}{\text{Weight of oven dry sample}} \times 100$$

The carbon contents were calculated from the organic matter assuming organic matter contains 58 per cent carbon.

$$\text{Carbon (\%)} = \frac{\text{Organic matter (\%)}}{1.724}$$

3.4.4 Total nitrogen content

Nitrogen content was measured by Macrokjeldahl digestion and distillation method following the procedure given by Jackson (1973). For determination of Nitrogen content of compost before cropping, samples were taken from each replication of different treatments. Collected samples were weighed, dried and prepared for Nitrogen content determination.

This method includes nitrogen in all forms such as inorganic nitrogen (NH_4), NO_3, urea etc. and also organic nitrogenous compounds like proteins, amino acids and other derivatives.

Reagents

a. Digestion mixture

K_2SO_4	20 parts
$CuSO_4.5H_2O$	1 part

$FeSO_4.7H_2O$	4 parts
Selenium di-oxide	2 parts
Selenium powder	2 parts

b. 45 per cent NaOH Solution

c. 0.1 N NaOH solution

d. 0.1 N HCl

e. Sulphuric acid concentrated

f. 4 per cent Boric acid solution

g. **Mixed indicator:** 0.5 g Bromocresol green + 0.1 g Methyl Red in 100 ml of 95 per cent of ethyl alcohol. Colour of the solution adjusted to bluish purple using dilute HCl or NaOH.

Procedure

Digestion

Nitrogen was estimated using 2 g dried and powdered sample. The sample was digested with 20 ml concentrate sulphuric acid and 10 g of digestion mixture in a 500 ml Kjelhdalh flask at 250-300°C for 4-6 hrs in a digestion fume hood until clear green solution was obtained. The solution was made up to 100 ml with distilled water.

Distillation

The distillation assembly was washed with hot water and finally with distilled water and dried before use. An amount of 10 ml of the digested aliquot was taken in a modified Markeham’s apparatus along with equal volume of 45 per cent NaOH. Hot steam allowed to pass through the mixture for 5-10 minutes and distillate was collected in 150 ml conical flask containing 20 ml of 4 per cent boric acid with one drop of mixed indicator. The tip of the condenser was always kept dipped into the boric acid solution during the distillation. The colour of the solution changed to greenish blue to green.

Titration

The distillate was titrated against 0.1N HCl till the end point is indicated by disappearance of blue colour and appearance of light pink colour. One blank was run without sample. One standard solution of ammonium chloride (1g/ml) was also titrated against 0.1 N HCl.

Calculation

$$\% N_2 = \frac{(X-Y) \times N \times 0.014 \times V_1}{V_2 \times W} \times 100$$

V1 = Volume of Aliquot (Total 100 ml)

W = Weight of Sample (2 g)

V2 = Aliquot taken (10 ml)

N = Normality of acid (0.1N)

X = Volume of HCl with sample

Y = Volume of HCl with Blank

3.4.5 Total potassium (K) and phosphorus (P)

For the estimation of P and K, 0.5 g grind sample of compost was digested in diacid mixture prepared by mixing nitric acid and perchloric acid (4:1) taking all relevant precautions as suggested by Piper (1966). The estimation of phosphorus content was determined by Vanado molybdo-phosphoric method (Heslop and Ramsey 1969). However, potassium was determined by flame-photometer (Jackson 1973).

3.4.6 Moisture content

Moisture content of compost was determined by Gravimetric method as per the standard procedure given by Black (1965). For determination of Moisture content of compost samples were drawn from each replication of different treatments before cropping. Collected samples were weighed and prepared for moisture content determination. The moisture content was estimated by drying the weighed sample up to a constant weight in hot air oven at 105°C

$$\text{Moisture }(\%) = \frac{(\text{Weight of fresh sample} - \text{Weight of dried sample})}{\text{Weight of fresh sample}} \times 100$$

3.4.7 Bulk density

Bulk density was measured following the method given by Singh (1980). For determination of bulk density of compost, samples were taken from each replication of different treatments. Collected samples were weighed and prepared for bulk density determination by pycnometer method.

$$\text{Bulk Density} = \frac{(\text{Weight of Pycnometer} + \text{Soil}) - \text{Weight of empty pycnometer})}{\text{Volume of pycnometer}}$$

3.4.8 Particle density

The particle density was determined by the pycnometer (specific-gravity flask) method. For the determination of particle density, 10 g of grinded compost samples from each replication were taken in the pycnometer and filled with water. Air was removed from the suspension by boiling the suspension (Rowell 1994). Particle density was calculated using the following formula.

$$\text{Particle Density} = \frac{\text{w (10)}}{\text{Wpw} + \text{w (10)} - \text{Wpwc}}$$

Weight of grind compost = w (10g)
Weight of water filled pycnometer = Wpw
Weight of pycnometer + water + compost = Wpwc

3.4.9 Porosity

Porosity was determined from bulk density and particle density values of composts (Allen 1974) using the following formulae.

$$\text{Porosity} = 1 - \frac{\text{Bulk density}}{\text{Particle density}} \text{X100}$$

3.4.10 Total microbial count

The compost was analyzed for total microbial counts. One gram of compost was taken in 9 ml of sterilized water blank and the soil suspension was diluted 10^{10} times and microbial count was assayed by standard pour plate technique on different media as described by Rao (1999). The population was expressed as colony forming units per gram of soil (cfu/g soil). The number of cfu was obtained by counting the number of colonies/plate and multiplying by dilution factor.

$$\text{Colony forming unit (cfu/g)} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume of culture plate}}$$

3.5 YIELD DATA AND GROWTH ASSESSMENT OF MUSHROOM

3.5.1 Days taken for spawn run

After spawning, the number of days taken for the mycelial colonization of compost was counted and reported as spawn run days.

3.5.2 Days taken for case run

After casing, the number of days taken for the complete spread of mushroom mycelium in the casing material was counted and reported as case run days.

3.5.3 Number of fruit bodies (per 100 kg compost)

The number of fruit bodies harvested per 100 kg compost was counted.

3.5.4 Length of fruit bodies

Length of the 25 fruit bodies from each treatment was measured with the help of a scale from the top of the mushroom cap to base of the mushroom stipe in centimetres (cm) and the average length was calculated.

3.5.5 Length of stipe of fruiting bodies

The length of the stipe of 25 fruit bodies from each treatment was measured with the help of a scale in centimetres (cm).

3.5.6 Width of stipe of fruiting bodies

The width of the stipe of 25 fruiting bodies from each treatment was measured with the help of a scale in centimetres (cm).

3.5.7 Average diameter of fruiting bodies cap

Average diameter of 25 fruiting bodies was measured with the help of ruler scale in centimeters (cm).

3.5.8 Mushroom yield

The mushroom yield (kg/100 kg weight of compost) was recorded for a period of 4 weeks.

3.5.9 Diseases /Pest prevalence/ Competitor moulds

Mushroom bags were observed daily for the prevalence of disease /pest/ competitor moulds.

3.5.10 Biological efficiency (B.E.)

The biological efficiency was calculated by the standard formula given by (Flegg et al. 1985).

$$\text{Biological efficiency (B.E) \%} = \frac{\text{Total fresh weight of mushrooms}}{\text{Fresh weight of compost}} \times 100$$

3.6 ISOLATION OF THERMOPHILIC FUNGI FROM THE PREPARED COMPOST FORMULATIONS

3.6.1 Sterilization

Glassware used during the experiments were washed thoroughly in detergent water, running tap water followed by rinsing in distilled water. Glasswares were sterilized in hot air over at 180ºC temperature for 20 min. All the media, water blanks etc. were sterilised in autoclave at 15 pounds per square inch pressure for 20 min. Laminar air flow chamber was sterilized by ethanol disinfectant followed by ultra violet (UV) radiation for 30 min before start of the work.

3.6.2 Isolation of thermophilic fungi

The thermophilic fungi were isolated from five different formulations of compost. One kg of compost was randomly sampled in 100g portions, mixed thoroughly and was used for isolation. Thermophilic fungi were isolated from different composting phase i.e. Phase-1 and Phase-2 using serial dilution method on Yeast Phosphate Soluble Starch (YPSS) agar media.

Composition of Yeast phosphate soluble starch agar

Yeast extract	:	4.0g
K_2HPO_4	:	1.0g
$MgSO_4.7H_2O$	:	0.5g
Soluble Starch	:	15g
Agar	:	20.0g
Distilled water	:	1000ml

10 g of compost sample from each treatment was taken in a 250ml Erlenmeyer flask containing 90 ml of sterile water and shaken on a rotary shaker for 1 hour. Various dilutions (10^{-2}, 10^{-3}, and 10^{-4}) were used for the isolation of themophilic fungi. Plates were incubated at 40- 45°C in the dark and were screened daily for up to 5 days. Representative isolates were purified and maintained on YPSS agar slants.

3.7 IDENTIFICATION OF THERMOPHILIC FUNGAL ISOLATES

3.7.1 Phenotypic characterization

Identification of thermophilic fungi was done on the basis of conventional morphological and physiological characterization of thermophilic fungi, as described by Cooney and Emerson (1964). Isolated thermophilic fungi were identified based on visual and microscopic characteristics such as colony morphology, color, culture growth, pigmentation, hyphae color, hyphae septation or non-septation, spores, shape and size etc.

3.7.2 Molecular identification

All the thermophillic fungal cultures were employed for molecular identification.

Reagents used

1. CTAB lysis buffer (For 100ml: CTAB - 2g, 1M Tris HCL - 7.5ml, 0.5M EDTA - 3.0ml, NaCl - 6.14g)
2. Extraction buffer (For 100ml: Chloroform + Isoamyl alcohol 24:1 w/w)
3. 70 per cent Ethanol
4. 100 per cent Isopropanol
5. 10Mm Ammonium acetate
6. RNAse (10mg/ml)
7. TE buffer
8. 3M Sodium acetate
9. TAE buffer (50X) (Tris - 242g, Acetic acid - 57.1g, 0.5M EDTA - 100ml make up 1000ml)
10. Ethidium bromide (10 mg/ml)
11. 6X gel loading dye (For 100ml: 0.25g Bromophenol, 0.25g Xylene cyanol, 60ml Glycerol, 40ml water)

Sample preparation

The pure fungal cultures were sub cultured under sterile conditions in 250 ml flasks containing 100 ml YPSS broth at 40- 45°C for eight days. The grown mycelia was filtered through Watchman filter paper No.1 and used for DNA extraction by using CTAB extraction method.

Extraction of DNA using CTAB method

The mycelial mats were vacuum dried for overnight and crushed in a sterilized mortar and pestle using a pinch of PVP and sterilized sea sand. 1 g of dried mycelial powdered was then placed in a 30 ml Oak Ridge tube and 15 ml pre-warm CTAB lysis buffer was added to it. The tubes were vortexed vigorously for 1-2 min and kept in water bath at 65°C for 60 minutes. Later, extraction was done using equal volume of Chloroform: Isoamyl alcohol (24:1) and mixed gently by inverting 10 minutes. Further, the tubes were centrifuged at 14000 rpm for 10 minutes. The aqueous phase was aspirated and transferred to a fresh tube. The aspirated liquid was re-extracted using same volume of Chloroform: Isoamyl alcohol (24:1) and mixed gently by inverting 10 minutes. Further, the tubes were centrifuged again at 14000 rpm for 10 minutes. The aqueous phase was aspirated and transferred to a fresh tube. Equal amount of chilled iso-propanol was added and allowed the DNA to settle down for 20 minutes to overnight at -20°C. The tubes were centrifuged at 13000 rpm for 5 minutes and the supernatant was discarded. The pellet was washed with 70 per cent ethanol twice followed by centrifugation at 1000 rpm for 10 minutes each time. The pellet was dissolved in 300-1000 µl of TE (1X) buffer and treated with RNase (10 mg/ml) @ 4 µl per 300 µl sample and incubated for 1 hr at 37°C. DNA was precipitated by adding 1/10 volume of sodium acetate (3M) and 2.5 times chilled ethanol. The tubes were centrifuged at 1000rpm for 10 minutes and supernatant was discarded. Finally, the pellet was air dried and re-dissolved in 500 µl 1X TE buffer and kept at -20°C until further use.

Extracted genomic DNA by agarose gel electrophoresis

For gel electrophoresis of high molecular weight genomic DNA, 0.8 per cent agarose gel was prepared in 1X TAE buffer prepared from 50X TAE stock solution. For making 120 ml of gel, 0.96 g of agarose was dissolved in 120 ml of 1 X TAE and the mixture was heated to dissolve agarose. The agarose solution was cooled to 60°C and 12 µl ethidium bromide solution was added to it. The solution was poured in gel casting tray. The comb was placed in the gel and allowed to solidify for 30-35 minutes at room temperature. After solidification, the gel tray was placed in the Bio-Rad gel electrophoresis tank and the comb was removed. A volume of 2 µl DNA, 1µl gel loading dye were mixed well and loaded in wells of the gel with the help of micropipette. The gel was run at constant voltage (50 volts) for 45-60 minutes. The electrophoresis was stopped when the samples had run the required distance and gel was visualized under the UV trans-illuminator and photographed using a Syngene gel documentation system.

PCR amplification

The DNA concentration was adjusted to 50 µg/µl by dilution and 5.8S rRNA gene was amplified by polymerase chain reaction (PCR) using universal primer pair of 0.5 µM of each primer i.e., ITS1 (5’-TCCGGTAGGTGAACCTGCGG-3’) and ITS4 (5’-TCCTCCGCTTATTGATATGC-3’). The 25 µl reaction mixture contained the following components: 2 µl template DNA, 2.5 µl of 1 X PCR buffer, 0.2 µl of Taq DNA polymerase (5U/ µl), 0.2 µl of dNTP mixture (100mM), 1.25 µl of $MgCl_2$ (25mM), 1 µl of 5% glycerol, 1 µl each of ITS-1 and ITS-4 primers, and sterile water 16.85 µl. Mix all the ingredients thoroughly for 1-2 seconds and place the PCR tubes in an Eppendorf thermocycler (Germany). Amplification was carried out for 34 cycles with initial denaturation step at 94°C for 1 minutes, followed by denaturation step at 94°C for 30 seconds, annealing at 55°C for 1 min 20 seconds, extension at 72°C for 1 minute and final extension at 72°C for 10 minutes. A volume of 5 µl of PCR product with 2 µl of loading dye was mixed and loaded on a 1.6 per cent agarose gel and PCR product were resolved at 70V for 45 min in a gel electrophoresis unit. The gel were visualized under UV light and photographed under Syngene gel doc imaging system.

ITS 5.8S rRNA Gene sequencing

PCR product was purified using Sigma definity tips and sent to Eurofins IT Solutions India Pvt. Ltd. Bengaluru for sequencing.

BLAST and Phylogenetic analysis tree

The 5.8S rDNA nucleotide sequence was obtained from sequencing and used to carry out BLAST (Basic Local Alignment Search Tool). BLAST of the obtained sequence was performed with NCBI database of gene bank and the 5.8S rDNA nucleotide sequence was subjected to Phylogenetic analysis using fast minimum evolution method.

3.8 ESTIMATION OF ENZYMES ACTIVITY IN DIFFERENT COMPOST FORMULATIONS

3.8.1 Cellulase assay

3.8.1.1 Carboxymethyl Cellulase (CMC) assay (Reese and Mandel 1963)

3.8.1.2 FPase assay (Reese and Mandel 1963)

3.8.1.3 β glycosidase assay (Bergheim and Patterson 1973)

3.8.1.1 Carboxymethyl Cellulase (CMC) assay (Reese and Mandel 1963)

Reagents

i) 1 per cent CMC in citrate buffer (0.05M, pH 5.0)

ii) Dinitrosalicylic acid (DNSA) Reagent: NaOH: 1.0 g, Phenol: 0.2 g, Sodium potassium tartarate: 20.0 g, Sodium sulphate: 0.05g, DNSA reagent: 1.0g, Distilled water: 100ml

iv) Standard solution of glucose (0.4mg/ml)

Procedure

The reaction mixture contained 0.5ml of 1 per cent CMC in citrate buffer (0.05M, pH-5.0) and 0.5ml of culture supernatant. Reaction mixture was incubated at 50°C for 30 min. After incubation 3ml of DNSA reagent was added. Tubes were immersed in boiling water bath and removed after 15 min when colour development was complete. Control was run with all the components except the enzyme. Tubes were cooled at room temperature and O.D was read at 540nm in spectrophotometer against the reagent blank i.e., 1ml of distilled water and 3ml of DNSA reagent. The standard curve was made from the stock solution of glucose (0.4 mg/ml) with concentration i.e., 100µl, 200µl, 300µl, 400µl, 500µl, 600µl, 700µl, 800µl, 900µl, 1000µl. The enzyme activity was expressed in terms of International Unit (IU). One International Unit (IU) of enzyme activity represents µ moles of glucose released/min/ml of enzyme.

$$\text{Enzyme activity} = \frac{\text{Product concentration} \times \text{Total ml}}{\text{Molecular weight} \times \text{ml of enzyme} \times \text{Incubation time}} \times \text{Dilution factor}$$

3.8.1.2 Filter Paperase (FPase) assay (Reese and Mandel 1963)

Reagents

i) Strips of filter paper (Whatman no.1)

ii) 0.05M citrate buffer (pH 5.0)

iii) Dinitrosalicylic acid (DNSA) Reagent

iv) Standard solution of glucose (0.4 mg/ml)

Procedure

To 50 mg of filter paper strips (Whatman no. 1), 0.5 ml of citrate buffer (0.05M, pH-5) and 0.5ml of culture supernatant was added. Reaction mixture was incubated at 50°C for 30 min. After incubation 3ml of DNSA reagent was added. Tubes were immersed in boiling water bath and removed after 15 min when colour development was complete. Control was run with all the components except the enzyme. Tubes were cooled at room temperature and O.D was read at 540nm in spectrophotometer against a reagent blank i.e., 1ml of distilled water and 3ml of DNSA reagent. The standard curve was prepared from the stock solution of glucose (0.4mg/ml) with concentration i.e. 100µl, 200µl, 300µl, 400µl, 500µl, 600µl, 700µl, 800µl, 900µl, 1000µl. The enzyme activity was expressed in terms of International Unit (IU). One International Unit (IU) of enzyme activity represents µmoles of glucose released/min/ml of enzyme.

$$\text{Enzyme activity} = \frac{\text{Product concentration} \times \text{Total ml}}{\text{Molecular weight} \times \text{ml of enzyme} \times \text{Incubation time}} \times \text{Dilution factor}$$

3.8.1.3 β-Glucosidase assay (Berghem and Petterson 1973)

Reagents

i) 1mM ρ-nitrophenyl β-D-glucopyranoside in 0.05M sodium acetate buffer of pH 5.0
ii) 1M sodium carbonate solution
iii) Standard solution of ρ-nitrophenol (80 µg/ml)

Procedure

Reaction mixture contained 1ml of 1mM of ρ-nitrophenyl β-D-glucopyranoside in 0.05M sodium acetate buffer (pH 5.0) and 100 µl of culture supernatant. After incubation at 40°C for 10 min, 2ml of 1M of Na_2CO_3 was added to the reaction mixture to stop the reaction. Reaction mixture was heated in boiling water bath for 15 min and after heating it was diluted to 10ml with distilled water. ρ-nitrophenol liberated was determined as the absorbance at 400nm against reagent blank i.e., 1ml of distilled water and 2ml of 1M of sodium carbonate. The standard curve was prepared from the stock solution of ρ-nitrophenol (80 µg/ml) with concentration i.e., 100µl, 200µl, 300µl, 400µl, 500µl, 600µl, 700µl, 800µl, 900µl, 1000µl. The enzyme activity was expressed in terms of International Unit (IU). One International Unit (IU) of enzyme activity represents µmoles of nitrophenyl ß-D-glucopyranoside released/min/ml of enzyme.

$$\text{Enzyme activity} = \frac{\text{Product concentration} \times \text{Total ml}}{\text{Molecular weight} \times \text{ml of enzyme} \times \text{Incubation time}} \times \text{Dilution factor}$$

3.8.2 Xylanase assay (Miller 1959)

Xylanase activity was determined by measuring the amount of reducing sugar released from xylan using (DNS) method (Miller 1959).

Reagents

i) 1.0 g Oatspelt xylan in 100 ml of 0.05 M citrate buffer, pH 4.0
ii) DNSA reagent
iii) Standard solution of xylose (0.4 mg/ml)

Procedure

0.8 ml of xylan solution (which was incubated overnight at 37°C) and 0.2 ml of culture supernatant was taken in a test tube. The control was run with 0.2 ml distilled water and 0.8 ml xylan solution except the enzyme (culture supernatant). The reaction mixture was incubated at 45°C for 10 min. After the incubation, 3 ml of DNSA reagent was added and the mixture was then heated in boiling water bath for 30 min. After cooling down at room temperature, absorbance of reaction mixture was recorded at 540 nm. The enzyme activity was expressed in terms of International Unit (IU). One International Unit of enzyme activity represents µmoles of glucose released/min/ml of enzyme.

$$\text{Enzyme activity} = \frac{\text{Product concentration} \times \text{Total ml}}{\text{Molecular weight} \times \text{ml of enzyme} \times \text{Incubation time}} \times \text{Dilution factor}$$

3.8.3 Laccase assay (Rehan et al. 2016)

The laccase assay was performed by the following method described by Rehan et al. (2016).

Reagent

i) 2mM Guaiacol
ii) 10mM Sodium acetate buffer (pH 5.5)

Procedure

Reaction mixture contained 1ml of 2mM of guaiacol in 3ml sodium acetate buffer (pH5.5) and 1ml of culture supernatant. The blank was also prepared with all components except the enzyme (culture supernatant). The reaction mixture was incubated at 30°C for 15 min. The absorbance was read at 450nm using UV spectrophotometer. The enzyme activity was expressed in terms of International Unit (IU). One International Unit (IU) of enzyme activity represents 1 µmoles guaiacol released/min/ml of enzyme.

The activity of laccase assay was calculated by using this formula:

$$\text{Laccase activity} = \frac{A \times V}{t \times e \times v}$$

Were,

A = Absorbance (O.D.)

V = Total volume of reaction mixture

T = Incubation time

E = Extinction coefficient of guaiacol at 450nm (0.6740 µM/cm)

V = Volume of culture supernatant

3.8.4 Manganese dependent peroxidase assay (MnP) (Paszczynski et al. 1986; Mata and Savoie 1998)

The activity of Manganese dependent peroxidase (MnP) was estimated according to method of Paszczynski et al. 1986; Mata and Savoie 1998, based on the rate of oxidation of Mn (II) to Mn (III) and hydrogen peroxide ions produced by fungi to degrade lignin substrate.

Reagent

i) 0.5M Sodium Tartrate buffer (pH 5.0)
ii) 1mM Guaiacol
iii) 1Mm Manganese sulphate ($MnSO_4$)
iv) 1Mm Hydrogen Peroxide (H_2O_2)

Procedure

Reaction mixture was contained 0.2ml of 0.5M Sodium Tartrate buffer, 0.25ml of 1mM Guaiacol, 0.1ml of 1Mm $MnSO_4$, 0.35ml of enzyme dilution (culture supernatant) and 0.1ml f 1mM H_2O_2 in test tube. After adding all these reagents to the test tube, the initial reading was taken at 465nm by using a spectrophotometer. The final reading was taken after

5 minutes of incubation at room temperature. The blank was also prepared with all components except the enzyme (culture supernatant). One International Unit (IU) of enzyme activity represents 1 µmoles guaiacol released/min/ml of enzyme. MnP activity calculated by using formula given below:

$$\text{MnP activity (U/L)} = \frac{(\Delta \text{ Absorbance}) \times 10}{\text{e} \times \text{ R} \times \text{ t}}$$

Where,

Δ Absorbance = Final absorbance- initial absorbance

e = Extinction coefficient of guaiacol substrate (26.6 mM^{-1} cm^{-1}.)

R = Amount of enzyme (reaction mixture) in broth (1ml)

T = Reaction Time (5min)

3.9 STUDIES ON IMPROVEMENTS IN *A. BISPORUS* COMPOST USING THERMOPHILIC FUNGI

The best evaluated compost formulation was inoculated with the two best isolated thermophilic fungi which showed the highest enzyme activities. Treatment one (T1) was inoculated with isolation 1, Treatment two (T2) with isolation 2, Treatment three (T3) was inoculated with consortium (mixture of 1 and 2 isolate) and T4 Control (uninoculated). Inoculation of thermophilllic fungi (pre grown on wheat grains) in compost was done on zero day @ 0.5% wet wt. basis. Standard procedure for compost preparation was followed.

Treatment		Formulation
T1	:	Isolated Fungi 1
T2	:	Isolated Fungi 2
T3	:	Consortium mixture of 1 and 2 isolate
T4	:	Control

No. of treatments: 4, No. of replication: 4, CRD (Completely Randomized Design)

OBSERVATIONS

3.9.1 Quality assessment of compost

3.9.1.1 pH of compost

pH of compost was measured as per the procedure given in section 3.4.1.

3.9.1.2 Electrical conductivity (EC)

EC of compost was measured as per the procedure given in section 3.4.2.

3.9.1.3 Organic carbon (OC)

OC of compost was measured as per the procedure given in section 3.4.3.

3.9.1.4 Nitrogen content

Nitrogen content of compost was measured as per the procedure given in section 3.4.4.

3.9.1.5 Total phosphorus (P) and potassium (K)

Total P and K content of compost was measured as per the procedure given in section 3.4.5.

3.9.1.6 Moisture content

Moisture content of compost was measured as per the procedure given in section 3.4.6.

3.9.1.7 Bulk density

Bulk density of compost was measured as per the procedure given in section 3.4.7.

3.9.1.8 Particle density

The particle density of compost was measured as per the procedure given in section 3.4.8.

3.9.1.9 Porosity

Porosity of compost was measured as per the procedure given in section 3.4.9.

3.9.1.10 Total microbial count

Total microbial count in compost was determined as per the procedure given in section 3.4.10.

3.9.2 Yield data and growth assessment of mushroom

3.9. 2.1 Days taken for spawn run

Days taken for spawn run were measured as per the procedure given in section 3.5.1.

3.9.2.2 Days taken for case run

Days taken for case run were measured as per the procedure given in section 3.5.2.

3.9.2.3 Number of fruit bodies (per 100kg compost)

The number of fruit bodies harvested per 100 kg compost was counted (3.5.3).

3.9.2.4 Length of fruit bodies

Length of the fruit bodies was measured as per the procedure given in section 3.5.4.

3.9.2.5 Length of stipe of fruiting bodies

The length of the stipe of fruit bodies was measured as per the procedure given in section 3.5.5.

3.9.2.6 Width of stipe of fruiting bodies

The width of the stipe of fruiting bodies was measured as per the procedure given in section 3.5.6.

3.9.2.7 Average diameter of fruiting bodies cap

Average diameter of fruiting bodies was measured as per the procedure given in section 3.5.7.

3.9.2.8 Mushroom yield

The mushroom yield (kg/100kg weight of compost) was measured as per the procedure given in section 3.5.8.

3.9.2.9 Diseases /Pest prevalence/Competitor moulds

Mushroom bags were observed daily for the prevalence of diseases, pest and competitor moulds 3.5.9.

3.9.2.10 Biological efficiency (B.E.)

The biological efficiency was calculated as per the procedure given in section 3.5.10.

3.10 NUTRITIONAL PARAMETERS OF MUSHROOM FRUITING BODIES

3.10.1 Total ash content

Total ash was determined by taking 5 g weight of dried samples in tarred silica crucibles. The crucibles were then placed in a muffle furnace at 550°C for 5-6 hours to obtain a carbon free white ash with a constant weight (Ranganna 2009). The ash content was expressed on dry weight basis.

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample taken}} \times 100$$

3.10.2 Total protein content

The total protein content of mushrooms is estimated by multiplying total nitrogen content with a conversion factor of 4.38 (Ulziijargal and Leun Mau 2011).

3.10.3 Phenol estimation

During the storage of button mushroom, variation in the quantity of Phenols were estimated following the method described by Malick and Singh 1980. The procedure followed was as followed

Solution used

1. 80 per cent Ethanol
2. 20 per cent Na_2CO_3
3. Folin Ciocalteu reagent
4. Standard Solution of Catechol (100 µg to 1000µg)

Extraction of Sample

A total of 0.5 g dry sample was taken in a mortar and pastle and grinded. The grinded materials were extracted using 5 ml of 80 per cent ethanol followed by centrifugation at 10,000 rpm for 20 minutes. Supernatant was taken. The residue was re-extracted using 5ml ethanol (80%) and supernatant was pooled. Further the supernatant was dried and re-dissolved in 5 ml of distilled water.

Estimation

Phenols were estimated taking 1 ml of solution in a test tube and 0.5 ml of Folin Ciocalteu reagent was added. The solution was incubated at room temperature for 3-4 minutes and 2 ml of 20 per cent Na_2CO_3 solution was added. Finally, the test tubes were kept in a boiling water bath for 1-2 minutes and cooled in cold water before reading absorbance by spectrophotometer (Hitachi, Japan) at 650 nm against a reagent blank.

Standard graph

Absorbance of standard samples containing 100mg to 1000mg catechol was taken by the above method and a standard graph was plotted taking concentration on X-axis and absorbance on Y-axis. The total quantity of phenol was estimated by placing the absorbance of the samples on the standard graph.

Flow Chart

0.5g. sample
↓
Grind in mortar and pestle
↓
10 times 80 per cent ethanol
↓
Centrifuge at 10000 rpm for 20 min.
↓
Take supernatant
↓
Re extract the residue with 5 times 80 per cent ethanol and pool supernatant
↓
Dry the supernatant
↓
Dissolve in 5ml of distilled water
↓
Take 1ml solution
↓
0.5ml Folin- Ciocalteu reagent
↓
Wait for 3 min
↓
Add 2ml of 20 per cent Na_2CO_3
↓
Mix and place tube in boil water bath for 1min and cool it
↓
O.D. at 650nm against a reagent blank

3.10.4 Total crude fibre content

Crude fibre was estimated following AOAC method (Cunniff 1995).

Reagents

1. 0.255N H_2SO_4: 7 ml Conc H_2SO_4 in 1000 ml water
2. 0.313N NaOH: 12.78 g of NaOH in 1000ml water

Procedure

A total of 2 g of dried sample (C gram) was taken for the analysis and added to boiling 200 ml of H_2SO_4 (0.255N) solution in a lipless beaker covered with a round bottom flask filled with water over the beaker. The solution was kept boiling for 30 minutes, cooled and filtered over a muslin cloth. The residue was washed with distilled water till free from acid. The filtrate was tested for acidity using pH paper. Further, the residue is added to 200 ml of boiling NaOH (0.313N) in the beaker with round bottom flask filled with water over the beaker and boiled for 30 min. The filtrate was filtered on muslin cloth and washed with distilled water till free from alkali. The filtrate was tested for alkalinity using pH paper. The residue is transferred to a silica crucible and dried overnight at 110°C. Weight of crucible along with filtrate was taken and designated as A gram. The crucible was placed in muffle furnace at 600°C till ashing and the weight was taken (B gram). The crude fibre per cent was calculated using the following formulae.

$$\text{Percent fibre} = \frac{A - B}{C} \times 100$$

3.10.5 Moisture content

Moisture content of composts was determined by Gravimetric method as per the standard procedure given by Black (1965). For determination of Moisture content, samples were taken from each replication of different treatments. Collected samples were weighed and prepared for moisture content determination. The moisture content was estimated by drying the weighed sample up to a constant weight in hot air oven at 105°C.

$$\text{Moisture (\%)} = \frac{\text{Weight of fresh sample} - \text{Weight of dried sample}}{\text{Weight of fresh sample}} \times 100$$

3.10.6 Total sugars (carbohydrate) by phenol sulphuric acid method

Total sugars (reducing and non-reducing) were estimated by Phenol sulphuric acid method following a method described by Dubois et al. (1956).

Reagents

1. 5 per cent Phenol in distilled water
2. 96 per cent Sulphuric acid
3. Standard glucose solutions (5 µg to 100 µg / ml)

Procedure

Extraction

100 mg grinded sample was taken in a test tube and added 5 ml of 2.5N Hydrochloric acid. The tubes were then kept in a boiling water bath for 3 hrs and cooled to room

temperature. The solution was neutralized by solid sodium carbonate until the effervescence ceases. The volume was made up to 100 ml with distilled water and centrifuged at 10,000 rpm for 10 minutes. Supernatant was collected and subjected to analysis.

Preparation of standard graph

Standard graph was plotted using solutions of different concentrations of glucose ranging 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg /ml. One ml of sugar solution was pipetted in the bottom of the test tubes (18 x 150 mm) followed by 1 ml phenol reagent. Finally 5.0 ml of 96 per cent sulphuric acid was directly pipetted with a fast flowing pipette. The solution was mixed and allowed to cool before the absorbance was read at 490 nm against a reagent blank. Standard graph was plotted taking concentrations on X-axis and absorbance on Y-axis.

Sugar estimation of samples

1 ml of the extract was taken in the test tube and the same procedure was followed. The quantity of the sugar was estimated by placing the absorbance of the unknown samples on the standard graph.

3.10.7 Energy value

Energy value was calculated by using following formula given by Tripathi et al. 2019.

$$\text{Energy (Kcal)} = 4 \times (\text{Protein g} + \text{Carbohydrate g}) + 9 \times (\text{Fat g})$$

Statistical Analysis

Results shall be statistically analysed by using Completely Randomised Design (CRD) as per Gomez and Gomez (1984).

1. ANOVA for CRD shall be as follows:

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	F_{cal}
Treatments	(t-1)	S_t	$M_t = \frac{S_t}{(t-1)}$	$\frac{M_t}{M_e}$
Error	(t)(r-1)	S_e	$M_e = \frac{S_e}{(r-1)(t-1)}$	
Total	(rt-1)	S_T		

Where,

T = Number of treatments

S_t = Sum of squares due to treatments

S_e = Sum of squares due to error

S_T = Total sum of squares

M_t = Mean sum of squares due to treatments

M_e = Mean sum of squares due to error

The replication and treatment mean sum of square shall be tested against error mean squares by ‘F’ test at (t-1), (t) (r-1) degree of freedom for CRD and at (r-1), (r-1) (t-1) and (t-1)at 5 per cent level of significance.

The calculated F-values shall be compared with tabulated F- value. When F- test will be found significant, critical difference will be calculated to find out the superiority of one treatment over the others.

2. Critical difference (CD) shall be calculated as follows:

For CRD:

$CD_{(0.05)} = S.E. (d) \times t_{(0.05)} (t) (r-1)$ df

For RBD:

$CD_{(0.05)} = S.E. (d) \times t_{(0.05)} (r-1) (t-1)$ df

$$SE (d) \pm = \sqrt{2\frac{M_e}{r}}$$

$$SE (m) \pm = \sqrt{\frac{M_e}{r}}$$

Where,

SE (m) ± = Standard error of mean

SE (d) ± = Standard error of difference of mean

$CD_{(0.05)}$ = Critical difference at 5 per cent level of significance

3. Path correlation analysis

Path correlation analysis was conducted for physical and chemical parameters of compost as independent variable and yield as dependable variable. Overall correlation of different variables was estimated and then the direct and indirect effects of the independent variables on yield per cent was analysed following Sheoran et al. (1998) using OPSTAT software.

Chapter-4

RESULTS AND DISCUSSION

Results of the present study entitled "**Studies on utilization of paddy straw for compost preparation of button mushroom (*Agaricus bisporus*)"** are described under following headings:

4.1 PREPARATION OF DIFFERENT COMPOST FORMULATIONS

4.1.1 Evaluation of compost and its effect on yield of mushroom

4.2 QUALITY ASSESSMENT OF COMPOST FORMED BY DIFFERENT COMPOST FORMULATIONS

4.2.1 Changes in physical properties of different compost formulations

4.2.2 Changes in chemical properties of different compost formulations

4.2.3 Silica content in different compost formulations

4.2.4 Thermophilic microbial count in different compost formulations

4.2.5 Estimation of degradative enzyme activities in different compost formulations

4.3 COMPARISON OF TWO-YEAR DATA OF MUSHROOM PRODUCTION

4.4 ISOLATION OF THERMOPHILIC FUNGI FROM THE COMPOST

4.5 ESTIMATION OF DIFFERENT ENZYME ACTIVITIES OF ISOLATED THERMOPHILIC FUNGI

4.6 EVALUATION OF COMPOST QUALITY AND MUSHROOM YIELD BY INOCULATING ISOLATED THERMOPHILIC FUNGI IN COMPOST

4.7 QUALITY ASSESSMENT OF COMPOST

4.7.1 Physical properties of composts made by different treatments

4.7.2 Chemical properties of composts made by different treatments

4.7.3 Silica content in different compost formation

4.7.4 Microbial count in different compost treatments by using thermophilic fungi

4.7.5 Changes of physico-chemical properties of treated compost at different stages

4.7.6 Estimation of degradative enzyme activities in different compost formulations upon thermophilic fungi inoculations

4.8 NUTRITIONAL ANALYSIS OF THE MUSHROOMS

4.1 PREPARATION OF DIFFERENT COMPOST FORMULATIONS

Paddy straw burning increased dramatically over the last decade, despite being banned in most rice- growing countries because of pollution and the associated health issues. So, it is important to look for sustainable solutions that can reduce the environmental footprint and upgrading the value chain of rice straw byproducts by using it as compost raw material for growing button mushroom. Thus, sustainable straw- management practices can influence farmers to avoid open-field burning, which can in turn reduce negative environmental and health consequences. Keeping the fact in view, use of paddy straw for compost production for button mushroom (*Agaricus bisporus*) cultivation was tried. The cultivation trial was laid at mushroom production unit of Department of Plant Pathology, Dr. Y.S. Parmar University of Horticulture and Forestry Nauni, Solan (H.P). There were five treatments in total with four replications mention below in table 4.1(Plate 1 and 2).

Table 4.1 Quantity of composting ingredients (kg) in different composting treatments

Treatments	**T1 (kg) PS:WS (1.5:1)**	**T2 (kg) PS: WS (3:1)**	**T3 (kg) PS:WS (1:1)**	**T4 (kg) PS**	**T5 (kg) WS (Control)**
Paddy straw (PS)	600	750	500	1000	-
Wheat straw (WS)	400	250	500	-	1000
Chicken manure	600	600	600	600	600
Wheat bran	100	100	100	100	100
Urea	15	15	15	15	15
Gypsum	30	30	30	30	30
Nitrogen (%)	1.68	1.69	1.66	1.72	1.60

No. of treatments: 5, No. of replications: 4, No. of bags per replication: 10, Bag size: 10kg, Design: CRD

4.1.1 Evaluation of compost and its effect on yield of mushroom

During evaluating different compost formulations, it was observed that treatment 3 took the minimum time for spawn run (14.00 days) followed by treatment 1 (15.02 days), which were statistically at par with each other. The maximum days for spawn run were taken by treatment 2 and 4 (16.01 days).

In case of case run, treatment 3 took minimum days (19.03 days) followed in treatment 5 (20.00 days). The maximum days for case run were taken in treatment 4

Plate 1. Different Compost formulations

1. Weighing of substrate 2. Addition of chicken manure and watering 3. Addition of wheat bran 4. Heap formation 5. Monitoring temperature 6. Turning 7. Adding gypsum 8. Heap formation 9. Shifting of compost to Phase-II

10. Filling into pasteurization tunnel 11. Spawning 12. Mushroom bags in cropping room (22-25°C) 13. Casing 14. Fruiting (14-18°C) 15. Harvesting

Plate 2. Steps involved in mushroom production by using different substrate

(21.02 days). For pin head formation also treatment 3 took minimum days (24.02 days) followed by treatment 4 (25.00 days) while the maximum days were taken by treatment 2 and 5 (26.04 days). The minimum days for first harvest was recorded in treatment 3 (28.02 days) followed by treatment 5 (29.00 days), which were statistically at par with each other. The maximum days for first harvest were taken by treatment 4 (32.08 days).

Out of all the five treatments, the maximum yield was obtained from the treatment 3 (19.98 kg/ 100kg compost) followed by treatment 1 (17.76 kg/100kg compost). The minimum yield was obtained from the treatment 4 (15.22 kg/ 100kg compost). Average fruit body weight was recorded the maximum in treatment 3 (15.05g) followed by treatment 1 (13.22g) while the minimum average fruit body weight was found in treatment 4 (11.30g). All the five treatments of different combinations were found free from any diseases or pest during cropping time (Table 4.2, Plate 3, 4 and 5). Yield of mushroom in T-5 (wheat straw control), which is generally used in cultivation of button mushroom worldwide at commercial level have shown lower productivity during the study. These results are unexpected and may be due to the fact that wetting period required for absorption of water by different straw varies considerably. It is reported that paddy straw normally absorbs water up to 70 per cent in a period of 18-22 hours of wetting while wheat straw takes almost 48 hours to absorb 70 per cent water. Since the experiment was conducted simultaneously for both wheat and paddy straw, it may have impacted the water absorption of wheat straw and optimum water absorption could not have achieved by the wheat straw in the case.

Kaur and Khanna (2001) reported a yield in the range of 17.9-23.7 kg/100kg compost using two synthetic compost preparations i.e., wheat straw+ paddy straw (1:1) and wheat straw+ paddy straw (1:2). According to Shandilya (1989) two formulations of paddy straw composts using horse manure (5:1 w/w) and chicken manure (2.5:1 w/w) produced a good yield of button mushrooms. Tewari and Sohi (1976) used paddy straw and maize stalks (1:1 w/w) as substitute for wheat straw for the cultivation of *Agaricus bisporus*. They prepared synthetic compost using maize stalks and paddy straw with other ingredients i.e., ammonium sulphate, super sulphate, urea, chalk, gypsum and rice bran with an average yield of 145.5 kg/tonne of compost. Two formulations of compost with WS+PS (1:1 w/w) and WS+PS (1:2 w/w) have been recommended by PAU for the growers (Khanna and Kapoor 2016). Kaur et al. (2019) evaluated composts made from paddy straw + maize stalks (1:1

Table 4.2 Effect of different compost treatments on the production of white button mushroom

Treatments	Days taken for				Yield (kg/100kg)	Average fruit body weight (g)
	Spawn run	Case run	Pinhead formation	First harvest		
T1 **Paddy straw+ Wheat straw (1.5:1)**	15.02	20.03	25.01	29.01	17.25	13.22
T2 **Paddy straw+ Wheat straw (3:1)**	16.01	21.01	26.04	30.01	16.75	12.80
T3 **Paddy straw+ Wheat straw (1:1)**	14.00	19.03	24.02	28.02	19.98	15.05
T4 **Paddy straw**	16.01	21.02	25.00	32.08	15.22	11.30
T5 **Wheat straw (Control)**	15.03	20.00	26.04	29.00	17.76	12.30
Mean	15.22	20.22	25.22	29.62	17.39	12.93
C.D. (0.05)	1.04	0.95	0.69	1.57	0.07	0.04
SE	0.34	0.31	0.23	0.52	0.02	0.01

w/w), paddy straw + maize stalk (2:1 w/w) and paddy straw alone. Yield data indicated a maximum yield of 13.6 kg/q compost in combination of paddy straw + maize stalk (1:1, w/w) with 1563 fruit bodies/ q compost. Days taken for spawn run, case run and fruiting bodies development was recorded the minimum in paddy straw + maize stalk (1:1, w/w) combination, while average fruit body weight and biological efficiency was also found to be the maximum in the combination. Uddin et al. (2012) evaluated wheat + paddy straw compost (1:1) along with paddy straw-based compost for production of *Agaricus bisporus*.

They studied number of fruiting bodies; number of primordia and yield was found better in the combination of wheat straw+ paddy straw (1:1) than paddy straw used alone. Singh et al. (2020) also studied the effect of wheat straw + paddy straw (1:1) formulations on growth and yield of button mushroom against wheat straw alone as control. They reported that days required for fruiting bodies development were less in combination of wheat straw + paddy straw while the yield and biological efficiency (11.2%) was recorded highest in combination of wheat straw + paddy straw.

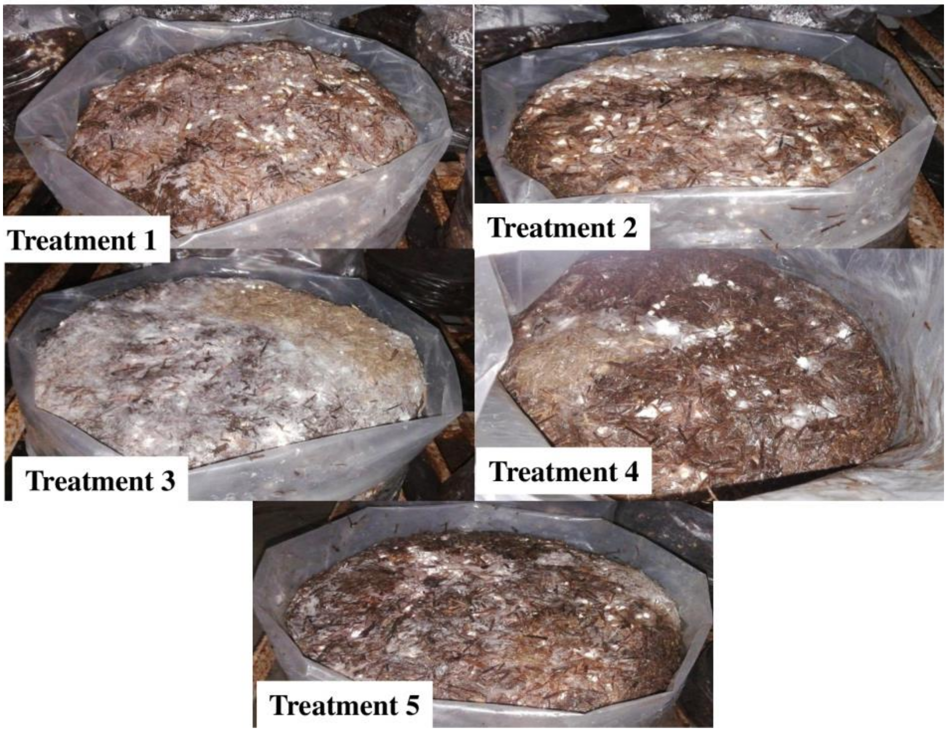


Plate 3. Effect of different compost treatments on the spawn run of button mushroom

1

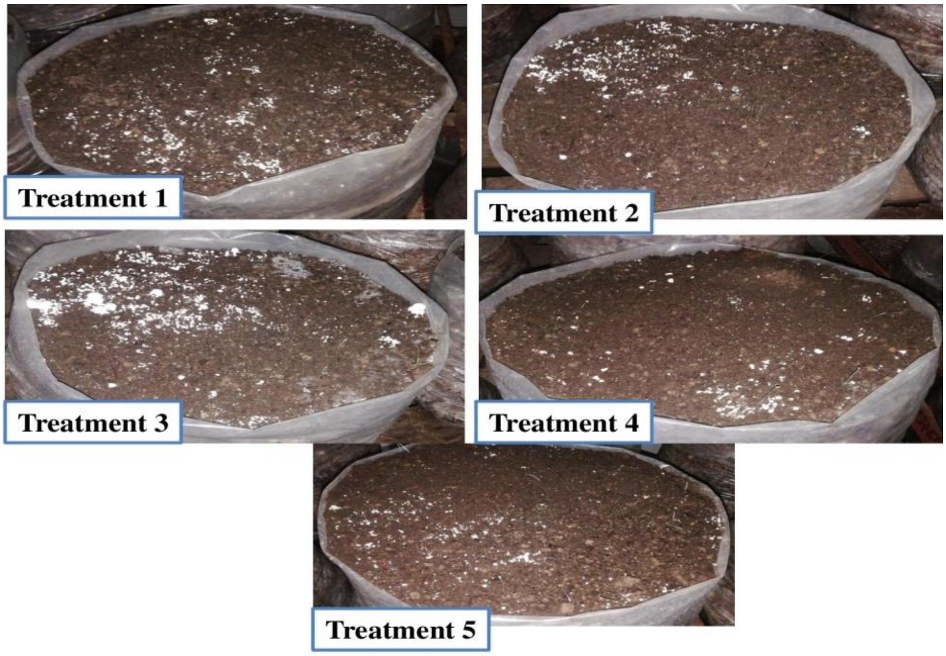


Plate 4. Effect of different compost treatments on the pinning of white button mushroom

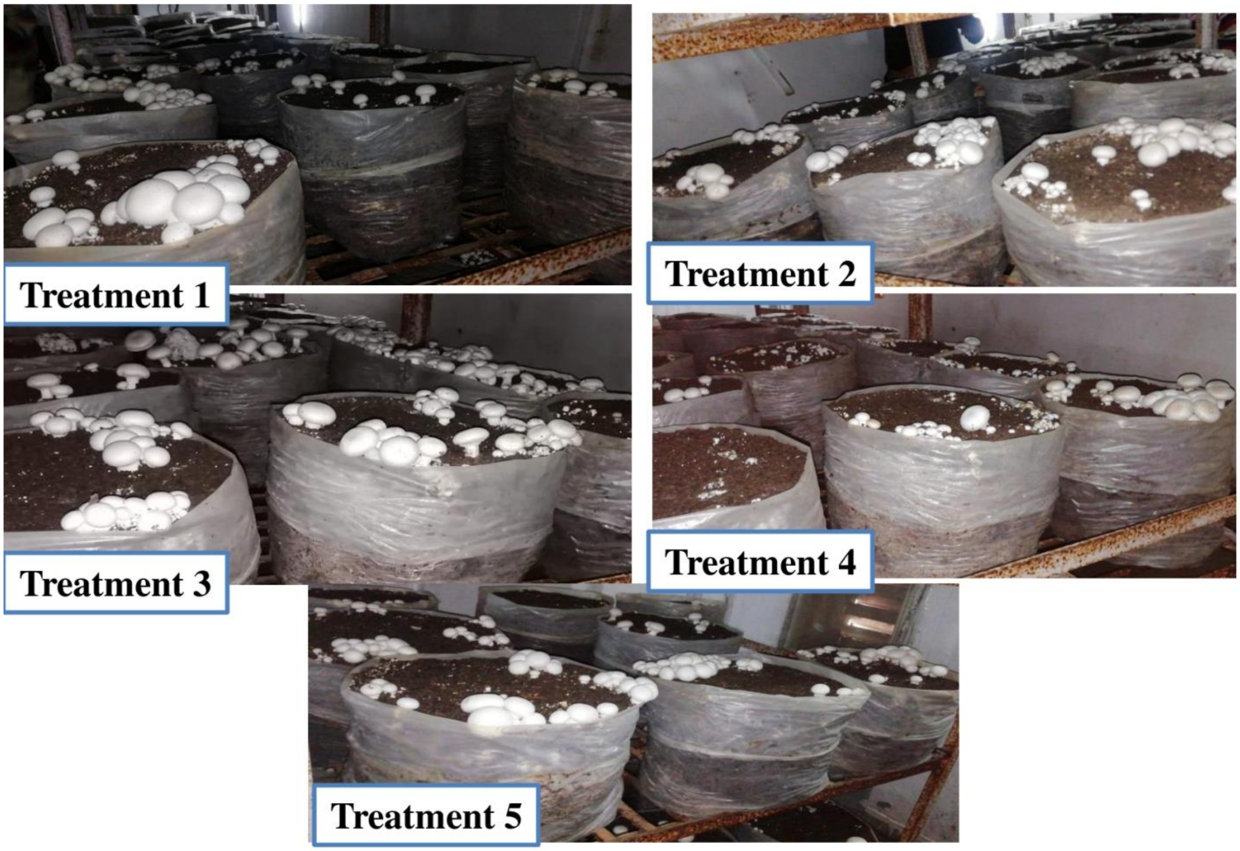


Plate 5. Effect of different compost treatments on the yield of button mushroom

4.1.2 Effect of different compost treatments on morphometric parameters of button mushroom

The observation recorded on morphometric parameters like diameter of pileus, thickness of pileus, length of stipe and diameter of stipe (Table 4.3). The observation revealed that diameter of pileus was found to be the highest in treatment 3 (3.90cm) followed by treatment 2 (3.80cm), whereas, the lowest pileus diameter was found in treatment 4 (3.40 cm). Thickness of pileus was recorded maximum in treatment 3 (1.80cm) followed by treatment 1 (1.50cm) and minimum pileus thickness was recorded in treatment 4 (1.10cm).

Maximum length of stipe was recorded in treatment 3 (2.60cm) followed by treatment 2 (2.44cm) and minimum length was found in treatment 4 (2.10cm). Diameter of stipe was recorded maximum in treatment 3 (1.50cm) followed by treatment 5 (1.40cm) and minimum diameter of stipe was recorded in treatment 2 (1.10cm).

Singh et al. (2020) studied the wheat straw + paddy straw (1:1) formulation for button mushroom production against wheat straw control. They studied the yield attributing characters i.e., stalk length, stalk diameter and pileus diameter. They reported a non-significant variation in the studied characters in both the formulations

Table 4.3 Effect of different compost treatments on morphometric parameters of button mushroom

Treatments	Diameter of pileus (cm)	Thickness of pileus (cm)	Length of stipe (cm)	Diameter of stipe (cm)
T1 **Paddy straw+ Wheat straw (1.5:1)**	3.60	1.50	2.33	1.20
T2 **Paddy straw+ Wheat straw (3:1)**	3.80	1.30	2.44	1.10
T3 **Paddy straw+ Wheat straw (1:1)**	3.90	1.80	2.60	1.50
T4 **Paddy straw**	3.40	1.10	2.10	1.30
T5 **Wheat straw (Control)**	3.50	1.12	2.33	1.40
Mean	3.64	1.36	2.36	1.30
C.D. (0.05)	0.27	0.21	0.06	0.15
SE	0.09	0.07	0.02	0.05

4.2 QUALITY ASSESSMENT OF COMPOSTS MADE BY DIFFERENTCOMPOST FORMULATIONS

The samples from composts prepared with five different formulations were drawn at the time of spawning following standard protocol. The samples were dried at 55-60º C in a hot air oven till constant weight, grinded and analyzed. The composts prepared with different combination were found to vary in their quality characteristics as shown below.

4.2.1 Changes in physical properties of different compost formulations

The composts prepared with different combinations were found to vary in their quality characteristics as shown in table 4.4. In physical properties, parameters evaluated were moisture content, pH, electric conductivity, organic matter, bulk density, particle density and porosity.

The highest moisture percent of compost was recorded in treatment 3 (69.83%) followed by treatment 5 (65.97%), which significantly varied to each other. Minimum moisture content was found in treatment 2 (60.83%). Miller et al. (1990) found that final compost had a moisture content of 66- 75 per cent. Kaur and Khanna (2001) reported that the moisture content of three compost combination wheat straw + paddy straw (1:1 w/w), wheat straw + paddy straw (1:2 w/w) and paddy straw alone was observed to be in the range of 59-72 per cent. The optimum moisture content for biodegradation has been reported to vary widely for different compost mixtures in the composting process ranging from 50 to 70 per cent on a wet basis (Richard et al. 2002). According to Almomany and Masaed (2019) the moisture content of compost ranged between 50 - 72 per cent. The moisture content of the composts was observed to be 65.2 - 67.3 per cent in paddy straw: maize Straw (2:1 and 1:1 w/w) (Kaur et al. 2019). All the finding ends support to our observations.

Hydrogen ion concentration (pH) of different compost formulations was found to vary in different treatment combinations. pH in treatment 1 (7.60), treatment 5 (7.59) and treatment 2 (7.49) were found statistically at par. Minimum pH was recorded in treatment 4 (7.03). Earlier workers have reported the optimum pH range for the composting process in the range of 5.8 to 7.2 (Dalzell et al. 1987). Kaur and Khanna (2001) reported pH range of 6.9 - 8.3 in four compost formulations comprising wheat straw alone, wheat straw + paddy straw (1:1 w/w), wheat straw + paddy straw (1:2 w/w), and paddy straw alone. The pH was recorded as 6.4-6.7 for paddy straw (PS)+maize straw (MS) (1:1 and 2:1 w/w) respectively at the start of the composting which gradually increased to 7.6-7.8 for paddy straw + maize straw (2:1 and 1:1 w/w) between the turnings and finally declined to 7.2-7.3 in PS:MS (2:1 and 1:1 w/w) at the time of spawning (Kaur et al. 2019). Electric conductivity (EC) of compost was recorded highest in treatment 1 (2.44 deci S m^{-1}) followed by the treatment 5 (2.42 deci S m^{-1}), which were statistically at par. Minimum EC was recorded in treatment 4 (1.91 deci S m^{-1}). According to Singh et al. (2000) electrical conductivity plays an important role in the production of *Agaricus bisporus*, but it is not the sole controlling factor. Shandilya and Hayes (1987) reported a decrease in the number of pin heads with an increase in the electrical conductivity. De Ger (2000) reported that an electrical conductivity value of more than 7 to 9 mho had a negative influence on the quality and especially quantity of mushrooms. Jarial and Shandilya (2004) also reported a negative correlation between electrical conductivity and mushroom yield indicating a decrease of 0.02 units in mushroom yield with every unit increase in electrical conductivity.

The organic matter content of compost prepared in this study ranged between 50- 69 per cent (dry weight basis) and it decreased with the time as a result of degradation and carbon utilization by the mushroom mycelium during growth. Out of all the five treatments, organic matter content of compost was found maximum in treatment 3 (67.24%) followed by treatment 4 (66.82%), which varied significantly to each other. Minimum organic matter was recorded in treatment 4 (63.43%). Organic carbon in all the treatments varied significantly. The decrease in organic matter may be due to the loss of carbon during the mycelia respiration. Similar kind of results was obtained by Almomany and Masaed (2019) they report organic matter content of compost ranged between 50- 69 per cent (dry weight basis) and an inverse relation to bulk density and organic matter, while a directly proportional relation in organic matter and the porosity. Organic carbon content in compost varied between 39.71 to 17.81 per cent. Chen et al. (2000) reported that organic matter contents of the substrates ranged from 55.0- 62.8 per cent. Fidanza et al. (2010) recorded organic matter content averaged 25.86 per cent (wet weight) or 60.97 per cent (dry weight). Organic matter and carbon are essential for enhancement of agriculture production (Davis et al. 2006 and Sinha et al. 2020). Almost similar results have been obtained in our findings also.

The bulk density of compost was recorded maximum in treatment 4 (0.47 g/cm^3) followed by treatment 2 (0.45 g/cm^3) which were significant to each other. Minimum bulk density was recorded in treatment 3 (0.39 g/cm^3). Particle density of the composts was found maximum in treatment 2 (0.97 g/cm^3) followed by treatment 3 and 4 (0.94 g/cm^3) out of all the five treatments. Minimum particle density was recorded in treatment 3 (0.89 g/cm^3). Porosity of

Table 4.4 Physical quality characteristics of the compost in different compost formulations

Treatments	Moisture (%)	pH	EC (deci S m^{-1})	Organic matter (%)	Bulk density (g/cm^3)	Particle density (g/cm^3)	Porosity (%)
T1 **Paddy straw+ Wheat straw (1.5:1)**	62.72	7.60	2.44	64.81	0.41	0.91	54.94
T2 **Paddy straw+ Wheat straw (3:1)**	60.83	7.49	2.26	66.62	0.45	0.97	53.61
T3 **Paddy straw+ Wheat straw (1:1)**	69.83	7.39	2.38	67.24	0.39	0.89	56.17
T4 **Paddy straw**	63.76	7.03	1.91	66.82	0.47	0.94	50.00
T5 **Wheat straw (Control)**	65.97	7.59	2.42	63.43	0.43	0.94	54.26
Mean	64.62	7.42	2.28	65.78	0.43	0.93	53.8
C.D. (0.05)	3.16	0.33	0.30	0.03	0.02	0.03	1.90
SE	1.04	0.11	0.10	0.01	0.01	0.01	0.63

all compost formulations were found to vary in different treatment combinations. Among all the five treatments, maximum porosity was recorded in treatment 3 (56.17%) followed by treatment 1 (54.94%), which showed significant variation to each other. Minimum porosity was recorded in treatment 4 (50%).

Miller et al. (1990) found that final compost had a bulk density 0.44 - 0.51 g/cm^3. Bulk density of the compost ranged between 0.58 g/cm^3 at the beginning of the composting process, which increased to 0.78 g/cm^3 at the end of the crop cycle (Almomany and Masaed 2019). These results are similar with our findings. In contrast, the porosity of the compost decreased with time. The porosity at the beginning of composting was 96 per cent, which reduced to 87 per cent at end of the cropping cycle. It was observed during the study that bulk density and porosity varied inversely to each other. Although it was reported that mushroom yield increased with the increase in porosity while decreased with the decrease in porosity (high bulk density).

Correlation analysis and path coefficient separation has been done for all the physical parameters with yield and results are given in table 4.5, 4.6 and 4.7.

The correlation analysis examines the effect of independent variables (all physical parameters of compost) on the dependent variables (yield). The standard error calculated in the analysis showed a significant effect of the moisture, organic matter, porosity on the yield per cent of white button mushroom (Table 4.5). The correlation analysis has shown a significantly positive effect of moisture and porosity on the yield per cent. This indicates that high moisture content along with high porosity in the compost has significantly affected the yield potential of the compost. It also implies that if moisture content becomes high with low porosity in the compost, it will have a negative effect on the yield per cent. It could also be inferred from the data analysis that pH, bulk density and particle density of the compost have significantly strong negative effect on the yield of button mushroom while electrical conductivity and organic matter of the compost have moderate positive effect on yield (Table 4.6).

The correlations coefficients were divided into direct and indirect effects and are depicted in table 4.7. The separation of direct and indirect effects of independent variable on dependent variable showed that the moisture *per se* does not have any significant direct effect on the yield but high moisture contributes to the high bulk and particle density with low porosity in the compost, which ultimately affects the yield per cent negatively. However, in the correlation analysis the moisture content with high porosity showed a positive effect on yield per cent of the button mushroom. The results showed that the organic matter content have direct significant effect on the yield, however, it also contributes to low bulk and particle density. The bulk density and particle density has shown a negative correlation with the yield implying the lower yield with higher bulk and particle density while the porosity has direct positive effect on yield per cent of white button mushroom.

Table 4.5. Means, standard deviation and standard error of correlation analysis of all physical parameters (independent variables) with yield (dependable variable)

Variables	**Mean**	**Standard deviation**	**Standard error**
Yield (%)	17.993	1.739	1.004
Moisture (%)	64.460	4.746	2.740
pH	7.493	0.105	0.061
EC (deci Sm-1)	2.360	0.092	0.053
Organic matter (%)	66.223	1.263	0.729
Bulk density (g/cm^3)	0.417	0.031	0.018
Particle density (g/cm^3)	0.923	0.042	0.024
Porosity (%)	54.907	1.280	0.739

Table 4.6. Correlation matrix of all physical parameters (independent variables) with yield (dependable variable)

Variables	**Yield (%)**	**Moisture (%)**	**pH**	**EC (deci Sm-1)**	**Organic matter (%)**	**Bulk density (g/cm^3)**	**Particle density (g/cm^3)**	**Porosity (%)**
Yield (%)	1.00							
Moisture (%)	**1.00**	1.00						
pH	**-0.77**	-0.73	1.00					
EC (deci Sm-1)	0.33	0.38	0.35	1.00				
Organic matter (%)	0.59	0.54	-0.97	-0.57	1.00			
Bulk density (g/cm^3)	**-0.84**	-0.87	0.30	-0.79	-0.06	1.00		
Particle density (g/cm^3)	**-0.79**	-0.82	0.21	-0.84	0.03	1.00	1.00	
Porosity (%)	**0.92**	0.94	-0.46	0.67	0.22	-0.99	-0.97	1.00

Table 4. 7. Path analysis of all physical parameters (independent variables) with yield (dependable variable)

Variables	Moisture (%)	pH	EC (deci Sm-1)	Organic matter (%)	Bulk density (g/cm^3)	Particle density (g/cm^3)	Porosity (%)	Correlation coefficient with yield
Moisture (%)	0.60	0.17	0.21	0.74	1.07	1.07	-1.65	**1.00**
pH	0.44	-0.23	0.19	-1.32	-0.37	-0.28	0.80	**-0.77**
EC (deci Sm-1)	-0.23	-0.08	0.55	-0.78	0.96	1.09	-1.18	0.33
Organic matter (%)	-0.32	0.23	-0.31	1.36	0.07	-0.04	-0.39	0.59
Bulk density (g/cm^3)	0.52	-0.07	-0.43	-0.08	1.22	1.29	-1.73	**-0.84**
Particle density (g/cm^3)	0.49	-0.05	-0.46	0.05	1.22	1.30	-1.70	**-0.79**
Porosity (%)	-0.56	0.11	0.37	0.31	-1.21	-1.25	1.76	**0.92**

4.2.2 Changes in chemical properties of different compost formulations

In chemical properties of compost macro and micronutrient analysis was done following standard procedures.

a) Macronutrients characteristics of the compost in different compost formulations

In macronutrients, organic carbon, nitrogen, phosphorous, potassium, calcium and magnesium were analyzed and data has been depicted in table 4.8. Organic carbon was recorded maximum in treatment 5 (41.99%) followed by treatment 3 (38.97%), which significantly differed to each other; whereas minimum carbon was recorded in treatment 1 (37.58%). In all the five treatments, nitrogen per cent was found maximum in treatment 3 (1.88%) followed by treatment 4 (1.78%) while minimum nitrogen (1.68%) was observed in treatment 1. Phosphorous analysis showed maximum concentration in treatment 3 (0.83%) followed by treatment 2 (0.81%), which were statistically at par to each other whereas, minimum phosphorous was recorded in treatment 4 (0.62%). Potassium was found maximum in the compost made by treatment 2 (2.76%) followed by treatment 1 (2.61%), which significantly differed to each other; whereas minimum potassium was recorded in treatment 4 (2.02%). All the treatments significantly varied to each other. Treatment 3 (3.49%) showed a maximum calcium concentration followed by treatment 2 (3.11%) in the study, which were significantly different to each other; whereas minimum was found in treatment 5 (2.11%). Magnesium was found maximum in treatment 3 (0.55%) followed by treatment 5 (0.54%), which were at par to each other. Minimum magnesium nutrient was recorded in treatment 1 (0.40%). Organic carbon content in compost is reported to vary between 39.71 - 17.81 per cent (Rupert 1995; Davis et al. 2006 and Sinha et al. 2020). Sinha et al. (2020) reported nitrogen content of composts in the range of 1.20 - 1.52 per cent. In general, composts have low nitrogen (N) content, typically in the 1 to 3 per cent range (Acquaah 2009). Fidanza et al. (2010) reported average total nitrogen content as 1.12 per cent (wet weight) or 2.65 per cent (dry weight). Micronutrients are an essential requirement for optimum growths of button mushroom mycelium and required in small amounts (Sinha et al. 2020).

Table 4. 8. Macronutrients composition in different compost formulations

Treatments	**Organic carbon (%)**	**Nitrogen (%)**	**Phosphorus (%)**	**Potassium (%)**	**Calcium (%)**	**Magnesium (%)**
T1 **Paddy straw+ Wheat straw (1.5:1)**	37.58	1.68	0.72	2.61	2.39	0.40
T2 **Paddy straw+ Wheat straw (3:1)**	38.63	1.71	0.81	2.76	3.11	0.52
T3 **Paddy straw+ Wheat straw (1:1)**	38.97	1.88	0.83	2.52	2.65	0.42
T4 **Paddy straw**	38.74	1.78	0.62	2.02	3.49	0.55
T5 **Wheat straw (Control)**	41.99	1.75	0.71	2.21	2.11	0.54
Mean	39.18	1.76	0.74	2.42	2.75	0.47
C.D. (0.05)	1.16	0.09	0.10	0.11	0.19	0.08
SE	0.38	0.03	0.03	0.04	0.06	0.03

They recorded phosphorus content in the compost as 0.73 per cent, calcium as 3.05 per cent and magnesium 0.185 per cent. Fidanza et al. (2010) reported average phosphorus (P) content as 0.29 per cent (wet weight) or 0.69 per cent (dry weight) and average potassium (K) content as 1.04 per cent (wet weight) or 2.44 per cent (dry weight). They also recorded that fresh mushroom compost contains the secondary macronutrients *viz.*, calcium (Ca) at 2.32 per cent (wet weight) or 5.38 per cent (dry weight), magnesium (Mg) at 0.36 per cent (wet weight) or 0.83per cent (dry weight).

b) Micronutrients characteristics of the compost in different compost formulations

In micronutrients copper, zinc, iron and manganese were analyzed by standard procedure. Data with respect to micronutrients has been depicted in table 4.9. Out of all the five treatments, copper was recorded maximum in treatment 4 (44.60ppm) followed by treatment 1 (31.50ppm) which differed significantly from each other, while copper was recorded minimum in treatment 2 (23.10ppm). Zinc was found maximum in treatment 3 (69.60 ppm) followed by treatment 5 (66.50ppm), which were significantly different to each other. Minimum zinc concentration was recorded in treatment 4 (57.50ppm) and all the treatments were significantly different from each other. Treatment 2 (2110ppm) showed maximum iron followed by treatment 3 (1670ppm) while minimum was found in treatment 4 (1096ppm). With respect to manganese, maximum concentration was recorded in treatment 2 (154 ppm) while minimum was recorded in treatment 4 (116ppm). Sinha et al. (2020) studied micronutrients Cu, Zn, Mn, Fe in mushroom compost. They reported changes in micronutrient concentration from day zero to final compost. They stated that Cu change from 11.08 to 16.22 ppm, Zn 0.96- 84.21 ppm, Mn 234.00- 150.20 ppm and Fe 51.61- 5120 ppm. Average micronutrients sequence was reported to be Cu < Zn < Mn < Fe. This order was arranged according to average content of micronutrients present in composting and cultivation of white button mushroom. The micronutrients must be present for optimum growth and are required in small amounts (Yawalkar et al. 2016).

Table 4.9 Micronutrients composition in different compost formulations

Treatments	**Copper (ppm)**	**Zinc (ppm)**	**Iron (ppm)**	**Manganese (ppm)**
T1-Paddy straw+ Wheat straw (1.5:1)	31.50	59.90	1,550.00	120.00
T2 -Paddy straw+ Wheat straw (3:1)	23.10	65.70	2,110.00	154.00
T3 -Paddy straw+ Wheat straw (1:1)	25.90	69.60	1,670.00	145.00
T4 -Paddy straw	44.60	57.50	1,096.00	116.00
T5 -Wheat straw (Control)	30.90	66.50	1,404.00	121.00
Mean	41.20	63.84	1566.00	131.20
C.D. $_{(0.05)}$	1.08	1.34	21.82	7.78
SE	0.36	0.44	7.17	2.56

c) Carbon and nutritional ratio of different compost formulations

The results of table 4.10 depicted that final C: N ratio of the compost was maximum in treatment 5 (23.59) and minimum in treatment 3 (20.73).

Table 4.10 Effect on carbon and nutrients ratio of compost by using different compost formulations

Treatments	C: N	C: P	C: K	N: P	N: K	P: K
T1 **Paddy straw+ Wheat straw (1.5:1)**	22.37	52.19	14.40	1.63	0.45	0.28
T2 **Paddy straw+ Wheat straw (3:1)**	22.59	47.69	14.00	1.42	0.42	0.29
T3 **Paddy straw+ Wheat straw (1:1)**	20.73	46.95	15.46	2.36	0.78	0.33
T4 **Paddy straw**	21.76	62.48	19.18	3.35	1.03	0.31
T5 **Wheat straw (Control)**	23.59	59.14	19.00	1.58	0.45	0.32
Mean	22.28	53.69	16.41	2.07	0.63	0.31
C.D. (0.05)	0.43	1.26	1.07	0.30	0.34	0.01
SE	0.14	0.41	0.35	0.10	0.11	0.02

C: P ratio was found to be highest in treatment 4 (62.48) followed by treatment 5 (59.14), which significantly differed to each other. The minimum C: P ratio was found in treatment 3 (46.95). C: K ratio varied from 14.00 to 19.18 in different compost formulations and recorded maximum in treatment 4 (19.18) followed by treatment 5 (19.00) and both were at par. In treatment 2, minimum C: K ratio (14.00) was observed. N: P ratio was in the range of 1.42 to 3.35 with a maximum in treatment 4 (3.35) followed by treatment 3 (2.36) and minimum in treatment 2 (1.42). N: K ratio varied from 0.42 to 1.03 with a maximum in treatment 4 (1.03) followed by the treatment 3 (0.78), treatment 1 and treatment 5 (0.45). Minimum N: K ratio was recorded in treatment 2 (0.42). P: K ratio was in the range of 0.28 to 0.33 with a maximum in treatment 3 (0.33) and minimum in treatment 1 (0.28).

The ideal C/N ratio for composting is generally considered to be around 30:1 or 30 parts carbon for each part nitrogen by weight. Pace et al. (1995) reported that the organic material consists of 20-30 parts of carbon for one part of nitrogen for good composting. To guarantee a good composting process, the starting mixture should have an adequate C:N ratio between 25-35:1 (Alsanius et al. 2016). It is generally recognized that a C/N ratio between 15:1 and 30:1 is ideal for composting (Guo et al. 2012). According to Kaur and Khanna (2001) the C:N ratio for compost made from wheat and paddy straw mixtures (1:1 (w/w), 1:2 (w/w)) and paddy straw alone ranged from 16.4 to 20.3:1. Fidanza and Beyer (2009) reported an ideal C:N between 15-21:1 for *A. bisporus* composting and not greater than 30:1. C:N ratio of compost was reported to vary in the range of 26.13:1 to 20.24:1 (Sinha et al. (2020). C:N ratio between 26-40:1 is recommended by many researchers for rapid and effective composting (Gaur and Mathur 1990). Fresh mushroom compost C:P ratio varied between 36.15 to 70.66 (Fidanza et al. 2010). The phosphate is important for a series of functions in the fungal metabolism and is one of the essential nutrients required for growth and development. P-deficient compost exhibit retarded mycelia growth and often shows a dark color (Yawalkar et al. 2016). Sinha et al. (2020) found that C:P ratio obtained from compost varied between 34.92 to 49.95 and suitable for white button mushroom cultivation. In fresh mushroom compost C:K ratio varied between 12.12 to 17.37 (Fidanza 2010). It benefits in the development of proteins. It is also reported by workers that an increased amount of nitrogen invites diseases and pest in the mushroom cultivation, therefore, a balanced ratio of N and K is essential in mushroom compost (Yawalkar et al. 2016).

Correlation analysis and path coefficient separation has been done for all the chemical parameters with yield and results are given in table 4.11, 4.12 and 4.13.

Table.4.11 Means, standard deviation and standard error of correlation analysis of all chemical parameters (independent variables) with yield (dependable variable)

Variables	Mean	Standard deviation	Standard error
Yield	17.99	1.74	1.00
OC	38.39	0.72	0.42
N	1.76	0.11	0.06
P	0.79	0.06	0.03
K	2.63	0.12	0.07
Ca	2.72	0.37	0.21
Mg	0.45	0.06	0.04
Cu	26.83	4.28	2.47
Zn	65.07	4.88	2.82
Fe	1776.67	294.85	170.23
Mn	139.67	17.62	10.17
C: N	21.90	1.02	0.59
C:P	48.94	2.84	1.64
C: K	14.62	0.75	0.44
N:P	1.80	0.49	0.29
N: K	0.55	0.20	0.12
P: K	0.30	0.03	0.02

Table 4.12. Correlation matrix of all chemical parameters (independent variables) with yield (dependable variable)

Variables	**Yield**	**OC**	**N**	**P**	**K**	**Ca**	**Mg**	**Cu**	**Zn**	**Fe**	**Mn**	**C: N**	**C: P**	**C: K**	**N: P**	**N: K**	**P: K**
Yield	1.00																
OC	0.58	1.00															
N	**0.96**	0.78	1.00														
P	0.52	1.00	0.74	1.00													
K	**-0.87**	-0.09	-0.69	-0.03	1.00												
Ca	-0.30	0.61	-0.02	0.66	0.74	1.00											
Mg	-0.49	0.43	-0.23	0.49	0.86	0.98	1.00										
Cu	-0.05	-0.84	-0.32	-0.88	-0.46	-0.94	-0.85	1.00									
Zn	**0.71**	0.99	0.88	0.97	-0.26	0.46	0.27	-0.74	1.00								
Fe	**-0.45**	0.47	-0.18	0.53	0.83	0.99	1.00	-0.87	0.31	1.00							
Mn	0.12	0.88	0.39	0.91	0.39	0.91	0.81	-1.00	0.78	0.83	1.00						
C: N	**-1.00**	-0.61	-0.97	-0.55	0.85	0.26	0.46	0.08	-0.74	0.41	-0.16	1.00					
C: P	-0.49	-0.99	-0.71	-1.00	-0.01	-0.69	-0.52	0.89	-0.96	-0.56	-0.93	0.52	1.00				
C: K	**0.99**	0.47	0.92	0.41	-0.92	-0.41	-0.59	0.08	0.62	-0.55	0.00	-0.99	-0.38	1.00			
N: P	**1.00**	0.52	0.94	0.46	-0.90	-0.37	-0.55	0.02	0.66	-0.51	0.05	-0.99	-0.43	1.00	1.00		
N: K	**1.00**	0.63	0.98	0.58	-0.83	-0.23	-0.43	-0.12	0.76	-0.38	0.19	-1.00	-0.55	0.98	0.99	1.00	
P: K	**0.95**	0.81	1.00	0.77	-0.66	0.03	-0.18	-0.37	0.90	-0.13	0.44	-0.96	-0.75	0.90	0.92	0.97	1.00

Table 4.13. Path analysis of all chemical parameters (independent variables) with yield (dependable variable)

Variables	**OC**	**N**	**P**	**K**	**Ca**	**Mg**	**Cu**	**Zn**	**Fe**	**Mn**	**C: N**	**C: P**	**C: K**	**N: P**	**N: K**	**P: K**	**Correlation coefficient with yield**
OC	-3.61	-0.32	0.85	0.11	-0.42	-1.98	-0.91	3.87	0.64	1.48	-0.31	1.72	-0.39	-2.44	-0.68	2.96	0.58
N	-2.83	0.41	0.63	0.83	0.01	1.04	-0.35	3.46	-0.24	0.66	-0.49	1.24	-0.76	-4.41	-1.06	3.63	**0.96**
P	-3.60	-0.30	0.85	0.03	-0.46	-2.24	-0.94	3.82	0.72	1.53	-0.28	1.73	-0.34	-2.17	-0.63	2.81	0.52
K	0.34	0.28	-0.02	-1.19	-0.52	-3.96	-0.49	-1.04	1.14	0.66	0.43	0.02	0.76	4.23	0.90	-2.38	**-0.87**
Ca	-2.19	0.01	0.56	-0.88	-0.70	-4.51	-1.01	1.81	1.34	1.53	0.13	1.19	0.34	1.71	0.25	0.11	-0.30
Mg	-1.55	0.09	0.41	-1.03	-0.69	-4.61	-0.91	1.04	1.36	1.35	0.23	0.90	0.49	2.58	0.46	-0.64	-0.49
Cu	3.04	0.13	-0.74	0.55	0.66	3.91	1.08	-2.89	-1.19	-1.67	0.04	-1.55	-0.06	-0.11	0.12	-1.35	-0.05
Zn	-3.55	-0.36	0.83	0.32	-0.32	-1.22	-0.79	3.93	0.43	1.32	-0.37	1.66	-0.51	-3.10	-0.82	3.28	**0.71**
Fe	-1.70	0.07	0.45	-0.99	-0.69	-4.61	-0.94	1.23	1.36	1.40	0.21	0.97	0.46	2.39	0.41	-0.47	**-0.45**
Mn	2.19	0.40	-0.47	-1.01	-0.19	-2.11	0.09	-2.89	0.56	-0.26	0.50	-0.90	0.81	4.67	1.08	-3.47	0.12
C: N	-3.17	-0.16	0.77	-0.47	-0.64	-3.72	-1.07	3.08	1.14	1.68	-0.08	1.60	0.00	-0.24	-0.20	1.60	**-1.00**
C: P	3.59	0.29	-0.85	0.02	0.48	2.41	0.96	-3.78	-0.77	-1.55	0.26	-1.73	0.31	2.00	0.59	-2.72	-0.49
C: K	-1.71	-0.38	0.35	1.10	0.29	2.74	0.08	2.43	-0.75	-0.01	-0.50	0.65	-0.82	-4.69	-1.06	3.26	**0.99**
N:P	-1.87	-0.38	0.39	1.07	0.26	2.53	0.03	2.59	-0.69	0.09	-0.50	0.74	-0.82	-4.70	-1.07	3.34	**1.00**
N: K	-2.28	-0.40	0.49	0.99	0.16	1.97	-0.12	2.98	-0.52	0.32	-0.50	0.95	-0.81	-4.65	-1.08	3.51	**1.00**
P: K	-2.94	-0.41	0.66	0.78	-0.02	0.81	-0.40	3.55	-0.18	0.74	-0.48	1.29	-0.74	-4.32	-1.04	3.63	**0.95**

The correlation analysis examines the effect of independent variables (all chemical parameters of compost) on the dependent variables (yield). The standard error calculated in the analysis showed a significant effect of the chemical parameters on the yield per cent of white button mushroom (Table 4.11). The correlation analysis has shown a significantly positive effect of nitrogen, zinc, phosphorus and organic carbon on the yield per cent while potassium and iron showed significantly strong negative effect on the yield of button mushroom (Table 4.12). It was also observed that the ratio of the two macro and microelements have also shown strong positive effect on the yield while the C: N and C: P ratio has negative correlation with the yield. It implies that the proper fermentation of the composting material is very important for the higher yield of the button mushroom. It helps to reduce C: N and C: P ratio by degradation of free sugars during composting and release of CO_2 which helps to enhance the selectivity of the compost.

The correlations coefficients were divided into direct and indirect effects and are depicted in table 4.13. The separation of direct and indirect effects of independent variable on dependent variable showed a direct positive effect of nitrogen, copper, zinc, iron and manganese while a direct negative effect was recorded with potassium, calcium and magnesium. The ratio of the two elements also showed significant effect on yield such as C: P, C: K, N: P and N: K ratio showed a direct negative effect on yield which implies the fact that higher free carbon in the compost has a negative effect on yield while higher phosphorus content has a positive effect on yield.

4.2.3 Silica content in different compost formations

It was recorded during the study that silica content varied in all the compost formulations. Data presented in table 4.14 revealed that the silica content decreased during spawning to post harvesting stage in the compost. Before spawning (BS) stage silica content varied from 9.65 to 14.23 per cent. In before spawning stage, maximum silica content was recorded in treatment 4 (14.23%) followed by treatment 2 (12.53%). Both the treatments were significantly different to each other whereas minimum silica content was observed in treatment 3 (9.65%).

In Fruit body development stage (FBD), silica content varied from 9.25 to 13.29 per cent. Maximum silica content was found in treatment 4 (13.29%) followed by treatment 2 (10.25%). The minimum silica content was observed in treatment 3 (7.80%). In after harvesting stage (AH), silica content varied from 7.8 to 10.78 per cent. Maximum was found in treatment 4 (10.78%) and minimum in treatment 3 (6.91%). Rice straw is unique in comparison to other cereal straws in being low in lignin and high in silica. Higher silica was reported in other agri-residues such as rice straw (13%), wheat straw (>4%) and oat straw (2-5%) (Van Soest 2006). The cell wall of rice straw is composed of approximately 40 per cent cellulose, 18 per cent hemicellulose, 5-12 per cent lignin, and 5-15 per cent silica (Oladosu et al. 2016; Nguyen and Dang 2020; Otero-Jimenez et al. 2021).

Table 4.14 Silica content in different compost formulations

	Silica content (%)		
Treatments	**Before spawning**	**Fruit body development**	**After harvesting**
T1 **Paddy straw+ Wheat straw (1.5:1)**	10.34	9.25	7.80
T2 **Paddy straw+ Wheat straw (3:1)**	12.53	10.25	9.18
T3 **Paddy straw+ Wheat straw (1:1)**	9.65	7.80	6.91
T4 **Paddy straw**	14.23	13.29	10.78
T5 **Wheat straw (Control)**	11.79	10.13	7.80
Mean	11.71	10.14	8.47
C.D. (0.05)	0.84	2.99	0.69
SE	0.28	0.98	0.23

Paddy straw contains high amount of silica (>9.0 %) and is therefore not suitable as animal feed. In northern India, surplus paddy straw residue is generally either left in the field or to a large extent burnt in open field. Burning of paddy straw resulting to greenhouse gases (GHG) emissions cause severe air pollution. According to one of the study, greenhouse gases emission through open-field burning of rice straw in India, Thailand, and the Philippines contributes 0.05, 0.18, and 0.56 per cent, respectively (Gadde et al. 2009). Alternatively, use of paddy straw for composting is one of the best alternative to manage this resource, along with its use for restoration of soil health (Gaind et al. 2008 and Sharma et al. 2014). Rice straw has the highest silica content (up to 16%), and this makes it a challenge for use in industrial applications (Llovera and Benjelloun-

Mlayah 2022). Wheat straw contains 2 to 10 per cent silica as small crystals embedded in the straw (Pekarovic et al. 2006).

4.2.4 Thermophilic microbial count in different compost formulations

Thermophilic microbial count in different compost formulations was taken at 10^4dilutions. Highest microbial count was recorded in treatment 3(19.75 cfu g^{-1} compost) followed by treatment 1 (15.25 cfu g-^{1} compost). Minimum microbial count was observed in treatment 4 (12.25 cfu g-^{1} compost) (Table 4.15).

In mushroom cultivation successive microbial community consists of a variety of microorganisms including bacteria, actinomycetes and fungi at first breakdown of the straw to form lignin humus complex and discharge the gases followed by cellulose and hemicellulose metabolism into compost microbial biomass. This decayed straw along with microbial biomass turns into an organic and inorganic nutrient source for the mushroom mycelium and these microflora play a main role during the different stages of composting and resist the growth of other competitor in the crop production.

Table 4.15 Thermophilic microbial count in different compost formulations

Microbial count (cfu g-^{1} compost)	
Treatments	**10^4 Dilution**
T1 **Paddy straw+ Wheat straw (1.5:1)**	15.25
T2 **Paddy straw+ Wheat straw (3:1)**	13.25
T3 **Paddy straw+ Wheat straw (1:1)**	19.75
T4 **Paddy straw**	12.25
T5 **Wheat straw (Control)**	13.00
Mean	14.70
C.D. (0.05)	3.62
SE	1.19

Colony forming units (cfu/g compost) among the different treatments before pasteurization was high as compare to after pasteurization. Vijay et al. (2007) recorded the cfu of thermophilic fungi in range 18.33- 26.00 before pasteurization and 11.00- 23.00 in different treatments after pasteurization. The thermophilic fungi in wheat straw + maize stalk (1:1) was maximum during 3rd (6.1 cfu) and 4th (6.9cfu) turning while in other compost preparations it was maximum in 4th turning (Kaur et al. 2019).

4.2.5 Estimation of degradative enzyme activities in different compost formulations

Quantitative estimation of enzymes like laccase, xylanase, cellulase and manganese dependent peroxides (MnP) was done at three different stages of button mushroom production i.e., before spawning (BS), fruit body development (FBD) and after harvest (AH) stage. Enzymes activities of the five different compost formulations were assayed. Observation depicted that enzymes activity increased from before spawning stage to during fruiting bodies development stage and decreased in after harvesting stage.

Lignin degradation is the primary step in lignocellulose degradation enabling the accessibility of cellulose and hemicellulose (Anderson et al. 2008 and Jurak et al. 2015). Ligninolytic microorganisms can degrade lignins *via* the secretion of oxidative enzymes, such as peroxidases and laccases or by producing a source of heterogeneous aromatics. Ligninolytic enzymes or ligninases are mainly comprised of laccases, (Lac), lignin peroxidases (LiPs,) manganese peroxidases (MnPs), versatile peroxidases (VPs) and dye decolorizing peroxidases (DyPs) (Scharf and Tartar 2008; Familoni et al. 2018) These enzymes display less substrate specificity than cellulases and hemicellulases (Scharf and Tartar 2008; Pollegioni et al. 2015 and Liang et al. 2019). Additionally, Lac, LiP and MnP and many other enzymes, such as aromatic acid reductase, aryl alcohol dehydrogenase, catalase aromatic aldehyde oxidase, dioxygenase, quinone oxidoreductase, vanillate hydroxylase, veratryl alcohol oxidase and versatile peroxidase, are also involved in lignin digestion (Pollegioni et al. 2015). Mushroom species are most frequently reported as Lac and MnP producers.

a) Quantitative estimation of ligninases in different compost formulations at different stages of mushroom production

Laccase enzyme

The quantitative estimation of laccase enzyme was done at three different stages of button mushroom production *viz*., before spawning stage (BS), fruit body development stage (FBD) and after harvest (AH) stage. The observation depicted that laccase activity increased during growth of mushroom mycelium in compost and decreased afterwards (Table 4.16).

During before spawning stage, treatment 3 showed maximum laccase enzyme activity (0.43 IU) followed by treatment 1 (0.41 IU), which were statistically at par to each other whereas, minimum enzyme activity was recorded in treatment 5 (0.32 IU). During fruit body development stage, maximum enzyme activity was recorded in treatment 3 (0.37 IU) followed by treatment 1 (0.34 IU). Minimum enzyme activity was observed in treatment 2 (0.28 IU). After harvesting stage, the laccase activity was reduced and the maximum enzyme activity was found in treatment 4 (0.31 IU) followed by treatment 3 and 5 (0.29 IU). Minimum enzyme activity was recorded in treatment 2 (0.22 IU). Overall, it was observed that treatment 3 had maximum laccase enzyme activity in all three.

Table 4.16 Quantitative estimation of laccase enzyme in different button mushroom compost formulation at different stages of mushroom production

Laccase enzyme activity (IU)			
Treatments	**Before spawning**	**Fruit body development**	**After harvesting**
T1 **Paddy straw+ Wheat straw (1.5:1)**	0.41	0.34	0.25
T2 **Paddy straw+ Wheat straw (3:1)**	0.34	0.28	0.22
T3 **Paddy straw+ Wheat straw (1:1)**	0.43	0.37	0.29
T4 **Paddy straw**	0.38	0.31	0.31
T5 **Wheat straw (Control)**	0.32	0.30	0.29
Mean	0.38	0.32	0.27
C.D. (0.05)	0.05	0.02	0.02
SE	0.02	0.01	0.01

IU (International unit = µM/min/ml**)**

Manganese peroxidase enzyme

During the present study, MnP enzyme activity was found to increase from before spawning stage to fruit body development stage and decreased afterwards (Table 4.17). In before spawning stage, MnP enzyme activity (2.92 IU) was maximum in treatment 1 followed by treatment 2 (2.00 IU). Minimum MnP enzyme activity was shown by treatment 5 (0.24 IU). In fruit body development stage, maximum enzyme activity recorded in treatment 3 (8.14 IU) followed by treatment 5 (7.31 IU) while minimum enzyme activity was observed in treatment 2 (2.55 IU). The MnP activity in post-harvest stage was maximum in treatment 3 (7.42 IU) followed by treatment 5 (6.44 IU) while minimum enzyme activity was recorded in treatment 1 (0.30 IU). Overall, treatment 3 showed maximum MnP enzyme activity.

Agricultural wastes are comprised of the raw and processed agricultural products. They are mainly obtained from the plants under field conditions and from industries during processing. They are mainly composed of 35-50 per cent cellulose, 25-35 per cent hemicellulose, 10-25 per cent lignin and rest with ash and others (Kumla et al. 2020).

Table 4.17 Quantitative estimation of MnP enzyme from the button mushroom different compost formulations

MnP enzyme activity (IU)			
Treatments	**Before spawning**	**Fruit body development**	**After harvesting**
T1 **Paddy straw+ Wheat straw (1.5:1)**	2.92	4.62	0.30
T2 **Paddy straw+ Wheat straw (3:1)**	2.00	2.55	2.16
T3 **Paddy straw+ Wheat straw (1:1)**	0.68	8.14	7.42
T4 **Paddy straw**	0.12	4.92	1.40
T5 **Wheat straw (Control)**	0.24	7.31	6.44
Mean	1.19	5.51	3.54
C.D. (0.05)	0.20	0.12	0.31
SE	0.07	0.04	0.10

IU (International unit = µM/min/ml**)**

The degradation of lignocellulosic biomass is achieved through cooperative activities of hydrolytic and oxidative enzymes (Lombard et al 2014; Lopez et al. 2016 and Madeira et al. 2017). The hydrolytic system is responsible for cellulose and hemicellulose degradations, whereas the oxidative system is known to participate in lignin degradation. Laccase is the key enzyme involved in the lignin degradation. Laccase is one of the major lignolytic enzyme produced by the basidiomycota fungus, which can be determined using guaiacol as substrate. Oxidation of guaiacol by laccase produces red color which is an indicator for production of laccase enzyme (Monssef et al. 2016). Laccase can be used for lignin removal in prehydrolysis of lignocellulosic biomass (Shi et al. 2014). Manganese peroxidase is an important enzyme associated with the lignin and organic pollutant degradation systems, for instance bioremediation (Khanongnuch et al. 2006). Manganese peroxidase (MnP) belongs to the family of oxidoreductases and cannot react directly with the lignin structure (Ardon et al. 1998). There are two groups: (1) Manganese dependent peroxidase is an extracellular enzyme that requires both H_2O_2 for lignin oxidation, Mn^{2+} as a co-factor and (2) Manganese independent peroxidase is an extracellular enzyme that requires H_2O_2 in lignin oxidation but does not need Mn^{2+} (Zhao et al. 2015).

b) Quantitative estimation of celluloses and hemicellulase in different button mushroom compost formulation at different stages of mushroom production

Filter paperase enzyme

Filter paperase is also known as C1 cellulase catalyzing the degradation of crystalline cellulose at carbon 1 of the glucose chain. During the course of investigations, it was observed that cellulase enzyme activity increased from before spawning stage to fruit body development stage and decreased afterward (Table 4.18).

Table 4.18 Quantitative estimation of C-1 cellulase enzyme from the different button mushroom compost formulation at different stages of mushroom production

Filter paperase enzyme activity (IU)			
Treatments	**Before spawning**	**Fruit body development**	**After harvesting**
T1 **Paddy straw+ Wheat straw (1.5:1)**	19.41	27.17	18.51
T2 **Paddy straw+ Wheat straw (3:1)**	18.18	23.65	18.95
T3 **Paddy straw+ Wheat straw (1:1)**	27.32	35.30	15.84
T4 **Paddy straw**	17.25	22.25	16.20
T5 **Wheat straw (Control)**	19.57	24.80	16.93
Mean	20.34	26.63	17.29
C.D. (0.05)	0.07	0.14	0.19
SE	0.02	0.05	0.06

IU (International unit = µM/min/ml**)**

In before spawning stage, treatment 3 showed maximum filter paperase activity (27.32 IU) followed by treatment 5 (19.57 IU) while minimum was recorded in treatment 4 (17.25 IU). In fruit body development stage, maximum enzyme activity was recorded in treatment 3 (35.30 IU) followed by treatment 1 (27.17 IU). The minimum enzyme activity was observed in treatment 4 (22.25 IU). After harvest stage, maximum enzyme activity was found in treatment 2 (18.95 IU) while minimum enzyme activity was observed in treatment 3 (15.84 IU). Overall, it was observed that treatment 3 showed maximum C1 cellulase enzyme activity.

Carboxymethyl cellulase enzyme (CMCase)

CMCase enzymes are type of cellulases catalyzing the degradation of cellulose at random places in the glucose chain producing oligosaccharides are called endocellulases. During the study, the CMCase enzyme activity increased from before spawning stage to fruit body development stage and decreased in after harvesting stage (Table 4.19).

Table 4.19 Quantitative estimation of CMCase enzyme from the different button mushroom compost formulation at different stages of mushroom production

CMCase enzyme activity (IU)			
Treatments	**Before spawning**	**Fruit body development**	**After harvesting**
T1 **Paddy straw+ Wheat straw (1.5:1)**	32.71	39.21	32.37
T2 **Paddy straw+ Wheat straw (3:1)**	43.21	35.21	28.29
T3 **Paddy straw+ Wheat straw (1:1)**	34.42	43.29	34.53
T4 **Paddy straw**	32.67	34.74	28.50
T5 **Wheat straw (Control)**	27.92	41.55	33.76
Mean	34.91	38.80	31.49
C.D. (0.05)	0.51	1.23	0.90
SE	0.17	0.41	0.30

IU (International unit = µM/min/ml**)**

In before spawning stage, treatment 2 showed maximum CMCase activity (43.21 IU) while minimum enzyme activity was recorded in treatment 5 (27.92 IU). In fruit body development stage, treatment 3 recorded the maximum enzyme activity (43.29 IU) followed by treatment 5 (41.55 IU). The minimum enzyme activity was observed in treatment 4 (34.74 IU). After the harvest of crop, maximum enzyme activity was found in treatment 3 (34.53 IU) followed by treatment 5 (33.76 IU) and minimum was in treatment 2 (28.29 IU). Overall, it was observed that treatment 3 was shown maximum CMCase enzyme activity.

β –Glucosidase enzyme

β-Glucosidase is also a type of cellulase enzyme catalyzing the degradation of oligosaccharides releasing monomers of glucose. The observation depicted that β-glucosidase enzyme activity increased from before spawning stage to fruit body development stage and decreased in after harvesting stage (Table 4.20).

In before spawning stage, treatment 2 showed maximum β-glucosidase enzyme activity (6.49 IU) followed by treatment 3 (6.32 IU) while minimum enzyme activity was recorded in treatment 1 (4.90 IU). In during fruit body development stage, maximum enzyme activity was recorded in treatment 2 (10.82 IU) followed by treatment 3 (8.73 IU). The minimum enzyme activity was observed in treatment 1 (5.99 IU). After harvest stage, maximum enzyme activity was found in treatment 3 (5.59 IU) followed by treatment 2 (5.55 IU) and minimum enzyme activity was observed by treatment 1 (4.60 IU).

Table 4.20 Quantitative estimation of β-glucosidase enzyme from the different button mushroom compost formulation at different stages of mushroom production

β -Glucosidase enzyme activity (IU)			
Treatments	**Before spawning**	**Fruit body development**	**After harvesting**
T1 **Paddy straw+ Wheat straw (1.5:1)**	4.90	5.99	4.60
T2 **Paddy straw+ Wheat straw (3:1)**	6.49	10.82	5.55
T3 **Paddy straw+ Wheat straw (1:1)**	6.32	8.73	5.59
T4 **Paddy straw**	5.22	6.49	5.02
T5 **Wheat straw (Control)**	5.11	6.09	5.03
Mean	5.61	7.62	5.16
C.D. (0.05)	0.22	0.05	0.26
SE	0.07	0.02	0.09

IU (International unit = µM/min/ml**)**

Xylanase enzyme

The observation depicted that xylanase enzyme activity increased from before spawning stage to during fruit body development stage after that decreased in after harvesting stage (Table 4.21).

In before spawning stage, treatment 1 showed maximum xylanase enzyme activity (5.25 IU) whereas, minimum enzyme activity was recorded in treatment 5 (3.33 IU). In fruit body development stage, maximum enzyme activity was recorded in treatment 3 (9.68 IU) followed by treatment 2 (6.64 IU) while the minimum enzyme activity was observed in treatment 5 (5.17 IU). After harvest stage, maximum enzyme activity was found in treatment 3 (5.06 IU) followed by treatment 4 (4.26 IU) and minimum enzyme activity was shown by treatment 5 (3.49 IU). Overall, it was observed that treatment 3 was shown maximum xylanase enzyme activity.

Table 4.21 Quantitative estimation of xylanase enzyme from the different button mushroom compost formulation at different stages of mushroom production

Xylanase enzyme activity (IU)			
Treatments	**Before spawning**	**Fruit body development**	**After harvesting**
T1 **Paddy straw+ Wheat straw (1.5:1)**	5.25	5.84	3.73
T2 **Paddy straw+ Wheat straw (3:1)**	4.20	6.64	4.06
T3 **Paddy straw+ Wheat straw (1:1)**	4.20	9.68	5.06
T4 **Paddy straw**	3.65	5.87	4.26
T5 **Wheat straw (Control)**	3.33	5.17	3.49
Mean	4.13	6.64	4.12
C.D. (0.05)	0.12	0.10	0.08
SE	0.04	0.03	0.03

IU (International unit = µM/min/ml**)**

Cellulase consists of three enzymes: β-glucosidase, endo-1,4-β-D-glucanase (CMCase) and exo-1,4-β-D-glucanase (filter paperase). These three enzymes are involved in the hydrolysis of cellulose by synergetic action for accomplished and effective hydrolysis of cellulose (Patel et al. 2019). These enzymes convert cellulose in order to oligosaccharides, cellobiose and glucose (Horn et al. 2012; Ritota and Manzi 2019). Endoglucanases preferentially hydrolyze internal β-1,4-glucosidic linkages in the cellulose chains, generating a number of reducing ends (Horn et al. 2012 and Sajith et al. 2016). This enzyme also acts on cellodextrins, which are the intermediate product of cellulose hydrolysis, and converts them to cellobiose and glucose. Exoglucanases release cellobiose from the reducing or the non-reducing end of the cellulose chain, facilitating the production of mostly cellobiose, which can readily be converted to glucose by β-glucosidases (Zhang et al. 2006; Yeoman et al. 2010 and Madeira et al. 2017). These enzymes may also act on cellodextrins and larger cello-oligosaccharides and are commonly named cellodextrinases (Saini et al. 2015). Oligosaccharides released as a result of these activities are converted to glucose by the action of cellodextrinases, whereas the cellobiose released mainly by the action of cellobiohydrolases is converted to glucose by β-glucosidases (Sajith et al. 2016). Ohmiya et al. (1997) reported that endoglucanase acts on inner sites of oligosaccharides found in carboxymethyl cellulose, cello-oligosaccharides or amorphous cellulose. Exoglucanase hydrolyzes non-reducing ends of crystalline cellulose and forms cellobiose or glucose as the major end products. β-glucosidase acts on non-reducing ends of cellobiose and cellodextrin.

In the process of composting, the production of extracellular hydrolytic enzymes by microbes plays an important role in starting stage of degradation and crop production. These hydrolytic enzymes degrade the lignocellulosic substrate to simple monomers. Carboxymethyl cellulase (CMCase) activity was found to be higher till 60 days and then declined in all the treatments till 90 days. β-1,4 exoglucanase (FPase) enzyme activity increased during the later phase (90 days) of composting in all the treatments (Gaind et al. 2008 and Pandey et al. 2009). The highest value of CMCase (0.43 IU/g) was observed till 60 days, whereas the highest activity of FPase (0.47 IU/g) was observed till 90 days in the treatment supplemented with compost inoculants (CI) and efficient microorganism (EM). β-1,4 Endoglucanase (CMCase) acts upon the native cellulose which provide reactive sites for the action of exoglucanase (FPase); so, this could be a possible reason for the high activity of CMCase till 60 days and FPase till 90 days. The activity of xylanase enzyme was highest in the initial stage of composting; then, a decline was observed in all the treatments at a later stage of composting. Pandey et al. (2009) also observed the same pattern of high activity of xylanase in the initial stages of composting.

4.3 COMPARISON OF TWO-YEAR DATA OF MUSHROOM PRODUCTION

Trail was laid out in the Department of Plant Pathology, Dr.Y.S.P. University of Horticulture and Forestry, Nauni, Solan (H.P) to evaluate the effect of different compost formulations on mushroom production. Data of two-year 2021 and 2022 was evaluated for comparison the mushroom production (Table 4.22).

Days taken for the formation of fruiting bodies

Days taken for spawn run during year 2021, was observed to be the minimum in treatment 3 (14.00 days) followed by treatment 1 (15.02 days), which were statistically at par to each other, whereas, maximum days were taken by treatment 2 (16.01 days). In year 2022 also, days taken for spawn run was observed minimum in treatment 3 (15.09 days) followed by treatment 1 and 4 (17.09 days) whereas, maximum days were taken by treatment 2 (18.07 days). Overall mean of both year 2021-2022 showed that treatment 3 taken minimum days for spawn run (14.55 days) followed by treatment 1 (16.05 days) whereas maximum days were in treatment 2 (17.04 days).

Days taken for case run during 2021, was observed minimum again in treatment 3 (19.03 days) followed by treatment 5 (20.00 days) which were significant to each other and the maximum days were taken by treatment 4 (21.02 days). Similar results were obtained in the year 2022 where days taken for case run was observed minimum in treatment 3 (21.09 days) followed by treatment 5 (21.35 days) but the maximum days was taken by treatment 2 (23.04 days) in this year. Overall mean of both year 2021-2022, showed that treatment 3 took minimum days for case run (20.06 days) followed by treatment 5 (20.67 days) whereas, the maximum days were taken by treatment 2 (22.03 days). Days taken for pin head formation during 2021, was observed to be the minimum in treatment 3 (24.02 days) followed by treatment 4 (25.00 days) whereas, maximum days were taken by treatment 2 (26.04 days). Similarly, in year 2022, days taken for pin head formation was observed minimum in treatment 3 (25.11 days) followed by treatment 1 (26.09 days). The maximum days were taken by treatment 2 (28.08 days). Overall mean of both year 2021-2022, showed that treatment 3 had minimum days for pin head formation (24.56 days) followed by treatment 1 (25.55 days) and maximum days were taken by treatment 2 (27.06 days).

Table 4.22 Comparative analysis of mushroom production by using different compost formulations

Treatments	Days taken for spawn run			Days taken for case run			Days taken for pin head formation			Days taken for first harvest		
	2021	**2022**	**Mean**	**2021**	**2022**	**Mean**	**2021**	**2022**	**Mean**	**2021**	**2022**	**Mean**
T1 Paddy straw+ Wheat straw (1.5:1)	15.02	17.09	16.05	20.03	21.37	20.70	25.01	26.09	25.55	29.01	30.12	29.57
T2 Paddy straw+ Wheat straw (3:1)	16.01	18.07	17.04	21.01	23.04	22.03	26.04	28.08	27.06	30.01	32.15	31.08
T3 Paddy straw+ Wheat straw (1:1)	14.00	15.09	14.55	19.03	21.09	20.06	24.02	25.11	24.56	28.02	29.09	28.55
T4 Paddy straw	16.00	17.09	16.55	21.02	22.04	21.53	25.00	27.12	26.06	32.08	33.10	32.59
T5 Wheat straw (Control)	15.03	17.12	16.07	20.00	21.35	20.67	26.04	27.10	26.57	29.00	30.14	29.57
Mean	15.22	16.89	16.05	20.22	21.78	20.99	25.22	26.70	25.96	29.62	30.92	30.27
C.D. (0.05)	1.04	0.07		0.95	1.00		0.69	0.09		1.57	0.09	
SE	0.34	0.02		0.31	0.33		0.23	0.03		0.52	0.03	

Days taken for first harvest during 2021, was observed minimum in treatment 3 (28.02 days) followed by treatment 5 (29.00 days) whereas, maximum days were taken by treatment 4 (32.08days). In year 2022, days taken first harvest was observed minimum in treatment 3 (29.09 days) followed by treatment 1 (30.12 days). The maximum days were taken by treatment 4 (33.10 days). Overall mean of both year 2021-2022, showed that treatment 3 was taken minimum days for first harvest (28.55 days) followed by treatment 1 and 5 (29.57 days), which significantly differed to each other. Maximum days for first harvest was taken by treatment 4 (32.59 days).

Fruiting body size

Data in table 4.23 depicts the morphometric parameters of button mushroom like diameter of pileus, thickness of pileus, length of stipe and diameter of stipe of the mushroom grown in different compost formulations during 2021 and 2022.

During 2021, the diameter of pileus was found to be the maximum in treatment 3 (3.91cm) followed by treatment 2 (3.80cm) whereas the minimum pileus diameter was found in treatment 4 (3.40 cm). In year 2022, it was found maximum in treatment 3 (4.08 cm) followed by treatment 5 (3.80 cm) and minimum in treatment 2 (3.40 cm). Overall mean of both years 2021-2022, showed that maximum pileus diameter (3.99 cm) was observed in treatment 3 followed by treatment 5 (3.65 cm) and minimum diameter was found in treatment 4 (3.50 cm).With respect to pileus thickness, maximum thickness was observed in treatment 3 (1.80cm) followed by treatment 1 (1.50cm) and minimum in treatment 4 (1.10cm) during 2021 while in the year 2022, maximum thickness was again observed in treatment 3 (1.9cm) followed by treatment 2 and 4 (1.4cm) and the minimum in treatment 1 and 5 (1.30 cm). Overall mean of both the years 2021-2022, pileus thickness of mushroom fruiting body was observed maximum in treatment3 (1.85 cm) followed by treatment 1 (1.4 cm) whereas minimum length was found in treatment 5 (1.21cm).

Stipe length was recorded to be the maximum in treatment 3 (2.60cm) followed by treatment 2 (2.44cm) and the minimum length was found in treatment 4 (2.10cm) during 2021 while maximum length during 2022 was again observed in treatment 3 (2.70 cm) followed by treatment 5 (2.60 cm). Overall, maximum length of stipe (2.65 cm) was recorded in treatment 3 and minimum length of stipe was found in treatment 1 (2.22 cm). In the year

Table 4.23 Comparative analysis of button mushroom fruiting bodies size by using different compost formulations.

	Diameter of pileus (cm)			**Thickness of pileus(cm)**			**Length of stipe(cm)**			**Diameter of stipe (cm)**		
Treatments	**2021**	**2022**	**Pooled data**	**2021**	**2022**	**Pooled data**	**2021**	**2022**	**Pooled data**	**2021**	**2022**	**Pooled data**
T1 **Paddy straw+ Wheat straw (1.5:1)**	3.60	3.50	3.55	1.50	1.30	1.40	2.33	2.10	2.22	1.20	1.30	1.25
T2 **Paddy straw+ Wheat straw (3:1)**	3.80	3.40	3.60	1.30	1.40	1.35	2.44	2.50	2.47	1.10	1.20	1.15
T3 **Paddy straw+ Wheat straw (1:1)**	3.91	4.08	3.99	1.80	1.90	1.85	2.60	2.70	2.65	1.50	1.70	1.60
T4 **Paddy straw**	3.40	3.60	3.50	1.10	1.40	1.25	2.10	2.40	2.25	1.30	1.20	1.25
T5 **Wheat straw (Control)**	3.50	3.80	3.65	1.12	1.30	1.21	2.33	2.60	2.47	1.40	1.50	1.45
Mean	3.64	3.68	3.66	1.36	1.46	1.41	2.36	2.46	2.46	1.30	1.38	1.34
C.D. $_{(0.05)}$	0.27	0.10		0.21	0.06		0.063	0.05		0.56	0.06	
SE	0.09	0.03		0.07	0.02		0.021	0.02		0.19	0.02	

2021, stipe diameter was recorded maximum in treatment 3 (1.50cm) followed by treatment 5 (1.40cm) while the minimum was recorded in treatment 2 (1.10cm). In the year 2022, treatment 3 showed maximum stipe diameter (1.70 cm) followed by treatment 5 (1.50 cm) whereas the minimum in treatment 2 and 4 (1.20 cm). Overall mean of both the years 2021-2022, diameter of mushroom fruiting body stipe was maximum in treatment 3 (1.60 cm) followed by treatment 5 (1.45 cm). The minimum diameter of stipe was recorded in treatment 2 (1.15 cm).

Mushroom Yield

Table 4.24 depicts the data of white button mushroom yield in different compost formulations during 2021 and 2022.The maximum yield was obtained in treatment 3 (19.98 kg/ 100kg compost) followed by treatment 1 (17.76 kg/100kg compost) during 2021. The minimum yield was obtained from treatment 4 (15.22 kg/ 100kg compost). In the year 2022, yield of mushroom was recorded to be maximum in treatment 3 (21.16 kg/100kg compost) followed by treatment 1 (16.96 kg/100kg compost). The minimum yield was recorded in treatment 4 (13.95 kg/100kg compost). Overall, the maximum yield was recorded in treatment 3 (20.57 kg/100kg compost) followed by treatment 1 (17.36 kg/100kg compost). The minimum yield was observed in treatment 4 (14.59 kg/100kg compost).

Table 4.24 Effect of different compost formulations on yield of white button mushroom

Treatments	**Total yield (kg/100kgcompost)**		
	2021	**2022**	**Mean**
T1 **Paddy straw+ Wheat straw (1.5:1)**	17.76	16.96	17.36
T2 **Paddy straw+ Wheat straw (3:1)**	16.75	14.54	15.65
T3 **Paddy straw+ Wheat straw (1:1)**	19.98	21.16	20.57
T4 **Paddy straw**	15.22	13.95	14.59
T5 **Wheat straw (Control)**	17.25	15.91	16.58
Mean	17.39	15.94	16.26
C.D. $_{(0.05)}$	0.07	0.21	
SE	0.02	0.07	

Table 4.25 **Cost benefit ratio of different compost formulations**

Compost formulations	**WS+PS (1.5:1)**	**WS+PS (3:1)**	**WS+PS (1:1)**	**PS**	**WS**	**Cost /kg**	**Cost involved (Rs)**				
	Quantity						**WS+PS (1.5:1)**	**WS+PS (3:1)**	**WS+PS (1:1)**	**PS**	**WS**
Paddy straw	600	750	500	1000	0	2.5	1500	1875	1250	2500	0
Wheat straw	400	250	500	0	1000	8	3200	2000	4000	0	8000
Chicken Manure	600	600	600	600	600	5	3000	3000	3000	3000	3000
Wheat Bran	100	100	100	100	100	10	1000	1000	1000	1000	1000
Urea	15	15	15	15	15	5.5	82.5	82.5	82.5	82.5	82.5
Gypsum	30	30	30	30	30	2	60	60	60	60	60
Electricity (Unit)	1200	1200	1200	1200	1200	5	6000	6000	6000	6000	6000
Labour (Rs)							1000	1000	1000	1000	1000
Spawn (kg)	25	25	25	25	25	80	2000	2000	2000	2000	2000
Casing (kg)	500	500	500	500	500	3	1500	1500	1500	1500	1500
Total cost (Rs)							**19342.50**	**18517.50**	**19892.50**	**17142.50**	**22642.50**
Yield (kg/100 kg compost)	17.25	16.75	19.98	15.22	17.76						
Compost prepared (kg)	2500	2500	2500	2500	2500						
Bags prepared (10 kg)	250	250	250	250	250						
Total mushroom produced (kg)	431.25	418.75	499.50	380.50	444.00						
Cost of mushroom Rs. per kg	100										
Cost of mushroom (Rs) (Total returns)						**100**	**43125**	**41875**	**49950**	**38050**	**44400**
Net Return or Net Profit (Rs)							**23782.50**	**23357.50**	**30057.50**	**20907.50**	**21757.50**
Benefit cost ratio							**1: 2.23**	**1: 2.26**	**1: 2.51**	**1: 2.22**	**1: 1.96**

Net Return or Net Profit (Rs) = Total returns- Total cost**, Benefit cost ratio=** Total return/Total cost

The cost-benefit ratio of the different compost formulations was calculated and it was concluded that the cost-benefit ratio of the compost formulation paddy straw + wheat straw (1:1) gave the maximum net return as compared to other compost formulations. So, on the basis of all the compost quality, yield parameters, and cost-benefit ratio, a compost formulation of paddy straw and wheat straw (1:1) can be recommended to the farmers to reduce the cost and increase the profit (Table 4.25).

4.4 ISOLATION OF THERMOPHILIC FUNGI FROM THE COMPOST

During the present study, a total of eighteen isolations of thermophilic and thermotolerant fungi were made from the mushroom compost (Table 4.26). Most of them represent the known thermophilic taxa. Higher dilutions (10^4) were used for the isolation of fungi in pure form. During phase-I isolation, it was found that most of the time; the plates were overcrowded with the ubiquitous *Aspergillus fumigatus.* Fungi isolated from Phase-II compost were *Myriococcum albomyces (Melanocarpus albomyces), Aspergillus fumigatus, Thermomyces lanuginosus, Scytalidium album, Talaromyces euchlorocarpius, Sordariales* sp., *Penicillium thomii, Chaetomium* sp. and *Thermomyces duponti* etc.

Table 4.26 Isolation of thermophillic fungi from different compost formulation of white button mushroom

Treatments	Thermophilic fungi isolated
T1 Paddy straw+ Wheat straw (1.5:1)	*Aspergillus fumigatus, Chaetomium* sp., *Thermomyces lanuginosus, Scytalidium album* and *Melanocarpus albomyces*
T2 Paddy straw+ Wheat straw (3:1)	*Aspergillus fumigatus, Thermomyces lanuginosus, Talaromyces euchlorocarpius, Melanocarpus albomyces, Humicola fuscoatra, Sordarials* sp., *Penicillium thomii* and *Aspergillus* sp.
T3 Paddy straw+ Wheat straw (1:1)	*Myriococcum albomyces (Melanocarpus albomyces*), *Aspergillus fumigatus, Thermomyces lanuginosus, Scytalidium album, Talaromyces euchlorocarpius, Thermomyces duponti, Humicola fuscoatra, Sordarials* sp., *Penicillium thomii* and *Aspergillus* sp.
T4 Paddy straw	*Aspergillus fumigatus, Penicillium thomii, Thermomyces duponti, Sordariales* sp., *Talaromyces euchlorocarpius* and *Melanocarpus albomyces*
T5 Wheat straw (Control)	*Aspergillus fumigatus, Thermomyces lanuginosus* and *Melanocarpus albomyces*

In the differential assessment of microbial population, *Aspergillus fumigatus, Chaetomium* sp., *Thermomyces lanuginosus, Scytalidium album* and *Melanocarpus albomyces* were the dominant flora in treatment 1, while *Aspergillus fumigatus, Thermomyces lanuginosus, Talaromyces euchlorocarpius, Melanocarpus albomyces, Humicola fuscoatra, Sordarials* sp., *Penicillium thomii* and *Aspergillus* sp. were present in treatment 2. Treatment 3 showed the dominance of *Myriococcum albomyces (Melanocarpus albomyces*), *Aspergillus fumigatus, Thermomyces lanuginosus, Scytalidium album, Talaromyces euchlorocarpius, Thermomyces duponti*, *Humicola fuscoatra, Sordarials* sp., *Penicillium thomii* and *Aspergillus* sp. while treatment 4 had *Aspergillus fumigatus, Penicillium thomii, Thermomyces duponti, Sordariales* sp., *Talaromyces euchlorocarpius* and *Melanocarpus albomyces* as the dominant thermophilic flora. Treatment 5 showed the dominance of *Aspergillus fumigatus*, *Thermomyces lanuginosus* and *Melanocarpus albomyces* species of themophilic fungi.

Compost production is the most important and integral part of white button mushroom cultivation. It is a product of aerobic fermentation brought about by succession of a variety of organisms, including bacteria, actinomycetes and fungi. These organisms convert and degrade the straw to form the lignin humus complex and also convert the soluble form of nitrogen into microbial cell substances (Waksman and Cordon 1939). The process of composting is governed by a carefully ordered changing population of organisms (Chang and Hudson 2001). Further, these mycoflora also play a key role towards selectivity and conditioning of the compost and make the growth of competitor organisms more difficult (Ross and Harris 1983). Various kinds of flora encountered during whole process of composting have different role to play. Mesophilic flora present in initial process of composting brings about the biodegradation of the straw and other ingredients resulting in production heat energy. This further result in the establishment of thermophilic flora in the compost, which later on govern the whole process of composting. Population of different organisms in compost and thermophilic flora in particular is very important since they decompose the straw and convert soluble form of nitrogen into microbial cell substances (Waksman and cordon 1939; Waksman et al. 1939 a and b). This decomposed straw along with microbial biomass both become a source of organic and inorganic nutrition for the mushroom mycelium (Wood and Fermor 1981).

Thermophilic fungi can grow at temperatures ranging from a minimum of 20°C to a maximum of 50°C or at even higher temperatures. Thermophilic species are present in the natural environment in composts, aquatic sediments, piles of hay, stored grains, wood chip piles, and other accumulations of organic matter wherein the conditions are warm, humid and aerobic (Cooney and Emerson 1964). A number of thermophilic fungi can survive in harsh conditions such as those with increased water pressure, an absence of oxygen, and under desiccation conditions (Mahajan et al. 1986). During the composting process, various organic materials are converted into simpler units of organic carbon and nitrogen. The overall efficiency of organic material break down depends on the microbes and their activities (Raut et al. 2008). Thermophilic fungi promote the degradation of organic materials by secreting various types of cellulolytic and xylanolytic enzymes. These fungi might have enzymes that maintain their activities at high temperatures. Enzymes from thermophilic fungi are often more stable at higher temperatures than the enzymes from mesophilic fungi, and some even show stability at 70- 80°C (Margaritis et al. 1986; Margaritis and Merchant 1983).

4.5 ESTIMATION OF DIFFERENT ENZYME ACTIVITY OF ISOLATED THERMOPHILIC FUNGI

Paddy straw consists of cellulose (35-40%), hemi-cellulose (20- 24%), lignin (8-12%), ash (14-16%) and extractives (10-12%), which are associated with each other (Saha 2003). Thermophiles are a good source of novel catalysts that are of great industrial interest and have more stable enzymes as compared to mesophiles (Li et al. 2005). Thermophilic enzymes are also active at low temperatures and they can reach to the peak of enzyme activities more quickly as compared to mesophiles. Moreover, the stability of obligate thermophiles increased with process temperature. Enzymes synthesized by thermophiles and hyper-thermophiles are known as thermozymes. These enzymes are typically thermostable or resistant to irreversible inactivation at high temperature. Thermozymes can be used in several industrial processes, in which they replace mesophilic enzymes or chemicals. The main advantages of performing process at higher temperature are reduced risk of microbial contamination, lower viscosity, improved transfer rates and improved solubility of substrates. Thermophilic fungi are potential sources of enzymes with scientific and commercial interests. Enzymes are useful as specific tools for elucidating the structure of plant cell walls and are also required for evolving biodegradative methods for the conversion of biomaterials containing cellulose and hemicellulose into monosaccharides from which single-cell protein, single-cell oil or ethanol could be produced (Flickinger 1980 and Fall et al. 1984). Thermophilic fungi have a powerful ability to degrade polysaccharide constituents of biomass. The properties of their enzymes show differences not only among species but also among strains of the same species. Some extracellular enzymes from thermophilic fungi are being produced commercially, and a few others have commercial prospects. Genes of thermophilic fungi encodes lipase, protease, xylanase and cellulase. Thermophilic fungi are the chief components of the microflora that develops in heaped masses of plant material, piles of agricultural and forestry products, and other accumulations of organic matter where in the warm, humid and aerobic environment. They constitute a heterogeneous physiological group of various genera in the Phycomycetes, Ascomycetes, Fungi Imperfecti and Mycelia Sterilia. Mandels (1975) observed that some species of thermophilic fungi degraded cellulose rapidly. Fungi are important sources of hemicellulases as they produce higher titers as compared to yeasts and bacteria (Krisana et al. 2005). Many thermophilic fungi like *Paecilomyces themophila, Malbranchea cinnamomea, Thermomyces lanuginosus, Scytalidium thermophilum, Sporotrichum thermophile, Rhizomucor* sp. and *Aspergillus* sp. have showed high production of xylanase using agro-industrial wastes (Yang et al. 2006; Maijala et al. 2012; Sadaf and Khare 2014; Robledo et al. 2015).

4.5.1 Qualitative and quantitative estimation of hydrolytic and oxidize enzyme of fungal isolates from different compost formulations

Eighteen species were isolated from the compost samples by serial dilution method. The aim of isolation of thermophilic fungi from compost was to evaluate the potential of thermostable enzymes for making button mushroom compost requiring high reaction temperatures. All these eighteen fungi were examined for qualitative and quantitative enzyme activities by different enzyme assays.

4.5.1.1 Qualitative estimation of fungal isolates for hydrolytic and oxidative enzymes production

All the isolates have produced varied hydrolysis zone (Table 4.27). With respect to cellulase enzyme, maximum hydrolytic zone was recorded in isolate TF4 (49.00 mm) followed by treatment TF6 (45.00 mm) whereas minimum hydrolytic zone was found in isolate TF1 and 12 (10mm) respectively. In xylanase enzyme estimation, maximum hydrolytic zone was observed in treatment TF4 (39mm) followed by treatment TF8 (36mm) while minimum hydrolytic zone was recorded in treatment TF3 and TF18. Treatment TF6 showed maximum laccase enzyme hydrolytic zone (29mm) followed by treatment TF4 (23mm) whereas minimum hydrolytic zone was observed in treatment TF10 (4mm). In MnP enzyme estimation maximum oxidative zone was formed in treatment TF6 (30mm) followed by treatment TF4 (25mm).

Ligninolytic fungal isolates of different compost formulations of button mushroom exhibited multiple hydrolytic and oxidative enzyme production i.e., cellulases, xylanase, laccase and MnP enzyme activity. Fungal isolates were selected on the basis of zone of hydrolysis surrounding fungal colonies. Many microorganisms, such as fungi, bacteria and yeast, can degrade hemicellulose by producing xylanases. Xylanase activities can be determined by several methods. The plate assay has been used for decades as a primary screening method to select xylanase producing strains.

Table 4.27 Qualitative estimation of fungal isolates for hydrolytic and oxidize enzyme production

Isolates	**Cellulase**	**Xylanase**	**Laccase**	**Manganese peroxidase**
	Zone size (mm)	**Zone size (mm)**	**Zone size (mm)**	**Zone size (mm)**
TF1 *Aspergillus fumigatus*	10.00	28.00	6.00	5.00
TF2 *Aspergillus* sp.	26.00	7.00	18.00	20.00
TF3 *Humicola fuscoatra*	43.00	5.00	5.00	6.00
TF4 *Thermomyces lanuginosus*	49.00	39.00	23.00	25.00
TF5 *Aspergillus fumigatus*	15.00	22.00	8.00	7.00
TF6- *Melanocarpus albomyces*	45.00	34.00	29.00	30.00
TF7 *Aspergillus fumigatus*	14.00	19.00	7.00	17.00
TF8 *Sordarials* sp.	29.00	36.00	7.00	15.00
TF9 *Talaromyces euchlorocarpius*	16.00	12.00	17.00	10.00
TF10 *Aspergillus fumigatus*	18.00	11.00	4.00	9.00
TF11 *Thermomyces duponti*	22.00	13.00	16.00	11.00
TF12 *Aspergillus* sp.	10.00	16.00	22.00	22.00
TF13 *Penicillium thomii*	19.00	8.00	8.00	13.00
TF14 *Chaetomium* sp.	13.00	14.00	5.00	12.00
TF15 *Sordarials* sp.	35.00	17.00	14.00	19.00
TF16 *Aspergillus fumigatus*	14.00	20.00	20.00	16.00
TF17 *Talaromyces euchlorocarpius*	17.00	26.00	15.00	13.00
TF18 *Scytalidium album*	15.00	5.00	6.00	9.00
C.D$_{(0.05)}$	3.98	3.06	3.36	3.38
SE	1.40	1.08	1.18	1.19

The strains are cultured on agar medium containing xylan as their carbon source until clear zones are observed (the xylan hydrolysis area) after being stained with congo red dye (Bajaj and Mahajan 2019) or gram’s iodine solution (Burlacu et al. 2016).

4.5.1.2 Quantitative estimation of fungal isolates for hydrolytic and oxidize enzyme production

Quantitative estimation of ligninolytic, cellulolytic, xylanolytic and manganese dependent peroxidase enzymes of fungal isolates from the button mushroom different compost formulations was done. The observations depicted that laccase enzyme activity was recorded maximum in isolate TF6 (2.51 IU) followed by isolate TF4 (2.19 IU), whereas, minimum activity was observed in isolate TF11 (0.22 IU). With respect to xylanase enzyme activity, maximum enzyme activity was recorded in isolate TF4 (39.52 IU) followed by isolate TF6 (35.05 IU). The MnP enzyme activity was observed to be the maximum in isolate TF6 (4.91 IU) followed by isolate TF14 (4.56 IU) whereas, minimum MnP enzyme activity was found in isolate TF10 (0.10 IU). CMCase enzyme assay depicted that the enzyme activity was recorded maximum in isolate TF4 (37.29 IU) followed by isolate TF3 (36.95 IU) whereas, minimum cellulase enzyme activity was found in isolate TF1 (5.76 IU).

Filterpapease activity was observed to be the maximum in isolate TF4 (12.52 IU) followed by isolate TF6 (10.82 IU), whereas minimum cellulase enzyme activity was found in isolate TF 3 and 17 (2.57 IU). β- Glucosidase assay was observed to be the maximum in isolate TF6 (19.83 IU) followed by TF4 (18.84 IU) and minimum in isolate TF16 (7.59 IU) (Table 4.28).

Thermophilic fungi have positive influence on the growth of *A. bisporus* by decreasing the ammonia concentration and it immobilizes the nutrients so that it is easily available to the mycelium of mushroom (Mahajan et al. 2021). Mushrooms are able to secrete lignin peroxides (LiP) and manganese peroxidase (MnP) and able to use the cellulose as sources of carbon. Mushrooms have the ability to completely degrade the lignin to the cellulose molecule using LiP, MnP and laccase and the cellulose fraction is exposed for the use of mushroom as a source of carbon (Lara et al. 2003 and Xu et al. 2012). Some reports indicate that fungal laccase take part in detoxification of phenolic compounds and sporophore development (Bollag et al. 1988; Zhao and Kwan 1999; Ohga and Royse 2001).

Table 4.28 Quantitative estimation of fungal isolates for hydrolytic and oxidative enzyme production

Enzyme activity (IU)						
Isolates	**Laccase**	**Xylanase**	**Manganese dependent peroxidase**	**Cellulase**		
				CMCase	**Filterpapease**	**β- Glucosidase**
TF1 *Aspergillus fumigatus*	1.90	19.03	0.13	5.76	10.70	18.29
TF2*Aspergillus* sp.	1.40	4.33	1.96	20.16	3.02	17.33
TF3*Humicola fuscoatra*	1.21	2.34	0.15	36.95	2.57	15.93
TF4*Thermomyces lanuginosus*	2.51	39.52	3.33	37.29	12.52	18.84
TF5*Aspergillus fumigatus*	0.82	17.45	0.15	9.49	7.79	10.76
TF6*Melanocarpus albomyces*	2.19	35.05	4.91	28.39	10.82	19.83
TF7*Aspergillus fumigatus*	0.61	12.66	1.20	10.41	6.25	17.73
TF8*Sordarials* sp.	0.21	24.04	1.05	25.14	9.31	18.61
TF9*Talaromyces euchlorocarpius*	1.13	3.73	0.20	13.62	5.57	18.25
TF10*Aspergillus fumigatus*	0.31	5.04	0.10	14.33	5.04	7.69
TF11*Thermomyces duponti*	0.22	22.23	0.50	18.44	5.76	8.50
TF12*Aspergillus* sp.	1.44	18.48	2.16	6.58	6.15	8.59
TF13*Penicillium thomii*	0.28	2.19	1.03	17.41	3.78	18.19
TF14 *Chaetomium* sp.	1.61	12.66	4.56	8.60	5.96	17.20
TF15*Sordarials* sp.	0.32	29.31	0.35	35.7	6.16	8.11
TF16*Aspergillus fumigatus*	1.24	14.04	1.60	8.58	6.26	7.59
TF17*Talaromyces euchlorocarpius*	1.12	2.25	0.93	13.83	8.06	18.09
TF18*Scytalidium album*	0.51	2.19	0.15	9.49	2.57	15.93
C.D$_{(0.05)}$	0.05	0.34	0.08	0.15	0.10	0.44
SE	0.02	0.12	0.03	0.05	0.03	0.16

IU (International unit = µM/min/ml)

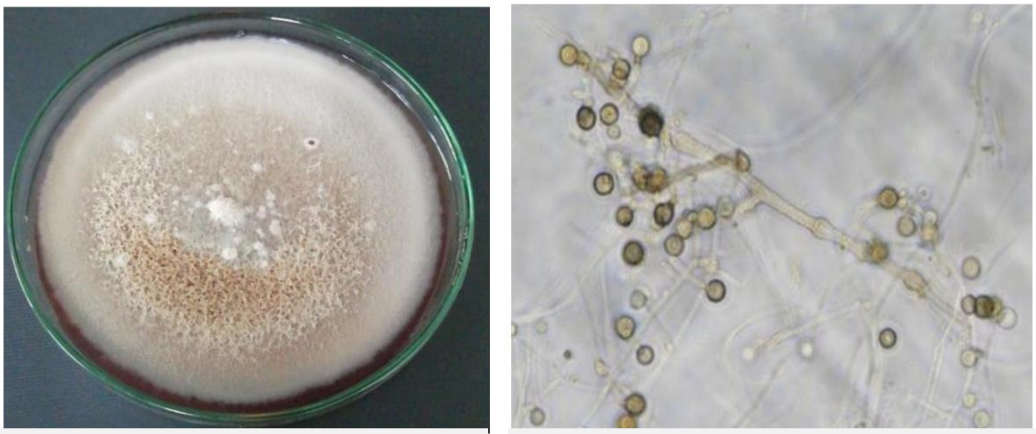
Out of eighteen, two thermophilic fungi showed highest enzyme activities which were selected for our next experiments i.e., TF1- *Thermomyces lanuginosus* and TF6-*Melanocarpus albomyces* which were identified morphologically, microscopically and molecularly (Plate 8 and 9). Four species of thermophilic fungi from self-heating hay: *Mucor pusillus, Thermomyces lanuginosus, Thermoidium sulfureum* and *Thermoascus aurantiacus* were isolated by Miehe (1930 and 1930a). Fungi isolated from Phase-II compost were *Chaetomium thermophile, Emericella nidulans, Thermoascus aurantiacus, Myriococcum albomyces, Humicola insolens, Malbranchea sulfurea, Torula thermophila, Stilbella thermophila* and *Thermomyces lanuginosus*. *Thermoascus aurantiacus* and *Thermomyces lanuginosus* belong to Eurotiales, Ascomycota and *Myriococcum thermophilum* belongs to Sordariomycetes, Ascomycota well-known thermophiles (Rajasekaran and Maheshwari 1993; Deacon 2006).

1) *Thermomyces lanuginosus*

Thermomyces lanuginosus is a species of thermophilic fungus that belongs to Thermomyces, a genus of hemicellulose degraders. It is classified as a deuteromycete (Cooney and Emerson 1964) and no sexual form has ever been observed. It is the dominant fungus of compost heaps, due to its ability to withstand high temperatures and use complex carbon sources for energy. As the temperature of compost heaps rises and the availability of simple carbon sources decreases, it is able to out compete pioneer microflora. It plays an important role in breaking down the hemicelluloses found in plant biomass due to the many hydrolytic enzymes that it produces, such as lipolase, amylase, xylanase, phytase and chitinase (Maheshwari 2016). These enzymes have chemical, environmental and industrial applications due to their hydrolytic properties.

Growth and morphology

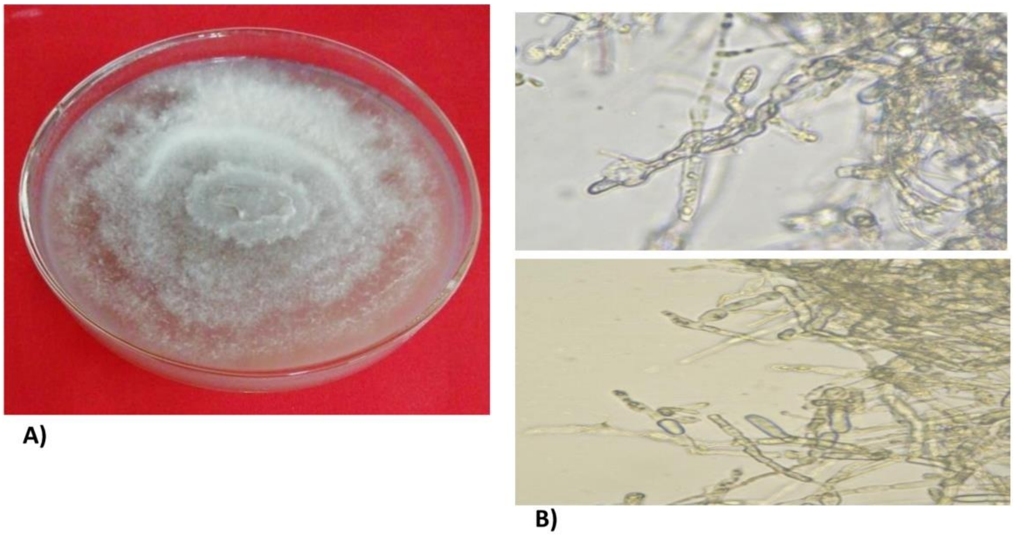
Thermomyces lanuginosus (Tsiklinskya 1899) is a synonym *Humicola lanuginosa* (Griffon and Maublanc 1911). Colonies on YpSs at 45°C appear white at first, but soon turn gray, beginning at the center of the colony. Gradually the colony turns purple brown, the agar stains deep pink or wine color may be due to the secretion of diffusible substances. Mature colonies appear dull dark brown to black. Hyphae were colorless and septate with 1.5 - 4 µm in diameter. Conidiogenous cell arise at right angle to the hyphae, 10-15 µm long, generally unbranched or rarely branched once or twice near the base forming clusters and often septate. Conidia single on each conidiogenous cell, colorless, spherical, smooth walled when young, at maturity turn dark brown and sculptured, 6-10 µm in diameter, separating easily from the conidiogenous cell and commonly retain a



A) *Thermomyces lanuginosus* colonies on yeast phosphate soluble starch agar (YPSS) at 40 ± 2 °C. and at 6-7 days incubation

B) Microscopic view of *Thermomyces lanuginosus* (40X magnification) with conidia and conidiophors.

Plate 6. TF4- *Thermomyces lanuginosus*



A) *Melanocarpus albomyces* colonies on yeast phosphate soluble starch agar (YPSS) at 40 ± 2 °C and at 6-7 days incubation

B) Microscope slide of *Melanocarpus albomyces* (40X magnification)

Plate 7. TF6- *Melanocarpus albomyces*

short attachment piece. Isolate TF4 was identified as *Thermomyces lanuginosus* on the basis of morphological and microscopical characters and on the basis of molecular identification by using ITS-1 and ITS-4 primer (Plate 6 and Fig. 1).

Thermomyces lanuginosus is classified as a thermophile, and experiences rapid growth at high temperatures (Cooney and Emerson 1964). Colonies are white and velvety at first, generally less than 1 mm high, but soon turn grey or greenish grey, starting from the center. Mature colonies are dull dark brown to black, often with pink or vivacious diffusing pigment secreted from the colony (Cooney and Emerson 1964; Howard 2002). Masses of developing aleuriophores can be seen on the fine, colourless hyphae of young colonies when viewed under a microscope. They are generally unbranched but occasionally branch once or twice near the base, appearing as a cluster. Septations may occur but are often difficult to observe. Aleuriospores are borne singly at tips of the aleuriophores (Cooney and Emerson 1964). Spores were colorless and smooth at first, but turn dark brown during maturation, and the thick exospore becomes wrinkled. Mature spores are spherical, irregularly shaped. Both immature and mature spores can be easily separated from the aleuriophore, which usually ruptures slightly below the point of attachment, so free spores may be found with the top portion still attached.

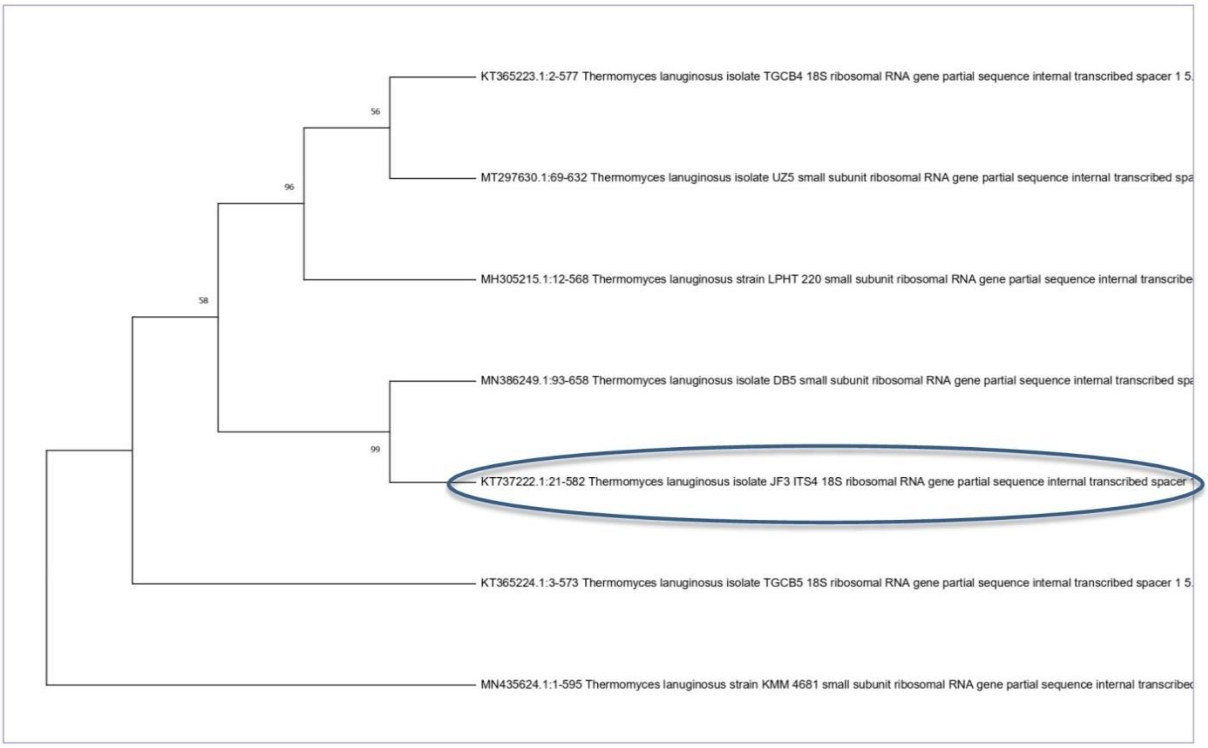


Figure 1. Phylogenetic tree of TF4- *Thermomyces lanuginosus* based on ITS sequences drawn by using the neighbour joining algorithm method

Thermophilic fungi are primarily compost fungi, though *Thermomyces lanuginosus* has also been found to thrive in spoil tips, senescent grass leaves, sewage, peat, bog soils and is the dominant species of thermophilic fungi in hot springs (Satyanarayana et al. 2013 and Maheshwari 2016). *Thermomyces lanuginosus* has two of the most important qualities required for being a compost colonizer - it is able to withstand high temperatures and use complex carbon sources for energy (Singh and Aneja 2012). It produces thermostable hemicellulases that degrade hemicellulose of plant biomass into simpler sugars (Zhang et al. 2015). As the temperature in compost systems rise, the pioneer flora disappears and thermophilic fungi become dominant (Satyanarayana et al. 2013). *Thermomyces lanuginosus* is a secondary sugar fungus and can participate in mutualistic relationships with some true cellulose decomposers of composts (Singh and Aneja 2012).

Uses of *Thermomyces lanuginosus*

Thermomyces lanuginosus has a number of different chemical, environmental and industrial applications, where hydrolytic processes are involved. Its regiospecificity allows the oleochemical industry to produce products such as cocoa butter equivalents, human milk fat substitutes, and other specific-structured lipids. There are a number of enzymes secreted by *Thermomyces lanuginosus viz*., lipase, amylase, xylanase, phytase, chitinase, glucoamylase, trehalase and invertase (Berikten and Kivanc 2014; Zhang et al. 2015; Maheshwari 2016 and Maheshwari et al. 2016). It can be used for hydrolysis of oils and fats, alcoholysis or trans-esterification’s of oils and fats, esterification of fatty acids and acidolysis and inter-esterification of oils. Large scale environmental applications include use in the degradation of polymers, treatment of wastewater from the meat industry, pretreatment of wool and a sensor of fat quality in large scale processing (Fernandez 2010).

2) *Melanocarpus albomyces*

Melanocarpus albomyces (Cooney and Emerson 1964) is Synonym to *Myriococcum albomyces* (Cooney and Emerson 1964) = *Thielavia albomyces* (Cooney and Emerson 1964; Malloch and Cain 1972). On YpSs agar, colony appear white and cottony in the early stages becoming grayish black by fourth day and grayish black pigment excreted in the agar medium. Two distinct types of hyphae: aerial septate hyphae variable in thickness (2-10 µm wide); prostrate hyphae constricted at the septa, forming branched chain like series of cylindrical or oval thick-walled cells that break apart easily. Ascocarps are superficial, dark

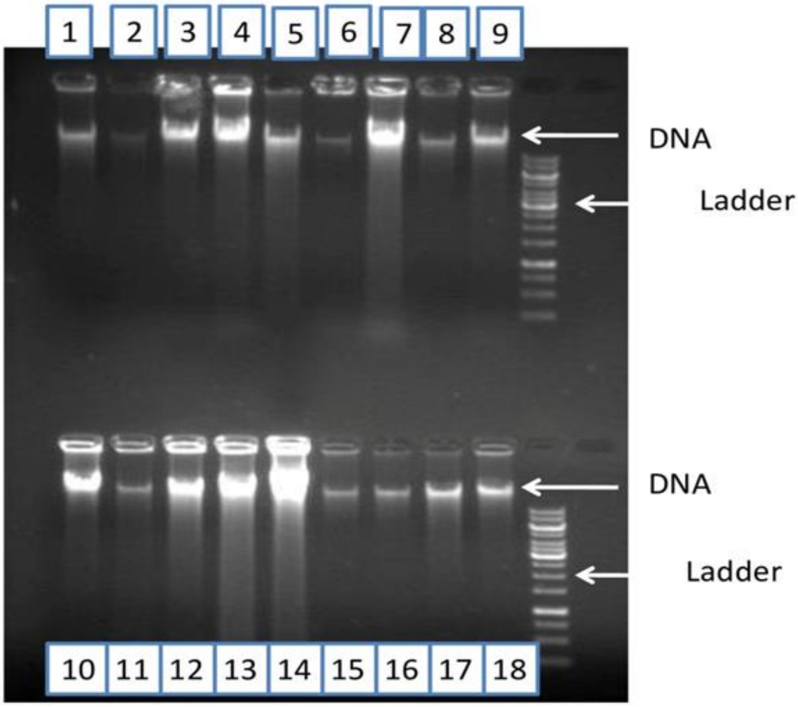


Plate 8. Gel image showing genomic DNA of thermophilic fungi

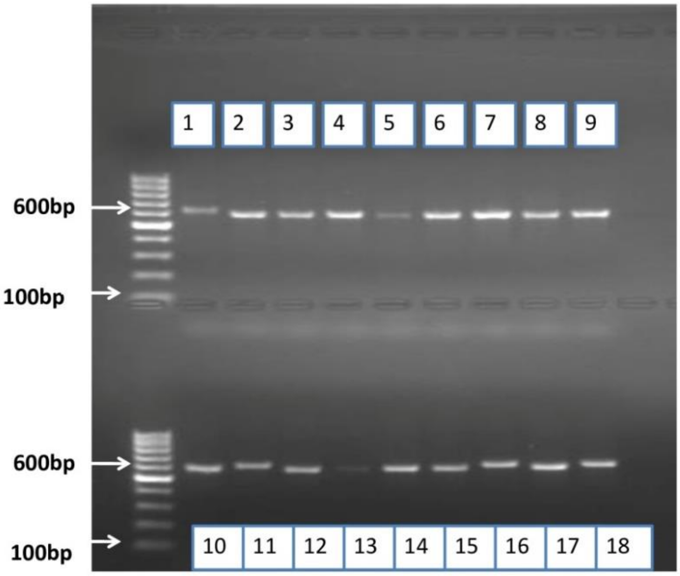


Plate 9. Molecular identification of thermophilic fungi using universal primer ITS-1 and ITS-4

brown, scattered or gregarious, globose, glabrous wall, non-ostiolate, 150-250 µm in diameter. Asci pyriform when young, at maturity irregularly oblong, 8-spored, ascus membrane simple, very thin, evanescent, 35-40 × 15-20 µm. Ascospores single celled, smooth, dark brown, globose or elliptical, with a single apiculus, irregularly distributed in the ascus, 10-15 µm in diameter. Isolate TF6 was identified as *Melanocarpus albomyces* on the basis of morphological and microscopical characters and on the basis of molecular identification by using ITS-1 and ITS-4 primer (Plate 7 and Fig.2).

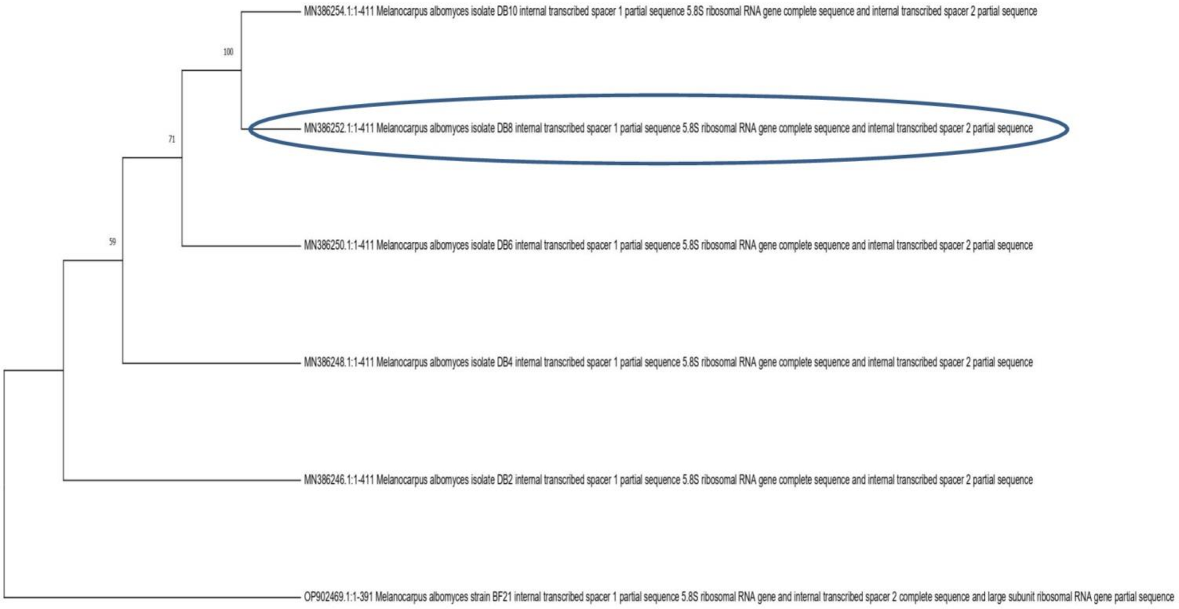


Figure 2. Phylogenetic tree of TF6- *Melanocarpus albomyces* based on ITS sequences drawn using by the neighbour joining algorithm method

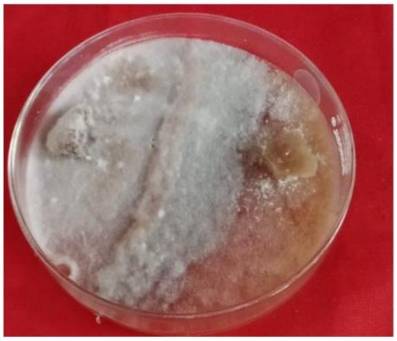
Thermophilic fungi with biodegradative capability, *Melanocarpus albomyces* (Cooney and Emerson 1964) von Arx (1975) was isolated for the first time from India. This fungus can rapidly produce high levels of extracellular xylanase. *Melanocarpus albomyces* is a thermostable, single-module, cellulose-degrading enzyme. A thermophilic ascomycete fungus *Melanocarpus albomyces* (formerly known also as *Myriococcum albomyces* or *Thielavia albomyces*) produces xylanases and cellulases with pronounced thermal stability (Maheshwari et al. 2000). Melanocarpus glycoside hydrolases have potential in various industrial applications, and three neutral cellulases have also been expressed at high levels. Novel laccase is also reported from the thermophilic fungus *Melanocarpus albomyces* (Kiiskinen et al. 2002). This laccase was shown to have very interesting properties relating to potential industrial applications (Laura et al. 2004).

These two fungal isolates, *Thermomyces lanuginosus* and *Melanocarpus albomyces* were selected and used singly and in combinations under *in vitro* condition to evaluate their effect on growth of *A. bisporus*. Before using these fungal isolates in combinations their compatibility was evaluated following dual culture. After checking their compatibility, these isolates were used as a consortium for further experiments.

4.5.1.3 *In vitro* testing of growth rates of *A. bisporus* mycelium on sterilized compost inoculated with test thermophilic fungi singly and in combinations

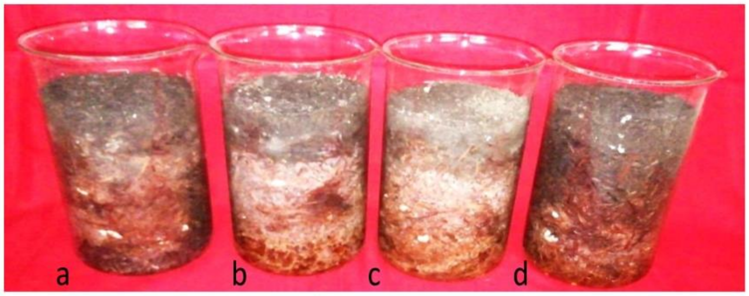
Thermomyces lanuginosus and *Melanocarpus albomyces* were used singly and in combinations to see their effect on the growth rates of *A. bisporus* on sterilized compost in beaker and petriplates under *in vitro* conditions. The linear growth rate of mycelium of *A. bisporus* on sterilized compost in beaker was found to be maximum (7.80 mm per day) in treatment 3 (consortium of isolate TF4 and TF6 of thermophilic fungi) in upward direction followed by treatment 2 (6.60 mm/day). The minimum mycelial growth rate was recorded in treatment 4 (control) (5.00 mm/day). Growth of *A. bisporus* in petri dishes on treatment 3 (compost inoculated with consortium of TF4+TF6 thermophilic fungi) showed maximum radial growth rate of 9.00 mm/day followed by 6.33 mm/day in treatment 2. The radial growth rate on control was observed to be the least in treatment 4 (4.50 mm/day). These two species of thermophilic fungi appeared to be the most promising and were used for more controlled preparation of the substrate for *A. bisporus* cultivation (Table 4.29 and Plate 10).

The effect of thermophilic fungi on growth rate of mushroom mycelia in sterilized compost is quite significant. Radial growth rate of mushroom mycelia on any laboratory medium never exceeds 3 mm/day (Last et al. 1974). Based on our experimental data, we could observe that inoculations of the thermophilic fungi provided a trigger for enhanced growth of *A. bisporus* mycelium. The study revealed that the results may be extrapolated to what actually happens during the preparation of mushroom compost. The high hyphal extension rates of *A. bisporus* on compost in the presence of thermophilic fungi may have an ecological significance that it might be able to grow as fast as possible, thereby colonizing as much substrate as possible. Once the substrate has been colonized with the mushroom mycelium, it prevents the colonization of compost by other microorganisms (Fermor and Wood 1981; Fermor and Grant 1985) or by excretion of carbon monoxide (Stoller 1978) which effectively inhibits growth of most competing organisms but inhibits the growth of the



In vitro compatibility test of *Thermomyces lanuginosus* and *Melanocarpus albomyces*

In vitro testing the radial growth of *Agaricus bisporus* on sterilized compost in petri-plates inoculated with isolated thermophilic fungi singly and as a consortia



a) Isolate1 (*Thermomyces lanuginosus)*
b)Isolate 2 (*Melanocarpus albomyces)*
c) Consortium of Isolates 1 & 2 *(Thermomyces lanuginosus + Melanocarpus albomyces*)
d) Control

In vitro testing the mycelial extension of *Agaricus bisporus* on sterilized compost in beaker inoculated with isolated thermophilic fungi singly and as a consortia

Plate 10. *In vitro* testing of *Thermomyces lanuginosus* and *Melanocarpus albomyces* for button mushroom mycelium growth

mushroom mycelium itself only partly (Derikx et al. 1990). Carbon dioxide concentrations in the range of 0.3 to 1.0 per cent generate a higher extension rate of mushroom mycelium (Wiegant et al. 1992). The probable reason of higher growth rate in culture beaker may be the ventilation in beaker.

Table.4.29 Growth rates of *A. bisporus* mycelium on sterilized compost inoculated with thermophilic fungi singly and in combinations

Compost treatments	**Mycelial extension rate in beaker (mm/day)**	**Radial growth rate in petri dishes (mm/day)**
Treatment 1 **Paddy Straw + Wheat Straw (1:1) inoculated with *Thermomyces lanuginosus***	6.20	5.40
Treatment 2 **Paddy Straw + Wheat Straw (1:1) inoculated with *Melanocarpus albomyces***	6.60	6.33
Treatment 3 **Paddy Straw + Wheat Straw (1:1) inoculated with consortium of *Thermomyces lanuginosus* + *Melanocarpus albomyces***	7.80	9.00
Treatment 4 **Control (Uninoculated)**	5.00	4.50
Mean	6.40	6.32
C.D. (0.05)	0.65	0.28
SE	0.21	0.09

Straatsma et al. (1989) pre-incubated the sterilized compost with isolates of thermophile *Scytalidium thermophilum*, *Myriococcum thermophilum* and the unidentified *Chaetomium* speciesand reported better growth of mushroom mycelium. Later, Straatsma et al. (1994a) tested other thermophilic fungi, i.e., *Chaetomium thermophilum*, an unidentified *Chaetomium* sp., *Malbranchea sulfurea, Myriococcum thermophilum, Symbiobacterium thermophilum, Stilbella thermophila, Thielavia terrestris* and two unidentified basidiomycetes, on sterilized compost, and found these promoted the mycelial growth of *A. bisporus*. *Mycothermus thermophilus* (Cooney and Emers 1964) Wang, Houbraken and Natvig (synonym of *Scytalidium thermophilum*), *Malbranchea cinnamomea* (Lib.) Oorschot and de Hoog (synonym *Malbranchea sulfurea*) and *Myriococcum thermophilum* (Fergus) Aa, were also found to stimulate the mycelial growth and production of *A. bisporus* (Straatsma et al. 1991, 1994; Sanchez 2007 and Sanchez et al. 2008) by reducing the concentration of ammonium, immobilizing nutrients and producing metabolites that can inhibit competing organisms. Straatsma and Samson (1993) tested the 54 isolates of *Symbiobacterium thermophilum* to promote the growth of *A. bisporus* at rates of about 7 mm/day. This finding indicates some specificity of the growth-promoting factor(s) *Chaetomium thermophilum, Chaetomium* sp., *Malbranchea sulfurea, Myriococcum thermophilum* and one of the unidentified basidiomycetes induced growth rates above 6 mm/ day (Straatsma et al. 1994).

4.6 EVALUATION OF COMPOST QUALITY AND MUSHROOM YIELD BY USING TWO SELECTED THERMOPHILIC FUNGI IN COMPOST

Out of five treatments of different compost formulations treatment 3 was evaluated as the best treatment and was used for further evaluation. Two themophilic fungi, which showed maximum enzyme activity, were used as inoculums to evaluate the effect of these fungi on compost preparation, quality parameters of compost and production of mushroom.

In this experiment, the treatments were given in best compost formulation i.e., treatment 3 of the previous experiment. Composition consisting of wheat straw 500 kg, Paddy straw 500 kg, chicken manure 600 kg, urea 15 kg, wheat bran 100 kg and gypsum 30kg was taken in the compounding mixture (Plate 11 and 12). There were four treatments used with four replications in the study. T-1- inoculated with 1st best isolate (TF4-*Thermomyces lanuginosus*), T-2 inoculated with 2nd best isolate (TF6-*Melanocarpus albomyces*), T-3 inoculated with consortium of TF4-*Thermomyces lanuginosus*+TF6-*Melanocarpus albomyces* and T-4 was the Control (uninoculated). These thermophilic fungi were grown on wheat grains as mushroom spawn as per standard procedure and incubated at 40±2°C for full growth (Plate 13).

4.6.1 Evaluation of compost with thermophilic fungi and its effect on yield of mushroom

The data in table 4.30 shows the days taken for spawn run, case run, pin head formation, first harvest, yield and average fruiting body weight of mushroom in different treatments. It was observed that treatment 3 took minimum time for spawn run (11.76 days) whereas, maximum days were taken by treatment 4 (14.22 days).

Days taken for case run was observed to be the least again in treatment 3 (16.05 days) while maximum was in treatment 1 (19.00 days). For pin head formation also treatment 3 took minimum days (23.09 days) and maximum days was in treatment 4 (24.08 days). In case of first harvest, out of all four treatments, treatment 3 took minimum days (26.01 days) followed by treatment 2 (26.02 days) whereas, maximum days were taken by treatment 4 (28.13 days).

Spawn preparation of thermophilic fungi on wheat grains at 40 ± 2 °C

Spawn of thermophilic fungi *Thermomyces lanuginosus*	**Spawn of thermophilic fungi** *Melanocarpus albomyces*	**Spawn of thermophilic fungi Consortium of** *Thermomyces lanuginosus* + *Melanocarpus albomyces*

Plate 11. Spawn of thermophilic fungi

Plate 12. Different compost treatments made by inoculums of thermophilic fungi

1.Weighing of substrate 2. Inoculum addition 3. Mixing of substrate and inoculum 4. Heap formation 5. After last turning 6. Filling into pasteurization chamber

7. Spawning 8. Mushroom bags in cropping room (22-25°C) 9. Casing 10.Fruiting (14-18°C) 11. Harvesting 12.Packing

Plate 13. Steps involved in making compost by using thermopilic fungi

Table 4.30 Evaluation of compost with thermophilic fungi and its effect on yield of mushroom

Compost treatments	**Days taken for**				**Yield (kg per 100kg)**	**Average weight (g)**
	Spawn run	**Case run**	**Pin head formation**	**First harvest**		
Treatment 1 **Paddy Straw + Wheat Straw (1:1) inoculated with *Thermomyces lanuginosus***	14.03	19.00	24.02	27.10	18.25	14.96
Treatment 2 **Paddy Straw + Wheat Straw (1:1) inoculated with *Melanocarpus albomyces***	13.08	18.03	24.01	26.02	20.75	15.11
Treatment 3 **Paddy Straw + Wheat Straw (1:1) inoculated with consortium of *Thermomyces lanuginosus* + *Melanocarpus albomyces***	11.76	16.05	23.09	26.01	23.98	17.18
Treatment 4 **Control (Uninoculated)**	14.22	17.99	24.08	28.13	16.22	14.12
Mean	13.27	17.77	24.05	26.81	19.86	15.34
C.D. (0.05)	0.93	0.98	0.09	0.98	1.13	0.99
SE	0.30	0.31	0.03	0.31	0.36	0.32

In case of mushroom yield, out of all four treatments, treatment 3 gave maximum yield (23.98 kg/q compost) followed by treatment 2 (20.75 kg/q compost). The minimum yield was recorded in treatment 4 (16.22 kg/q compost). Average fruit body weight was observed maximum in treatment 3 (17.18 g) and minimum average fruit body weight was found in treatment 4 (14.12 g). All the four treatments of different combinations were found free from the any diseases or pest during cropping time (Plate 14, 15 and 16). Previous reports also showed that the mushroom yield in the compost prepared using *Malbranchea sulfurea* + *Torula thermophila* was significantly higher (1990 g/5 kg of compost), which was about twice as much as the pasteurized control compost (1020 g/5 kg of compost) and also promoted the growth of *A. bisporus in vitro* to 7.7 mm/day in petriplates (Salar and Aneja 2007). According to Gerben et al. (1995) presence of theromophilic fungus in compost has a positive correlation to the yield of mushrooms. Several workers suggested the role of these thermophilic fungi in making compost selective and nutritive for *A. bisporus* (Waksman et al. 1939b; Pope et al. 1962; Straatsma et al. 1989 and Vijay 1996). Straatsma et al. (1994) recorded that *Scytalidium thermophilum* and *Myriococcum thermophilum* grew well on pasteurized compost; both species were effective on the crop yield of *A. bisporus* mushrooms. The yield of mushrooms on inoculated composts was almost twice that of the pasteurized control. This finding is of relevance for the environmentally controlled production of high-yielding compost. Thermophilic fungi in particular *Torula thermophila* and *Malbranchea sulfurea* provides a trigger for enhanced growth of *A. bisporus* acting by an unknown mechanism (Salar and Aneja 2007). All these finding end support to the present investigations.

4.6.2 Effect of different compost treatments on morphometric parameters of button mushroom

The observations were recorded on morphometric parameters of button mushroom grown on different compost treatments like diameter of pileus, thickness of pileus, diameter of stipe and length of stipe (Table 4.31).

The observations revealed that the diameter of pileus was also observed to be the maximum in treatment 3 (5.02cm) while the lowest was in treatment 2 (4.00 cm). Maximum thickness of pileus of mushroom fruiting bodies was recorded in treatment 3 (2.50cm) followed by treatment 2 (2.30cm) while the minimum was recorded in treatment 4 (2.10cm). Diameter of mushroom stipe was recorded to be the maximum in treatment 3 (1.70cm) followed by treatment 2 (1.50cm) and minimum was found in treatment 1 (0.90cm). Length

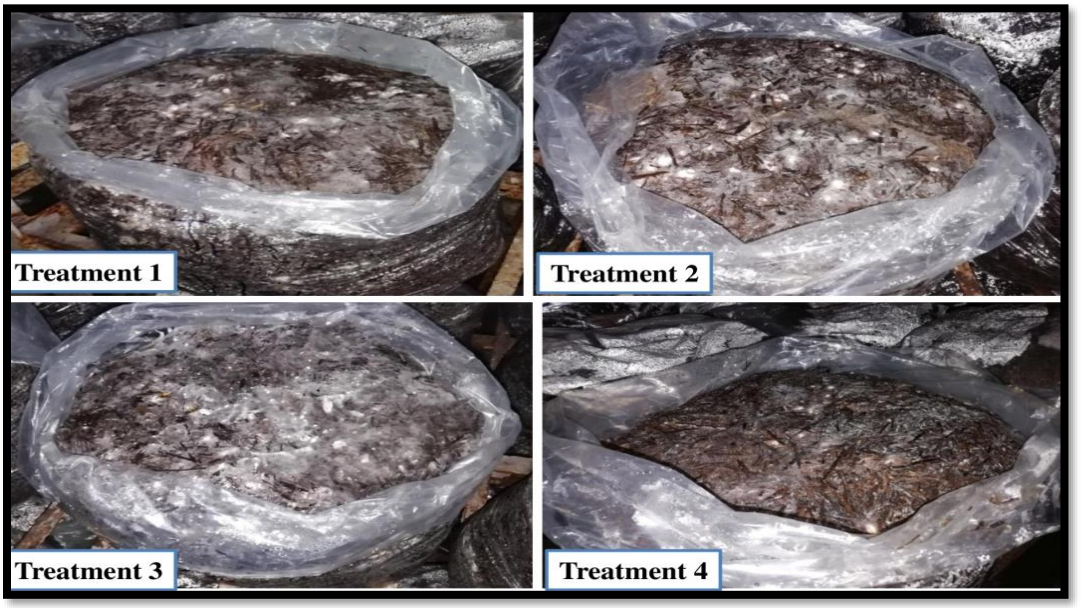


Plate 14. Effect of thermophilic fungi inoculum on spawn run of button mushroom

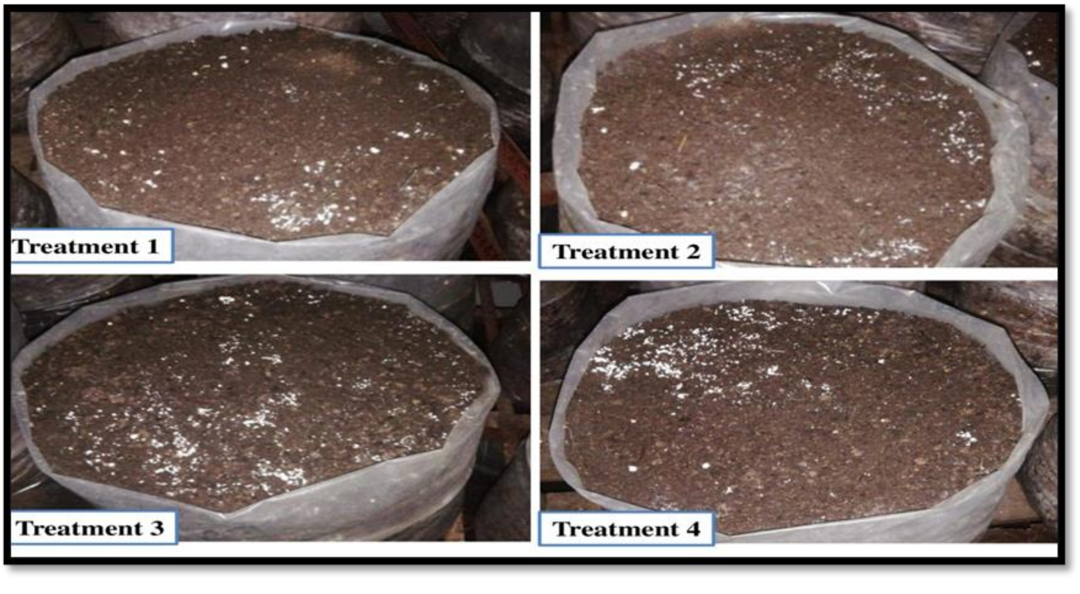


Plate 15. Effect of thermophilic fungi inoculum on pinning of button mushroom

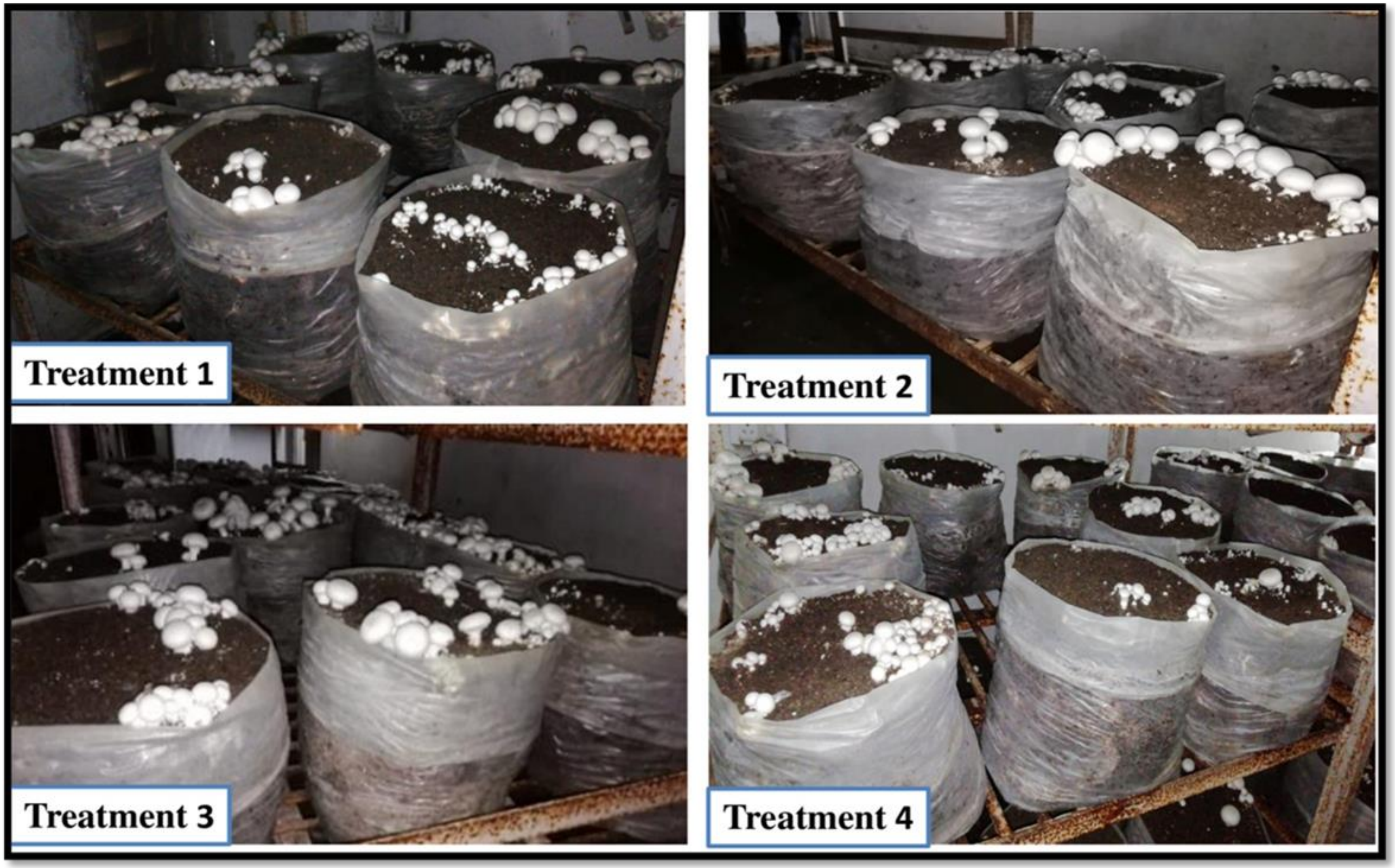


Plate 16. Effect of inoculation of thermophilic fungi on yield of button mushroom

of stipe was maximum in treatment 3 (1.90cm) whereas, minimum was recorded in treatment 1 (1.20cm).

Table 4.31 Effect of different compost treatments on morphometric parameters of button mushroom by inoculating thermophilic fungi

Compost treatments	**Diameter of pileus (cm)**	**Thickness of pileus (cm)**	**Diameter of stipe (cm)**	**Length of stipe (cm)**
Treatment 1 **Paddy Straw + Wheat Straw (1:1) inoculated with *Thermomyces lanuginosus***	4.20	2.20	0.90	1.20
Treatment 2 **Paddy Straw + Wheat Straw (1:1) inoculated with *Melanocarpus albomyces***	4.00	2.30	1.50	1.70
Treatment 3 **Paddy Straw + Wheat Straw (1:1) inoculated with consortium of *Thermomyces lanuginosus* + *Melanocarpus albomyces***	5.02	2.50	1.70	1.90
Treatment 4 **Control (Uninoculated)**	4.81	2.10	1.30	2.10
Mean	4.90	2.04	1.20	1.40
C.D. $_{(0.05)}$	0.61	0.27	0.55	0.41
SE	0.20	0.09	0.18	0.13

Vijay and Pathak (2014) reported a significant role of thermophilic fungi especially, *Scytalidium thermophilum*, *Humicola insolens* and *Humicola grisea* in selectivity and productivity of the compost. They artificially inoculated these fungi on zero day in long method of composting (LMC) and recorded a reduced composting period to 20 days. Inoculation of *Scytalidium thermophilum* significantly increased the button mushroom yield. Inoculation of these fungi in Phase-II of short method of composting could bring down the period from seven to five days. High conversion of compounding mixture to compost was found in inoculated treatments with increased yield over control. Highest degradation of cellulose, hemicellulose and carbon was observed in inoculated pile, which contributed in good spawn run and higher yield.

4.7 QUALITY ASSESSMENT OF COMPOST

The composts made using different treatments were analyzed for different physical and chemical properties using optimized compost formulations. The samples of composts prepared with four different treatments were drawn at the time of spawning following standard protocol.

The samples were dried at 55-60º C in a hot air oven, grinded and then analyzed. The composts prepared with different combination were found to vary in their quality characteristics.

4.7.1 Physical properties of composts made by different treatments

Moisture content of the composts was recorded highest in treatment 3 (67.13%). The minimum moisture content was observed in treatment 2 (62.98%). pH of the compost recorded highest in treatment 4 (7.41) and minimum pH was recorded in treatment 1 (7.05). Electric conductivity (EC) of the composts were recorded highest in treatment 2 (3.50 deci Sm-^{1}) followed by the treatment 4 (3.12 deci Sm^{-1}) whereas minimum EC was recorded in treatment 3 (2.64 deci Sm-^{1}). Out of all the four treatments, organic matter content of compost was found to be the maximum in treatment 1 (71.52%) followed by treatment 4 (69.00%). The minimum organic matter was found in treatment 2 (65.04%). The bulk density of compost was recorded the maximum in treatment 1 (0.44 g/cm^{3}) followed by treatment 2 and 4 (0.41 g/cm^{3}) while the minimum bulk density was recorded in treatment 3 (0.37 g/cm^{3}). Particle density of compost in all the four treatments, recorded to be the maximum in treatment 2 (0.94 g/cm^{3}) followed by treatment 1 (0.93 g/cm^{3}). The minimum particle density was recorded in treatment 3 (0.87 g/cm^{3}).

Porosity of all compost treatments were observed to vary in different treatment combinations. It varied from 52.69 per cent in treatment 1 to 57.47 per cent in treatment 3. The maximum porosity of compost was recorded in treatment 3 (57.47%) followed by treatment 2 (56.38%). The minimum porosity was observed in treatment 1 (52.69%) (Table 4.32). Thai et al. (2022) recorded that the moisture content of the compost increased during the initial bale wetting stage to 70 per cent (w/w) and was maintained at 70–80 per cent during Phase I, decreasing to 60-70 per cent during Phase II and during the spawn run. Kaur et al. (2007) reported that thermophile inoculation helped in reducing the electrical conductivity of the compost significantly. Electrical conductivity, a measure of dissolved salts, ranged between 1.3 m S and 2.4 m S and was the safe limit below 3 m S. Fungal inoculation resulted in a lowering of the pH of compost up to the neutral range, which is an acceptable value for the final product of all the treatments compared to the control.

All the physical parameters (independent variables) were analyzed for the correlation with the yield per cent (dependent variable) of white button mushroom using path analysis. The mean value, standard deviation and standard error were calculated and given in Table 4.33.

Table 4.32 Physical quality characteristics of the compost in different compost treatments inoculated with thermophilic fungi

Compost treatments	Moisture (%)	pH	EC (deci Sm^{-1})	Organic matter (%)	Bulk density (g/cm^3)	Particle density (g/cm^3)	Porosity (%)
Treatment 1 **Paddy Straw + Wheat Straw (1:1) inoculated with *Thermomyces lanuginosus***	66.30	7.05	2.82	71.52	0.44	0.93	52.69
Treatment 2 **Paddy Straw + Wheat Straw (1:1) inoculated with *Melanocarpus albomyces***	62.98	7.36	3.50	65.04	0.41	0.94	56.38
Treatment 3 **Paddy Straw + Wheat Straw (1:1) inoculated with consortium of *Thermomyces lanuginosus* +*Melanocarpus albomyces***	67.13	7.32	2.64	68.03	0.37	0.87	57.47
Treatment 4 **Control (Uninoculated)**	63.32	7.41	3.12	69.00	0.41	0.88	54.55
Mean	64.93	7.28	3.02	68.40	0.41	0.91	55.27
C.D. $_{\mathbf{(0.05)}}$	0.93	0.21	0.38	4.16	0.01	0.01	0.35
SE	0.30	0.07	0.12	1.34	0.01	0.01	0.11

The correlation analysis examines the effect of independent variables (all physical parameters of compost) on the dependent variables (yield). The standard error calculated in the analysis showed significant variations (Table 4.33). The correlation analysis has shown a strong significantly positive effect of pH and porosity on the yield per cent while moisture percent has shown positive effect on yield along with porosity. This indicates that high porosity with high pH in the compost has significantly affected the yield potential of the compost. It also implies that if moisture content becomes high with low porosity in the compost, it will have a negative effect on the yield per cent. It could also be inferred from the data analysis that bulk density and particle density of the compost have significantly strong negative effect on the yield of button mushroom while electrical conductivity and organic matter of the compost have moderate negative effect on yield (Table 4.34). Further, the correlations coefficients were divided into direct and indirect effects and are depicted in table 4.35. The separation of direct and indirect effects of independent variable on dependent variable showed that high moisture contributes to the high bulk density with low porosity in the compost and bulk density is negatively correlated with yield. Thus, higher moisture and bulk density ultimately affects the yield per cent negatively. The particle density has also shown a negative correlation with the yield implying the lower yield with higher particle density while the porosity has indirect positive effect on yield per cent of white button mushroom through bulk density. The whole analysis showed that the bulk density is affecting the yield negatively through various other interdependent physical parameters of the compost.

Tables 4.33. Means, standard deviation and standard error of different physical parameters analyzed during the study by using thermophilic fungi

Variables	Mean	Standard deviation	Standard error
Yield (%)	20.99	2.87	1.66
Moisture (%)	65.47	2.20	1.27
Ph	7.24	0.17	0.10
EC (deci Sm-1)	2.99	0.45	0.26
Organic matter (%)	68.20	3.24	1.87
Bulk density (g/cm^3)	0.41	0.04	0.02
Particle density (g/cm^3)	0.91	0.04	0.02
Porosity (%)	55.51	2.51	1.45

Tables 4.34. Correlation matrix of different physical parameters (independent variables) with yield per cent (dependent variable) analyzed during the study by using thermophilic fungi

Variables	**Yield (%)**	**Moisture (%)**	**pH**	**EC (deci Sm-1)**	**Organic matter (%)**	**Bulk density (g/cm^3)**	**Particle density (g/cm^3)**	**Porosity (%)**
Yield (%)	1.00							
Moisture (%)	0.26	1.00						
pH	**0.75**	-0.44	1.00					
EC (deci Sm-1)	**-0.27**	-1.00	0.43	1.00				
Organic matter (%)	**-0.48**	0.73	-0.94	-0.72	1.00			
Bulk density (g/cm^3)	**-1.00**	-0.27	-0.75	0.28	0.47	1.00		
Particle density (g/cm^3)	**-0.84**	-0.75	-0.27	0.76	-0.09	0.84	1.00	
Porosity (%)	**0.93**	-0.11	0.94	0.10	-0.77	-0.93	-0.57	1.00

Tables 4.35. Path correlation matrix of different physical parameters (independent variables) with yield per cent (dependent variable) analyzed during the study by using thermophilic fungi

Variables	**Moisture (%)**	**pH**	**EC (deci Sm-1)**	**Organic matter (%)**	**Bulk density (g/cm^3)**	**Particle density (g/cm^3)**	**Porosity (%)**	**Correlation coefficient with Yield**
Moisture (%)	1.06	-0.16	-0.27	-0.44	0.25	-0.23	0.06	0.26
pH	-0.47	0.38	0.12	0.57	0.70	-0.08	-0.46	**0.75**
EC (deci Sm-1)	-1.06	0.16	0.27	0.44	-0.26	0.23	-0.05	-0.27
Organic matter (%)	0.77	-0.35	-0.20	-0.61	-0.43	-0.03	0.37	-0.48
Bulk density (g/cm^3)	-0.29	-0.28	0.08	-0.29	-0.93	0.26	0.45	**-1.00**
Particle density (g/cm^3)	-0.80	-0.10	0.21	0.05	-0.78	0.30	0.28	**-0.84**
Porosity (%)	-0.12	0.35	0.03	0.47	-0.86	-0.18	0.49	**0.93**

4.7.2 Chemical properties of composts made by different treatments

In chemical properties of compost macro and micronutrient were analysis by standard procedures.

4.7.2.1 Macronutrients status in the compost made by using different treatments

Macronutrients i.e., organic carbon (OC), nitrogen (N), phosphorous (P), potassium (K), calcium (Ca) and magnesium (Mg) in the compost made using different treatments were analyzed during the study and data is depicted in table 4.36. Organic carbon was recorded maximum in treatment 2 (35.96%) followed by treatment 4 (35.17%) while minimum carbon was recorded in treatment 3 (34.56%). Nitrogen per cent was found maximum in treatment 3 (1.96%) and minimum in treatment 1 (1.89%).

Phosphorous was recorded to the maximum in treatment 2 and treatment 3 (0.85%), which were statistically at par to each other while minimum was recorded in treatment 1 (0.73%). Potassium was recorded to be the maximum in treatment 3 (3.57%) followed by treatment 1 (3.34%) and minimum was recorded in treatment 2 (3.00%). Maximum calcium was observed in treatment 4 (3.77%) followed by treatment 2 (3.34%). Magnesium was recorded to be the maximum in treatment 2 (0.83%).

Kaur et al. (2007) recorded that fungal inoculation resulted in a reduction in total organic carbon (TOC) content, which was observed in all the composts during the study. Ghaly et al. (2012) tested the effectiveness of inoculating the compost with thermophilic-cellulolytic microorganisms (*Thermomonospora curvata, Thermomonospora fusca* and *Thermoascus aurantiacus*) and they recorded a decrease in moisture content from initial to final compost. The breakdown of organic nitrogen into ammonium caused an initial increase in pH due to production of ammonium hydroxide, which later decreased due to the formation of organic acid from the decomposition of fats and the loss of ammonia as exhaust gases.

Table 4.36. Macronutrients composition of the compost by inoculated with thermophilic fungi

Compost treatment	**OC (%)**	**N (%)**	**P (%)**	K (%)	**Ca (%)**	**Mg (%)**
Treatment 1 **Paddy Straw + Wheat Straw (1:1) inoculated with *Thermomyces lanuginosus***	34.77	1.89	0.73	3.34	2.73	0.70
Treatment 2 **Paddy Straw + Wheat Straw (1:1) inoculated with *Melanocarpus albomyces***	35.96	1.93	0.85	3.00	3.34	0.83
Treatment 3 **Paddy Straw + Wheat Straw (1:1) inoculated with consortium of *Thermomyces lanuginosus +Melanocarpus albomyces***	34.56	1.96	0.85	3.57	2.49	0.77
Treatment 4 **Control (Uninoculated)**	35.17	1.90	0.74	3.01	3.77	0.42
Mean	35.12	1.92	0.79	3.23	3.08	0.68
C.D. (0.05)	0.26	0.09	0.09	0.23	0.08	0.08
SE	0.08	0.03	0.03	0.08	0.03	0.03

4.7.2.2 Micronutrients status in the compost made by using different treatments

In micronutrients, copper, zinc, iron and manganese were analyzed by standard procedures and the data is depicted in table 4.37. While studying four treatment, copper micronutrient was recorded to be the maximum in treatment 1 (50.90ppm) followed by treatment 3 (47.60ppm) whereas minimum was recorded in treatment 4 (45.00 ppm).

Table 4.37 Micronutrients composition of the compost inoculated with thermophilic fungi

Compost treatment	Copper (ppm)	Zinc (ppm)	Iron (ppm)	Manganese (ppm)
Treatment 1 **Paddy Straw + Wheat Straw (1:1) inoculated with *Thermomyces lanuginosus***	50.90	169.00	1740.00	285.00
Treatment 2 **Paddy Straw + Wheat Straw (1:1) inoculated with *Melanocarpus albomyces***	45.30	165.00	1900.00	464.00
Treatment 3 **Paddy Straw + Wheat Straw (1:1) inoculated with consortium of *Thermomyces lanuginosus + Melanocarpus albomyces***	47.60	180.00	2070.00	480.00
Treatment 4 **Control (Uninoculated)**	45.00	164.00	2030.00	398.00
Mean	47.20	169.50	1,935.00	406.75
C.D. (0.05)	1.37	7.84	40.94	11.52
SE	0.44	2.52	13.14	3.70

Zinc was found maximum in treatment 3 (180 ppm) followed by treatment 1 (169 ppm) and minimum in treatment 4 (164 ppm). Iron was recorded maximum in treatment 3 (2070 ppm). Manganese was recorded maximum in treatment 3 (480 ppm) followed by treatment 2 (464 ppm).

4.7.2.3 Carbon and nutritional ratio of the compost inoculated with thermophilic fungi

The results of the study are depicted in table 4.38. C: N ratio was recorded to be the maximum in treatment 2 (18.60) followed by the treatment 4 (18.51). C: P ratio was observed to be the maximum in treatment 1 (47.63) while minimum was observed in treatment 3 (40.65).

C: K ratio significantly varied in different compost treatments from 9.68 in treatment 3 to 11.97 in treatment 2. N: P ratio was observed to be the maximum in treatment 1 (2.59) while minimum was recorded in treatment 2 (2.27). N: K ratio varied significantly from 0.55 in treatment 3 to 0.64 in treatment 2. P: K ratio varied from 0.22 to 0.28. Maximum was recorded in treatment 2 (0.28) followed by the treatment 4 (0.25). The minimum P: K ratio was recorded in treatment 1 (0.22).

Table 4.38 Ratio of carbon with macronutrients of the compost inoculated with thermophilic fungi

Compost treatments	**C: N**	**C: P**	**C: K**	**N: P**	**N: K**	**P: K**
Treatment 1 **Paddy Straw + Wheat Straw (1:1) inoculated with *Thermomyces lanuginosus***	18.40	47.63	10.41	2.59	0.57	0.22
Treatment 2 **Paddy Straw + Wheat Straw (1:1) inoculated with *Melanocarpus albomyces***	18.60	42.24	11.97	2.27	0.64	0.28
Treatment 3 **Paddy Straw + Wheat Straw (1:1) inoculated with consortium of *Thermomyces lanuginosus* + *Melanocarpus albomyces***	17.63	40.65	9.68	2.31	0.55	0.24
Treatment 4 **Control (Uninoculated)**	18.51	47.53	11.68	2.57	0.63	0.25
Mean	18.30	44.07	10.94	2.43	0.60	0.25
C.D. $_{\mathbf{(0.05)}}$	0.18	3.30	0.03	0.02	0.04	0.01
SE	0.06	1.06t	0.01	0.01	0.01	0.01

4.7.3 Silica content in different compost formation

Silica content was found to decrease after adding the inoculums of thermophilic fungi in compost (Table 4.39). Out of four treatments, least silica content was recorded in treatment 3 (4.51%) followed by the treatment 2 (5.36%). The maximum silica content was recorded in treatment 4 (5.88%).

Kumar et al. (2008) isolated 10 thermophilic fungi from wheat straw, farmyard manure, and soil. Out of these, only three showed the highest cellobiase, carboxymethyl cellulase, xylanase and FPase activities. They were identified as *Aspergillus nidulans* (Th4), *Scytalidium thermophilum* (Th5) and *Humicola* sp. (Th10). A fungal consortium of these three fungi was used to compost a mixture (1:1) of silica-rich paddy straw and lignin-rich soybean trash. The fungal consortium was effective in converting high-silica paddy straw into nutritionally rich compost there, leading to economical and environmentally friendly disposal of this crop residue.

Table 4.39 Effect of inoculation of thermophilic fungi on silica content in different compost treatments

Compost treatments	Silica content (%)
Treatment 1 **Paddy Straw + Wheat Straw (1:1) inoculated with *Thermomyces lanuginosus***	5.44
Treatment 2 **Paddy Straw + Wheat Straw (1:1) inoculated with *Melanocarpus albomyces***	5.36
Treatment 3 **Paddy Straw + Wheat Straw (1:1) inoculated with consortium of *Thermomyces lanuginosus +Melanocarpus albomyces***	4.51
Treatment 4 **Control (Uninoculated)**	5.88
Mean	5.30
C.D. (0.05)	0.33
SE	0.11

All the chemical parameters (independent variables) were analyzed for the correlation with the yield per cent (dependent variable) of white button mushroom using path analysis. The mean value, standard deviation and standard error were calculated and has been given in Table 4.40

Table 4.40. Means, standard deviation and standard error of different chemical parameters analyzed during the study by using thermophilic fungi

Variables	Mean	Standard deviation	Standard error
Yield	20.99	2.87	1.66
OC	35.10	0.76	0.44
N	1.93	0.04	0.02
P	0.81	0.07	0.04
K	3.30	0.29	0.17
Ca	2.85	0.44	0.25
Mg	0.77	0.07	0.04
Cu	47.93	2.82	1.63
Zn	171.33	7.77	4.49
Fe	1903.33	165.03	95.28
Mn	409.67	108.26	62.50
C: N	18.21	0.51	0.30
C: P	43.51	3.66	2.11
C: K	10.69	1.17	0.68
N: P	2.39	0.17	0.10
N: K	0.59	0.05	0.03
P: K	0.25	0.03	0.02
Si	5.10	0.52	0.30

The correlation analysis examines the effect of independent variables (all chemical parameters of compost) on the dependent variables (yield). The standard error calculated in the analysis showed significant variations (Table 4.40). The correlation analysis has shown a significantly positive effect of nitrogen, zinc, phosphorus, manganese and iron on the yield per cent while silica has strong negative correlation with yield percent (Table 4.41). It was also observed that the ratio of the two macro and microelements have also shown strong negative effect on the yield of the button mushroom. C: N, C: P and N: P ratio has negative correlation with the yield. It implies that the proper fermentation of the composting material is very important for the higher yield of the button mushroom. It helps to reduce C: N and C: P ratio by degradation of free sugars during composting and release of CO_2 which helps to enhance the selectivity of the compost. The correlations coefficients were divided into direct and indirect effects and are depicted in table 4.41. The separation of direct and indirect effects of independent variable on dependent variable showed a direct positive effect of nitrogen, phosphorus, zinc, iron and manganese while a direct negative effect was recorded with potassium, calcium and magnesium. The organic carbon showed a negative effect on total yield of mushroom but organic carbon *per se* does not have a direct effect on yield but as the organic carbon is negatively correlated with zinc, iron and manganese, which overall negatively affected the yield per cent.

The silica content is strongly negatively correlated with yield, which may have direct and indirect correlation both with yield as the silica content is also positively correlated with C:N ratio. Thus, the higher silica content is associated with higher C: N ratio, which may have a negative effect on the yield. The ratio of the two elements also showed significant effect on yield such as C: P, C: K, N: P and N: K ratio showed a direct negative effect on yield which implies the fact that higher free carbon in the compost has a negative effect on yield while higher phosphorus content has a positive effect on yield. (Table 4.42).

Table 4.41. Correlation matrix of different physical parameters (independent variables) with yield per cent (dependent variable) analyzed during the study by using thermophilic fungi

Variables	**Yield**	**OC**	**N**	**P**	**K**	**Ca**	**Mg**	**Cu**	**Zn**	**Fe**	**Mn**	**C: N**	**C: P**	**C: K**	**N: P**	**N: K**	**P: K**	**Si**
Yield	1.00																	
OC	-0.21	1.00																
N	**0.99**	-0.06	1.00															
P	**0.83**	0.38	0.90	1.00														
K	0.47	-0.96	0.32	-0.11	1.00													
Ca	-0.34	0.99	-0.19	0.24	-0.99	1.00												
Mg	0.48	0.76	0.61	0.89	-0.56	0.66	1.00											
Cu	-0.53	-0.72	-0.65	-0.91	0.51	-0.62	-1.00	1.00										
Zn	**0.76**	-0.80	0.65	0.26	0.93	-0.87	-0.21	0.16	1.00									
Fe	**1.00**	-0.16	1.00	0.86	0.42	-0.29	0.52	-0.57	0.72	1.00								
Mn	**0.87**	0.31	0.93	1.00	-0.04	0.17	0.85	-0.88	0.33	0.89	1.00							
C: N	**-0.80**	0.76	-0.70	-0.32	-0.91	0.84	0.15	-0.09	-1.00	-0.76	-0.39	1.00						
C: P	**-0.93**	-0.16	-0.98	-0.98	-0.11	-0.03	-0.77	0.80	-0.46	-0.95	-0.99	0.52	1.00					
C: K	-0.38	0.98	-0.23	0.21	-1.00	1.00	0.63	-0.59	-0.89	-0.33	0.13	0.86	0.01	1.00				
N: P	**-0.76**	-0.48	-0.85	-0.99	0.22	-0.35	-0.93	0.95	-0.15	-0.79	-0.98	0.21	0.95	-0.32	1.00			
N: K	-0.28	1.00	-0.13	0.31	-0.98	1.00	0.71	-0.67	-0.84	-0.23	0.23	0.80	-0.09	1.00	-0.41	1.00		
P: K	0.26	0.89	0.40	0.76	-0.73	0.82	0.97	-0.96	-0.44	0.31	0.71	0.38	-0.60	0.80	-0.83	0.85	1.00	
Si	**-0.93**	0.55	-0.86	-0.57	-0.76	0.66	-0.12	0.18	-0.94	-0.91	-0.63	0.96	0.73	0.69	0.47	0.61	0.11	1.00

Tables 4.42. Path correlation matrix of different physical parameters (independent variables) with yield per cent (dependent variable) analyzed during the study by using thermophilic fungi

Variables	**OC**	**N**	**P**	**K**	**Ca**	**Mg**	**Cu**	**Zn**	**Fe**	**Mn**	**C: N**	**C: P**	**C: K**	**N: P**	**N: K**	**P: K**	**Si**	**Correlation coefficient with yield**
OC	2.73	0.03	0.23	0.41	2.05	0.09	0.55	-3.38	-0.03	-0.29	0.59	0.17	-0.55	-0.26	0.15	-2.63	-0.08	-0.21
N	-0.16	-0.55	0.57	-0.14	-0.40	0.07	0.50	2.75	0.22	-0.90	-0.54	1.00	0.13	-0.47	-0.02	-1.19	0.12	**0.99**
P	1.02	-0.50	0.63	0.05	0.51	0.11	0.70	1.10	0.19	-0.96	-0.25	1.00	-0.11	-0.54	0.05	-2.23	0.08	**0.83**
K	-2.63	-0.18	-0.07	-0.43	-2.05	-0.07	-0.39	3.95	0.09	0.04	-0.70	0.11	0.56	0.12	-0.15	2.17	0.10	0.47
Ca	2.71	0.11	0.15	0.43	2.07	0.08	0.47	-3.70	-0.06	-0.17	0.65	0.03	-0.56	-0.19	0.15	-2.42	-0.09	-0.34
Mg	2.08	-0.34	0.56	0.24	1.37	0.12	0.77	-0.91	0.11	-0.82	0.12	0.79	-0.35	-0.51	0.11	-2.87	0.02	0.48
Cu	-1.97	0.36	-0.57	-0.22	-1.28	-0.12	-0.77	0.67	-0.12	0.85	-0.07	-0.83	0.33	0.52	-0.10	2.83	-0.03	-0.53
Zn	-2.18	-0.36	0.16	-0.40	-1.81	-0.03	-0.12	4.24	0.16	-0.32	-0.77	0.48	0.50	-0.08	-0.13	1.29	0.13	**0.76**
Fe	-0.43	-0.55	0.54	-0.18	-0.60	0.06	0.44	3.05	0.22	-0.86	-0.59	0.98	0.18	-0.43	-0.04	-0.92	0.13	**1.00**
Mn	0.83	-0.52	0.62	0.02	0.36	0.10	0.67	1.40	0.19	-0.96	-0.30	1.02	-0.07	-0.54	0.04	-2.08	0.09	**0.87**
C: N	2.07	0.38	-0.20	0.39	1.74	0.02	0.07	-4.23	-0.17	0.38	0.77	-0.53	-0.48	0.12	0.12	-1.11	-0.13	**-0.80**
C: P	-0.45	0.54	-0.61	0.05	-0.06	-0.09	-0.62	-1.97	-0.21	0.95	0.40	-1.03	-0.01	0.52	-0.01	1.76	-0.10	**-0.93**
C: K	2.69	0.13	0.13	0.43	2.07	0.08	0.45	-3.78	-0.07	-0.13	0.67	-0.01	-0.56	-0.17	0.15	-2.35	-0.10	-0.38
N: P	-1.31	0.47	-0.62	-0.10	-0.73	-0.11	-0.73	-0.63	-0.17	0.94	0.16	-0.97	0.18	0.55	-0.06	2.44	-0.06	**-0.76**
N: K	2.72	0.07	0.19	0.42	2.07	0.09	0.51	-3.56	-0.05	-0.23	0.62	0.09	-0.55	-0.23	0.15	-2.52	-0.08	-0.28
P: K	2.43	-0.22	0.47	0.32	1.70	0.12	0.73	-1.85	0.07	-0.68	0.29	0.61	-0.44	-0.45	0.13	-2.95	-0.02	0.26
Si	1.51	0.48	-0.35	0.33	1.37	-0.02	-0.14	-4.00	-0.20	0.60	0.75	-0.75	-0.39	0.26	0.09	-0.33	-0.14	**-0.93**

4.7.4 Microbial count in different compost treatments by using thermophilic fungi

Microbial count was assayed in different thermophilic fungi inoculated compost formulations at dilution 10^4 (Table 4.43). It was observed that microbial count increased after inoculating the thermophilic fungi in compost. At 10^4dilutions, the maximum microbial count was found in treatment 3 (27.75 cfu g-^{1} compost) and minimum microbial count was observed in treatment 4 (14.75 cfu g-^{1} compost). Thermophilic fungus, *Mycothermus thermophilus* (syn. *Scytalidium thermophilum*/ *Humicola insolens*) aids the re-assimilation of ammonia into the compost (Vos et al. 2017) and stimulates growth of the button mushroom mycelium. In the presence of *Mycothermus thermophilus*, hyphal elongation of *A. bisporus* doubles (Wiegant 1992 and Straatsma et al. 1994) and fungal competitors of *A. bisporus*, such as *Chaetomium globosum*, are suppressed (Vos et al. 2017).

Table 4.43 Effect of inoculation of thermophilic fungi on microbial count

Microbial count (cfu g-^{1} compost)	
Compost treatments	**10^4 dilution**
Treatment 1 **Paddy Straw + Wheat Straw (1:1) inoculated with *Thermomyces lanuginosus***	16.50
Treatment 2 **Paddy Straw + Wheat Straw (1:1) inoculated with *Melanocarpus albomyces***	20.75
Treatment 3 **Paddy Straw + Wheat Straw (1:1) inoculated with consortium of *Thermomyces lanuginosus +Melanocarpus albomyces***	27.75
Treatment 4 **Control (Uninoculated)**	14.75
Mean	19.93
C.D. (0.05)	4.29
SE	1.37

Mycothermus thermophilus is the dominant fungal taxon in Phase II compost and makes up most of the microbial biomass in the compost (Vieira and Pecchia 2018), but it is just one player in a multifaceted microbial community. The density of *Scytalidium thermophilum* in compost was found to be positively correlated with mushroom yield (Straatsma et al. 1989) and *Scytalidium thermophilum* strongly stimulated the extension rate of growth of mushroom mycelium (Straatsma et al. 1991).

4.7.5 Changes of physico-chemical properties of treated compost at different stages.

Temperature profiles of different treatments during composting process in compost piles

The temperature profile of four compost treatments was recorded from the three zones at different turnings. In all the four composts, the temperature of middle zone was found to be more than the upper and lower zones.

Table 4.44 Temperature profile during composting process of different compost piles

Compost treatments	Zero day (°C)	First turning (°C)	Second turning (°C)	Third turning (°C)	Fourth turning (°C)	Before spawning (°C)
Treatment 1 **Paddy Straw + Wheat Straw (1:1) inoculated with *Thermomyces lanuginosus***	37.01	51.01	64.03	70.04	68.01	22.02
Treatment 2 **Paddy Straw + Wheat Straw (1:1) inoculated with *Melanocarpus albomyces***	35.01	62.00	65.01	70.00	70.00	23.00
Treatment 3 **Paddy Straw + Wheat Straw (1:1) inoculated with consortium of *Thermomyces lanuginosus +Melanocarpus albomyces***	42.00	50.06	66.01	72.01	71.02	24.03
Treatment 4 **Control (Uninoculated)**	38.01	52.00	64.03	68.01	70.01	23.01
Mean	38.01	53.77	64.77	70.02	69.76	23.01
C.D. (0.05)	0.15	0.12	0.10	0.16	0.11	0.10
SE	0.05	0.04	0.03	0.05	0.04	0.03

It was found to rise gradually from the start of the composting and reached its peak value on third to fourth turnings. The maximum temperature recorded was 70-72°C in the middle zone. Highest temperature was observed in treatment 3 in all the stages of button mushroom compost (Table 4.44). The temperature of different composts increased from zero day to third turning and after that, the temperature started to fall.

Moisture content

The moisture content of different composts was the maximum at the initial stages of the composting process; it ranged from 75.24- 78.98 per cent. It was found to reduce gradually during the process and finally stabilized at 62.98 - 67.13 per cent (Table 4.45). Maintaining a proper moisture content in the compost stack is an important parameter since dry matter loss is strictly moisture content and temperature dependent (Laborde et al.1993).

Table 4.45 Moisture percentage of different compost piles at different intervals

Compost treatments	**Zero day (%)**	**First turning (%)**	**Second turning (%)**	**Third turning (%)**	**Fourth turning (%)**	**Before spawning (%)**
Treatment 1 **Paddy Straw + Wheat Straw (1:1) inoculated with *Thermomyces lanuginosus***	78.98	77.23	75.34	72.34	69.34	66.30
Treatment 2 **Paddy Straw + Wheat Straw (1:1) inoculated with *Melanocarpus albomyces***	76.66	75.28	73.12	71.67	68.48	62.98
Treatment 3 **Paddy Straw + Wheat Straw (1:1) inoculated with consortium of *Thermomyces lanuginosus +Melanocarpus albomyces***	77.87	72.67	71.98	70.98	69.95	67.13
Treatment 4 **Control (Uninoculated)**	75.24	73.85	72.98	71.98	68.56	63.32
Mean	77.19	74.76	73.36	71.74	69.08	64.93
C.D. (0.05)	0.09	0.05	0.10	0.07	0.06	0.93
SE	0.03	0.02	0.03	0.02	0.02	0.30

pH estimation

The pH of different compost piles was recorded at different turnings during the composting process as shown in table 4.46. The pH profile showed a different trend in all four compost stacks inoculated with thermophilic fungi. It was low at start, gradually increased between turnings and then stabilized towards the end of the composting. The pH was found to be in the range of 6.85-8.27. The pH stabilized between 7.05-7.41 at the end of the process. The rise in pH was due to the release of ammonia during the initial stages of composting which subsequently declined with the addition of gypsum.

Carbon percentage

The four different compost piles were analyzed for their carbon percentage at initial and mature stage of composting and sharp decline in carbon percent was observed from zero day to final stage, which attributes a good compost for the growth of *A. bisporus*. Narrowing down of carbon was due to the organic matter loss accompanied with the growth of thermophilic fungi in compost. Highest degradation was observed in treatment 3 followed by treatment 2 (Table 4.47).

Table-4.46: pH at different turning of compost piles

Compost treatments	**Zero day**	**First turning**	**Second turning**	**Third turning**	**Fourth turning**	**Before spawning**
Treatment 1 **Paddy Straw + Wheat Straw (1:1) inoculated with *Thermomyces lanuginosus***	6.98	7.83	8.27	7.34	7.24	7.05
Treatment 2 **Paddy Straw + Wheat Straw (1:1) inoculated with *Melanocarpus albomyces***	7.01	8.03	7.94	7.65	7.47	7.35
Treatment 3 **Paddy Straw + Wheat Straw (1:1) inoculated with consortium of *Thermomyces lanuginosus* + *Melanocarpus albomyces***	7.02	7.86	7.89	7.60	7.56	7.32
Treatment 4 **Control (Uninoculated)**	6.85	7.56	8.03	7.44	7.50	7.41
Mean	6.97	7.82	8.03	7.51	7.44	7.28
C.D. (0.05)	0.14	0.15	0.06	0.06	0.04	0.21
SE	0.05	0.05	0.02	0.02	0.01	0.07

Table-4.47: Carbon percentage in different plies

Compost treatments	**Zero day (%)**	**Before spawning (%)**
Treatment 1 **Paddy Straw + Wheat Straw (1:1) inoculated with *Thermomyces lanuginosus***	41.89	34.77
Treatment 2 **Paddy Straw + Wheat Straw (1:1) inoculated with *Melanocarpus albomyces***	44.07	35.96
Treatment 3 **Paddy Straw + Wheat Straw (1:1) inoculated with consortium of *Thermomyces lanuginosus* +*Melanocarpus albomyces***	45.10	34.56
Treatment 4 **Control (Uninoculated)**	42.67	35.17
Mean	43.43	35.12
C.D. (0.05)	0.77	0.26
SE	0.25	0.08

Nitrogen percentage

The nitrogen percentage of the four different compost piles was estimated at the initial and mature stages of the composting process using the kjeldahl method. Nitrogen was observed in an increasing trend from the initial stage to the mature stage, and the highest nitrogen was recorded in third (consortium) treatment followed by the control. It was assumed that a consortium of thermophilic fungi utilized a marginal amount of nitrogen as compared to other treatments as a microbial protein source. N per cent at zero day and final compost is shown in table 4.48. The reduction in carbon contents in compost was accompanied by corresponding increase in nitrogen levels from 1.47-1.96 in treatment 3; 1.59-1.93 in treatment 2, 1.51-1.90 in treatment 4 (control) and 1.49-1.89 in treatment 1.

Table 4.48 Nitrogen percentage in different compost piles

Compost treatments	**Zero day (%)**	**Before spawning (%)**
Treatment 1 **Paddy Straw + Wheat Straw (1:1) inoculated with *Thermomyces lanuginosus***	1.49	1.89
Treatment 2 **Paddy Straw + Wheat Straw (1:1) inoculated with *Melanocarpus albomyces***	1.59	1.93
Treatment 3 **Paddy Straw + Wheat Straw (1:1) inoculated with consortium of *Thermomyces lanuginosus +Melanocarpus albomyces***	1.47	1.96
Treatment 4 **Control (Uninoculated)**	1.51	1.90
Mean	1.52	1.92
C.D. (0.05)	0.06	0.09
SE	0.02	0.03

C/N ratio

The C/N ratio of the four compost piles narrowed down from the beginning towards the end of composting (Table 4.49). This narrowing down of the C/N ratio was due to the organic matter loss and corresponding relative increase in nitrogen contents. The substrate decomposition has been reflected in the loss of carbon contents in compost in different stacks from initial to final turnings. The C/N ratio in the initial stage ranges from 26.84 to 30.68 and is reduced to the final stage up to a range of 17.63 to 18.60.

Table 4.49: C/N ratio for different compost piles

Compost treatments	**Initial C/N**	**Final C/N**
Treatment 1 **Paddy Straw + Wheat Straw (1:1) inoculated with *Thermomyces lanuginosus***	28.11	18.40
Treatment 2 **Paddy Straw + Wheat Straw (1:1) inoculated with *Melanocarpus albomyces***	29.19	18.60
Treatment 3 **Paddy Straw + Wheat Straw (1:1) inoculated with consortium of *Thermomyces lanuginosus* + *Melanocarpus albomyces***	30.68	17.63
Treatment 4 **Control (Uninoculated)**	26.84	18.51
Mean	28.70	18.30
C.D. $_{(0.05)}$	0.01	0.18
SE	0.01	0.06

4.7.6 Estimation of degradative enzyme activities in different compost formulations upon thermophilic fungi inoculation

Compost is a selective medium which is produced by solid substrate fermentation by various microorganisms such as bacteria, actinomycetes and fungi. Thermophilic fungi utilize different components of compost as nutritive sources by secreting a series of cellulolytic and lignolytic enzymes which act in a synergetic manner to degrade the lignocellulosic materials of compost. Crystalline cellulose which is the main cellulolytic component of straw is degraded by chain of various enzymes *viz*, filterpaperase (FPase), carboxymethyl cellulase (CMCase) and β-glucosidase along with other lignin degrading enzymes *viz*, peroxidase and laccase enzymes. Laccase enzymes are secreted more as compared to other lignin degrading enzymes in straw-based medium which allow various microbes to proliferate in compost and are also responsible for fastest growth of *A. bisporus*.

These enzymes break down the polymers of cellulose and lignin in simple monomers forms which are readily utilized by thermophilic fungi as carbon and nitrogen sources and through which they proliferate in compost. Dominance of thermophilic fungi in compost make compost more selective and nutritive for the growth of *A. bisporus* and also causes inhibitory effect on other competitive fungi which are present in compost at spawning.

Quantitative estimation of enzymes like laccase, xylanase, cellulase and manganese dependent peroxides (MnP) was done at three different stages of button mushroom production i.e., before spawning (BS), fruit body development (FBD) and after harvest (AH) stage. Enzymes activities of the four different compost formulations were assayed. Observation depicted that the enzyme activity of compost was increased by inoculating the thermophilic fungi as compared to uninoculated treatment (control).

Laccase enzyme

The quantitative estimation of laccase enzyme was done at three different stages of button mushroom production like before spawning (BS), fruit body development (FBD) and after harvest (AH) stage. Results obtained are depicted in table 4.50.

Table 4.50 Effect of thermophilic fungi inoculation on laccase assay in different compost formulations

Laccase enzyme activity (IU)			
Compost treatments	**Before spawning**	**Fruit body development**	**After harvest**
Treatment 1 **Paddy Straw + Wheat Straw (1:1) inoculated with *Thermomyces lanuginosus***	0.53	0.44	0.35
Treatment 2 **Paddy Straw + Wheat Straw (1:1) inoculated with *Melanocarpus albomyces***	0.50	0.48	0.41
Treatment 3 **Paddy Straw + Wheat Straw (1:1) inoculated with consortium of *Thermomyces lanuginosus* +*Melanocarpus albomyces***	0.60	0.57	0.49
Treatment 4 **Control (Uninoculated)**	0.48	0.40	0.30
Mean	0.53	0.47	0.39
C.D. $_{(0.05)}$	0.04	0.01	0.02
SE	0.01	0.01	0.01

IU= International unit (µM/min/ml)

Before spawning stage, treatment 3 showed maximum laccase enzyme activity (0.60 IU), followed by treatment 1 (0.53 IU), whereas minimum enzyme activity was recorded in treatment 4 (0.48 IU). During the fruit body development stage, maximum enzyme activity was recorded in treatment 3 (0.57 IU), followed by treatment 2 (0.48 IU). The minimum enzyme activity observed in treatment 4 (0.40 IU). After the harvest stage, the laccase activity was reduced and the maximum enzyme activity was found in treatment 3 (0.49 IU), followed by treatment 2 (0.41 µM/min/ml), whereas the minimum laccase activity was observed in treatment 4 (0.30 IU).

Laccase (benzenediol: oxygen oxidoreductase) is a blue-copper oxidoreductase that catalyze the oxidation of wide range of substrates including phenolic compounds with the concomitant reduction of molecular oxygen to water (Nunes and Kunamneni 2018). Around 150 laccases have been fully characterized. The most studied have been isolated from fungi capable of destroying wood, especially white-rot fungi as: *Pleurotus pulmonarius, Pleurotus ostreatus*, *Agaricus bisporus*, *Trametes versicolor* etc. (Bertrand et al. 2016; Nunes and Kunamneni 2018). Generally, the enzyme is produced during the fungi secondary metabolism (Brijwani et al. 2010). In nature, laccase is secreted by the fungus to access carbohydrates (cellulose and hemicellulose) in the wood through the degradation of lignin (Osma et al. 2010). Lignin, which is a phenylpropanoid biopolymer, is considered the most abundant polymer in nature (Chio et al. 2019). Lignin has an extremely complex structure; it is recalcitrant and difficult to degrade. In its natural state it has practically no applications, however, it can be burned to get energy. That is why one of the great challenges to allow the use of lignin is its depolymerization. The production of laccase enzyme has been reported to be higher during spawn run stage while it decreases towards the fruit body formation stages in button mushroom. Thus, the higher laccase production is an indicator of better spawn run. Laccase has been used as a growth marker for *A. bisporus* mycelium (Wood 1979).

Manganese peroxidase enzyme

During the present study, MnP enzyme activity was found to increase from before spawning stage to fruit body development stage and decreased afterwards (Table 4.51). In before spawning stage, MnP enzyme activity was recorded maximum in treatment 1 (4.91 IU) followed by treatment 4 (4.12 IU). The minimum MnP enzyme activity was observed in treatment 3 (3.68 IU). In fruit body development stage, maximum enzyme activity was recorded in treatment 3 (10.14 IU) followed by treatment 1 (7.62 IU) while minimum enzyme activity was observed in treatment 2 (6.54 IU). The MnP activity in post-harvest stage was maximum in treatment 3 (6.42 IU) followed by treatment 1(5.30 IU) while minimum enzyme activity was recorded in treatment 4 (3.41 IU). Manganese peroxidase (MnP) is a haeme glycoprotein belonging to the extracellular oxidase II family, similar to LiPs (Welinder 1992; Morgenstern et al. 2008 and Pollegioni et al. 2015). MnPs are abundantly secreted proteins in most wood-decayed basidiomycetes fungi. Recently, the productivity of MnP was shown to be more efficient in terms of H_2O_2 consuming capacity than a cofactor metal ion and carbon sources in the solid-state medium of *A. bisporus* (Vos et al. 2017). H_2O_2 when used as a cofactor for peroxidase, is considered a limiting factor for the ligninolytic activity and productivity of lignin degradation. MnP oxidizes phenolic compounds indirectly *via* the oxidation of Mn ions and secretion of Mn^{3+} to the extracellular environment (Wariishi et al. 1992).

Table 4.51 Effect of thermophilic fungi inoculation on Manganese peroxidase assay in different compost formulations

MnP enzyme activity (IU)			
Compost treatments	**Before spawning**	**Fruit body development**	**After harvest**
Treatment 1 **Paddy Straw + Wheat Straw (1:1) inoculated with *Thermomyces lanuginosus***	4.91	7.62	5.30
Treatment 2 **Paddy Straw + Wheat Straw (1:1) inoculated with *Melanocarpus albomyces***	4.01	6.54	5.15
Treatment 3 **Paddy Straw + Wheat Straw (1:1) inoculated with consortium of *Thermomyces lanuginosus* +*Melanocarpus albomyces***	3.68	10.14	6.42
Treatment 4 **Control (Uninoculated)**	4.12	6.92	3.41
Mean	4.18	7.81	5.07
C.D. (0.05)	0.02	0.02	0.01
SE	0.01	0.02	0.01

IU= International unit (µM/min/ml)

c) Quantitative estimation of celluloses and hemicellulase in different button mushroom compost formulation upon thermophilic fungi inoculations

Filter paperase enzyme

Filter paperase is also known as C1 cellulase catalyzing the degradation of crystalline cellulose at carbon 1 of the glucose chain. During the present studies, it was observed that cellulase enzyme activity increased from before spawning stage to fruit body development stage and decreased afterward (Table 4.52). Treatment 3 showed maximum filter paperase activity (29.02 IU) at spawning stage followed by treatment 1 (21.11 IU) while minimum was recorded in treatment 4 (18.07 IU). During fruit body development stage, maximum enzyme activity was also recorded in treatment 3 (37.11 IU) followed by treatment 1 (29.16 IU). The minimum enzyme activity was observed in treatment 4 (24.05 IU). After harvest of mushroom also, the maximum enzyme activity was recorded in treatment 3 (22.75 IU) while minimum enzyme activity was observed in treatment 4 (17.50 IU).

Table 4.52 Effect of inoculation of thermophilic fungi on C-1 cellulase assay in different compost formulation

Filter paperase enzyme activity (IU)			
Compost treatments	**Before spawning**	**Fruit body development**	**After harvest**
Treatment 1 **Paddy Straw + Wheat Straw (1:1) inoculated with *Thermomyces lanuginosus***	21.11	29.16	20.10
Treatment 2 **Paddy Straw + Wheat Straw (1:1) inoculated with *Melanocarpus albomyces***	19.10	25.22	19.43
Treatment 3 **Paddy Straw + Wheat Straw (1:1) inoculated with consortium of *Thermomyces lanuginosus* +*Melanocarpus albomyces***	29.02	37.11	22.75
Treatment 4 **Control (Uninoculated)**	18.07	24.05	17.50
Mean	21.83	28.89	19.95
C.D. $_{(0.05)}$	0.04	0.03	0.02
SE	0.01	0.01	0.01

IU= International unit (µM/min/ml)

Carboxymethyl cellulase enzyme (CMCase)

During the study, the CMCase enzyme activity increased from spawning stage to fruit body development stage and decreased in after harvesting stage (Table 4.53). In before spawning stage, treatment 3 showed maximum CMCase activity (49.42 IU) while minimum enzyme activity was recorded in treatment 4 (34.66 IU). In fruit body development stage, treatment 2 recorded the maximum enzyme activity (54.19 IU) followed by treatment 3 (50.28 IU). The minimum enzyme activity was observed in treatment 4 (43.74 IU). After the harvest of crop, maximum enzyme activity was found in treatment 3 (39.53 IU) followed by treatment 1 (38.36 IU) and minimum was in treatment 2 (30.29 IU).

Table 4.53 Effect of inoculation of thermophilic fungi on CMCase assay in different compost formulations

CMCase enzyme activity (IU)			
Compost treatments	**Before spawning**	**Fruit body development**	**After harvest**
Treatment 1 **Paddy Straw + Wheat Straw (1:1) inoculated with *Thermomyces lanuginosus***	34.71	45.21	38.36
Treatment 2 **Paddy Straw + Wheat Straw (1:1) inoculated with *Melanocarpus albomyces***	47.21	54.19	30.29
Treatment 3 **Paddy Straw + Wheat Straw (1:1) inoculated with consortium of *Thermomyces lanuginosus +Melanocarpus albomyces***	49.42	50.28	39.53
Treatment 4 **Control (Uninoculated)**	34.66	43.74	36.49
Mean	41.50	48.36	36.17
C.D. $_{(0.05)}$	0.02	0.03	0.02
SE	0.01	0.01	0.01

IU= International unit (µM/min/ml)

β –Glucosidase enzyme

The observation depicted that β-glucosidase enzyme activity also increased from before spawning stage to fruit body development stage and decreased in after harvesting stage (Table 4.54).

In before spawning stage, treatment 3 showed maximum β-glucosidase enzyme activity (8.32 IU) followed by treatment 2 (7.47 IU) while minimum enzyme activity was recorded in treatment 1 (5.91 IU). In during fruit body development stage, maximum enzyme activity recorded in treatment 3 (14.72 IU) followed by treatment 2 (11.83 IU). The minimum enzyme activity observed in treatment 4 (9.48 IU). After harvest stage, maximum enzyme activity was found in treatment 3 (10.58 IU) followed by treatment 1 (8.55 IU) and minimum enzyme activity was observed by treatment 4 (8.06 IU).

Commonly, cellulose hydrolysis requires a combination of three main types of cellulase: endo-1,4-β-d-glucanase (endoglucanase), exo-1,4-β-d-glucanase or cellobiohydrolases (exoglucanase) and β-glucosidase (β-d-glucoside glucanhydrolase), in order to convert cellulose into oligosaccharides, cellobiose, and glucose (Horn et al. 2012; Ritota and Manzi 2019). Endoglucanases preferentially hydrolyze internal β-1, 4-glucosidic linkages in the cellulose chains, generating a number of reducing ends (Horn et al. 2012 and Sajith et al. 2016).

Table 4.54 Effect of inoculation of thermophilic fungi on β-glucosidase enzyme in different compost formulations

β -Glucosidase enzyme activity (IU)			
Compost treatments	**Before spawning**	**Fruit body development**	**After harvest**
Treatment 1 **Paddy Straw + Wheat Straw (1:1) inoculated with *Thermomyces lanuginosus***	5.91	10.99	8.55
Treatment 2 **Paddy Straw + Wheat Straw (1:1) inoculated with *Melanocarpus albomyces***	7.47	11.83	7.57
Treatment 3 **Paddy Straw + Wheat Straw (1:1) inoculated with consortium of *Thermomyces lanuginosus +Melanocarpus albomyces***	8.32	14.72	10.58
Treatment 4 **Control (Uninoculated)**	6.21	9.48	8.06
Mean	6.98	11.76	8.69
C.D. $_{(0.05)}$	0.03	0.05	0.23
SE	0.01	0.02	0.07

IU= International unit (µM/min/ml)

This enzyme also acts on cellodextrins, which are the intermediate product of cellulose hydrolysis and converts them to cellobiose and glucose. Exoglucanases release cellobiose from the reducing end or the nonreducing end of the cellulose chain, facilitating the production of mostly cellobiose which can readily be converted to glucose by β-glucosidases (Zhang et al. 2006; Pollegioni et al. 2015 and Madeira et al. 2017). These enzymes may also act on cellodextrins and larger cello-oligosaccharides, in which case they are commonly named cellodextrinases (Saini et al. 2015). Oligosaccharides released as a result of these activities are converted to glucose by the action of cellodextrinases, whereas the cellobiose released mainly by the action of cellobiohydrolases is converted to glucose by β-glucosidases (Sajith et al. 2016).

Cellulases are produced in a wide range of organisms such as plants, some animals and certain microorganisms including protozoans, bacteria, and fungi. Among these organisms, fungi have been studied extensively for their cellulase producing capabilities, such as the genera Aspergillus, Penicillium, Rhizopus and Trichoderma (Prasanna et al. 2016). However, mushrooms are the most potent degraders of natural lignocellulosic waste. They are mostly grown on litter, dead wood, or in soil and nature-rich cellulose. Several previous reports have found that various mushrooms species can produce cellulase *via* solid state fermentation (SSF) of agricultural or natural lignocellulosic waste (Ellila et al. 2017). Many agricultural or natural lignocellulosic solid waste, especially different kinds of straw (wheat, sorghum and rice) and sawdust (oak and pine), were used as a substrate or source for mushroom growth and cellulases production (Pandey and Singh 2014). Furthermore, other forms of lignocellulosic waste, such as peanut hulls, mandarin peels, cotton waste, corn stovers and tree leaves (*Fagus sylvatica*), have also been used as substrates to determine cellulase activity (Pandey and Singh 2014; Cardoso et al. 2018). The high-value potential of these forms of waste is encouraging as they can be sources that support the growth and cellulases production of different mushroom species, namely Ganoderma, Grifola, Lentinula, Lentinus, Pleurotus, Piptoporus and Trametes by SSF (Wu and Shin 2016).

Cellulase activity is mainly tested using a reducing sugar assay to determine cellulase hydrolysis activity at the end of the production process (Philippoussis and Diamantopoulou 2011). The common enzyme activity assays consist of total cellulase assays, endoglucanase assays, exoglucanase assays and β-glucosidase assays (Ghose 1987). Filter paper assay (FPA) is widely used to determine total cellulase activity. The degree of filter paper activity is determined as the micromole of glucose equivalent liberated per minute of culture filtrate under assay conditions (Dashtban et al. 2010). Endoglucanase activity can be measured using the carboxymethyl cellulose (CMC) as a substrate. This carboxymethyl cellulase (CMCase) is mainly measured by examining the reducing sugars of enzymatic reactions with CMC based on the procedure described by (Ghose 1987). The exoglucanase activity mainly uses commercial avicel as a substrate for measuring the activity (Philippoussis and Diamantopoulou 2011). The β-glucosidase assay can be measured based on the procedure of Kubicek (Mandels et al. 1976) using chromogenic and nonchromogenic substrates such as p-nitrophenol-β-glucoside (pNPG) and cellobiose, respectively (Korotkova et al. 2009). Moreover, various reducing sugar assays, for instance, 3,5-dinitrosalicylic acid (DNS), glucose oxidase (GOD) and high-performance liquid chromatography were also used.

Wood and Goodenough (1977), reported that the cellulase activity remains low until after the first pins were seen and then increases some 10-fold. The activity remains high for some time and then declines during the later cycles. The authors showed that there was an increase of cellulase activity, with a steady increase in activity till 10 days of casing, a relatively high activity during the subsequent 10 days and then a sharp decline in activity within 2 - 4 days, a period that spans a time for two to three flushes in a regular crop. Claydon et al. (1988) did describe that cellulase activity levels rose and fell in direct proportion to harvested fruit body mass.

Xylanase enzyme

Our experimental observations revealed that xylanase enzyme activity increased from before spawning stage to during fruit body development stage after that decreased in after harvesting stage (Table 4.55). In before spawning stage, treatment 3 showed maximum xylanase enzyme activity (7.21 IU) whereas, minimum enzyme activity was recorded in treatment 4 (4.65 IU). In fruit body development stage, maximum enzyme activity was recorded in treatment 3 (11.68 IU) followed by treatment 1 (7.84 IU) while the minimum enzyme activity was observed in treatment 4 (6.87 IU). After harvest stage, maximum enzyme activity was found in treatment 3 (9.08 IU) followed by treatment 1 (6.73 IU) and minimum enzyme activity was shown by treatment 4 (5.26 IU).

Table 4.55 Effect of inoculation of thermophilic fungi on xylanase assay in different compost formulations

Xylanase enzyme activity (IU)			
Compost treatments	**Before spawning**	**Fruit body development**	**After harvest**
Treatment 1 **Paddy Straw + Wheat Straw (1:1) inoculated with *Thermomyces lanuginosus***	6.25	7.84	6.73
Treatment 2 **Paddy Straw + Wheat Straw (1:1) inoculated with *Melanocarpus albomyces***	6.21	7.65	6.05
Treatment 3 **Paddy Straw + Wheat Straw (1:1) inoculated with consortium of *Thermomyces lanuginosus* + *Melanocarpus albomyces***	7.21	11.68	9.08
Treatment 4 **Control (Uninoculated)**	4.65	6.87	5.26
Mean	6.08	8.51	6.78
C.D. (0.05)	0.02	0.01	0.04
SE	0.01	0.01	0.01

IU= International unit (µM/min/ml)

Hemicelluloses are usually classified based on the backbone sugars present in the structural polymer with typical glucose galactose, xylose, mannose and arabinose. The principal hemicelluloses are comprised of xyloglucans, xylans, mannans, glucomannans and mixed linkage

-glucans (Sorensen et al. 2013). In order to digest hemicellulose, microorganisms need to be able to produce a variety of enzymes to hydrolyze complex substrates with a synergistic action. Hemicellulolytic enzymes or hemicellulases are glycoside hydrolases or carbohydrate esterases that are responsible for polysaccharide degradation. The enzymes include xylanase, xylosidase, arabinofuranosidase glucuronidase and mannosidases (Lombard et al. 2014).

Xylan is a heteropolysaccharide and a major hemicellulose. The main chain of xylan consists of 1,4-linked d-xylopyranosyl residues, which are partially replaced with O-acetyl, l-arabinosyl and 4-O-methyl-d-glucuronic acid. The xylan backbone is substituted by different side chains with l-arabinose, d-galactose, d-mannoses, and glucouronic acid linked by glysosidic bonds and ester bonds with ferulic acid (Vos et al. 2018). Biodegradation of xylan requires diverse modes of action of hydrolytic enzymes. Xylanases are a group of glycoside hydrolase enzymes that break down hemicelluloses through the degradation of the linear polysaccharide xylan into xylose by catalyzing the hydrolysis of the glycosidic linkage (1,4) of xylosides. The xylanolytic enzyme system includes a mixture of endo-1,4-xylanases also called endo-xylanases, xylosidases, arabino-furanosidases, glucuronidases and acetylxylanases, which attach to the specific site of xylan (Dos Santos et al. 2018). Endo-xylanases randomly hydrolyze-1,4-xylanopyranosyl linkages of xylan to form xylo-oligosaccharides, xylotriose, xylobiose and xylose. The hydrolysis of xylans is not attacked randomly but depends upon the degree of branching, chain length and presence of substituents in the substrate molecule (Bajaj and Mahajan 2019).

Multifunctional xylanolytic enzyme system is relatively common in fungi, actinomycetes and bacteria (Azeri et al. 2010). A large variety of industrial xylanase enzymes are produced from various kind of microorganisms (Driss et al. 2012). SSF with batch processing has been used for the utilization of agro-industrial waste (Hatanaka 2012). However, very few studies have reported on the xylanolytic enzymes obtained from mushroom on SSF. These potential outcomes provide opportunities for scientists to explore the hydrolytic potential of xylanase for the efficient saccharification of lignocellulosic biomass from mushroom cultivation. For effective utilization of lingo–cellulosic residues, several physical and chemical pre-treatments are required, that may not be convenient for farmers having small holdings. To make the process of lignin degradation economically viable, inoculation with lignocellulolytic microorganisms may prove beneficial. Since no single organism produces all the enzymes necessary for bioconversion of lignocellulose to optimum level, there is need to use a consortium of lignocellulolytic microorganism which can act synergistically for rapid bioconversion of agricultural residues without any chemical pre-treatment. The decomposition rate is most rapid during the thermophilic stage, achieved with in first week of composting. Though, there may be some natural thermophilic microorganisms present in the substrate mixture, but they may not be able to execute the desired degradation of substrate due to lack of specific enzymes for cellulose and lignin degradation to optimum level. Therefore, there is need to inoculate thermophilic microorganisms capable of producing cellulolytic and lignolytic enzymes to degrade the crop waste like paddy straw (Kumar et al. 2008). *Scytalidium thermophilum* (synonyms: *Humicola grisea* var. *thermoidea)*, *Humicola insolens*, *Torula thermophila* and *Chaetomium thermophilum* grow fast and degrade cellulose strongly.

4.8 NUTRITIONAL ANALYSIS OF MUSHROOMS

Nutritional analysis data of button mushrooms using different treatments of compost with thermophilic fungi is depicted in table 4.56. In nutritional analysis, different parameters were evaluated, like carbohydrate, protein, ash, fat, crude fibre, phenol and energy.

The carbohydrate content of mushrooms was highest in treatment 3 (59.44%), followed by treatment 4 (59.39%). The minimum carbohydrate was observed in treatment 2 (51.02%). The protein content of mushrooms was highest in treatment 2 (34.34%), followed by treatment 1 (29.43%). The minimum protein was observed in treatment 4 (28.21%). The ash content of mushrooms was highest in treatment 1 (9.40%), followed by treatment 2 (8.35%). The minimum ash was observed in treatment 3 and 4 (7.70%). The fat content of mushrooms was highest in treatment 2 (2.35%), followed by treatment 1 (1.95%). The minimum fat was observed in treatment 4 (0.85%). The crude fibre content of mushrooms was highest in treatment 2 (3.95%), followed by treatment 4 (3.85%). The minimum crude fibre was observed in treatment 1(2.95%). The phenol content of mushrooms was highest in treatment 2 (41.07mg/g), followed by treatment 3 (34.80 mg/g). The minimum phenol content was observed in treatment 4 (29.47 mg/g). The energy of mushrooms was recorded highest in treatment 2 (362.59 Kcal/100g), followed by treatment 1 (360.35 Kcal/100g). The minimum energy was observed in Treatment 4 (358.05 Kcal/100g).

Mushrooms have a high nutritional value. They are a good source of proteins, dietary fibres, vitamins, minerals and phenolic compounds with antioxidant activity.

Furthermore, mushrooms have a low-fat content (composed mostly by unsaturated fatty acids) and a low energetic density (Guillamon et al. 2010). Sinha et al. (2020b) recorded that white button mushroom contains a wide range of nutritional components: ash (7.01 -

Table 4.56 Effect of inoculation of thermophilic fungi on nutritional profile of button mushroom

Compost treatments	Carbohydrate (%)	Protein (%)	Ash (%)	Fat (%)	Crude fibre (%)	Phenol (mg/g)	Energy (Kcal/100g)
Treatment 1 **Paddy Straw + Wheat Straw (1:1) inoculated with *Thermomyces lanuginosus***	56.27	29.43	9.40	1.95	2.95	30.23	360.35
Treatment 2 **Paddy Straw + Wheat Straw (1:1) inoculated with *Melanocarpus albomyces***	51.02	34.34	8.35	2.35	3.95	41.07	362.59
Treatment 3 **Paddy Straw + Wheat Straw (1:1) inoculated with consortium of *Thermomyces lanuginosus* + *Melanocarpus albomyces***	59.44	28.36	7.70	0.90	3.60	34.80	359.30
Treatment 4 **Control (Uninoculated)**	59.39	28.21	7.70	0.85	3.85	29.47	358.05
Mean	56.53	30.09	8.29	1.51	3.85	33.89	360.07
C.D. $_{(0.05)}$	0.06	0.06	0.37	0.37	0.10	0.09	3.42
SE	0.02	0.02	0.12	0.12	0.03	0.03	1.10

17.92 %), fibers (15.42- 29.02 %), proteins (18.31 - 41.06 %), fats (1.54 -5.38 %) and carbohydrates (28.38 – 34.88 %). Kaur et al. (2007) recorded the highest production of phenol (1422.12 µg g^{-1}) in unamended fungal inoculated paddy straw compost. Mushrooms secrete enzymes to digest surrounding foodstuff to get nutrients from organic matter contained in compost. In other terms, they are grown by bioconversion of agricultural wastes into edible food (Goyal et al. 2006). As a result, their nutritional value largely depends on the chemical composition of the compost (Tshinyangu 1996 and Gothwal et al. 2012). It was observed that the lowest mushroom protein contents were obtained with lowest (hors-ban: 0-100 with 1.6% N) and highest was obtained with (hors-chic: 0-100 with 2.3% N). Different works has also reported different protein content in mushroom with varying levels of nitrogen and its source (Sadiq et al. 2008; Muszynska et al. 2011; Mohiuddin et al. 2015 and Ahlawat et al. 2016).

In general, the total substitution of the traditional compost or its supplementation by the various types of tested wastes has allowed the production of mushrooms with qualitative attributes highly demanded by consumers such as low fat and carbohydrates contents and good composition in dietary fibers which play a key role in healthy properties of mushrooms (Cheung 2009). Furthermore, mushrooms are known to be an excellent accumulator of minerals from the environment in which they grow (Atilda et al. 2017). The mineral composition of the button mushroom is undoubtedly affected by the composition of growth substrate (Bakowski et al. 1986) and is differently presented by various authors (Beelman and Edwards 1989; Mattila and Konko 2001; Vetter 2003). It seemed that the utilization of chicken manure as a main substrate or as a supplement for the traditional compost has increased sodium, potassium, calcium and iron contents in fruits which is convenient for consumers with calcium deficiencies or anemia but is not suitable for consumers with blood pressure problems.

Chapter-5

SUMMARY AND CONCLUSION

The present investigations entitled "**Studies on utilization of paddy straw for compost preparation of button mushroom (*Agaricus bisporus*)"** was carried out during 2020-2023. Five different formulations of short method compost using paddy straw, wheat straw and their combinations were evaluated for production of *Agaricus bisporus*. The formulations were analyzed for various quality parameters. The thermophilic fungi were isolated from the compost formulations.

The cultivation trial was laid at mushroom production unit of the Department of Plant Pathology, Dr. Y.S Parmar University of Horticulture and Forestry Nauni, Solan (H.P). There were five treatments in total with four replications: Treatment-1 was (1.5:1) 600 kg paddy straw and 400 kg wheat straw, treatment-2 (3:1) consisted of 750 kg paddy straw and 250 kg wheat straw, treatment- 3 was 500 kg of paddy straw and 500 kg wheat straw (1:1), treatment-4 consisted only paddy straw 1000 kg and treatment-5 served as control with 1000 kg of wheat straw alone.

The physical quality parameters of the compost were tested and the highest moisture percent of compost was recorded in treatment 3 (69.83%) while highest pH (7.60) and electrical conductivity (2.44 deci S m^{-1}) of compost was recorded in treatment 1. The maximum organic matter content of compost was found in treatment 3 (67.24%), bulk density in treatment 4 (0.47 g/cm^3), particle density in treatment 2 (0.97 g/cm^3) and porosity in treatment 3 (56.17%).

Organic carbon was recorded maximum in treatment 5 (41.99%), nitrogen and phosphorous in treatment 3 (1.88% and 0.83%) while potassium in treatment 2 (2.76%), calcium in treatment 2 (3.11%) and magnesium in treatment 3 (0.55%). Copper was recorded maximum in treatment 4 (44.60 ppm), zinc in treatment 3 (69.60 ppm), iron (2110 ppm) and manganese (154 ppm) in treatment 2. Final C: N ratio of the compost was maximum in treatment 5 (23.59), C: P ratio in treatment 4 (62.48), C: K ratio in treatment 4 (19.18) and N: P ratio in treatment 4 (3.35). N: K ratio ranged from 0.42 to 1.03 with a maximum in treatment 4 (1.03) while P: K ratio in treatment 3 (0.33). Thermophilic microbial count in different compost formulations was taken at dilutions 10^4. Highest microbial count was recorded in treatment 3 (19.75 cfu g^{-1} compost).

Silica content in paddy straw plays an important role in productivity of the compost and it was observed that silica content decreased from spawning to post harvesting stage in the compost. In before spawning (BS) stage silica content varied from 9.65 to 14.23 per cent in different compost formulations. In before spawning stage, maximum silica content was recorded in treatment 4 (14.23%) and minimum was in treatment 3 (9.65%). In Fruit body development stage (FBF), silica content varied from 9.25 to 13.29 per cent. Maximum silica content was found in treatment 4 (13.29%) and minimum in treatment 3 (7.80%). In after harvesting stage (AH), silica content varied from 7.8 to 10.78 per cent. Maximum was found in treatment 4 (10.78%) and minimum in treatment 3 (6.91%).

It was observed that treatment 3 took the minimum days for spawn run (14.00 days), case run (19.03 days), pin head formation (24.02 days) and first harvest (28.02 days). The maximum yield was also obtained from the treatment 3 (19.98 kg/ 100kg compost) followed by treatment 1 (17.76 kg/100kg compost). Average fruit body weight was recorded to be the maximum in treatment 3 (15.05g). Diameter of pileus and thickness of pileus was recorded maximum in treatment 3 (3.90 cm and 1.80 cm). Length of stipe and diameter of stipe was also recorded to be maximum in treatment 3 (2.60 cm and 1.50 cm).

Correlation of the physical and chemical properties of compost as independent variables was analyzed with yield as dependable variable and its partitioning into direct and indirect effect was also analyzed using path correlation studies. A significantly positive effect of moisture and porosity together on the yield was observed. It could also be inferred from the data analysis that pH, bulk density and particle density of the compost have significantly strong negative effect on the yield of button mushroom. Partitioning of correlation coefficient into direct and indirect effects showed that the moisture *per se* does not have any significant direct effect on the yield but high moisture contribute to the high bulk and particle density with low porosity in the compost, which ultimately affects the yield per cent negatively. However, in the correlation analysis the moisture content with high porosity showed a positive effect on yield per cent of the button mushroom. The bulk density and particle density has shown a negative correlation with the yield implying the lower yield with higher bulk and particle density while the porosity has direct positive effect on yield per cent of white button mushroom.

Quantitative estimation of laccase, MnP, cellulase and xylanase enzyme was done at three different stages of button mushroom production *viz*., before spawning stage (BS), fruit body development stage (FBD) and after harvest (AH) stage. The observation revealed that irrespective of treatment, laccase activity increased during growth of mushroom mycelium and decreased afterwards. Maximum laccase enzyme activity was recorded in treatment 3 during spawn run and case run stage while it reduced after harvesting stage. MnP enzyme activity was also found to increase from before spawning stage to fruit body development stage and decreased afterwards. Maximum MnP enzyme activity was observed in treatment 3 and it followed the same trend as in case of laccase enzyme. Both the ligninase enzymes are normally associated with mycelial growth of *A. bisporus*, thus higher laccase enzyme production also indicates faster mycelial colonization in the compost.

Cellulase enzymes was estimated by three enzyme assays i.e Filter paperase enzyme, Carboxymethyl cellulase enzyme (CMCase) and β –Glucosidase at three different stages of button mushroom production *viz*., before spawning stage (BS), fruit body development stage (FBD) and after harvest (AH) stage. It was observed that total cellulase enzymes activity increased from before spawning stage to fruit body development stage and decreased afterward.

Filter paperase (C1 cellulase) catalyzes the degradation of crystalline cellulose at carbon 1 of the glucose chain. In before spawning stage and fruit development stage treatment 3 showed maximum filter paperase activity (27.32 IU and 35.30 IU) while at after harvest stage, maximum enzyme activity was found in treatment 2 (18.95 IU). CMCase enzymes (Cx cellulase) catalyze the degradation of cellulose at random places in the glucose chain producing oligosaccharides and called as endocellulases. In before spawning stage, treatment 2 showed maximum CMCase activity (43.21 IU) while in fruit body development stage and after harvest stage, treatment 3 recorded the maximum enzyme activity (43.29 IU and 34.53 IU). β-Glucosidase (cellobiase) catalyzes the degradation of oligosaccharides releasing monomers of glucose. In before spawning and fruit body development stage, treatment 2 showed maximum β-Glucosidase enzyme activity (6.49 IU and 10.82 IU) while at after harvest stage, maximum enzyme activity was found in treatment 3 (5.59 IUl). Xylanase enzyme activity, in before spawning stage was recorded maximum in treatment 1 (5.25 IU) and in fruit body development and after harvest stage, maximum enzyme activity was recorded in treatment 3 (9.68 IU and 5.06 IU).

To evaluate different compost formulations with respect to mushroom production, trials were conducted at the Department of Plant Pathology, Dr.YSP University of Horticulture and Forestry, Nauni, Solan (H.P) for two consecutive years 2021 and 2022. Overall mean of both year 2021-2022 showed minimum days for spawn run (14.55 days), case run (20.06 days), pin head formation (24.56 days), first harvest (28.55 days) and maximum yield (20.57 kg/100kg compost) in T3 treatment. Maximum pileus diameter (3.99 cm), pileus thickness (1.85 cm), length of stipe (2.65 cm) and stipe diameter (1.60 cm) was also observed in treatment 3.

During the present study, a total of eighteen isolations of thermophilic and thermotolerant fungi were made from the mushroom compost at dilution (10^{-4}) i.e., *Myriococcum albomyces (Melanocarpus albomyces*), *Aspergillus fumigatus, Thermomyces lanuginosus, Scytalidium album, Talaromyces euchlorocarpius, Thermomyces duponti, Chaetomium* sp. *Humicola fuscoatra, Sordarials* sp., *Penicillium thomii* and *Aspergillus* sp. Qualitative and quantitative estimation of fungal isolates was done for hydrolytic and oxidative enzymes production (ligninolytic, cellulolytic, xylanolytic and manganese dependent peroxidise enzymes). All the isolates produced varied hydrolysis zone. With respect to cellulase enzyme, maximum hydrolytic zone was recorded in isolate TF4 (49.00 mm) followed by treatment TF6 (45.00 mm). In xylanase enzyme estimation, maximum hydrolytic zone was observed in treatment TF4 (39mm) followed by treatment TF8 (36mm). Treatment TF6 showed maximum laccase enzyme hydrolytic zone (29mm) followed by treatment TF4 (23mm). In MnP enzyme estimation maximum oxidative zone was formed in treatment TF6 (30mm) followed by treatment TF4 (25mm).

Quantitative estimation of ligninolytic, cellulolytic, xylanolytic and manganese dependent peroxidise enzymes of fungal isolates showed maximum laccase and MnP enzyme activity in isolate TF6. Filterpaperase, CMCase and Xylanase enzyme activity was recorded maximum in isolate TF4 followed by isolate TF6. β- Glucosidase assay was observed to be the maximum in isolate TF6 followed by TF4. Out of eighteen thermophilic fungi isolated, two thermophilic fungi showed highest enzyme activities and were selected for further experimentations i.e., TF4- *Thermomyces lanuginosus* and TF6 - *Melanocarpus albomyces,* which were identified morphologically, microscopically and molecularly.

These two fungal isolates, *Thermomyces lanuginosus* and *Melanocarpus albomyces* were used singly and in combinations under *in vitro* condition to evaluate their effect on growth of *A bisporus* mycelium on sterilized compost in beaker and petriplates under *in vitro* conditions. Before using these fungal isolates in combinations their compatibility were also evaluated following dual culture technique. The linear growth rate of mycelium of *A. bisporus* on sterilized compost in beaker was found to be maximum (7.80 mm per day) in treatment 3 (consortium of thermophilic fungi isolate TF4 and TF6) in upward direction. Growth of *A. bisporus* in petri dishes on treatment 3 (compost inoculated with consortium of thermophilic fungi TF4 and TF6) showed maximum radial growth rate of 9.00 mm/day.

These two fungi were inoculated in compost (PS+WS, 1:1) singly and in combinations. Composition consisting of wheat straw 500 kg, Paddy straw 500 kg, chicken manure 600 kg, urea 15 kg, wheat bran 100 kg and gypsum 30 kg was taken in the compounding mixture. Four treatments were used with four replications in the study. T-1 inoculated with 1st best isolate (*T. lanuginosus* TF4), T-2 inoculated with 2nd best isolate (*M. albomyces* TF6), T-3 inoculated with consortium (*T. lanuginosus* TF4 and *M. albomyces* TF6) and T-4 was the Control (uninoculated). It was observed that minimum time for spawn run (11.76 days), case run (16.05 days), pin head formations (23.09 days), first harvest (26.01 days) and maximum yield (23.98 kg/q compost) was recorded in treatment 3.

Average fruit body weight, pileus diameter, pileus thickness, stipe diameter and stipe length were also observed to be the maximum in treatment 3. Moisture content of the composts was recorded highest in treatment 3 (67.13%) while pH of the compost recorded highest in treatment 4 (7.41). Electric conductivity (EC) of the composts was recorded highest in treatment 2 (3.50 deci S m-^{1}). Organic matter content of compost was found to be the maximum in treatment 1 (71.52%). The bulk density of compost was recorded the maximum in treatment 1 (0.44 g/cm^{3}). Particle density of compost in all the four treatments, recorded to be the maximum in treatment 2 (0.94 g/cm^{3}). Porosity of all compost treatments were varied from 52.69 per cent in treatment 1 to 57.47 per cent in treatment 3.

All the physical parameters (independent variables) were analyzed for the correlation with the yield per cent (dependent variable) of white button mushroom using path analysis. The correlation analysis has shown a strong significantly positive effect of pH and porosity on the yield per cent while moisture percent has shown positive effect on yield along with porosity. This indicates high porosity with high pH in the compost has significantly affected the yield potential of the compost. It could also be inferred from the data analysis that bulk density and particle density of the compost have significantly strong negative effect on the yield of button mushroom while electrical conductivity and organic matter of the compost have moderate negative effect on yield. The separation of direct and indirect effects of independent variable on dependant variable showed that high moisture contributes to the high bulk density with low porosity in the compost and bulk density is negatively correlated with yield. Thus, higher moisture and bulk density ultimately affects the yield per cent negatively. The particle density has also shown a negative correlation with the yield implying the lower yield with higher particle density while the porosity has indirect positive effect on yield per cent of white button mushroom through bulk density.

Organic carbon was recorded maximum in treatment 2 (35.96%) while nitrogen per cent was found maximum in treatment 3 (1.96%). Phosphorous was recorded to be the maximum in treatment 2 and treatment 3 (0.85%). Potassium was recorded to be the maximum in treatment 3 (3.57%), calcium was maximum in treatment 4 (3.77%) and magnesium was recorded to be the maximum in treatment 2 (0.83%). Copper micronutrient was recorded to be the maximum in treatment 1 (50.90ppm). Zinc (180ppm), iron (2070ppm) and manganese (480ppm) were found maximum in treatment 3. C: N ratio was recorded to be the maximum in treatment 2 (18.60), C: P ratio was observed to be the maximum in treatment 1 (47.63). C: K ratio significantly varied in different compost treatments from 9.68 in treatment 3 to 11.97 in treatment 2. N: P ratio was observed to be the maximum in treatment 1 (2.59). N: K ratio varied significantly from 0.55 in treatment 3 to 0.64 in treatment 2. P: K ratio varied from 0.22 to 0.28. Maximum was recorded in treatment 2 (0.28). At 10^4 dilution, the maximum microbial count was found in treatment 3 (27.75 cfu g^{-1} compost) and minimum microbial count was observed in treatment 4 (14.75 cfu g^{-1} compost). Silica content was found to decrease after adding the inoculums of thermophilic fungi in compost. Out of four treatments least silica content was recorded in treatment 3 (4.51%) followed by the treatment 2 (5.36%). While the maximum silica content recorded in treatment 4 (5.88%).

The correlation analysis has shown a significantly positive effect of nitrogen, zinc, phosphorus, manganese and iron on the yield per cent while silica has strong negative correlation with yield percent. C: N, C: P and N: P ratio has negative correlation with the yield. It implies that the proper fermentation of the composting material is very important for the higher yield of the button mushroom. It helps to reduce C: N and C: P ratio by degradation of free sugars during composting and release of CO_2 which helps to enhance the selectivity of the compost. The separation of direct and indirect effects of independent variable on dependant variable showed a direct positive effect of nitrogen, phosphorus, zinc, iron and manganese while a direct negative effect was recorded with potassium, calcium and magnesium. The silica content is strongly negatively correlated with yield, which may have direct and indirect correlation both with yield as the silica content is also positively correlated with C: N ratio. Thus, the higher silica content is associated with higher C: N ratio, which may have a negative effect on the yield.

The temperature profile of four compost treatments was recorded from the three zones at different turnings. In all the four composts, the temperature of middle zone was found to be more than the upper and lower zones. The temperature of different composts increased during zero day to third turning, and after which it started to fall. The moisture content of different composts was the maximum at the initial stages of the composting process. It ranged from 75.24-78.98 per cent. It was found to reduce gradually during the process and finally stabilized at 62.98- 67.13 per cent. pH was low at start, gradually increased between turnings and then stabilized towards the end of the composting. The pH was found to be in the range of 6.85– 8.27. The pH stabilized between 7.05 – 7.41 at the end of the process. Carbon percentage at initial and mature stage of composting and sharp decline in carbon percent was observed from zero day to final stage, which attributes in good compost for the growth of *A. bisporus*. Nitrogen was observed in an increasing trend from the initial stage to the mature stage, and the highest nitrogen was recorded in third (consortium) treatment. The C/N ratio of the four compost piles narrowed down from the beginning towards the end of composting. The C/N ratio in the initial stage ranges from 26.84 to 30.68 and it reduced to 17.63 to 18.60 upto final stage.

The quantitative estimation of laccase, MnP, cellulase, xylanase enzyme was done at three different stages of button mushroom production like before spawning stage (BS), fruit body development stage (FBD) and after harvest (AH) stage. Treatment 3 showed the maximum laccase activity in all the stages i.e. before spawning, fruit body development and after harvesting stage. In before spawning stage MnP enzyme activity was recorded maximum in treatment 1 (4.91 IU). In fruit body development stage and after harvesting stage maximum enzyme activity was recorded in treatment 3 (10.14 IU and 6.42 IU). Treatment 3 showed maximum filter paperase activity at spawning stage, fruit body development and after harvest stage (29.02 IU, 37.11 IU and 22.75 IU). CMCase activity was maximum in before spawning stage in treatment 3 (49.42 IU) while in fruit body development stage, treatment 2 recorded the maximum enzyme activity (54.19 IU) and at after the harvest of crop, maximum enzyme activity was found in treatment 3 (39.53 IU). β-Glucosidase enzyme activity in before spawning stage, fruit body development and after harvesting stage was recorded maximum in treatment 3 (8.32 IU, 14.72 IU and 10.58 IU). Xylanase enzyme activity in before spawning stage, fruit body development and after harvest stage was recorded maximum in treatment 3 (7.21 IU, 11.68 IU and 9.08 IU). Overall, it could be concluded from the studies that the compost made using consortium of TF-4 and TF-6 showed maximum enzyme activities required for growth and development of white button mushroom.

The carbohydrate content of mushrooms was highest in treatment 3 (59.44%). The protein content of mushrooms was highest in treatment 2 (34.34%), the ash content was highest in treatment 1 (9.40%). The fat content, crude fibre, phenol content and energy of mushrooms was highest in treatment 2 (2.35%, 3.95%, 41.07mg/g and 362.59 Kcal/100g). Overall, it was recorded that paddy straw and wheat straw combinations in the ratio 1:1 gave promising results. The cost-benefit ratio of the compost formulation of paddy straw and wheat straw (1:1) gave the maximum net return as compared to other compost formulations. So, on the basis of all the compost quality, yield parameters and cost-benefit ratio, a compost formulation of paddy straw wheat straw (1:1) was found to be the best as it reduced the cost and increased the profit. The compost formulation inoculated with thermophilic fungi, namely *T. lanuginosus* (TF4) and *M. albomyces* (TF6) improved the compost quality as well as the yield of button mushrooms.

LITERATURE CITED

Abdurrahman IM, Chaki S, Saini G. 2020. Stubble burning: effects on health and environment, regulations and management practices. *Environmental Advances*. 2: 1-12.

Abercrombie J. 1779. In the garden mushroom, its nature and cultivation. Lockyer Davis, London. 54p.

Acquaah G. 2009. Horticulture, principles and practices Prentice Hall Upper Saddle River, NJ. 816p.

Ajonina AS and Tatah LE. 2012. Growth performance and yield of oyster mushroom (*Pleurotus ostreatus*) on different substrates composition in Buea South West. *The Journal of Biochemistry*. 3: 39-145.

Adams PR. 1990. Mycelial amylase of thermophilic species of Rhizomucor, Humicola and Papulospora. *Mycopathologia*. 112: 35-37.

Ahlawat OP, Manikandan K and Singh M. 2016. Proximate composition of different mushroom varieties and effect of UV light exposure on vitamin D content in *Agaricus bisporus* and *Volvariella volvacea*. *Mushroom Research.* 25(1): 1-8.

Ahlawat OP and Verma RN. 2001. *In vitro* interactions studies between bacteria and Pleurotus: a tool for differentiating *Pleurotus* spp. and selecting fruiting including bacterium. In: process of International Conference on New Horizons in Biotechnology, Thiruvananthapuram, India. 78p.

Ahlawat OP and Vijay B. 2001. Role of microbes in the management of the environmental pollution. R P Tewari et al (eds). APH publishing corporation, 5Ansari Road, Daryagnag, New Delhi. 83-88.

Ahlawat OP and Vijay B. 2010. Potential of thermophilic bacteria as microbial inoculants for commercial scale white button mushroom (*Agaricus bisporus*) compost production. *Journal of Scientific and Industrial Research.* 69: 948-955.

Ajonina AS and Tatah LE. 2012. Growth performance and yield of oyster mushroom (*Pleurotus ostreatus*) on different substrates composition in Buea South West Cameroon. *Science Journal of Biochemistry.* 10: 1-6

Alispahic A, Sapcanin A, Salihovic M, Ramic E, Dedic A and Pazalja M. 2015. Phenolic content and antioxidant activity of mushroom extracts from Bosnian market. *Bulletin of the Chemists and Technologist of Bosnia and Herzegovina*. 44: 5-8.

Allen SE. 1974. Chemical analysis of ecological materials, Blackwell Scientific Publications, Oxford. 565p.

Allison DS, Rey MW, Berka RM, Armstrong G and Dunn-Coleman NS. 1992. Transformation of the thermophilic fungus *Humicola grisea* var *thermoieda* and over production of *Humicola glucoamylase*. *Current Genetics.* 21: 225-229.

Almomany AM and Masaed NK. 2019. Impact of physical properties of substrate on mycelial growth and yield of two strains of *Agaricus bisporus*. International conference on Agriculture, Food, Veterinary and Pharmacy Sciences. At: Turkey -Trabzon. 1-15.

Alsanius BW, Blok C, Cuijpers WJ, Franca SC, Fuchs JG, Janmaat L and vander Wurff AW. 2016. Handbook for composting and compost use in organic horticulture. *BioGreenhouse COST Action FA.* 1105.

Anandh K and Prakasam V. 2002. *Tricholoma lobayense*, a new edible mushroom for commercial exploitation. Abstract of paper presented in 3rd Indian Mushroom Conference held during 6- 7, March-2002 at Tamil Nadu Agricultural University. 135p.

Anasonye F, Winquist E, Rasanen M, Kontro J and Bjorklof K. 2015. Bioremediation of TNT contaminated soil with fungi under laboratory and pilot scale conditions. *International Biodeterioration and Biodegradation.* 105:7-12.

Anastasi A, Coppola T, Prigione V and Varese GC. 2009. Pyrenedegradation and detoxification in soil by a consortium of basidiomycetes isolated from compost: role of laccases and peroxidases. *Journal of Hazardous Materials.* 165:1229-3399.

Anderson WF and Akin DE. 2008. Structural and chemical properties of grass lignocelluloses related to conversion for biofuels. *Journal of Industrial Microbiology and Biotechnology*. 35:355-366.

Andrade MC, Jesus JP, Vieira FR, Viana SR, Spoto MH and Minhoni T. 2014. Effect of gamma irradiation on the nutritional quality of *Agaricus bisporus* strains cultivated in different composts. *Anais da Academia Brasileira deCiencias*. 86 (2): 897-906.

Anwar Z, Gulfraz M and Irshad M. 2014. Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: A brief review. *Journal of Radiation Research and Applied Sciences.*7:163-173.

Ardon O, Kerem Z and Hadar Y. 1998. Enhancement of lignin degradation and laccase activity in *Pleurotus ostreatus* by cotton stalk extract. *Canadian Journal of Microbiology*. 44: 676-680.

Arx JAV. (1975). On Thielavia and some similar genera of ascomycetes. *Studies in Mycology*. 8: 1-29.

Atilda F, Owaid MN and Shariati MA. 2017. The nutritional and medical benefits of *Agaricus bisporus*: A review. *Journal of Microbiology, Biotechnology and Food Science*.7(3): 281-286.

Atkey PT and Wood DA. 1983. An electron microscope study of wheat straw composted as a substrate for the cultivation of edible mushroom (*Agricus bisporus*). *Journal of Applied Bacteriology*. 55: 293-304.

Azeri C, Tamer AU and Oskay M. 2010. Thermoactivecellulase-free xylanase production from alkaliphilic Bacillus strains using various agro-residues and their potential in biobleaching of kraft pulp. *African Journal of Biotechnology*. 9: 63–72

Bahl N, Kapoor JN and Chowdhry PN. 1989. Dominant microflora of wheat straw compost. *Indian Phytopathology*. 42: 496-498.

Bajaj P and Mahajan R. 2019. Cellulase and xylanase synergism in industrial biotechnology. *Applied Microbiology and Biotechnology*. 103: 8711- 8724.

Bąkowski J, Kosson R and Horbowicz M. 1986. Nutritional value of different species of mushrooms (*Agaricus bisporus*). 39(1): 111-121.

Beelman RB and Edwards CG. 1989. Variability in the composition and nutritional value of the cultivated mushroom *Agaricus bisporus*. *Mushroom News*. 37: 17-26

Berghem LER and Pettersson LG. 1973. Mechanism of enzymatic cellulose degradation and purification of a cellulolytic enzyme from *Trichoderma viride* active on highly ordered cellulose. *Journal of Biochemistry*. 37:21-30.

Berikten D and Kivanc M. 2014. "Optimization of solid-state fermentation for phytase production by *Thermomyces lanuginosus* using response surface methodology". *Preparative Biochemistry and Biotechnology*. 44 (8): 834-848.

Bernal MP, Alburquerque JA and Moral R. 2009. Composting of animal manures and chemical criteria for compost maturity assessment: A review. *Bioresource Technology*. 100 (22): 5444-5453.

Bertrand B, Mayolo-Deloisa K, Gonzalez-Gonzalez M, Tinoco-Valencia R, Serrano-Carreon L and Martinez-Morales F. 2016. *Pleurotus ostreatus* laccase recovery from residual compost using aqueous two-phase systems. *Journal of Chemical Technology and Biotechnology*. 91: 2235- 2242.

Beyer DM. 2003. Basic procedures for Agaricus mushroom growing Pennsylvania state University, Coop. Ext University Park.1-8.

Bhattacharya A and Pletschke BI. 2014. *Thermophilic Bacilli* and their enzymes in composting.In book: Composting for Sustainable Agriculture.*Springer*.103-124.

Bisht IS and Singh RN. 1986. Prospects of active mycelium spawning technique in commercial cultivation of button mushroom, *Agaricus bisporus*. *Indian Journal of Mycology and Plant Pathology*. 16:30p.

Black CA. 1965. Methods of Soil Analysis. *American Society of Agronomy*. 2:1572 p.

Blanco A and Pastor FIJ. 1993. Characterization of cellulase-free xylanase from the newly isolated *Bacillus* sp. strain BP-23. *Canadian Journal of Microbiology*. 39: 1162-1166.

Bollag JM, Shuttleworth KL and Anderson DH. 1988. Laccase mediated detoxification of phenolic compounds. *Applied and Environmental Microbiology*. 54: 3086-3091.

Bonnen AM, Anton LH and Orth AB. 1994. Lignin degrading enzymes of the commercial button mushroom, *Agaricus bisporus*. *Applied and Environmental Microbiology*. 60: 960-965.

Brijwani K, Rigdon A and Vadlani PV. 2010. Fungal laccases: production, function, and applications in food processing. *Enzyme Research*. 1-10.

Burlacu A, Cornea CP and Israel-Roming F. 2016. Screening of xylanase producing microorganisms. *Research Journal of Agricultural Sciences*. 48: 8-15.

Burrows S. 1951. The chemistry of mushroom composts I- General introduction and methods of investigation. *Journal of the Science of Food and Agriculture*. 2: 395.

Callow E. 1831. Observations on methods now in use for the artificial growth of mushrooms, with full explanation of an improved mode of culture. Fellowes, London. (Reprinted 1965 by WS Maney and Son Ltd., Hudson Road Leeds). 1-13.

Canellas LP, Piccolo A, Dobbss LB, Spaccini R, Olivares FL, Zandonadi DB and Facanha AR. 2010. Chemical composition and bioactivity properties of size-fractions separated from a vermicompost humic acid. *Chemosphere.* 78: 457–466.

Cardoso WS, Queiroz PV, Tavares GP, Santos FA, Soares FEDF, Kasuya MCM and Queiroz JHD. 2018. Multi-enzyme complex of white rot fungi in saccharification of lignocellulosic material. *Brazilian Journal of Microbiology.* 49: 879–884

Carrasco J, Zied D, Pardo J, Preston G and Pardo-Gimenez A. 2018b. Supplementation in mushroom crops and its impact on yield and quality. *AMB Express.* 8:146p.

Carrasco J, Tello ML, Perez-Clavijo M and Preston G. 2018a. Biotechnological requirements for the commercial cultivation of macrofungi: substrate and casing layer. Chapter 7. In Biology of Macrofungi. Singh B and Chhakchhuak L (eds). Berlin, Germany: *Springer Nature*. 159-175.

Chakravarty B. 2011. Trends in mushroom cultivation and breeding. *American Journal of Agricultural Economics*. 2(4):102-109.

Chang S and Miles PG. 2004. Mushrooms: cultivation, nutritional value, medicinal effect and environmental impact. 2nd edition, published location: Boca Raton, CRC Press.480p

Chang Y and Hudson HJ. 2001. The fungi of wheat straw compost I. Ecological studies. *Transactions of the British Mycological Society.* 50: 649-666.

Chen Y, Chefetz B, Rosario R, Van HJ, Romaine CP and Hatcher PG. 2000. Chemical nature and composting of compost during mushroom growth. *Compost Science and Utilization Journal.* 8:4p.

Cheung PCK. 2009. Mushrooms as functional foods (ed Cheung PCK), John Wiley & Sons, Inc., Hoboken, NJ, USA. 296p.

Chio C, Sain M and Qin W. 2019. Lignin utilization: a review of lignin depolymerization from various aspects. *Renewable and Sustainable Energy Reviews.* 107: 232-249.

Claydon N, Allan M and Wood D. 1988. Fruit body biomass regulated production of extracellular endocellulase during periodic fruiting by *Agaricus bisporus*. *Transactions of the British Mycological Society.* 90:85-90.

Cooney DG and Emerson R. 1964. Thermophilic fungi. An account of their biology, activities, and classification. 188 p.

Crisan EV. 1959. The isolation and identification of thermophilic fungi. MSc Thesis, Purdue University, Lafayette, India. 107p.

Cunniff P. 1995. Official methods of analysis of AOAC International (16th ed., Vol. 1). Virginia, U.S. AOAC International. 1-49.

da Silva LL. 2016. Adding value to agro-Industrial wastes. *Industrial Chemistry.* 2:2p.

Dalzell HW, Biddlestone AJ, Gary KR and Thurairajan K. 1987. Soil management: Compost production and use in tropical and subtropical environments FAO Soils Bulletin No 56. Andhra Pradesh, India. Medak Agricultural Centre. 193p.

Dashtban M, Maki M, Leung KT, Mao C and Qin W. 2010. Cellulase activities in biomass conversion: Measurement methods and comparison. *Critical Reviews in Biotechnology*. 30: 302–309.

Davis DD, Kuhns LJ and Harpster TL. 2006. Use of mushroom compost to suppress artillery fungi. *Journal of Environmental Horticulture.* 24: 212- 215.

De Ger JE. 2000. In: Science and cultivation of edible fungi (Van Griensven 00.). Balkema, Rottardam. 931-934.

Deacon JW. 2006. Fungal biology. Malden: Wiley-Blackwell. 384p.

De-Bertoldi M, Vallini G and Pera A. 1983. The biology of composting: a review. *Waste Management and Research.* 1(2):157–176.

Deherain PP. 1889. Exposition Internationale de 1889. Travaux de la Station Agronomique de lEcole d Agriculture (French). Publisher- Hachette Livre – BNF. 86p.

Demelon A, Burgevin H and Marcel M. 1937. Culture du champignon de couchesur fumier artificiel. *Annales des Sciences Naturelles; Botanique.* 19: 141-153.

Demirer T, Rock-Okuyucu B and Ozer I. 2005. Effect of different types and doses of nitrogen fertilizers on yield and quality characteristics of mushrooms (*Agaricus bisporus*) cultivated on wheat straw compost. *Journal of Agriculture and Rural Development in the Tropics and Subtropics*. 106(1):71-77.

Derikx PJL, Op Den Camp HJM, van der Drift C, van Griensven LJLD and Vogels GD. 1990. Biomass and biological activity during the production of compost used as a substrate in mushroom cultivation. *Applied and Environmental Microbiology.* 56: 3029-3034

Dhamodharan G and Mirunalini S. 2010. A novel medicinal characterization of *Agaricus bisporus* (white button mushroom). *Pharmacologyonline*. 2: 456-463.

Dhar BL and Munjal RL. 1976. Micoflora of synthetic compost for *Agaricus bisporus. Indian Journal of Mushrooms.* 2: 21-25.

Diaz LF, Savage GM, Eggerth LL and Golueke CG. 1993. Composting and recycling municipal solid waste. United State of America: Lewis Publishers. CRC Press. 318p.

Dos Santos CR, de Giuseppe PO, de Souza FHM, Zanphorlin LM, Domingues MN, Pirolla RAS, Honorato RV, Tonoli CCC, de Morais MAB, Martins VPM and Murakami MT. 2018. The mechanism by which a distinguishing arabinofuranosidase can cope with internal di-substitutions in arabinoxylans. *Biotechnology for Biofuels and Bioproducts*. 11: 223p.

Driss D, Bhiri F, Elleuch L, Bouly N, Stals I, Miled N, Blibech M, Ghorbel R and Chaabouni SE. 2012. Purification and properties of an extracellular acidophilic endo-1,4-β-xylanase, naturally deleted in the “thumb”, from *Penicillium occitanis* Pol6. *Process Biochemistry*. 46: 1299-1306.

Dubeau H, Chahal DS and Ishaque M. 1987. Xylanase of *Chaetomium cellulolyticum*: its nature of production and cellulolytic potentials. *Biotechnology Letters.* 9: 275-280.

Dubois M, Gilles KA, Hamilton JK, Rabers PA and Smith F. 1956. Calorimetric method for determination of sugars and related substances. *Analytical Chemistry*. 26: 350p.

Eddy BP and Jacobs L. 1976. Mushroom compost as a source of food for *Agaricus bisporus. Mushroom Journal*. 38:56-59.

Edwards RL. 1949. MRA report on synthetic compost. *Mushroom Growers Association Bulletin*. 15:84-88.

Edwards RL. 1950. International Conference of Mushroom Science, Yaxley, Peterborough. *Mushroom Science.* 1 (39): 62p.

Edwards RL. 1974. Why do we add gypsum to mushroom compost. *Mushroom Journal*. 16:150-153.

Eicker A, Peng JT and Chen ZC. 1991. A survey of pathogenic fungi and weed moulds of cultivated mushrooms in Taiwan. Mushroom-Science-XIII-Volume-1- Proceedings-of-the-13th-International-congress-on-the-science-and-cultivation-of-ediblefungi-Dublin,-Irish-Republic,-1-6-September. 425-429.

Eicker A. 1977. Thermophilic fungi associated with the cultivation of *Agaricus bisporus*. *Journal of South African Botany.* 43: 193-207.

Ellila S, Fonseca L, Uchima C, Cota J, Goldman GH, Saloheimo M, Sacon V and Siika-aho M. 2017. Development of a low-cost cellulase production process using *Trichoderma reesei* for Brazilian biorefineries. *Biotechnology for Biofuels and Bioproduct*.10: 1-17.

Fall R, Phelps P and Spindler D. 1984. Biocon-version of xylan to triglycerides by oil-rich yeasts. *Applied and Environmental Microbiology*.47: 1130-1134.

Familoni TV, Ogidi CO, Akinyele BJ and Onifade AK. 2018. Evaluation of yield, biological efficiency and proximate composition of *Pleurotus* species cultivated on different wood dusts. *Czech Mycology.* 70: 33–45.

FAOSTAT. 2022. Food and Agriculture Organization of the United Nations Statistics Database. Available online at: http://www.fao.org/faostat/en/#data (accessed June 2022).

Fergus CL and Sinden JW. 1969. A new thermophilic fungus from mushroom compost:*Thielavia thermophila* spec. nov. *Canadian Journal of Botany.* 47: 1635-1637.

Fermor TR and Grant WD. 1985. Degradation of fungal and actinomycete mycelia by *Agaricus bisporus. Journal of General Microbiology.* 131: 1729-1734

Fermor TR and Wood DA. 1981. Degradation of bacteria by *Agaricus bisporus* and other fungi. *Journal of General Microbiology.* 126: 377-387.

Fermor TR, Smith JF and Spencer DM. 1989. The microflora of experimental mushroom composts. *Journal of Horticultural Sciences.* 54: 137-147.

Fernandez LR. 2010. "Lipase from *Thermomyces lanuginosus:* Uses and prospects as an industrial biocatalyst". *Journal of Molecular Catalysis B: Enzymatic*. 62 (3-4): 197–212.

Fidanza MA and Beyer DM. 2009. Plant nutrients and fresh mushroom compost. *Mushroom News*.12: 78-83.

Fidanza MA, Sanford DL, Beyer DM and Aurentz DJ. 2010. Analysis of fresh mushroom compost. *HortTechnology*. 20 (2): 449-453.

Filho EXF, Puls J and Coughlan MP. 1993. Physiochemical and catalytic properties of low molecular weight endo-1, 4-β-D-xylanase from *Myrothecium verrucaria. Enzyme and Microbial Technology*. 15: 529-540.

Finstein MS and Morris ML. 1975. Microbiology of municipal solid waste composting. *Advances in Applied Microbiology*. 19: 113-151.

Flannigan B and Sellars PN. 1977. Amylase, beta-glucosidase and beta-xylosidase activity of thermotolerant and thermophilic fungi isolated from barley. *Transactions of the British Mycological Society*. 69: 316-317.

Flegg BP, Spencer MD and Wood AD. 1985. The biology and technology of the cultivated mushrooms. Chapter 10- Crop productivity (Flegg BP). 179-193.

Flegg PB and Loughton A. 1961. Cereal straws used for mushroom composts. *Experimental Horticulture*. 5: 50-52.

Flegg PB and Randle PE. 1980. Effect of the duration of composting on the amount of compost produced and yield of mushrooms. *Scientia Horticulturae*. 12: 351-359.

Flegg PB and Randle PE. 1981. Relation between the initial nitrogen content of mushroom compost and the duration of composting. *ScientiaHorticulturae*. 15(1): 9-15.

Flickinger MC. 1980. Current biological research in conversion of cellulosic carbohydrates into liquidfuels: how far have we come? *Biotechnology and Bioengineering*. 23:27-48.

Gadde B, Menke C and Wassmann R. 2009. Rice straw as a renewable energy source in India, Thailand and the Philippines: overall potential and limitations for energy contribution and greenhouse gas mitigation. *Biomass and Bioenergy*. 33(11):1532- 1546.

Gaind S, Nain L and Patel VB. 2008. Quality evaluation of co-composted wheat straw, poultry droppings and oil seed cakes. *Biodegradation*. 20(3):307–317.

Gao M, Liang F, Yu A, Li B and Yang L. 2010. Evaluation of stability and maturity during forced-aeration composting of chicken manure and sawdust at different C/N ratios. *Chemosphere*. 78(5):614-619.

Garg P. 2014. Use of paddy straw during composting to bring down the cost of mushroom production. *Indian Journal of Scientific Research*. 3:11p.

Gaur AC and Mathur RS. 1990. Organic manures. In soil fertility and fertilizer use. IV. V Kumar et al., (Eds.) IFFCO, New Delhi.140-159.

Gea FJ, Martinez-Carrasco A and Navarro MJ. 2009. Efecto de la suplementación del sustrato sobre la cosecha de setas. *Horticulture International*. 67: 32-40.

Gerben S, Robert AS, Tineke W, Olijnsma, Huub JM, Den Camp OP, JAN PG, Gerrits, LEO JLD and Griensven VAN. 1994a. Ecology of thermophilic fungi in mushroom compost, with emphasis on *Scytalidium thermophilum* and growth stimulation of *Agaricus bisporus* mycelium. *Journal of Applied and Environmental Microbiology.* 60: 454-458.

Gerben S, Robert AS, Tineke WO, Gerrits PG, Jan Huub JM, Camp O, Leo JL and Van Griensven D.1995. Bioconversion of cereal straw into mushroom compost. *Canadian Journal of Botany.* 73:1019-1024.

Gerben S, Tineke WO, Jan PGG, Jos GMA, Huub J M OP Den Camp and Leo J LD Van Griensven. 1994. Inoculation of *Scytalidium thermophilum* in button mushroom compost and its effect on yield. *Applied and Environmental Microbiology*. 60(9): 3049-3054.

Gerrits JPG, Bels-Koning HC and Muller EM. 1967. Changes in mushroom compost constituents during composting, pasteurization and cropping. *Mushroom Science.* 6: 225-243.

Gerrits JPG. 1972. The influence of water in mushroom compost. *Mushroom Science*. 8: 4-57.

Gerrits JPG. 1974. Development of synthetic compost for mushroom growing based on wheat straw and chicken manure. *Netherlands Journal of Agricultural Science*. 25: 288-302.

Gerrits JPG. 1977.The significance of gypsum applied to mushroom compost, in particular in relation to the ammonia content.25: 4p.

Gerrits JPG. 1988a. Compost treatment in bulk for mushroom growing. *Mushroom Journal.* 182: 471-475.

Gerrits JPG. 1988b. Nutrition and compost. In:The cultivation of mushrooms. Van Griensven LJLD (eds). Darlington mushroom laboratories Ltd, Peterborough. 29-72.

Ghaly AE, Dave D and Zhang B. 2012. Augmenting composting microbial community with thermophilic cellulytic organisms for enhanced degradation of phenolic compounds in creosote treated wood waste. *Journal of Bioremediation and Biodegradation.* 3:3-6.

Ghosal D, Ghosh S, Dutta TK and Ahn Y. 2016. Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs). *Frontiers in Microbiology.* 7:1369p.

Ghose TK. 1987. Measurement of cellulase activities. *Pure and Applied Chemistry*. 59: 257-268.

Gomez KA and Gomez AA. 1984. Statistical procedure for agricultural research. 2nd edition. John Wiley and Sons, New York. 680p.

Gonawala SS and Jardosh H. 2017. Organic waste in composting: A brief review. *International Journal of Current Engineering and Technology.* 8(1):36-38.

Gothwal R, Gupta A, Kumar A, Sharma S and Alappat BJ. 2012. Feasibility of dairy waste water (DWW) and distillery spent wash (DSW) effluents in increasing the yield potential of *Pleurotus flabellatus* (PF 1832) and *Pleurotus sajor-caju* (PS 1610) on bagasse. 3 *Biotechnology*. 2: 249-257.

Gowda NAN and Manvi D. 2019. Agro-residues disinfection methods for mushroom cultivation: A review. *Agricultural Revolution.* 40(2): 93-103.

Goyal R, Grewal RB and Goyal RK. 2006. Nutritional attributes of *Agaricus bisporus* and *Pleurotus sajor caju* mushrooms. *Nutrition and Health*. 18:179-184.

Griffon E and Maublanc A. 1911. Deuxmoisissures thermophiles. *Bulletin of the Society of Mycology*, France. 27: 68-74.

Grimm D and Wosten HAB. 2018. Mushroom cultivation in the circular economy. *Applied Microbiology and Biotechnology*. 102:7795–7803.

Guillamon E, Garcia-Lafuente A, Lozano M, Arrigo MD, Rostagno MA, Villares A and Martinez JA.2010. Edible mushrooms: Role in the prevention of cardiovascular diseases. *Fitoterapia*. 81(7): 715-23.

Guo R, Li G, Jiang T, Schuchardt F, Chen T, Zhao Y and Shen Y. 2012. Effect of aeration rate, C/N ratio and moisture content on the stability and maturity of compost. *Bioresource Technology*. 112:171-178.

Han NS, Ahmad WANW and Ishak WRW. 2016. Quality characteristics of *Pleurotus sajor-caju* powder: Study on nutritional compositions, functional properties and storage stability. *Sains Malaysiana*. 45(11):1617-1623.

Hatanaka K. 2012. Incorporation of fluorous glycosides to cell membrane and saccharide chain elongation by cellular enzymes. Top. *Current Chemistry*.308: 291–306.

Hayashida S and Yoshioka H. 1980. The role of carbohydrate moiety on thermostability of cellulases from *Humicola insolens* YH-8. *Agricultural and Biological Chemistry*. 44:481-487.

Hayashida S, Ohta K and Mo K. 1988. Cellulases of *Humicola insolens* and *Humicola grisea*. *Methods in Enzymology*. 160:323-332.

Hayes WA and Randle PE. 1968. The use of water soluble carbohydrates and methyl bromide in the preparation of mushroom composts. *MGA Bulletin*. 218: 81-97.

Hayes WA and Randle PE. 1969. Use of molasses as an ingredient of wheat straw mixtures used for the preparation of mushroom composts. *Glasshouse Crops Research Institute*. 142-147.

Hayes WA and Shandilya TR. 1977. Casing soil and compost substrates used in the artifical culture of *Agaricus bisporus*, the cultivated mushroom. *Indian Journal of Mycology and Plant Pathology*. 7: 5-10.

Hayes WA. 1981. Interrelated studies of physical, chemical and biological factors in casing soils and relationships with productivity in commercial culture of *Agaricus bisporus*. *Mushroom Science*. 11: 103-129.

Hebert A. 1892. Annales de Stratigraphie Et de Paleontologie Du Laboratoire de Geologie de la Faculte Des Sciences de Paris, Volume no. 1 (French). 456p.

Heleno SA, Barros L, Sousa MJ, Martins A and Ferreira ICFR. 2010. Tocopherols composition of Portuguese wild mushrooms with antioxidant capacity. *Food Chemistry*.119: 1443-1450.

Heslop BR and Ramsey CA.1969. The solvent extraction of vanadomolybdophosphoric acid as the basis of a substoichiometric method for the determination of phosphorus. *Analytica Chimica Acta*. 47 (2): 305-314.

Hongzhang C. 2016. Biotechnology of lignocellulose: theory and practice. Springer New York¸USA. 1-510.

Horn SJ, Vaaje-Kolstad G, Westereng B and Eijsink V. 2012. Novel enzymes for the degradation of cellulose. *Biotechnology for Biofuels and Bioproducts.* 5:45p.

Howard DH. 2002. Pathogenic fungi in humans and animals. CRC Press. 808p.

Hu Y, Mortimer PE, Hyde KD, Kakumyan P and Thongklang N. 2021. Mushroom cultivation for soil amendment and bioremediation. *Circular Agricultural Systems.* 1: 11.

Imbeah M. 1998. Composting piggery waste: A review. *Bioresource Technology*.63:197-203.

Jackson ML. 1973. Soil Chemical Analysis. Printice Hall of India Private Limited New Delhi. 111-126.

Jain SK, Gujral GS, Vasudevan P and Jha NK. 1989. Uptake of heavy metals by *Azolla pinnata* and their translocation into the fruit bodies of *Pleurotussajor-caju. Journal of Fermentation and Bioengineering.* 68(1):64–67.

Jaradat AA. 2010. Genetic resources of energy crops: Biological systems to combat climate change. *Australian Journal of Crop Science.* 4: 309-323.

Jarial RS and Shandilya TR. 2004. Physico-chemical parameters of different casing materials and their relationship with the yield of *Agaricus bisporus* (Lange) Imbach. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences*. 6: 93-98.

Jurak E, Punt AM, Arts W, Kabel MA and Gruppen H. 2015. Fate of carbohydrates and lignin during composting and mycelium growth of *Agaricus bisporus* on wheat straw based compost. *PLoS One.* 10(10): 1-16.

Jusoh MLC, Manaf LA and Latiff PA. 2013. Composting of rice straw with effective microorganisms (EM) and its influence on compost quality. *Iranian Journal of Environmental Health Science and Engineering.*10:17p.

Kalac P. 2013. A review of chemical composition and nutritional value of wild-growing and cultivated mushrooms. *Journal of the Science of Food and Agriculture.* 93(2):209-218.

Kalamdhad AS, Singh YK, Ali M, Khwairakpam M and Kazmi AA. 2009. Rotary drum composting of vegetable waste and tree leaves. *Bioresource Technology.* 100(24): 6442-6450.

Kalberer PP. 1990. Water relations of the mushroom culture *Agaricus bisporus*: Study of a single break. *Scientia Horticulturae.* 41(3): 277-2783.

Kalberer PP. 1991. Water relations of the mushroom culture (*Agaricus bisporus*): Influence on the crop yield and on the dry matter content of the fruit bodies. In: Proceedings of the 13th International Congress on the science and cultivation of edible fungi. Maher MJ and Balkema AA (eds). Rotterdam. 269-274.

Kalmis E, Azbar N, Yıldız H and Kalyoncu F. 2008. Feasibility of using olive mill effluent (OME) as a wetting agent during the cultivation of oyster mushroom. *Bioresource Technology*. 99(1):164–169.

Kalmis E, Nuri A, Hasan Y and Fatin K. 2008. Feasibility of using olive mill effluent (OME) as a wetting agent during the cultivation of oyster mushroom, *Pleurotus ostreatus* on wheat straw. *Bioresource Technology.* 99:164-169.

Kalra YP and Maynard DG. 1991. Forestry Canada, Northwest region, Northern forestry centre, Edmonton, Alberta. Information report NOR-X-319E. 116p.

Karthika A, Seenivasagan R and Vasanthy M. 2022. A review on technological approach for obtaining nutrient from solid waste. Part of the book series: Emerging contaminants and associated treatment technologies (ECAT). *Organic Pollutants*. *Springer*. 475–502.

Kaur H and Khanna PK. 2001. Physicochemical and microbiological characteristics of paddy straw based compost for *Agaricus bisporus* production. *Indian Journal of Mushrooms*. 2: 15-20.

Kaur J, Chadha BS, Kumar BA, Kaur GS and Saini HS. 2007. Purification and characterization of β-glucosidase from *Melanocarpus* sp. MTCC3922. *Electronic Journal of Biotechnology*. 10:260-270.

Kaur S, Kaur M, Devi R and Kapoor S. 2019. Paddy straw and maize stalks compost for cultivation of *Agaricus bisporus*. *International Journal of Current Microbiology and Applied Sciences.* 8(01): 2418-2428.

Kayode RMO, Olakulehin TF, Adedeji BS, Ahmed O, Aliyum TH and Badmos AHA. 2015. Evaluation of amino acid and fatty acid profiles of commercially cultivated oyster mushroom (*Pleurotus sajor-caju*) grown on gmelina wood waste. *Nigerian Food Journal.* 33(1):18-21.

Keles A, Koca I and Genccelep H. 2011. Antioxidant properties of wild edible mushrooms. *Journal of Food Processing and Technology*.2:130p.

Kertesz M, Safianowicz K and Bell T. 2016. New insights into the microbial communities and biological activities that define mushroom compost. In proceedings of the 19th International Society for mushroom science conference.161–165.

Kertesz MA and Thai M. 2018. Compost bacteria and fungi that influence growth and development of *Agaricus bisporus* and other commercial mushrooms. *Applied Microbiology and Biotechnology.*102:1639–1650.

Khanna PK and Kapoor S. 2016. Mushroom growing bulletin. Punjab Agricultural University, Ludhiana. 78p.

Khanongnuch C, Sanguansook C and Lumyong S. 2006. Nutritive quality of β-mannanase treated copra meal in broiler diets and effectiveness on some fecal bacteria. *International Journal of Poultry Science.* 5: 1087-1091.

Kiiskinen LL, Viikari L and Kruus K. 2002. Purification and characterization of a novel laccase from the ascomycete *Melanocarpus albomyces. Applied Microbiology and Biotechnology.* 59:198–204.

Knob A, Forthamp D, Prolo T, Izidoro SC and Almeida JM. 2014. Agro-residues as alternative for xylanase production by filamentous fungi. *Bioresources.* 9:5738-5773.

Korotkova OG, Semenova MV, Morozova VV, Zorov IN, Sokolova LM, Bubnova TM, Okunev ON and Sinitsyn AP. 2009. Isolation and properties of fungal beta-glucosidases. *Biochemistry.* 74:569-577.

Krisana A, Rutchadaporn S, Jarupan G, Lily E, Sutipa T and Kanyawim K. 2005. Endo-1,4-β-xylanase from *Aspergillus niger* BCC14405 isolated in Thailand: purification, characterization and gene isolation. *Journal of Biochemistry and Molecular Biology*. 38:17-23.

Kumar A, Gaind S and Nain L. 2008. Evaluation of thermophilic fungal consortium for paddy straw composting. *Biodegradation*. 19:395-402

Kumari D, Reddy MS and Upadhyay RC. 2011. “Nutritional composition and antioxidant activities of 18 different wild Cantharellus mushrooms of Northwestern Himalayas,” *Food Science and Technology International*. 17(6): 557-567.

Kumari J and Jaiswal RR. 2023. Scientific research and its approach towards the mushroom production and industrial developments. *International Journal of Home Science.* 9(1): 157-162.

Kumla J, Suwannarach N, Sujarit K, Penkhrue W, Kakumyan P, Jatuwong K, Vadthanarat S and Lumyong S. 2020. Cultivation of mushrooms and their lignocellulolytic enzyme production through the utilization of agro-industrial waste. *Molecules*. 25(12):2811p.

Kurtzman RHJR. 1995. *Agaricus bisporus* (Lge.) Imb. casing layer, II: porosity, the most important character. *International Journal of Mushroom Science*. 1: 11-17.

Laborde J and Delmas J. 1969. La preparation rapide de substrats. *Bulletin de la federation nationale des syndicatsagricoles des cultivateurs de champignons*. 184: 2093–2109.

Laborde J, Lanzi G, Francescutti B and Giordani E. 1993 Indoor composting: General principles and large scale development in Italy. In mushroom biology and mushroom products. 93-113.

Lacey J. 1973. Actinomycetes in soils, composts and fodders, in *Actinomycetales: Characteristics and Practical Importance*. Sykes G and Skinner FA (eds) Academic Press, London. 231–251.

Lambert EB. 1941. Studies on the preparation of mushroom compost. *Journal of Agricultural Research*. 62: 415-422.

Lanyi K. 2010. Assessment of the relations between the spectroscopic characteristics of soils and their ability to adsorb organic pollutants. *Microchemical Journal.* 79: 249-256.

Lara MA, Rodriguez-Malaver AJ, Rojas OJ, Holmuist O, Gonzalez AM and Bullon J. 2003. Black liquor lignin biodegradation by *Trametes elegans*. *International Biodeterioration and Biodegradation.* 52: 167–73.

Last FT, Holdings M and Stone OM. 1974. Effects on cultural conditions on the mycelia growth of healthy and virus infected cultivated mushroom, *Agaricus bisporus*. *Annals of Applied Biology*. 76: 99-111.

Laura LK, Kristiina K, Michael B, Erkko Y, Matti S and Markku S. 2004. Expression of *Melanocarpus albomyces* laccase in *Trichoderma reesei* and characterization of the purified enzyme. *Microbiology*.150: 3065-3074.

Levanon D, Dosoretz C and Motro B. 1983. Chemical and biological qualification of synthetic composts for mushrooms (*Agaricus bisporus*). *Mushroom News*. 16-19.

Li WF, Zhou XX and Lu P. 2005. Structural features of thermozymes. *Biotechnology*. 23:271-281

Liang C, Das CK and McClendon WR. 2003. The influence of temperature and moisture contents regimes on the aerobic microbial activity of a biosolids composting blend. *Bioresource Technology*. 86(2): 131-137.

Liang CH, Wu CY, Lu PL, Kuo YC, Liang ZC. 2019. Biological efficiency and nutritional value of the culinary-medicinal mushroom *Auricularia* cultivated on a sawdust basal substrate supplement with different proportions of grass plants. *Saudi Journal of Biological Sciences*. 26: 263-269.

Liu Y, Huang F, Yang H, Ibrahim SA, Wang Y and Huang W. 2014. Effects of preservation methods on amino acids and 5-nucleotides of *Agaricus bisporus* mushrooms. *Food Chemistry*. 149: 221-225.

Llovera L and Benjelloun-Mlayah B. 2022. "Silica extraction from organosolv pretreated wheat straw." *BioResources*. 17(2): 3130-3147.

Lombard V, Golaconda RH, Drula E, Coutinho PM and Henrissat B. 2014. The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Research*. 42:490-495.

Lopez-Mondejar R, Zuhlke D, Becher D, Riedel K and Baldrian P. 2016. Cellulose and hemicellulose decomposition by forest soil bacteria proceeds by the action of structurally variable enzymatic systems. *Scientific Reports*. 6: 1-12.

Madeira JV, Jr Contesini FJ, Calzado F, Rubio MV, Zubieta MP, Lopes DB and de Melo RR. 2017. Agro-industrial residues and microbial enzymes: An overview on the eco-friendly bioconversion into high value-added products. In biotechnology of microbial enzymes; brahmachari G, Ed, Elsevier: Amsterdam, The Netherlands. 475- 511.

Mahajan K, KumarS, Rao A and Mahajan KA. 2021. Microbial diversity and their role in *Agaricus bisporus* production. *International Journal of Current Microbiology and Applied Sciences*. 10(07): 405-413.

Mahajan MK, Johri BN and Gupta RK. 1986. Influence of desiccation stress in a xerophilic thermophile *Humicola* sp. *Current Science*. 55:928-930.

Maheshwari R, Bharadwaj G and Mahakingesgwara KB. 2000. Thermophilic fungi: Their physiology and enzymes. *Microbiology and Molecular Biology Reviews*. 64: 461-488.

Maheshwari R. 2016. Fungi: experimental methods in biology, 2nd edition. CRC Press. 358p.

Maijala P, Kango N, Szijarto N and Viikari L. 2012. Characterization of hemicellulases from thermophilic fungi. Anton Van Leeuwenhoek. 101:905-917.

Major J, Lehmann J, Rondon M and Goodale C. 2010. Fate of soil-applied black carbon:

Downward migration, leaching and soil respiration. *Global Change Biology*. 16:1366-1379.

Malick CP and Singh MB. 1980. In plant enzymology and histo enzymology. Kalyani publisher, New Delhi. 286p.

Malloch D and Cain RF. 1972. The Trichocomataceae: Ascomycetes with Aspergillus, Paecilomyces, and Penicillium imperfect states. *Canadian Journal of Botany*. 50: 2613-2628.

Mandels M, Andreotti RE and Roche C. 1976. Measurement of saccharifying cellulase. *Biotechnology and Bioengineering Symposium.* 6: 21-33.

Mandels M. 1975. Microbial sources of cellulase. *Biotechnology and Bioengineering Symposium.* 5:81-105.

Manning K and Wood DA. 1983. Production and regulation of cellulase of *A. bisporus*. *The Journal of General Microbiology.* 129: 1839-1847.

Margaritis A and Merchant R. 1983. Production and thermal stability characteristics of cellulase and xylanase enzymes from *Thielavia terrestris*. *Biotechnology and Bioengineering Symposium.*13:426–428.

Margaritis A, Merchant RF and Yaguchi M. 1986. Thermostable cellulases from thermophilic microorganisms. *Critical Reviews in Biotechnology.* 4:327-367.

Martinez-Carrera D. 1989. Simple technology to cultivate Pleurotus on coffee pulp in the tropics. *Mushroom Science*. 12 (II): 169-178.

Mata G and Savoie JM.1998. Extracellular enzyme activities in six *Lentinula edodes* strains during cultivation in wheat straw. *World Journal of Microbiology and Biotechnology*. 14: 513 - 519.

Mattila P and Konko K. 2001. Content of vitamins, mineral elements and some phenolic compounds in cultivated mushrooms. *Journal of Agricultural and Food Chemistry*. 49: 2343-2348.

McCartney D and Tingley J. 1998. Development of a rapid method for compostmaterials. *Compost Science and Utilization*. 6:14-25.

McGee CF, Byrne H, Irvine A and Wilson J. 2017. Diversity and dynamics of the DNA and cDNA-derived bacterial compost communities throughout the *Agaricus bisporus* mushroom cropping process. *Annals of Microbiology*. 67:751-761.

McKinley VL, Vestal JR and Erlap AE. 1985. Microbial activity in composting. *Biocycle*. 26: (7):47-50.

Miehe H. 1930. Die warmebildung von reinkulturenimhinblick auf die atiologie der selbsterhitzungpflanzlicherstoffe. *Archives of Microbiology*. 1:78-118.

Miehe H. 1930a. Uber die selbsterhitzung des heues. arbdtschlandwirtsch-gesellsch berlin.111:76-91.

Miller FC, Harper ER, Macaunly BJ and Gulliver A. 1990. Composting based on moderately thermophillic and aerobic conditions for the production of commercial mushroom growing compost. *Australian Journal of Experimental Agriculture*. 30(2): 287-298.

Miller FC. 1992. Composting as a process based on the control of ecologically selective factors. *Soil Microbial Ecology*. 18(3):515-543.

Miller GL. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*. 31:426-428.

Mohan D, Sarswat A, Ok YS and Pittman JCU. 2014. Organic and inorganic contaminants removal from water with biochar, a renewable, low cost and sustainable adsorbent-A critical review. *Bioresource Technology*. 160:191- 202.

Mohiuddin KM, Alam M, Arefin T and Ahmed I. 2015. Assessment of nutritional composition and heavy metal content in some edible mushroom varieties collected from different areas of Bangladesh. *Asian Journal of Medical and Biological Research*. 1(3):495-501.

Monssef R.Abd Hassan A, Enas A and Ramadan ME. 2016. Production of laccase enzyme for their potential application to decolorize fungal pigments on aging and paper parchment. *Annals of Agricultural Sciences.* 61:145-154.

Morgenstern I, Klopman S and Hibbett DS. 2008. Molecular evolution and diversity of lignin degrading heme peroxidases in the Agaricomycetes. *Annals of Agricultural Sciences.* 66: 243-257.

Mosher D and Anderson RK. 1977. Composting sewage sludge by high-rate suction aeration techniques-the process as conducted at Bangor, ME, and some guidelines of general applicability. Interim report number SW-614d. US government printing office, Washington, DC. 1-6.

Murugkar AD and Subbulakshmi G. 2005. Nutritional value of edible wild mushrooms collected from the Khasi hills of Meghalaya. *Food Chemistry.* 89: 599-603.

Muszynska B, Kisielewska AG, Kała K and Argasinska JG. 2017. Anti-inflammatory properties of edible mushrooms: A review. *Food Chemistry.* 243:373-381.

Muszynska B, Sulkowska-Ziaja K and Ekiert H. 2011. Indole compounds in fruiting bodies of some edible basidiomycota species. *Food Chemistry*. 125: 1306-1308.

Naraian R, Sahu RK, Kumar S, Garg SK, Singh CS and Kanaujia RS. 2009. Influence of different nitrogen rich supplements during cultivation of *Pleurotus florida* on corn cob substrate. *Environmentalist.* 29:1–7.

Narain R, Sahu RK, Kumar S, Garg SK, Singh CS and Kanaujiaet RS. 2009. Influence of different nitrogen rich supplements during cultivation of *Pleurotus florida* on maize cobs substrate. *Environmentalist.* 29(1):1–7.

Nguyen DV and Dang LH. 2020. Fresh rice straw silage affected by ensiling additives and durations and its utilization in beef cattle diets. *Asian Journal of Animal Sciences.* 14: 6p.

Nunes CS and Kunamneni A. 2018. “Chapter 7- Laccases properties and applications,” in enzymes in human and animal nutrition, Cambridge, Academic Press. 133-161.

Nunes DM, Luz RMJ, Paes AS, Ribeiro OJJ, Silva SCM and Kasuya MCM. 2012. Nitrogen supplementation on the productivity and the chemical composition of oyster mushroom. *Journal of Food Research.* 1(2):113-117.

Ogundero VW. 1979. Thermophilic and thermotolerant fungi from poultry droppings in Nigeria. *The Journal of General Microbiology.*115: 253-254.

Ohga S and Royse DJ. 2001. Transcriptional regulation of laccase and cellulase genes during growth and fruiting of *Lentinula edodes* on supplemented sawdust. *FEMS Microbiology Letters.* 201: 111-115.

Ohmiya K, Sakka K, Kimura T and Karita S. 1997. Structure of cellulases and their applications. *Biotechnology and Genetic Engineering Reviews.* 14:365-414.

Oladosu Y, Rafii MY, Abdsullah N, Magaji U, Hussin G, Ramli A and Miah G. 2016. Fermentation quality and additives: A case of rice straw silage. *BioMed Research International.* 1–14.

Onwosi CO, Igbokwe VC, Odimba JN, Eke IE, Nwankwoala MO, Iroh IN and Ezeogu LI. 2017. Composting technology in waste stabilization: On the methods, challenges and future prospects. *Journal of Environmental Management.* 190:140-157.

Osma JF, Toca-Herrera JL and Rodriguez-Couto S. 2010. Uses of laccases in the food industry. *Enzyme Research.* 1-8.

Otero-Jimenez V, Carreno-Carreno JDP, Barreto-Hernandez E, van Elsas JD and Uribe-Velez D. 2021. Impact of rice straw management strategies on rice rhizosphere microbiomes. *Applied Soil Ecology*. 167: 1-11.

Overstijns APA. 1964. Fundamenteleproblemen en synthetische compost in 1964. *Champignon Cultuur.* 8: 304-306.

Pabhabraom MV, Sopanrao PS, Ahmed SA and Vaseem BMM. 2007. Bioconversion of low quality lignocellulosic agricultural waste into edible protein by *Pleurotus sajor-caju* (Fr.) Singer. *Journal of Zhejiang University-Science B.* 8:745-751.

Pace CN, Vajdos F, Fee L, Grimsley G and Gray T. 1995. How to measure and predict the molar absorption coefficient of a protein. *Protein Science*. 4 (11): 2411-2423.

Pandey AK, Gaind S, Ali A and Nain L. 2009. Effect of bioaugmentation and nitrogen supplementation on composting of paddy straw. *Biodegradation.* 20(3): 293-306.

Pandey VK and Singh MP. 2014. Biodegradation of wheat straw by *Pleurotus ostreatus* . *Cellular and Molecular Biology.* 60:29-34

Paszczynski A, BV Huyn and R Crawford. 1986. Comparison of ligninase I and peroxidase-M2 from the white rot fungus *Phanerochaete chrysosporium. Archives of Biochemistry and Biophysics*. 244: 750p.

Patel AK, Singhania RR, Sim SJ and Pandey A. 2019. Thermostable cellulases: Current status and perspectives. *Bioresource Technology.* 279:385-392.

Patel Y, Naraian R and Singh VK. 2012. Medicinal properties of *Pleurotus* species (oyster mushroom) – A review. *World Journal of Fungal and Plant Biology.* 3(1):1-12.

Pavel K. 2009. Chemical composition and nutritional value of european species of wild growing mushrooms: A review. *Food Chemistry*. 113: 9-16.

Pekarovic J, Pekarovicova A and Fleming PD. 2006. “Two-step straw processing- a new concept of silica problem solution,” In: Engineering, pulping and environmental conference, Atlanta, USA.1-6.

Philippoussis A and Diamantopoulou P. 2011. Agro-food industry wastes and agricultural residues conversion into high value products by mushroom cultivation. In proceedings of the 7th International Conference on mushroom biology and mushroom products, Institute National de la Recherche Agronomique (INRA), Arcachon, France. 339-351.

Piper CS. 1966. Soil chemical analysis. Asia Publishing House, Bombay. 408 p.

Pizer NH. 1937. Investigations into the environment and initiation of the cultivated mushroom. Part 1. Some properties of composts in relation to growth of mycelium. *Journal of Agricultural Science*. 27: 349-376.

Pollegioni L, Tonin F and Rosini E. 2015. Lignin-degrading enzymes. *FEBS Journal*. 282:1190-1213.

Pope S, Knaust HC and Knaust K. 1962. Production of compost by thermophilic fungi. *Mushroom Science.* 5: 125-126.

Prasanna HN,Ramanjaneyulu G and Rajasekhar Reddy B. 2016. Optimization of cellulase production by *Penicillium* sp. 3 *Biotech*. 6: 1-11.

Rajarathnam S, ZakhiaBano Z and Steinkraus and Keith H. 1989. *Pleurotus* mushrooms. Part III. Biotransformations of natural lignocellulosic wastes: Commercial applications and implications. *Critical Reviews in Food Science and Nutrition.* 28(1): 31-113.

Rajasekaran AK and Maheshwari R. 1993. Thermophilic fungi: an assessment of their potential for growth in soil. *Journal of Biosciences.* 18:345-354.

Rana RS. 1998. Compost and composting for white button mushroom. Lecture compendium of recent advances in mushroom cultivation. Academy of agricultural research and education management, HAU, Hisar. 21-33.

Randle PE and Flegg PB. 1985. The effects of duration of composting on compost density and the yield of mushrooms. *Scientia Horticulturae*. 27(1-2): 21-31.

Randle PE. 1974. Compost. Report of the Glasshouse Crops Research Institute. 82–84.

Rangabhashiyam S and Balasubramanian P. 2019. The potential of lignocellulosic biomass precursors for biochar production: Performance, mechanism and wastewater application - A review. *Industrial Crops and Products.* 405-423.

Ranganna S. 2009. Handbook of analysis and quality control of fruits and vegetables products.7th ed. Tata McGrow Hill Publishing Company Limited, New Delhi, India. 1-9.

Rao NSS. 1999. Soil microorganism and plant growth. Oxford and IBH publishing Company, New Delhi. 252 p.

Raut MP, Prince WSP, Bhattacharyya JK, Chakrabarti T and Devotta S. 2008. Microbial dynamics and enzyme activities during rapid composting of municipal solid waste: A compost maturity analysis perspective. *Bioresource Technolnogy*. 99: 6512-6519.

Reese ET and Mandel M. 1963. Enzymatic hydrolysis of cellulose and its derivatives In: Method carbohydrate chemistry (Whistles R L eds.). Academic Press London. 139-143 p.

Rehan AM, Hassan E and Ramadan E. 2016. Production of laccase enzyme for their potential application to decolorize fungal pigments on aging paper and parchment. *Annals of Agricultural Sciences*. 61:1-11.

Rich N, Bharti A and Kumar S. 2018. Effect of bulking agents and cow dung as inoculants on vegetable waste compost quality. *Bioresource Technology*. 252: 83-90.

Richard TL, Hamelers HVM, Veeken A and Silva T. 2002. Moisture relationships in composting processes. *Composte Science and Utilization*. 10(4): 286-302.

Ritota M and Manzi P. 2019. *Pleurotus* spp. cultivation on different agri-food by-products: example of biotechnological application. *Sustainability*. 11: 5049.

Robledo A, Aguilar CN, Belmares-Cerda RE, Flores-Gallegos AC, Contreras-Esquivel JC, Montañez JC and Mussatto SI. 2015. Production of thermostable xylanase by thermophilic fungal strains isolated from maize silage. *Cyta Journal of Food*. 14(2): 302-308.

Ross RC and Harris PJ. 1983. The significance of thermophilic fungi in mushroom compost preparation. *Scientia Horticulturae.* 20: 61-70.

Rowell DL. 1994. Soil science: Methods and applications. Longman Group Ltd. U.K. 1-350 p.

Royse DJ and Chalupa W. 2009. Effects of spawn, supplement, and phase II compost additions and time of re-casing second break compost on mushroom (*Agaricus bisporus*) yield and biological efficiency. *Bioresource Technology.* 100(21): 5277-5282.

Royse DJ. 2010. Effects of fragmentation, supplementation and the addition of phase II compost to 2nd break compost on mushroom (*Agaricus bisporus*) yield. *Bioresource Technology.* 101(1):188–192.

Rupert DR. 1995. Use of spent mushroom substrate in stabilizing disturbed and commercial sites. *Compost Science and Utilization.* 3: 80-83.

Sadaf A and Khare SK. 2014. Production of *Sporotrichum thermophile* xylanase by solid state fermentation utilizing deoiled *Jatrophacurcas* seed cake and its application in xylooligosachharide synthesis. *Bioresource Technology*. 153:126-130.

Sadiq S, Bhatti HN and Hanif MA. 2008. Studies on chemical composition and nutritive evaluation of wild edible mushrooms. *Iran Journal of Chemistry and Chemical Engineering*. 27(3): 151-154.

Sadler M. 2003. Nutritional properties of edible fungi. *Journal of Industrial Microbiology and Biotechnology.* 28: 305-308.

Saha BC. 2003. Hemicellulose bioconversion. *Journal of Industrial Microbiology and Biotechnology*. 30: 279-291.

Saini KJ, Saini R and Lakshmi Tewari L. 2015. Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: Concepts and recent developments. *Biotechnolgy*. 5: 337-353.

Sajith S, Priji P, Sreedevi S and Benjamin S. 2016. An overview on fungal cellulases with an industrial perspective. *Journal of Nutrition and Food Sciences*. 6: 461.

Salar RK and Aneja KR. 2007. Significance of thermophilic fungi in mushroom compost preparation: effect on growth and yield of *Agaricus bisporus* (Lange) Sing. *International Journal of Agricultural Technology.* 3(2): 241-253.

Sanchez C. 2004. Modern aspects of mushroom culture technology. *Applied Microbiology and Biotechnology*. 64: 756-762.

Sanchez C. 2009. Lignocellulosic residues: Biodegradation and bioconversion by fungi. *Biotechnology Advances*. 27: 185-194.

Sanchez C. 2017. Reactive oxygen species and antioxidant properties from mushrooms. *Synthetic and Systems Biotechnology*. 2: 13-22.

Sanchez JE, Mejia L and Royse DJ. 2008. Pangola grass colonized with *Scytalidium thermophilum* for production of *Agaricus bisporus*. *Bioresource Technology*. 99: 655-62.

Sanchez JE. 2007. Uso de hongos termófilos para la preparacion de sustratos. In: Sanchez JE, Royse DJ, Leal Lara H. (eds.), Cultivo, mercadotecnia e inocuidad alimenticia de *Agaricus bisporus.* El Colegio de la Frontera Sur, Tapachula. 65-74 p.

Sarkar PS and Chanda S .2016. Optimization of a vegetable waste composting process with a significant thermophilic phase. *Procedia Environmental Sciences*. 35: 435-440.

Satyanarayana T, Littlechild J and Kawarabayasi Y. 2013. Thermophilic microbes in environmental and industrial biotechnology: Biotechnology of thermophiles. *Science and Business Media*. 10:1-4.

Satyanarayana T. 1978. Thermophilic microorganism and their role in composting process. Ph.D. Thesis. University of Saugar. 213p.

Sayara T, Basheer-Salimia R, Hawamde F and Sanchez A. 2020. Recycling of organic wastes through composting: Process performance and compost application in agriculture. *Agronomy*. 10: 1838p.

Scharf ME and Tartar A. 2008. Termite digestomes as sources for novel lignocellulases. *Biofuels, Bioproducts and Biorefining.* 2: 540-552.

Schülein M. 1997. Enzymatic properties of cellulases from *Humicola insolens. Journal of Biotechnology.* 57: 71-81.

Schulze KL. 1962. Continuous thermophilic composting. *Applied Microbiology*. 10: 108-122.

Scialabba N, Gomez I and Thivant L. 2017. Training manual of organic agriculture. United Book Prints. 52-55 p.

Seal KJ and Eggins HOW. 1976. The upgrading of agricultural wastes by thermophilic fungi. In food from wastes (eds G.G. Birch, K.J. Parker and J.T. Wargan). *Applied SciencePublishers*, London. 58-78 p.

Shandilya TR and Agarwal RK 1983. Evaluating farmyard manure and spent compost as casing substrate in the cultivation of *Agaricus bisporus*. *Indian Journal of Plant Pathology*. 1: 34-37.

Shandilya TR and Hayes WA. 1987. Availabe element in some casing soils. *Indian Journal of Mushroom Science*. 2: 10-15.

Shandilya TR. 1989. Studies on casing soil media during the cultivation of *Agaricus bisporus*. *Composites Science and Technology*. 4: 6-17.

Shandilya TR. 1989. Studies on casing soil media during the cultivation of *Agaricus bisporus*. *Compost Science and Utilization*. 4: 6-17.

Sharma A, Sharma R, Arora A, Shah R, Singh A, Pranaw K and Nain L. 2014. Insights into rapid composting of paddy straw augmented with efficient microorganism consortium. *International Journal of Recycling of Organic Waste in Agriculture*. 3(54): 14-20.

Sharma AR, Kharol SK, Badarinath KVS and Singh D. 2010. Impact of agriculture crop residue burning on atmospheric aerosol loading – A study over Punjab state, India. *Annals of Geophysics.* 28: 367-379.

Sharma SR. 1991. Viruses in mushrooms-A review. *Advance Journal of Mushroom Science*. 61p.

Sharma VP and Kumar S. 2011. Spawn production technology. In: Mushroom cultivation, marketing and consumption, ed. M Singh, B Vijay, S Kamal and GC Wakchaure. Directorate of Mushroom Research (ICAR), Chambaghat, Solan. 31- 42 p.

Sharma VP, Annepu SK, Gautam Y, Singh M and Kamal S. 2017. Status of mushroom production in India. *Mushroom Research.* 2: 111-120.

Sheoran OP, Tonk DS, Kaushik LS, Hasija RC and Pannu RS. 1998. Statistical software package for agricultural research workers. Recent advances in information theory, statistics and computer applications by DS Hooda and RC Hasija, Department of Mathematics Statistics, CCS HAU, Hisar. 139-143.

Shi H, Ding H, Huang Y, Wang L, Zhang Y, Li X and Wang F. 2014. Expression and characterization of a GH43 endo-arabinanase from *Thermotoga thermarum*. *Biotechnology*.14: 35p.

Sinden JW and Hauser E. 1950. The short method of mushroom composting. *Mushroom Science*. 1: 52-59.

Sinden JW and Hauser E. 1953. The nature of the composting process and its relation to short composting. *Mushroom Science*. 2: 123-131.

Sinden JW. 1938. Synthetic compost for mushroom growing. *Bulletin of the Agricultural Experiment Station of Nebraska.* 365: 1-27.

Singh C, Pathak P, Chaudhary N, Rathi A, Dehariya P and Vyas D. 2020. Mushrooms and mushroom composts in integrated farm management. *Research Journal of Agricultural Sciences.* 11(6): 1436-1443.

Singh H. 1991. Mushrooms: The art of cultivation. Sterling publisher’s private limited. 120p.

Singh J and Aneja KR. 2012. From ethnomycology to fungal biotechnology: Exploiting fungi from natural resources for novel products (1^{st} edition). *Springer.* 293p.

Singh KH, Kerketta A and Shukla SC. 2020. Effect of compost and growing structures on growth and yield of button mushroom (*Agaricus bisporus* lange imbach). *Mushroom Research.* 29(1): 27-30.

Singh M, Singh RP and Chaube HS. 2000. Impact of physiochemical properties of casing on yield of *Agaricus bisporus* (Lange) Imbach. (Van. Griensven ed.) In: Science and cultivation of edible fungi Balkema, Rotterdam. 441- 446.

SinghRA.1980.Soilphysicalanalysis.NewDelhi:KalyaniPublishers.165p.

Sinha KS, Upadhyay KT, Sharma KS and Sharma M. 2020. A review: Bacterial diversity, physicochemical factors and quality of compost for white button mushroom cultivation. *Bulletin UASVM series Agriculture.* 77(2): 69-80.

Siwulski M, Budka A, Rzymski P, Gąsecka M, Kalac P, Budzynska S, Magdziak Z, Niedzielski P, Mleczek P and Mleczek M. 2020. Worldwide basket survey of multielemental composition of white button mushroom *Agaricus bisporus. Chemosphere.* 239:1-10.

Smith DW. 1978. Water relations of microorganisms in nature. In DJ Kushner ed. Microbial life in extreme environments. Academic Press, New York, NY. 369-380.

Smith JF, Wood DA and Thurston CF. 1995. Growth measurement of Agaricus mycelium in composted substrates as an indicator of compost selectivity and mushroom productivity. *Mushroom Science*. 14: 293-301.

Smith JF. 1990. Composted substrates used in the cultivation of the white button mushroom, *Agaricus bisporus. Aspects of Applied Biology*. 24: 131-143.

Smith JL and Collins HP. 2007. Composting. In: Soil microbiology, ecology and biochemistry (3rd edition), EA Paul, (Ed), Academic Press, Burlington. 483-486.

Sofi B, Mushtaq A and Khan M. 2014. Effect of different grains and alternate substrates on oyster mushroom (*Pleurotus ostreatus*) production. *African Journal of Microbiology Research.* 8: 1474-1479.

Sofi JA, Bangroo SA and Chesti MUH. 2016. Practical manual for analysis of soil, water fertilizer and manure. Daya Publishing House. 1-168.

Sorensen A, Lubeck M, Lubeck PS and Ahring BK. 2013. Fungal beta-glucosidases: a bottleneck in industrial use of lignocellulosic materials. *Biomolecules*. 3(3): 612–631.

Sparling GP, Femor TR and Wood DA. 1982. Measurement of the microbial biomass in composted wheat straw and the possible contribution of the biomass to the nutrition of *Agaricus bisporus. Soil Biology and Biochemistry*. 14: 609-611.

Stoller BB. 1943. Preparation of synthetic composts for mushroom culture. *Plant Physiology*. 18: 397-414.

Stoller BB. 1952. Studies on the function of the casing for mushroom beds. *MGA Bulletin*. 34: 289-297.

Stoller BB. 1978. Detection and evaluation of carbon monoxide, ethylene and oxidants in mushroom beds. *Mushroom Science*. 10: 445-449.

Straatsma G and Samson RA. 1993. Taxonomy of *Scytalidium thermophilum*, an important thermophilic fungus in mushroom compost. *Mycological Research*. 97: 321-328.

Straatsma G, Gerrits JPG, Augustijn MPAM, Opden Camp HJM, Vogels GD and Van Griensven L. 1989. Population dynamics of *Scytalidium thermophilum* in mushroom compost and stimulatory effects on growth rate and yield of *Agaricus bisporus*. *The Journal of General Microbiology*. 135: 751-759.

Straatsma G, Gerrits JPG, Gerrits TM, Op denCamp HJM and Van Griensven L. 1991. Growth kinetics of *Agaricus bisporus* mycelium on solid substrate (Mushroom compost). *The Journal of General Microbiology*. 137: 1471-1477.

Straatsma G, Olijnsma JW, Gerrits JPG, Amsing JGM, Op Den Camp HJM and Van Griensven L. 1994a. Inoculation of *Scytalidium thermophilum* in button mushroom compost and its effect on yield. *Applied and Environmental Microbiology*. 3049-3054

Straatsma G, Samson RA, Olijnsma TW, Op den Camp HJM, Gerrits JPG and Van Griensven L. 1994. Ecology of thermophilic fungi in mushroom compost, with emphasis on *Scytalidium thermophilum* and growth stimulation of *Agaricus bisporus* mycelium. *Applied and Environmental Microbiology*. 60: 454-58.

Sumner JL, Morgan ED and Evans HC. 1969. The effect of growth temperature on fatty acid composition of fungi in the order Mucorale. *Canadian Journal of Microbiology*. 15: 515-520.

Sunar NM, Stentiford EI, Stewart DI and Fletcher LA. 2009. The process and pathogen behaviour in composting: A review. Proceeding UMT-MSD 2009 post graduate seminar 2009. University Malaysia Terengganu, Malaysian student department UK & Institute for transport studies university of Leeds. 78- 87.

Suwannarach N, KumlaJ, Zhao Y and Kakumyan P. 2022. Impact of cultivation substrate and microbial community on improving mushroom productivity: A review. *Biology*. 11(4): 569p.

Tansey MR. 1971. Agar-diffusion assay of cellulolytic ability of thermophilic fungi. *Archives of Microbiology*. 77: 1-11.

Tewari RP and Ahlawat OP. 2007. Recycling of agro-wastes from microbial protein production through mushroom production. Souvenir cum Abstracts - International Conference on mushrooms and biotechnology held at National Research Centre for Mushroom, Solan on Feb 10-11, 2007. *Mushroom Society of India*. 85p.

Tewari RP and Sohi HS. 1976. Studies on the use of paddy straw and maize stalks as substitutes for wheat straw to prepare synthetic compost for cultivation of European mushroom *Agaricus bisporus*. *Indian Journal of Mushrooms*. 2(2): 18-20.

Thai M, Safianowicz K and Bell TL. 2022. Dynamics of microbial community and enzyme activities during preparation of *Agaricus bisporus* compost substrate. *International Society for Microbial Ecology*. 2: 88p.

Thapa CD, Munjal RL and Seth PK. 1977. The mushroom nematodes and their control. *Indian Journal of Mushrooms.* 3: 1-11.

Till O. 1962. Champignonkultur auf sterilisiertemnahrsubstrat und die wiederverwendung von abgetragenemkompost. *Mushroom Science*. 5: 127-33.

Tiquia SM, Tam NFY and Hodgkiss IJ. 1996. Microbial activities during composting of spent-manure sawdust litter at different moisture content. *Bioresource Technology.* 55: 201-206.

Tournefort J. 1707. Observations sur la naissance et sur la culture des champignons. *Academie Royale des Sciences*. 58- 66.

Tripathi J, Kesharwani L and Mishra MK. 2019. Quality assessment of carbohydrate, protein, and energy in protein powder as claimed constituents. *Journal of Pharmacognosy and Phytochemistry.* 8(4): 2559-2563

Tshinyangu KK. 1996. Effect of grass hay substrate on nutritional value of *Pleurotus ostreatus* var. *Columbinus Die Nahrung*. 40: 79-83.

Tsiklinskaya P. 1899. Sur les mucedinees thermophiles. *Annales de l'Institut Pasteur*, Paris 13: 500-505.

Tuomela M, Vikman M, Hatakka A and Itavaara M. 2000. Biodegradation of lignin in a compost environment: A review. *Bioresource Technology*. 72: 169-183.

Uddin MJ, Haque S, Haque ME, Bilkis S and Biswas AK. 2012. Effect of different substrate on growth and yield of button mushroom. *Journal of Environmental Sciences and Natural Resources.* 5(2): 177-180.

Ulziijargal E and Leun Mau J. 2011. Nutrient compositions of culinary-medicinal mushroom fruiting bodies and mycelia. *International Journal of Medicinal Mushrooms*. 13(4): 343-349.

Upadhyay CR, Verma NR, Singh KS and Yadav CM. 2002. Effect of organic nitrogen supplementation in *pleurotus* species. The 4th ICMBMP. National research centre for mushroom, Chambaghat, Sola, HP- India. 1- 11.

Upadhyay RC, Kaur A, Kumari D, Semwal KC and Gulati A. 2008. New records and taxonomy of Agaricales from North-Western Himalaya. *Journal of Mycology and Plant Pathology.* 38: 24-39.

Vajna B, Szili D, Nagy A and Marialigeti K. 2012. An improved sequence aided TRFLP analysis of bacterial succession during oyster mushroom substrate preparation. *Microbial Ecology*. 64(3): 702- 713.

Van Elsas JD, Chiurazzi M, Mallon CA, Elhottova D, Kristufek V and Salles JF. 2012. Microbial diversity determines the invasion of soil by a bacterial pathogen. *Proceedings of the National Academy of Sciences*. 109: 1159-1164

Van Soest PJ. 2006. Rice straw, the role of silica and treatments to improve quality. *Animal Feed Science and Technology*. 130(130): 137-171.

Verma BN, Prasad PK and Sahu KK. 2013. Mushrooms: Edible and medicinal. Daya Publishing House. 1-34.

Vetter J. 2003. Chemical composition of fresh and conserved *Agaricus bisporus* mushroom. *European Food Research and Technology*. 217: 10-12.

Vieira FR and Pecchia JA. 2018. An exploration into the bacterial community under different pasteurization conditions during substrate preparation (composting- Phase II) for *Agaricus bisporus* cultivation. *Microbial Ecology*. 75: 318-330.

Vijay B and Pathak A. 2014. Exploitation of thermophilic fungi in compost production for white button mushroom (*Agaricus bisporus*) cultivation - A review. Proceedings of 8th International conference on mushroom biology and mushroom products, New Delhi, India. 62p.

Vijay B, Mediratta V and Ahlawat OP. 2007. Evaluation of different compost formulations for white button mushroom cultivation. National Research Centre for Mushroom, Chambhaghat Solan H.P. *Journal of Mushroom*. 25- 29.

Vijay B. 1996. Investigations on compost microflora and crop improvement in *Agaricus bisporus* (Lange) Sing. Ph.D. H.P.U., Shimla, India. 216p.

Vos AM, Heijboer A, Boschker HTS, Bonnet B, Lugones LG and Wosten HAB. 2017. Microbial biomass in compost during colonization of *Agaricus bisporus*. *AMB Express*. 7: 12p.

Vos AM, Jurak E, de Gijsel P, Ohm RA, Henrissat B, Lugones LG, Kabel MA and Wosten HAB. 2018. Production of α-1, 3-l-arabinofuranosidase active on substituted xylan does not improve compost degradation by *Agaricus bisporus*. *Plos One*. 13 (7): 1-12.

Waksman SA and Cordon TC. 1939. Thermophilic decomposition of plant residues in compost by pure and mixed cultures of micro-organisms. *Soil Science*. 47:217-224.

Waksman SA and Reneger CA. 1934. Artificial manure for mushroom production. *Mycologia*. 26: 38-45.

Waksman SA, Cordon TC and Hulpio EN. 1939a. Influence of temperature upon the micro-biological population and decomposition processes in composts of stable manure. *Soil Science*. 47: 81-112.

Waksman SA, Unbreit WW and Cordon TC. 1939b. Thermophilic actinomycetes and fungi in soil and in composts. *Soil Science*. 47: 37-60.

Wang L, Mao J, Zhao H, Li M, Wei Q, Zhou Y and Shao H. 2016. Comparison of characterization and microbial communities in rice straw- and wheat straw-based compost for *Agaricus bisporus* production. *Journal of Industrial Microbiology and Biotechnology*. 43: 1249-1260.

Wani BA, Bodha RH and Wani AH. 2010. Nutritional and medicinal importance of mushrooms. *Journal of Medicinal Plants Research*. 4: 2598-2604.

Wariishi H, Valli K and Gold MH. 1992. Manganese (II) oxidation by manganese peroxidase from the basidiomycete *Phanerochaete chrysosporium*. Kinetic mechanism and role of chelators. *Journal of Biological Chemistry*. 267: 23688-23695.

Wasser KS. 2002. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Applied Microbiology and Biotechnology*. 60: 258-274.

Weber J, Chen Y, Jamroz E and Miano T. 2018. Preface: Humic substances in the environment. *Journal of Soils and Sediments.* 18: 2665-2667.

Welinder KG. 1992. Super family of plant, fungal and bacterial peroxidases. *Current Opinion in Structural Biology*. 2: 388-393.

Wiegant WM, Wery J, Buitenhuis ET and De Bont JA. 1992. Growth promoting effect of thermophilic fungi on the mycelium of the edible mushroom, *Agaricus bisporus*. *Applied and Environmental Microbiology.* 58: 2654-2659.

Wiegant WM. 1992. Growth characteristics of the thermophilic fungus *Scytalidium thermophilum* in relation to production of mushroom compost. *Applied and Environmental Microbiology.* 58(4): 1301-1307.

Wood DA and Fermor TR. 1981.Nutrition of *Agaricus bisporus* in Compost. *The International Society for Mushroom Science.* 11(2): 6.

Wood DA and Goodenough PW. 1977. Fruiting of *Agaricus bisporus. Archives of Microbiology*. 114(2): 161-165

Wood DA. 1979. A method for estimating biomass of *Agaricus bisporus* in a solid substrate, composted wheat straw. *Biotechnology Letters*. 1: 255-260.

Wu Y and Shin H. 2016. Cellulase from the fruiting bodies and mycelia of edible mushrooms: a review. *Journal of Mushroom*. 14: 127-135.

Wuest PJ and Fahy HK. 1992. Spent mushroom compost: its origins and components and impact on water quality. *Mushroom News*. 40: 27-33.

Wuest PJ. 1982. Penn State handbook for commercial mushroom growers Pennsylvania state University Press University Park, PA.129p.

Xu ZJ, Zhang LJ, Hu HK and Zhang GW. 2012. The relationship between lignin peroxidase and manganese peroxidase production capacities and cultivation periods of mushrooms. *Microbial Biotechnology*. 6: 241-247.

Yan K, Liu ZN, Cheng PY and Zhao HY. 2019. Status quo of agricultural waste resource utilization in China and its prospects. *Agricultural Outlook.* 15(7): 62-65.

Yang SQ, Yan QJ, Jiang ZQ, Li LT, Tian HM and Wang YZ. 2006. High-level of xylanase production by the thermophilic *Paecilomyces themophila* J18 on wheat straw in solid state fermentation. *Bioresource Technology.* 97: 1794-1800.

Yawalkar, KS, Agarwal, JP and Bokde S. (2016). Manures and Fertilizers. Agri-Horticultural Pub. House. 332p.

Yeo SG and Hayes WA. 1979. A new medium for casing mushroom beds. *Mushroom Science*. 10: 217-229.

Yeoman CJ, Han Y, Dodd D, Schroeder CM, Mackie RI and Cann IK. 2010. Thermostable enzymes as biocatalysts in the biofuel industry. *Advances in Applied Microbiology*. 70: 1-55.

Zhang M, Puri A K, Govender A, Wang Z, Singh S and Permaul K. 2015. The multi-chitinolytic enzyme system of the compost-dwelling thermophilic fungus *Thermomyces lanuginosus*. *Process Biochemistry*. 50 (2): 237-244.

Zhang Y, Chen M, GuoJ, Liu N, Yi W, Yuan Z and Zeng L. 2022. Study on dynamic changes of microbial community and lignocellulose transformation mechanism during green waste composting. *Engineering in Life Sciences*. 22(5): 376-390.

Zhang YHP, Himmel ME and Mielenz JR. 2006. Outlook for cellulase improvement, screening and selection strategies. *Biotechnology Advances*. 24: 452-481.

Zhao J and Kwan HS. 1999. Characterization, molecular cloning, and differential expression analysis of laccase genes from the edible mushroom *Lentinula edodes*. *Applied Environmental Microbiology*. 65: 4908-4913.

Zhao M, Zhang C, Zeng G, Huang D, Xu P and Cheng M. 2015. Growth, metabolism of *Phanerochaete chrysosporium* and route of lignin degradation in response to cadmium stress in solid-state fermentation. *Chemosphere*. 138: 560-567.

Zheng BX, Ibrahim M, Zhang DP, Bi QF, Li HZ, Zhou GW and Yang XR .2018. Identification and characterization of inorganic-phosphate-solubilising bacteria from agricultural fields with a rapid isolation method. *AMB express*. 8(1): 47.

Zheng FQ, Yang RC and Liu RX. 1995. Effects of C/N ratios in compost on nutrient transformation and yield and quality of *Agaricus bisporus*. *Acta Agriculture*. 11: 33-38.

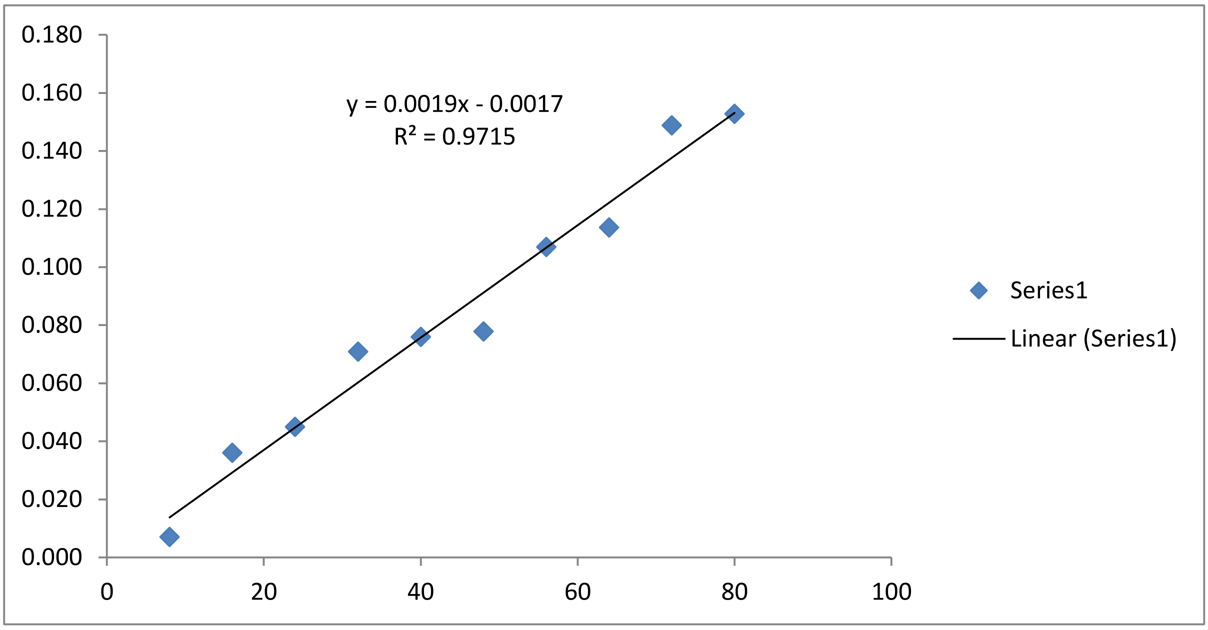
Zhou GX, Xu XF and Qiu XM. 2019. Biochar influences the succession of microbial communities and the metabolic functions during rice straw composting with pig manure. *Bioresource Technology*. 272: 10-18.

APPENDIX – I

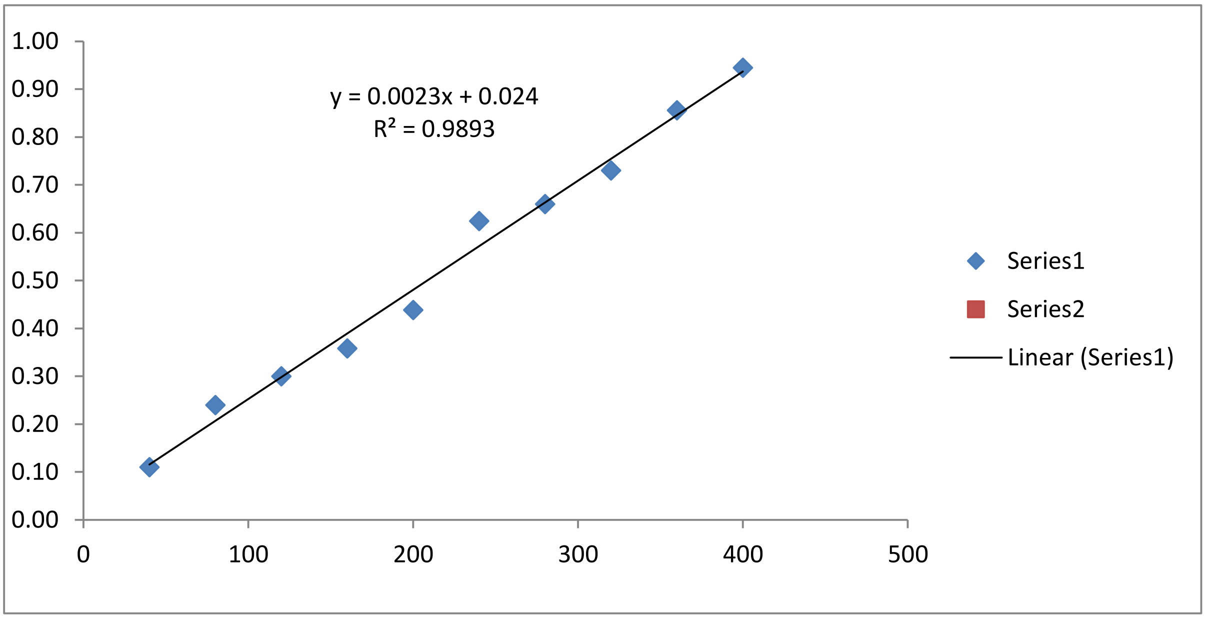
COMPOSITION OF DIFFERENT SOLID MEDIA

Constituents	Quantity (g)
Potato Dextrose Agar (PDA)	
Pealed potato	250
Dextrose	20
Agar	20
Distilled water	1000ml
Malt Extract Agar (MEA)	
Malt Extract	20
Glucose	20
Peptone	1
Agar	20
Distilled water	1000ml
Yeast Phosphate Soluble Starch Agar	
Yeast extract	4
$K_2 HPO_4$	1
$MgSO_4.7H_2O$	0.5
Soluble Starch	15
Agar	20
Distilled water	1000ml
Laccase hydrolysis media	
Guaiacol	0.04%
Potato(peeled)	200
Dextrose	20
Agar	20
pH	1000
Distilled water	5.5
Cellulose/xylan hydrolysis media	
Cellulose	1%
Beef extract	3
NaCl	5
Peptone	5
Agar	20
Distilled water	1000ml
*For xylan hydrolysis 1% of xylan was added in place of cellulose powder	

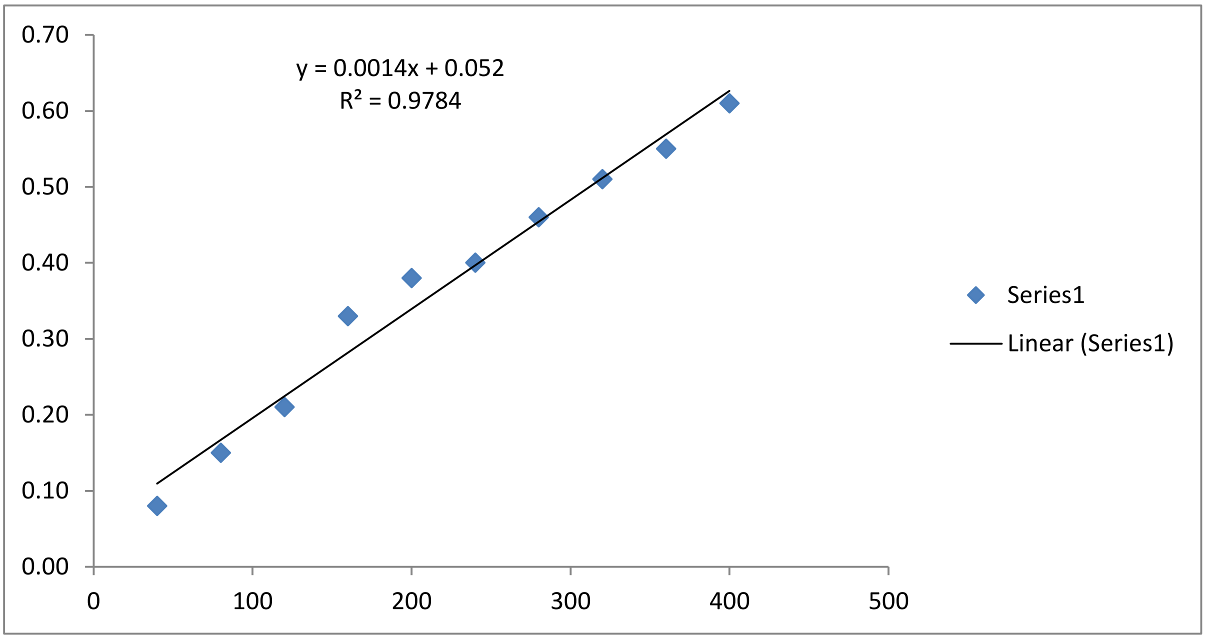
APPENDIX- II



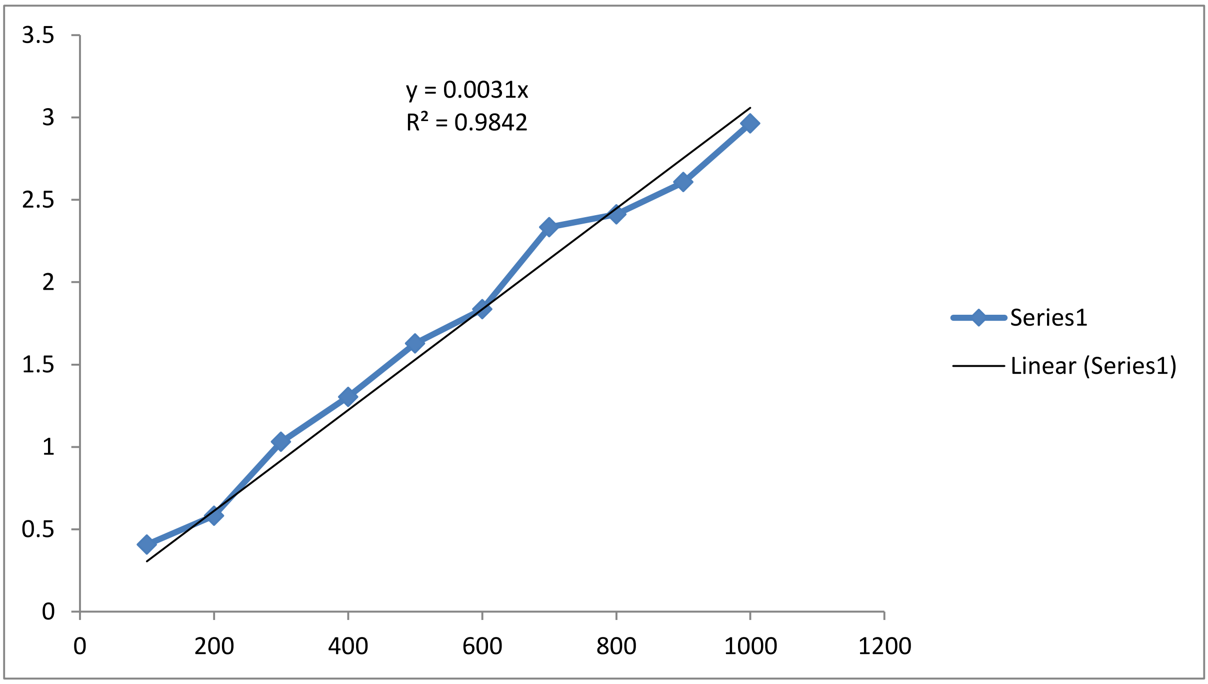
Standard curve of p-nitrophenol (80 µg/ml) for β-Glucosidase assay (cellulase enzyme estimation)



Standard curve of glucose (0.4mg/ml) for CarboxymethylCellulase (CMC) and Filter Paperase (FPase) assay (cellulase enzyme estimation)



Standard curve of Xylose (0.4 mg/ml) for Xylanase enzyme assay



Standard curve of Catechol (1000mg/ml) for Phenol estimation

APPENDIX- III

Sequence of TF4- *Thermomyces lanuginosus*

ACAAACTGCTCCGGAAGGGTCCAACCTTCTGGCAAGAATTTGACCTTTCAGCAGGCTGTGCTCCCGGGCCGGGACCCCTCCTTGAGCGCGCCACAGAGCCCCATACGCTCGAGGACCGGACGCCACGCCGCCGCTGCCTTTCAGGCCCGTCCTCTCCAAACAAGGTGACGGCGAGTCCAACACACAAGCCGAGCTTGAGGATTCGCAATGACGCTCGTTACAGGCATGCCCCCCGGAATACCAGAGGGCGCTATGTACGTTCGAAGATTCCATGATTCACTGAATTCTGCAATTCACATTATTATCGCATTTCGCTGCCTTCTTCAGCGATGCCGGAACCTAGAGATCCATTGTTGAAAGTTTGAACGATTGCATTTCGTCACTCAGACTATCCTCTGCATTCACAAAACCGCTCACATTGAGTGCCTCTGCCTGACACGGGCCCCGGGGGCACACCGCGCCCCGGGACGACCCCCCCGTCTGAACACCGCACGAGTGGGCCCGCCAAAGCAACACTAGGCTGTGTAGACACGTTCGTCAGATGTGACCCGACTGCATTCCCGCACTCGGTAATGATCCTTCCGCATGTCACCCTACAGAAATTACGAGTGCGGGAACCCAGTCGGCTCCATTCCCCCCGTTTTACCCCTTTTTTTTTTTTGGGGCCCCCCCTGTTTTCCCCGGGGTTCCCCCCCCGGGGCCCCCCCCCCCCCCCCCCCCCCCCTCCCTTTTTTTTTTTTTTTTATTTTTTTTGTGAAAAAAAAAAAAA

Sequence of TF6- *Melanocarpus albomyces*

ATCAACCAAGGGAGCAACTTGGCCTTTCCCAACCGGGTGCTTTCGACGGGTTCGGGCCGGCCCGGCCTTGGAGCGAGGATACGAGTGCCTACTACGCTCGGAGTGACAGCGAGCCCGCCACGGTTTTCAGGGCCTGCGGGCAGCCGCAGATCCCCAACACAAGCCCGGGGCTTGATGGTTGAATGACGCTCGAACAGGCATGCCCGCCAGAATACTGGCGGGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCAGAACCAAGAGATCCGTTGTTGAAAGTTTTGACTTATTCAGTACAGAGACTCAGAGAGGCGTACAAGTGCCAAGGAGTTTTGTGTACCTCCGGCGGGCCGCCCCCTCCACGGGGGCGAAGGGGCCCCCCCCGGCCGAGGCCGGGGCGGCCCGAACCCGCCGAACCACCGGCTGTAAGGTATGTTCACGATGGTGGGAGGGAGTTTTGCAACCCTGTAATAATCCCCCCGCAGGATAAACTAAGAG

APPENDIX- IV

Estimation of Nitrogen in different compost formulations

		Quantity (kg)	**Moisture (%)**	**Dry Wt (Kg)**	**Nitrogen (%)**	**Nitrogen (kg)**
T1 (PS+WS) (1.5:1)	Paddy straw (PS)	600	10	540	0.60	3.24
	Wheat straw (WS)	400	10	360	0.40	1.44
	Chicken Manure	600	20	480	2.50	12
	Wheat Bran	100	10	90	2	1.8
	Urea	15	0	15	46	6.9
	Gypsum	30	0	30	0	0
	Total	1745		1515		25.38
	Total Nitrogen %	**1.68**				
T2 (PS+WS) (3:1)	Paddy straw	750	10	675	0.60	4.05
	Wheat straw	250	10	225	0.40	0.9
	Chicken Manure	600	20	480	2.50	12
	Wheat Bran	100	10	90	2	1.8
	Urea	15	0	15	46	6.9
	Gypsum	30	0	30	0	0
	Total	1745		1515		25.65
	Total Nitrogen %	**1.69**				
T3 (WS+PS) (1:1)	Paddy straw	500	10	450	0.60	2.7
	Wheat straw	500	10	450	0.40	1.8
	Chicken Manure	600	20	480	2.50	12
	Wheat Bran	100	10	90	2	1.8
	Urea	15	0	15	46	6.9
	Gypsum	30	0	30	0	0
	Total	1745		1515		25.2
	Total Nitrogen %	**1.66**				
T4 (PS)	Paddy straw	1000	10	900	0.60	5.4
	Wheat straw	0	10	0	0.40	0
	Chicken Manure	600	20	480	2.50	12
	Wheat Bran	100	10	90	2	1.8
	Urea	15	0	15	46	6.9
	Gypsum	30	0	30	0	0
	Total	1745		1515		26.1
	Total Nitrogen %	**1.72**				
T5 (WS) Control	Paddy straw	0	10	0	0.60	0
	Wheat straw	1000	10	900	0.40	3.6
	Chicken Manure	600	20	480	2.50	12
	Wheat Bran	100	10	90	2	1.8
	Urea	15	0	15	46	6.9
	Gypsum	30	0	30	0	0
	Total	1745		1515		24.3
	Total Nitrogen %	**1.60**				

PS= Paddy straw, WS= Wheat straw

Total Nitrogen % = $\frac{\text{Nitrogen (Kg)}}{\text{Dry weight of compost}}$ X100

APPENDIX- V

ANOVA TABLES

ANOVA 1: Analysis of variance for evaluation of different compost formulation for number of days taken for completion of spawn run of *Agaricus bisporus* during year 2021 (Table 4.2)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	11.211	2.803	6.011	0.00430
Error	15	6.994	0.466		
Total	19	18.205			

ANOVA 2: Analysis of variance for evaluation of different compost formulation for number of days taken for completion of case run of *Agaricus bisporus* during year 2021 (Table 4.2)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	11.048	2.762	7.145	0.00199
Error	15	5.798	0.387		
Total	19	16.846			

ANOVA 3: Analysis of variance for evaluation of different compost formulation for number of days taken for pinhead formation of *Agaricus bisporus* during year 2021 (Table 4.2)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	11.552	2.888	13.869	0.00006
Error	15	3.124	0.208		
Total	19	14.675			

ANOVA 4: Analysis of variance for evaluation of different compost formulation for number of days taken for first harvest of *Agaricus bisporus* during year 2021 (Table 4.2)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	38.073	9.518	8.955	0.00067
Error	15	15.944	1.063		
Total	19	54.017			

ANOVA 5: Analysis of variance for evaluation of different compost formulation for yield of *Agaricus bisporus* during year 2021 (Table 4.2)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	85.692	21.423	10,307.297	0.00000
Error	15	0.031	0.002		
Total	19	85.723			

ANOVA 6: Analysis of variance for evaluation of different compost formulation for average fruit body weight of *Agaricus bisporus* during year 2021 (Table 4.2)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	70.612	17.653	21,361.817	0.00000
Error	15	0.012	0.001		
Total	19	70.625			

ANOVA 7: Analysis of variance for evaluation of different compost formulation for diameter of *Agaricus bisporus* pileus (Table 4.3)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	0.830	0.207	6.403	0.00326
Error	15	0.486	0.032		
Total	19	1.316			

ANOVA 8: Analysis of variance for evaluation of different compost formulation for thickness of *Agaricus bisporus* pileus (Table 4.3)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	1.317	0.329	16.814	0.00002
Error	15	0.294	0.020		
Total	19	1.611			

ANOVA 9: Analysis of variance for evaluation of different compost formulation for length of *Agaricus bisporus* stipe (Table 4.3)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	0.534	0.133	78.775	0.00000
Error	15	0.025	0.002		
Total	19	0.559			

ANOVA 10: Analysis of variance for evaluation of different compost formulation for diameter of *Agaricus bisporus* stipe (Table 4.3)

Source of Variation	**DF**	**Sum of Squares**	**Mean Squares**	**F-Calculated**	**Significance**
Treatment	4	2.123	0.531	3.879	0.02339
Error	15	2.052	0.137		
Total	19	4.175			

ANOVA 11: Analysis of variance for evaluation of different compost formulation for moisture content (Table 4.4)

Source of Variation	**DF**	**Sum of Squares**	**Mean Squares**	**F-Calculated**	**Significance**
Treatment	4	190.646	47.662	11.021	0.00023
Error	15	64.870	4.325		
Total	19	255.516			

ANOVA 12: Analysis of variance for evaluation of different compost formulation for pH (Table 4.4)

Source of Variation	**DF**	**Sum of Squares**	**Mean Squares**	**F-Calculated**	**Significance**
Treatment	4	0.874	0.219	4.669	0.01198
Error	15	0.702	0.047		
Total	19	1.576			

ANOVA 13: Analysis of variance for evaluation of different compost formulation for EC (Table 4.4)

Source of Variation	**DF**	**Sum of Squares**	**Mean Squares**	**F-Calculated**	**Significance**
Treatment	4	0.770	0.192	4.924	0.00976
Error	15	0.586	0.039		
Total	19	1.356			

ANOVA 14: Analysis of variance for evaluation of different compost formulation for organic matter (Table 4.4)

Source of Variation	**DF**	**Sum of Squares**	**Mean Squares**	**F-Calculated**	**Significance**
Treatment	4	41.551	10.388	24,731.167	0.00000
Error	15	0.006	0.000		
Total	19	41.558			

ANOVA 15: Analysis of variance for evaluation of different compost formulation for bulk density (Table 4.4)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	0.016	0.004	40.465	0.00000
Error	15	0.001	0.000		
Total	19	0.017			

ANOVA 16: Analysis of variance for evaluation of different compost formulation for particle density (Table 4.4)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	0.015	0.004	7.937	0.00121
Error	15	0.007	0.000		
Total	19	0.022			

ANOVA 17: Analysis of variance for evaluation of different compost formulation for porosity (Table 4.4)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	84.977	21.244	13.600	0.00007
Error	15	23.431	1.562		
Total	19	108.409			

ANOVA 18: Analysis of variance for evaluation of different compost formulation for organic carbon (Table 4.8)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	43.921	10.980	18.936	0.00001
Error	15	8.698	0.580		
Total	19	52.619			

ANOVA 19: Analysis of variance for evaluation of different compost formulation for nitrogen (Table 4.8)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	0.095	0.024	6.514	0.00302
Error	15	0.055	0.004		
Total	19	0.150			

ANOVA 20: Analysis of variance for evaluation of different compost formulation for phosphorus (Table 4.8)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	0.115	0.029	7.076	0.00208
Error	15	0.061	0.004		
Total	19	0.176			

ANOVA 21: Analysis of variance for evaluation of different compost formulation for potassium (Table 4.8)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	1.463	0.366	67.067	0.00000
Error	15	0.082	0.005		
Total	19	1.545			

ANOVA 22: Analysis of variance for evaluation of different compost formulation for calcium (Table 4.8)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	4.906	1.226	83.162	0.00000
Error	15	0.221	0.015		
Total	19	5.127			

ANOVA 23: Analysis of variance for evaluation of different compost formulation for magnesium (Table 4.8)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	0.080	0.020	7.014	0.00216
Error	15	0.043	0.003		
Total	19	0.122			

ANOVA 24: Analysis of variance for evaluation of different compost formulation for copper (Table 4.9)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	14,453.623	3,613.406	7,155.551	0.00000
Error	15	7.575	0.505		
Total	19	14,461.197			

ANOVA 25: Analysis of variance for evaluation of different compost formulation for zinc (Table 4.9)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	397.622	99.406	129.095	0.00000
Error	15	11.550	0.770		
Total	19	409.172			

ANOVA 26: Analysis of variance for evaluation of different compost formulation for iron (Table 4.9)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	2,216,608.000	554,152.000	2,691.801	0.00000
Error	15	3,088.000	205.867		
Total	19	2,219,696.000			

ANOVA 27: Analysis of variance for evaluation of different compost formulation for manganese (Table 4.9)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	4,683.200	1,170.800	44.801	0.00000
Error	15	392.000	26.133		
Total	19	5,075.200			

ANOVA 28: Analysis of variance for evaluation of different compost formulation for C: N ratio (Table 4.10)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	17.032	4.258	53.295	0.00000
Error	15	1.198	0.080		
Total	19	18.230			

ANOVA 29: Analysis of variance for evaluation of different compost formulation for C: P ratio (Table 4.10)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	762.694	190.673	278.272	0.00000
Error	15	10.278	0.685		
Total	19	772.972			

ANOVA 30: Analysis of variance for evaluation of different compost formulation for C: K ratio (Table 4.10)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	100.460	25.115	50.740	0.00000
Error	15	7.425	0.495		
Total	19	107.885			

ANOVA 31: Analysis of variance for evaluation of different compost formulation for N: P ratio (Table 4.10)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	10.309	2.577	65.849	0.00000
Error	15	0.587	0.039		
Total	19	10.896			

ANOVA 32: Analysis of variance for evaluation of different compost formulation for N: K ratio (Table 4.10)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	1.160	0.290	5.902	0.00465
Error	15	0.737	0.049		
Total	19	1.897			

ANOVA 33: Analysis of variance for evaluation of different compost formulation for P: K ratio (Table 4.10)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	0.007	0.002	95.839	0.00000
Error	15	0.000	0.000		
Total	19	0.007			

ANOVA 34: Analysis of variance for evaluation of different compost formulation for silica content before spawning stage (Table 4.14)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	52.614	13.153	42.981	0.00000
Error	15	4.590	0.306		
Total	19	57.204			

ANOVA 35: Analysis of variance for evaluation of different compost formulation for silica content during fruit development stage (Table 4.14)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	64.872	16.218	4.208	0.01759
Error	15	57.812	3.854		
Total	19	122.684			

ANOVA 36: Analysis of variance for evaluation of different compost formulation for silica content after harvest stage (Table 4.14)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	37.342	9.335	46.041	0.00000
Error	15	3.041	0.203		
Total	19	40.383			

ANOVA 37: Analysis of variance for evaluation of different compost formulation for microbial count (Table 4.15)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	147.200	36.800	6.494	0.00307
Error	15	85.000	5.667		
Total	19	232.200			

ANOVA 38: Analysis of variance for evaluation of different compost formulation for laccase enzyme activity before spawning stage (Table 4.16)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	0.018	0.004	32.016	0.00000
Error	15	0.002	0.000		
Total	19	0.020			

ANOVA 39: Analysis of variance for evaluation of different compost formulation for laccase enzyme activity during fruit development stage (Table 4.16)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	0.078	0.019	20.283	0.00001
Error	15	0.014	0.001		
Total	19	0.092			

ANOVA 40: Analysis of variance for evaluation of different compost formulation for laccase enzyme activity after harvest stage (Table 4.16)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	0.020	0.005	34.884	0.00000
Error	15	0.002	0.000		
Total	19	0.022			

ANOVA 41: Analysis of variance for evaluation of different compost formulation for MnP enzyme activity before spawning stage (Table 4.17)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	27.734	6.933	406.133	0.00000
Error	15	0.256	0.017		
Total	19	27.990			

ANOVA 42: Analysis of variance for evaluation of different compost formulation for MnP enzyme activity during fruit development stage (Table 4.17)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	127.149	31.787	4,956.671	0.00000
Error	15	0.096	0.006		
Total	19	127.245			

ANOVA 43: Analysis of variance for evaluation of different compost formulation for MnP enzyme activity after harvest stage (Table 4.17)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	164.064	41.016	1,013.063	0.00000
Error	15	0.607	0.040		
Total	19	164.672			

ANOVA 44: Analysis of variance for evaluation of different compost formulation for Filter paperase enzyme activity before spawning stage (Table 4.18)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	257.606	64.401	29,392.005	0.00000
Error	15	0.033	0.002		
Total	19	257.638			

ANOVA 45: Analysis of variance for evaluation of different compost formulation for Filter paperase enzyme activity during fruit development stage (Table 4.18)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	612.967	153.242	18,368.837	0.00000
Error	15	0.125	0.008		
Total	19	613.093			

ANOVA 46: Analysis of variance for evaluation of different compost formulation for Filter paperase enzyme activity after harvest stage (Table 4.18)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	163.995	40.999	2,567.098	0.00000
Error	15	0.240	0.016		
Total	19	164.235			

ANOVA 47: Analysis of variance for evaluation of different compost formulation for CMCase enzyme activity before spawning stage (Table 4.19)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	529.389	132.347	1,163.087	0.00000
Error	15	1.707	0.114		
Total	19	531.096			

ANOVA 48: Analysis of variance for evaluation of different compost formulation for CMCase enzyme activity during fruit development stage (Table 4.19)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	88.648	22.162	33.728	0.00000
Error	15	9.856	0.657		
Total	19	98.504			

ANOVA 49: Analysis of variance for evaluation of different compost formulation for CMCase enzyme activity after harvest stage (Table 4.19)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	822.914	205.729	582.364	0.00000
Error	15	5.299	0.353		
Total	19	828.213			

ANOVA 50: Analysis of variance for evaluation of different compost formulation for β -Glucosidase enzyme activity before spawning stage (Table 4.20)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	20.730	5.183	243.201	0.00000
Error	15	0.320	0.021		
Total	19	21.050			

ANOVA 51: Analysis of variance for evaluation of different compost formulation for β -Glucosidase enzyme activity during fruit development stage (Table 4.20)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	98.976	24.744	28,606.773	0.00000
Error	15	0.013	0.001		
Total	19	98.989			

ANOVA 52: Analysis of variance for evaluation of different compost formulation for β -Glucosidase enzyme activity after harvest stage (Table 4.20)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	3.484	0.871	30.351	0.00000
Error	15	0.431	0.029		
Total	19	3.915			

ANOVA 53: Analysis of variance for evaluation of different compost formulation for xylanase enzyme activity before spawning stage (Table 4.21)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	8.539	2.135	331.441	0.00000
Error	15	0.097	0.006		
Total	19	8.635			

ANOVA 54: Analysis of variance for evaluation of different compost formulation for xylanase enzyme activity during fruit development stage (Table 4.21)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	50.450	12.612	3,126.233	0.00000
Error	15	0.061	0.004		
Total	19	50.510			

ANOVA 55: Analysis of variance for evaluation of different compost formulation for xylanase enzyme activity after harvest stage (Table 4.21)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	15.208	3.802	1,557.069	0.00000
Error	15	0.037	0.002		
Total	19	15.245			

ANOVA 56: Analysis of variance for evaluation of different compost formulation for number of days taken for completion of spawn run of *Agaricus bisporus* during year 2022 (Table 4.22)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	19.044	4.761	2,036.733	0.00000
Error	15	0.035	0.002		
Total	19	19.080			

ANOVA 57: Analysis of variance for evaluation of different compost formulation for number of days taken for completion of case run of *Agaricus bisporus* during year 2022 (Table 4.22)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	9.947	2.487	5.756	0.00517
Error	15	6.480	0.432		
Total	19	16.427			

ANOVA 58: Analysis of variance for evaluation of different compost formulation for number of days taken for pinhead formation of *Agaricus bisporus* during year 2022 (Table 4.22)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	20.613	5.153	1,625.471	0.00000
Error	15	0.048	0.003		
Total	19	20.661			

ANOVA 59: Analysis of variance for evaluation of different compost formulation for number of days taken for first harvest of *Agaricus bisporus* during year 2022 (Table 4.22)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	43.387	10.847	2,917.180	0.00000
Error	15	0.056	0.004		
Total	19	43.442			

ANOVA 60: Analysis of variance for evaluation of different compost formulation for diameter of *Agaricus bisporus* pileus during year 2022 (Table 4.23)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	1.165	0.291	74.791	0.00000
Error	15	0.058	0.004		
Total	19	1.224			

ANOVA 61: Analysis of variance for evaluation of different compost formulation for thickness of *Agaricus bisporus* pileus during year 2022(Table 4.23)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	1.008	0.252	141.036	0.00000
Error	15	0.027	0.002		
Total	19	1.035			

ANOVA 62: Analysis of variance for evaluation of different compost formulation for length of *Agaricus bisporus* stipe during year 2022 (Table 4.23)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	0.848	0.212	227.128	0.00000
Error	15	0.014	0.001		
Total	19	0.862			

ANOVA 63: Analysis of variance for evaluation of different compost formulation for diameter of *Agaricus bisporus* stipe during year 2022(Table 4.23)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	0.752	0.188	106.814	0.00000
Error	15	0.026	0.002		
Total	19	0.778			

ANOVA 64: Analysis of variance for evaluation of different compost formulation for yield of *Agaricus bisporus* during year 2022 (Table 4.24)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	68.467	17.117	929.909	0.00000
Error	15	0.276	0.018		
Total	19	68.743			

ANOVA 65: Analysis of variance for qualitative estimation of fungal isolates for cellulose enzyme production (Table 4.27)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	17	10,412.444	612.497	78.007	0.00000
Error	54	424.000	7.852		
Total	71	10,836.444			

ANOVA 66: Analysis of variance for qualitative estimation of fungal isolates for xylanase enzyme production (Table 4.27)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	17	7,569.778	445.281	96.181	0.00000
Error	54	250.000	4.630		
Total	71	7,819.778			

ANOVA 67: Analysis of variance for qualitative estimation of fungal isolates for laccase enzyme production (Table 4.27)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	17	3,850.278	226.487	40.632	0.00000
Error	54	301.000	5.574		
Total	71	4,151.278			

ANOVA 68: Analysis of variance for qualitative estimation of fungal isolates for manganese peroxide enzyme production (Table 4.27)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	17	3,032.000	178.353	31.474	0.00000
Error	54	306.000	5.667		
Total	71	3,338.000			

ANOVA 69: Analysis of variance for quantitative estimation of fungal isolates for laccase enzyme production (Table 4.28)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	17	0.030	0.002	153.167	0.00000
Error	54	0.001	0.000		
Total	71	0.030			

ANOVA 70: Analysis of variance for quantitative estimation of fungal isolates for xylanase enzyme production (Table 4.28)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	17	503.284	29.605	6,545.681	0.00000
Error	54	0.244	0.005		
Total	71	503.528			

ANOVA 71: Analysis of variance for quantitative estimation of fungal isolates for manganese dependent peroxides enzyme production (Table 4.28)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	17	88.775	5.222	1,825.285	0.00000
Error	54	0.154	0.003		
Total	71	88.929			

ANOVA 72: Analysis of variance for quantitative estimation of fungal isolates for CMCase enzyme production (Table 4.28)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	17	7,678.818	451.695	43,373.687	0.00000
Error	54	0.562	0.010		
Total	71	7,679.380			

ANOVA 73: Analysis of variance for quantitative estimation of fungal isolates for Filterpapease enzyme production (Table 4.28)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	17	9,562.190	562.482	9,819.191	0.00000
Error	54	3.093	0.057		
Total	71	9,565.283			

ANOVA 74: Analysis of variance for quantitative estimation of fungal isolates for β-Glucosidase enzyme production (Table 4.28)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	17	61.106	3.594	37.376	0.00000
Error	54	5.193	0.096		
Total	71	66.299			

ANOVA 75: Analysis of variance for mycelial extension rate of *A. bisporus* on sterilized compost inoculated with thermophilic fungi singly and in combinations in beaker (mm/day) (Table 4.29)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	16.000	5.333	30.770	0.00001
Error	12	2.080	0.173		
Total	15	18.080			

ANOVA 76: Analysis of variance for radial growth rate of *A. bisporus* on sterilized compost inoculated with thermophilic fungi singly and in combinations in Petri dishes (mm/day) (Table 4.29)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	46.447	15.482	492.146	0.00000
Error	12	0.378	0.031		
Total	15	46.824			

ANOVA 77: Analysis of variance for evaluation of compost formulation for number of days taken for completion of spawn run of *Agaricus bisporus* by using thermophilic fungi (Table 4.30)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	15.226	5.075	14.319	0.00029
Error	12	4.253	0.354		
Total	15	19.480			

ANOVA 78: Analysis of variance for evaluation of compost formulation for number of days taken for completion of case run of *Agaricus bisporus* by using thermophilic fungi (Table 4.30)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	10.816	3.605	9.118	0.00203
Error	12	4.745	0.395		
Total	15	15.560			

ANOVA 79: Analysis of variance for evaluation of compost formulation for number of days taken for pin head formation of *Agaricus bisporus* by using thermophilic fungi (Table 4.30)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	8.231	2.744	10.566	0.00110
Error	12	3.116	0.260		
Total	15	11.346			

ANOVA 80: Analysis of variance for evaluation of compost formulation for number of days taken for first harvest of *Agaricus bisporus* by using thermophilic fungi (Table 4.30)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	40.326	13.442	3,456.463	0.00000
Error	12	0.047	0.004		
Total	15	40.373			

ANOVA 81: Analysis of variance for evaluation of compost with thermophilic fungi and its effect on yield of mushroom (Table 4.30)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	50.415	16.805	31.886	0.00001
Error	12	6.324	0.527		
Total	15	56.740			

ANOVA 82: Analysis of variance for evaluation of compost with thermophilic fungi and its effect on average weight of mushroom (Table 4.30)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	21.512	7.171	17.419	0.00011
Error	12	4.940	0.412		
Total	15	26.452			

ANOVA 83: Analysis of variance for evaluation of compost with thermophilic fungi and its effect on diameter of pileus (cm) of mushroom (Table 4.31)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	2.720	0.907	5.978	0.00985
Error	12	1.820	0.152		
Total	15	4.540			

ANOVA 84: Analysis of variance for evaluation of compost with thermophilic fungi and its effect on thickness of pileus (cm) of mushroom (Table 4.31)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	0.350	0.117	3.889	0.03741
Error	12	0.360	0.030		
Total	15	0.710			

ANOVA 85: Analysis of variance for evaluation of compost with thermophilic fungi and its effect on diameter of stipe (cm)) of mushroom (Table 4.31)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	1.400	0.467	3.705	0.04271
Error	12	1.512	0.126		
Total	15	2.912			

ANOVA 86: Analysis of variance for evaluation of compost with thermophilic fungi and its effect on length of stipe (cm) of mushroom (Table 4.31)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	1.360	0.453	6.476	0.00745
Error	12	0.840	0.070		
Total	15	2.200			

ANOVA 87: Analysis of variance for evaluation of compost formulation for moisture content by using thermophilic fungi (Table 4.32)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	52.444	17.481	49.171	0.00000
Error	12	4.266	0.356		
Total	15	56.710			

ANOVA 88: Analysis of variance for evaluation of compost formulation for pH by using thermophilic fungi (Table 4.32)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	0.306	0.102	5.760	0.01118
Error	12	0.212	0.018		
Total	15	0.518			

ANOVA 89: Analysis of variance for evaluation of compost formulation for EC by using thermophilic fungi (Table 4.32)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	1.699	0.566	9.608	0.00164
Error	12	0.707	0.059		
Total	15	2.407			

ANOVA 90: Analysis of variance for evaluation of compost formulation for organic matter by using thermophilic fungi (Table 4.32)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	85.984	28.661	4.012	0.03430
Error	12	85.726	7.144		
Total	15	171.710			

ANOVA 91: Analysis of variance for evaluation of compost formulation for bulk density by using thermophilic fungi (Table 4.32)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	0.011	0.004	192.116	0.00000
Error	12	0.000	0.000		
Total	15	0.011			

ANOVA 92: Analysis of variance for evaluation of compost formulation for particle density by using thermophilic fungi (Table 4.32)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	0.015	0.005	373.473	0.00000
Error	12	0.000	0.000		
Total	15	0.015			

ANOVA 93: Analysis of variance for evaluation of compost formulation for porosity by using thermophilic fungi (Table 4.32)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	52.986	17.662	342.647	0.00000
Error	12	0.619	0.052		
Total	15	53.604			

ANOVA 94: Analysis of variance for evaluation of compost formulation for organic carbon by using thermophilic fungi (Table 4.36)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	4.598	1.533	56.810	0.00000
Error	12	0.324	0.027		
Total	15	4.922			

ANOVA 95: Analysis of variance for evaluation of compost formulation for nitrogen by using thermophilic fungi (Table 4.36)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	0.759	0.253	80.794	0.00000
Error	12	0.038	0.003		
Total	15	0.797			

ANOVA 96: Analysis of variance for evaluation of compost formulation for phosphorus by using thermophilic fungi (Table 4.36)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	0.053	0.018	5.803	0.01091
Error	12	0.037	0.003		
Total	15	0.090			

ANOVA 97: Analysis of variance for evaluation of compost formulation for potassium by using thermophilic fungi (Table 4.36)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	0.916	0.305	13.481	0.00038
Error	12	0.272	0.023		
Total	15	1.188			

ANOVA 98: Analysis of variance for evaluation of compost formulation for calcium by using thermophilic fungi (Table 4.36)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	4.057	1.352	513.528	0.00000
Error	12	0.032	0.003		
Total	15	4.089			

ANOVA 99: Analysis of variance for evaluation of compost formulation for magnesium by using thermophilic fungi (Table 4.36)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	0.394	0.131	44.818	0.00000
Error	12	0.035	0.003		
Total	15	0.430			

ANOVA 100: Analysis of variance for evaluation of compost formulation for copper by using thermophilic fungi (Table 4.37)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	89.204	29.735	38.381	0.00000
Error	12	9.297	0.775		
Total	15	98.501			

ANOVA 101: Analysis of variance for evaluation of compost formulation for zinc by using thermophilic fungi (Table 4.37)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	644.000	214.667	8.474	0.00271
Error	12	304.000	25.333		
Total	15	948.000			

ANOVA 102: Analysis of variance for evaluation of compost formulation for iron by using thermophilic fungi (Table 4.37)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	266,000.000	88,666.667	128.347	0.00000
Error	12	8,290.000	690.833		
Total	15	274,290.000			

ANOVA 103: Analysis of variance for evaluation of compost formulation for manganese by using thermophilic fungi (Table 4.37)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	94,171.000	31,390.333	574.213	0.00000
Error	12	656.000	54.667		
Total	15	94,827.000			

ANOVA 104: Analysis of variance for evaluation of compost formulation for C: N ratio by using thermophilic fungi (Table 4.38)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	3.431	1.144	87.642	0.00000
Error	12	0.157	0.013		
Total	15	3.588			

ANOVA 105: Analysis of variance for evaluation of compost formulation for C: P ratio by using thermophilic fungi (Table 4.38)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	408.935	136.312	30.449	0.00001
Error	12	53.721	4.477		
Total	15	462.657			

ANOVA 106: Analysis of variance for evaluation of compost formulation for C: K ratio by using thermophilic fungi (Table 4.38)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	14.001	4.667	10,118.341	0.00000
Error	12	0.006	0.000		
Total	15	14.006			

ANOVA 107: Analysis of variance for evaluation of compost formulation for N: P ratio by using thermophilic fungi (Table 4.38)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	0.340	0.113	1,178.734	0.00000
Error	12	0.001	0.000		
Total	15	0.341			

ANOVA 108: Analysis of variance for evaluation of compost formulation for N: K ratio by using thermophilic fungi (Table 4.38)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	0.026	0.009	1,163.489	0.00000
Error	12	0.000	0.000		
Total	15	0.027			

ANOVA 109: Analysis of variance for evaluation of compost formulation for P: K ratio by using thermophilic fungi (Table 4.38)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	0.008	0.003	35.377	0.00000
Error	12	0.001	0.000		
Total	15	0.009			

ANOVA 110: Analysis of variance for evaluation of compost formulation for silica content by using thermophilic fungi (Table 4.39)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	3.891	1.297	48.437	0.00000
Error	12	0.321	0.027		
Total	15	4.213			

ANOVA 111: Analysis of variance for evaluation of compost formulation for microbial count by using thermophilic fungi (Table 4.43)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	401.688	133.896	17.608	0.00011
Error	12	91.250	7.604		
Total	15	492.938			

ANOVA 112: Analysis of variance for evaluation of compost formulation for temperature profiles in compost piles at zero day by using thermophilic fungi (Table 4.44)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	103.698	34.566	3,789.791	0.00000
Error	12	0.109	0.009		
Total	15	103.808			

ANOVA 113: Analysis of variance for evaluation of compost formulation for temperature profiles in compost piles at first turning by using thermophilic fungi (Table 4.44)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	368.968	122.989	20,917.394	0.00000
Error	12	0.071	0.006		
Total	15	369.038			

ANOVA 114: Analysis of variance for evaluation of compost formulation for temperature profiles in compost piles at second turning by using thermophilic fungi (Table 4.44)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	10.780	3.593	871.920	0.00000
Error	12	0.049	0.004		
Total	15	10.829			

ANOVA 115: Analysis of variance for evaluation of compost formulation for temperature profiles in compost piles at third turning by using thermophilic fungi (Table 4.43)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	32.124	410.708	997.743	0.00000
Error	12	0.129	0.011		
Total	15	32.252			

ANOVA 116: Analysis of variance for evaluation of compost formulation for temperature profiles in compost piles at fourth turning by using thermophilic fungi (Table 4.44)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	19.043	6.348	1,306.866	0.00000
Error	12	0.058	0.005		
Total	15	19.102			

ANOVA 117: Analysis of variance for evaluation of compost formulation for temperature profiles in compost piles before spawning by using thermophilic fungi (Table 4.44)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	8.138	2.713	682.433	0.00000
Error	12	0.048	0.004		
Total	15	8.186			

ANOVA 118: Analysis of variance for evaluation of compost formulation for moisture content in compost piles at zero day by using thermophilic fungi (Table 4.45)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	30.995	10.332	3,407.033	0.00000
Error	12	0.036	0.003		
Total	15	31.031			

ANOVA 119: Analysis of variance for evaluation of compost formulation for moisture content in compost piles at first turning by using thermophilic fungi (Table 4.45)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	46.270	15.423	14,144.651	0.00000
Error	12	0.013	0.001		
Total	15	46.283			

ANOVA 120: Analysis of variance for evaluation of compost formulation for moisture content in compost piles at second turning by using thermophilic fungi (Table 4.45)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	24.134	8.045	1,806.491	0.00000
Error	12	0.053	0.004		
Total	15	24.187			

ANOVA 121: Analysis of variance for evaluation of compost formulation for moisture content in compost piles at third turning by using thermophilic fungi (Table 4.45)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	4.008	1.336	597.938	0.00000
Error	12	0.027	0.002		
Total	15	4.035			

ANOVA 122: Analysis of variance for evaluation of compost formulation for moisture content in compost piles at fourth turning by using thermophilic fungi (Table 4.45)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	5.832	1.944	1,581.460	0.00000
Error	12	0.015	0.001		
Total	15	5.846			

ANOVA 123: Analysis of variance for evaluation of compost formulation for moisture content in compost piles before spawning by using thermophilic fungi (Table 4.45)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	52.444	17.481	49.171	0.00000
Error	12	4.266	0.356		
Total	15	56.710			

ANOVA 124: Analysis of variance for evaluation of compost formulation for pH in compost piles at zero day by using thermophilic fungi (Table 4.46)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	0.086	0.029	3.511	0.04922
Error	12	0.098	0.008		
Total	15	0.184			

ANOVA 125: Analysis of variance for evaluation of compost formulation for pH in compost piles at first turning by using thermophilic fungi (Table 4.46)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	0.442	0.147	17.062	0.00013
Error	12	0.104	0.009		
Total	15	0.546			

ANOVA 126: Analysis of variance for evaluation of compost formulation for pH in compost piles at second turning by using thermophilic fungi (Table 4.46)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	0.341	0.114	89.993	0.00000
Error	12	0.015	0.001		
Total	15	0.356			

ANOVA 127: Analysis of variance for evaluation of compost formulation for pH in compost piles at third turning by using thermophilic fungi (Table 4.46)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	0.246	0.082	58.485	0.00000
Error	12	0.017	0.001		
Total	15	0.263			

ANOVA 128: Analysis of variance for evaluation of compost formulation for pH in compost piles at fourth turning by using thermophilic fungi (Table 4.46)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	0.238	0.079	119.679	0.00000
Error	12	0.008	0.001		
Total	15	0.246			

ANOVA 129: Analysis of variance for evaluation of compost formulation for pH in compost piles before spawning by using thermophilic fungi (Table 4.46)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	0.306	0.102	5.760	0.01118
Error	12	0.212	0.018		
Total	15	0.518			

ANOVA 130: Analysis of variance for evaluation of compost formulation for carbon percent in compost piles at zero day by using thermophilic fungi (Table 4.47)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	24.565	8.188	33.572	0.00000
Error	12	2.927	0.244		
Total	15	27.492			

ANOVA 131: Analysis of variance for evaluation of compost formulation for carbon percent in compost piles before spawning by using thermophilic fungi (Table 4.47)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	4.598	1.533	56.810	0.00000
Error	12	0.324	0.027		
Total	15	4.922			

ANOVA 132: Analysis of variance for evaluation of compost formulation for nitrogen percent in compost piles at zero day by using thermophilic fungi (Table 4.48)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	0.033	0.011	6.588	0.00701
Error	12	0.020	0.002		
Total	15	0.054			

ANOVA 133: Analysis of variance for evaluation of compost formulation for nitrogen percent in compost piles before spawning by using thermophilic fungi (Table 4.48)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	0.759	0.253	80.794	0.00000
Error	12	0.038	0.003		
Total	15	0.797			

ANOVA 134: Analysis of variance for evaluation of compost formulation for C: N ratio in compost piles at zero day (initial stage of compost) by using thermophilic fungi (Table 4.49)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	31.886	10.629	361,615.649	0.00000
Error	12	0.000	0.000		
Total	15	31.887			

ANOVA 135: Analysis of variance for evaluation of compost formulation for C: N ratio in compost piles before spawning (Final stage of compost) by using thermophilic fungi (Table 4.49)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	3.431	1.144	87.642	0.00000
Error	12	0.157	0.013		
Total	15	3.588			

ANOVA 136: Analysis of variance for evaluation of compost formulation for laccase enzyme activity before spawning stage by using thermophilic fungi (Table 4.50)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	0.036	0.012	20.169	0.00006
Error	12	0.007	0.001		
Total	15	0.043			

ANOVA 137: Analysis of variance for evaluation of compost formulation for laccase enzyme activity during fruit development stage by using thermophilic fungi (Table 4.50)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	0.045	0.015	1,541.273	0.00000
Error	12	0.000	0.000		
Total	15	0.045			

ANOVA 138: Analysis of variance for evaluation of compost formulation for laccase enzyme activity after harvest stage by using thermophilic fungi (Table 4.50)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	0.051	0.017	73.981	0.00000
Error	12	0.003	0.000		
Total	15	0.054			

ANOVA 139: Analysis of variance for evaluation of compost formulation for MnP enzyme activity before spawning stage by using thermophilic fungi (Table 4.51)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	3.244	1.081	10,600.610	0.00000
Error	12	0.001	0.000		
Total	15	3.245			

ANOVA 140: Analysis of variance for evaluation of compost formulation for MnP enzyme activity during fruit development stage by using thermophilic fungi (Table 4.51)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	31.517	10.506	928,362.715	0.00000
Error	12	0.000	0.000		
Total	15	31.517			

ANOVA 141: Analysis of variance for evaluation of compost formulation for MnP enzyme activity after harvest stage by using thermophilic fungi (Table 4.51)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	18.585	6.195	421,428.055	0.00000
Error	12	0.000	0.000		
Total	15	18.585			

ANOVA 142: Analysis of variance for evaluation of different compost formulation for Filter paperase enzyme activity before spawning stage by using thermophilic fungi (Table 4.52)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	295.004	98.335	161,495.204	0.00000
Error	12	0.007	0.001		
Total	15	295.012			

ANOVA 143: Analysis of variance for evaluation of different compost formulation for Filter paperase enzyme activity during fruit development stage by using thermophilic fungi (Table 4.52)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	417.828	139.276	531,512.376	0.00000
Error	12	0.003	0.000		
Total	15	417.831			

ANOVA 144: Analysis of variance for evaluation of different compost formulation for Filter paperase enzyme activity after harvest stage by using thermophilic fungi (Table 4.52)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	56.385	18.795	113,713.438	0.00000
Error	12	0.002	0.000		
Total	15	56.387			

ANOVA 145: Analysis of variance for evaluation of compost formulation for CMCase enzyme activity before spawning stage by using thermophilic fungi (Table 4.53)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	752.708	250.903	2,067,792.938	0.00000
Error	12	0.001	0.000		
Total	15	752.710			

ANOVA 146: Analysis of variance for evaluation of different compost formulation for CMCase enzyme activity during fruit development stage by using thermophilic fungi (Table 4.53)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	275.798	91.933	305,758.941	0.00000
Error	12	0.004	0.000		
Total	15	275.801			

ANOVA 147: Analysis of variance for evaluation of compost formulation for CMCase enzyme activity after harvest stage by using thermophilic fungi (Table 4.53)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	203.100	67.700	585,011.106	0.00000
Error	12	0.001	0.000		
Total	15	203.101			

ANOVA 148: Analysis of variance for evaluation of compost formulation for β - Glucosidase enzyme activity before spawning stage by using thermophilic fungi (Table 4.54)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	15.109	5.036	17,480.735	0.00000
Error	12	0.003	0.000		
Total	15	15.113			

ANOVA 149: Analysis of variance for evaluation of compost formulation for β - Glucosidase enzyme activity during fruit development stage by using thermophilic fungi (Table 4.54)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	58.141	19.380	19,116.573	0.00000
Error	12	0.012	0.001		
Total	15	58.153			

ANOVA 150: Analysis of variance for evaluation of by using thermophilic fungi. compost formulation for β -Glucosidase enzyme activity after harvest stage by using thermophilic fungi (Table 4.54)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	20.982	6.994	325.958	0.00000
Error	12	0.257	0.021		
Total	15	21.239			

ANOVA 151: Analysis of variance for evaluation of compost formulation for xylanase enzyme activity before spawning stage by using thermophilic fungi (Table 4.55)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	13.470	4.490	26,268.910	0.00000
Error	12	0.002	0.000		
Total	15	13.472			

ANOVA 152: Analysis of variance for evaluation of compost formulation for xylanase enzyme activity during fruit development stage by using thermophilic fungi (Table 4.55)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	55.649	18.550	849,369.141	0.00000
Error	12	0.000	0.000		
Total	15	55.649			

ANOVA 153: Analysis of variance for evaluation of compost formulation for xylanase enzyme activity after harvest stage by using thermophilic fungi (Table 4.55)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	32.513	10.838	20,863.542	0.00000
Error	12	0.006	0.001		
Total	15	32.519			

ANOVA 154: Analysis of variance for evaluation of compost formulation for carbohydrate content in white button mushroom by using thermophilic fungi (Table 4.56)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	321.465	107.155	63,547.468	0.00000
Error	12	0.020	0.002		
Total	15	321.486			

ANOVA 155: Analysis of variance for evaluation of compost formulation for protein content in white button mushroom by using thermophilic fungi (Table 4.56)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	196.961	65.654	42,796.315	0.00000
Error	12	0.018	0.002		
Total	15	196.980			

ANOVA 156: Analysis of variance for evaluation of compost formulation for ash content in white button mushroom by using thermophilic fungi (Table 4.56)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	7.715	2.572	46.613	0.00000
Error	12	0.662	0.055		
Total	15	8.377			

ANOVA 157: Analysis of variance for evaluation of compost formulation for fat in white button mushroom by using thermophilic fungi (Table 4.56)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	6.879	2.293	40.962	0.00000
Error	12	0.672	0.056		
Total	15	7.550			

ANOVA 158: Analysis of variance for evaluation of compost formulation for crude fibre content in white button mushroom by using thermophilic fungi (Table 4.56)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	2.428	0.809	214.501	0.00000
Error	12	0.045	0.004		
Total	15	2.473			

ANOVA 159: Analysis of variance for evaluation of compost formulation for phenol content in white button mushroom by using thermophilic fungi (Table 4.56)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	341.251	113.750	35,132.268	0.00000
Error	12	0.039	0.003		
Total	15	341.290			

ANOVA 160: Analysis of variance for evaluation of compost formulation for energy produce by white button mushroom by using thermophilic fungi (Table 4.56)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	65.188	21.729	4.515	0.02433
Error	12	57.750	4.813		
Total	15	122.938			

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Title of Thesis : Studies on utilization of paddy straw for compost preparation of button mushroom (*Agaricus bisporus*)
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ABSTRACT

The compost for white button mushroom is generally been prepared using wheat straw all over the world but complete dependency on wheat straw is restricting the growth of mushroom industry severely. Thus it is imperative to search for alternative agriculture residue for white button mushroom compost preparation. During the study, we have tried to develop new composting formulations using paddy straw and supplementing wheat straw with paddy straw in different proportions. During the study, a combination of wheat straw and paddy straw (1:1) proved promising formulation taking minimum days for first harvest (28.02 days) and maximum yield (19.98 kg/ 100kg compost). To enhance the process of composting and make the compost more selective and productive, a total of eighteen thermophilic fungi were isolated from the different compost formulations. The thermophilic fungi were screened for enzyme production potential (laccase, manganese peroxide, cellulase, and xylanase) responsible for composting, growth and development of button mushroom. Two selected thermophiles showing maximum enzyme activities (TF4 *Thermomyces lanuginosus* and TF6 *Melanocarpus albomyces*) were identified based on morphological and molecular tools. The optimized compost formulae i.e., T3 (PS: WS, 1:1) was inoculated with the selected thermophilic fungi, singly and in combination. Inoculation of consotium of *Thermomyces lanuginosus* and *Melanocarpus albomyces* in the compost of paddy straw + wheat straw (1:1) took the minimum days for first harvest (26.01) and gave maximum yield (23.98 kg/100 kg compost). The consortium of isolates also increased the availability of macronutrients (N, P, K, Ca and Mg) and micronutrients (Cu, Fe, Zn and Mn) due to enhanced enzyme activities. It also helped in decreasing the silica content in compost. Nutritional value of the mushroom produced was also recorded be better with compost inoculated with *Thermomyces lanuginosus* and *Melanocarpus albomyces*. Overall, it was recorded that paddy straw and wheat straw combinations with inoculated thermophiles (TF4 and TF6) gave promising results.

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