DEVELOPMENT OF COMPOST FORMULATION BASED ON PADDY STRAW AND MAIZE STALKS FOR CULTIVATION OF *Agaricus bisporus*

Thesis

Submitted to the Punjab Agricultural University
in partial fulfilment of the requirements
for the degree of

MASTER OF SCIENCE
in
MICROBIOLOGY
(Minor Subject: Biochemistry)

By

Sukhmandeep Kaur
(L-2016-BS-296-M)

Department of Microbiology
College of Basic Sciences and Humanities

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CERTIFICATE-I

This is to certify that the thesis entitled, **"Development of compost formulation based on paddy straw and maize stalks for cultivation of *Agaricus bisporus*"** submitted for the degree of **Master of Science**, in the subject of **Microbiology** (**Minor subject: Biochemistry)** of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Sukhmandeep Kaur (L-2016-BS-296-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

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

(Dr. S. Kapoor)
Major Advisor
Senior Mycologist
Department of Microbiology
Punjab Agricultural University
Ludhiana-141 004, Punjab

CERTIFICATE – II

This is to certify that the thesis entitled, "**Development of compost formulation based on paddy straw and maize stalks for cultivation of *Agaricus bisporus*"** submitted by **Sukhmandeep Kaur (L-2016-BS-296-M)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Master of Science** in the subject of **Microbiology (Minor subject: Biochemistry)** has been approved by the Student's Advisory Committee along with External Examiner after an oral examination on the same.

_____
(Dr. S. Kapoor)
Major Advisor

_____
Dr. S. S. Kanwar
External Examiner
Directorate of Research
CSK, Himachal Pradesh
Agricultural University,
Palampur-176062 (H.P.)

_____
(Dr. S. Kapoor)
Head of the Department

_____
(Dr. Gurinder Kaur Sangha)
Dean Postgraduate Studies

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Abstract

Composting is carried out under aerobic thermogenesis to prepare a selective substrate for *Agaricus bisporus* production. Conventionally, wheat straw based synthetic compost is used for its production. Use of paddy straw as such tends to make the composting process anaerobic leading to low quality compost and low yields. In the present study, an alternative high yielding compost formulation based on paddy straw + maize stalks as a substitute to the existing wheat straw based compost has been made. The physico-chemical characteristics of the composts revealed their moisture, pH, temperature, C:N ratio to be within the range of 58.7-67.3%, 6.4-7.9, 42.1-76.4°C, 37.7:1-16.6:1 respectively. During different stages of mushroom cultivation i.e at zero day of compost, final grade compost, pin head stage, after first flush and at crop termination, the straw was analysed for proximate composition. NDF, ADF, cellulose and hemicellulose content was significantly reduced upto pin head stage and ranged from (70.20-46.20%), (52.41-33.72%), (36.37-20.69%), (17.59-8.57%) respectively and stable thereafter in all the compost. Total ash content was showed increment and ranged from 10.90-21.37%. There was no decline in the lignin content during different stages of composting and crop production. The yield data indicated that maximum yield (13.6 kg/q compost) in paddy straw + maize stalk (1:1) compost with 1563 fruit bodies/q compost. It was observed that paddy straw + maize stalk (1:1, w/w) compost was better degraded than paddy straw + maize stalk (2:1,w/w) and paddy straw composts. From the present study, it concluded that paddy straw + maize stalk (1:1, w/w) compost was the best formulation which could be further exploited for large scale production of mushrooms for small and marginal farmers.

Key words: *Agaricus bisporus*, paddy straw, maize stalk, composting, proximate analysis.

Signature of Major Advisor **Signature of the Student**

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CHAPTER I

INTRODUCTION

Mushrooms have been considered as ingredient of gourmet cuisine across the globe; especially for their unique flavor and have been valued by humankind as a culinary wonder. Generally, edible mushrooms are used not only for their nutritional value but are now in demand for their medicinal properties and therapeutic attributes (Chang 1996, Ribeiro and Salvadori 2003, Borchers *et al* 2004). At the same time, nutritionists also regard mushrooms since they are low in calories, fats, essential fatty acids and high in vegetable proteins, vitamins and minerals (Murugkar and Subbulakshim 2005). Moreover to reduce hunger and malnutrition in a world of rising food prices, cultivation of mushrooms is a very reliable and advantageous option (Lakshmipathy *et al* 2017). The ever increasing population, shrinking of agricultural land, environmental issues, water availability and quality food demands is going to be the issues of concern during coming decades. To meet these challenges diversification in food portfolio in areas like horticulture is of vital importance. Utilizing these wastes for growing mushrooms can enhance income and impart higher level of sustainability (Tewari and Pandey 2002).

Undoubtedly, India has remarkable potential for the growth of agro based industries and mushroom production with processing is an emerging area with an excellent export potential (Kumar *et al* 2018). More than 2000 species of mushrooms exist in nature, but only around 25 are widely accepted as food and few are commercially cultivated (Valverde *et al* 2015). Among various mushrooms *Agaricus bisporus* (button mushroom), *Calocybe indica* (milky mushroom), *Pleurotus ostreatus* (oyster mushroom), *Volvariella* spp. (Paddy Straw Mushroom), *Lentinula edodes* (Shiitake mushroom) and *Auricularia polytricha* (Jew's ear mushroom) are commonly available edible mushrooms in Punjab. Cultivation of edible mushrooms is still considered the only profitable way to utilize lignocellulosic waste material from agriculture and forestry. Consequently, a better understanding of lignocellulosic degradation and ecological adaptation in ***A. bisporus*** may offer opportunities to improve mushroom yields or simplify substrate preparation for commercial cultivation (Gonaus *et al* 2015). Some of them have been shown to possess wide beneficial properties such as antioxidant, anticancer, antimicrobial. The world production of mushrooms has increased from about from a meager one million tons in 1978 to about 27 million tons in 2012. The current production of mushrooms in India has approximately more than 1.30 lakh ton mark with the annual growth rate of more than 15% (Royse 2014).

The button mushroom (*Agaricus bisporus*) is the most widely cultivated and consumed mushroom throughout the world contributing about 40% of total world mushroom production and about 80% of total mushrooms produced in India (Royse 2014). The button

mushroom is grown on composted substrates and a variety of substrates have been used in composting the world over. The main purpose of composting is to release the nutrients in the straw and supplements and to transform them in such a way that they are suitable for the nutrition of this mushroom. These composted substrates show huge variation in the yield of button mushroom indicating thereby the role of various physicochemical factors and the compositional changes in the substrate (Sharma 1991) as these are important factors contributing to the composting process and hence to yield. The nitrogen available in the ingredients is converted into proteins of the microorganisms and further a lignin humus complex is also formed during composting both of which later on are utilized by the mushroom mycelium as food. Kaur and Khanna (2002) discovered carbon: nitrogen (C:N) proportion 16.4-20.3:1 for compost prepared as mixes of wheat straw and paddy straw in the proportion of 1:1 (w/w), 1:2 (w/w) and paddy straw alone. Andrade *et al* (2008) revealed ideal C:N ratio for *A. bisporus* compost supplemented with perfect compost has C:N ratio in the of 15 to 21:1% and not greater than 30:1. Blanco and Almandros (2002) examined that wheat straw with 5% dry weight horse manure acclimated to C: N ratio of 23:1 with urea was required during progressive stages of wheat straw composting.

Compost for cultivation of the white button mushroom (*A. bisporus*) is prepared from a mixture of organic materials subjected to a composting process making it selective for growth of *A.bisporus* (Colak 2004). *A. bisporus* have a requirement for a separate ‘‘casing layer’’ that has specific physical, chemical and microbiological properties which stimulate and promote the initiation of primordia (Taherzadeh *et al* 2013). Mushroom mycelial growth and mushroom development is not only related to genetic factors but also depends on environmental, chemical, and microbiological conditions (Pardo *et al* 2004). Casing soil protects the compost against desiccation and supports the mushroom against pests and diseases and provides support for developing sporophores and gas exchange for development and growth of mushrooms (Colauto *et al* 2011). Casing layer provide an environment change in which the mushroom shifts from a vegetative stage to a reproductive one. This happens due to microorganisms present in the casing soil (Gulser and Peksen 2003). Bacteria in the casing soil influence productivity, product quality and uniformity (Barman *et al* 2017).

World production of agricultural wastes is about 4664.63 million tons, of which about 600 million tons is produced in India. According to a recent report, 5 per cent of the global biomass is burnt in India alone, contributing to ozone depletion (Tewari and Ahlawat 2007). Rice is one of the major crops grown in India and produces 23 per cent of rice straw by its weight and it is the most abundant lignocellulosic waste materials in the world (Singh *et al* 2013). Every kilogram of grain harvested is accompanied by production of 1-1.5 kg of the straw. This ratio would give around 650-975 million tons of rice straw per year globally. It is estimated that about 15 million tons of paddy straw is burnt in Punjab. The options for the

disposal of rice straw are limited due to its low bulk density, slow degradation in soil, harboring of rice stem diseases, and high mineral content (Goel and Wati 2016). 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. It represents one of the most efficient biological ways by which these residues can be recycled. 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 ratios (Rana 1998). The already recommended paddy straw formulations by PAU (Khanna and Kapoor 2016) include wheat straw (WS) paddy straw (PS) (1:1, w/w) and WS:PS (1:2, w/w). Ample amount of the paddy straw is available in Punjab in the season of September-March which is favourable for the growth of *Agaricus bisporus.* Therefore, it can solve the problem of storage of wheat straw for use in winter. Moreover, wheat straw is not available easily in areas where paddy is grown due to heavy transportation costs e.g. Southern India. Therefore, paddy straw could be used for compost preparation in button mushroom.

Effective production of button mushroom in synthetic compost from agricultural waste was derived (Todd *et al* 2003). The most broadly utilized agro-waste for the production of eatable mushrooms are wheat straw, rice dust, saw dust or wood chip, sugarcane bagasse, cotton waste, cottonseed body, corn cob, rice or wheat grain, poultry litter, cotton stalks and soybean straw (Pabhabraom *et al* 2007), coffee mash (Martinez and Carrera 1989) have also been utilized for growing edible mushrooms in a few countries. Shandilya (1989) discovered two formulations of paddy straw compost with horse manure (5:1, w/w) and chicken manure (2.5:1, w/w) as high yielding compost formulations. Singh and Solomon (1995) discovered the development of *A. bisporus* on sugarcane lignocellulosic deposits. Mohan (1997) utilized sugarcane waste in a blend with wheat straw and paddy straw for making compost and recorded most elevated yield of 16.8 kg/100 kg compost on WS + sugarcane junk (3:1, w/w) trailed by PS + sugarcane waste (3:1, w/w). Singh (1997) used diverse extents of wheat straw and paddy straw with sugarcane bagasse during composting. Grape stalks and vine shoots as base materials for growing *A. bisporus* were financially feasible and naturally favorable (Pardo *et al* 2007). Municipal source separated food wastage (MSSFW) were likewise considered as a decent substrate for growing *A. bisporus* (Stoknes *et al* 2008) to get a yield of 27.9%. Animal beddings and residues, which are normally high in supplements, have been utilized for mushroom development (Noble *et al* 2002).

Maize is the third most important food crops after rice and wheat. Huge quantities of biomass are generated after harvest of the cobs and farmers have the practice of burning it in the field itself. This residue can be exploited for mushroom cultivation as maize stalks are rich in cellulose (44%) and lignin (14.34%) which can be used by button mushroom for its growth. Maize stalks can also be utilized for making compost, which in turn can be applied to the field to enrich the organic matter of the soil. Sharma and Gupta (1993) detailed that around 80 percent of the agricultural wastage are made out of cellulose, hemicellulose, and lignin that are hard to degrade. Majority of the consumable fungi has enzymatic frameworks that can break these complex substances. Mushroom mycelial growth and primordial development relies upon the lignocellulosic materials and different factors particularly the C:N proportion (Narain *et al* 2008). Ajonia and Tatah (2012) expressed that the biological yield shifted essentially because of substrate composition in various flushes. Further, they discovered that presence of right proportion of alpha cellulose and lignin was the reason of higher rate of mycelium running. Further, paddy straw + chopped maize stalks in combination could provide the desirable physicochemical characteristics required for compost preparation for button mushroom production.

Therefore, the present study has been planned to develop high yielding compost formulation based on paddy straw + maize stalks for cultivation of button mushroom with the following objectives:

- Development of compost formulations based on different combination of paddy straw and maize stalks
- Optimization of physico-chemical characteristics of the developed formulations for production of quality compost
- Evaluation of the developed compost formulations using commercial strains of *A. bisporus*

CHAPTER II

REVIEW OF LITERATURE

Mushroom cultivation represents one of the appealing techniques for changing over the agricultural residues into protein-rich sustenance. *Agaricus bisporus* is the most widely cultivated mushroom in the world. Research throughout the decades has concluded that nitrogen, carbon, phosphorus, sulphur, iron and vitamins are required for white button mushroom development. All the ingredients that contain these compounds are mixed in a fixed proportion and fermented (decomposed) in a set pattern to form compost, which is selective in value for supporting the growth of *Agaricus bisporus* mycelium (Rana 2000).

High quality substrate for growing *A. bisporus* has alluring compound for maintaining physical and biological properties that improves the marketability of the substrate (Suess and Curties 2007). The fruiting bodies of *A. bisporus* degrade the compost to satisfy their supplements requirement (Barr and Parandi 2006). The compost ingredients vary widely in their characteristics, which causes variation in physico-chemical characteristics of composted substrate (Wakchaure *et al* 2013).

The edible fungi under cultivation for the production of sporocarps principally fall into two noteworthy groups: *Ascomycetes* and *Basidiomycetes. Basidiomycetes* are the biggest gathering which contains mushrooms under the order *Agaricales.* The sporocarp demonstrates great diversity in size, shape and consistency. *Agaricus bisporus* follows the taxonomic classification:

Class	Basidiomycetes
Subclass	Holobasidiomycetidae
Order	Agaricales
Family	Agaricaceae
Class	*Agaricus*
Species	*bisporus*

The significant writing identified with the present investigation on *A.bisporus* has been discussed under the following headings:

2.1 Use of substrates for making compost

2.2 Physiological factors responsible for composting

2.2.1 pH, moisture, temperature

2.2.2 C: N

2.3 Proximate analysis

2.1 Use of substrates for making compost

Compost production is the most vital component of *A. bisporus* cultivation process (Vijay and Ahlawat 2002). Wheat straw, straw-bedded with horse manure, chicken manure and gypsum are used for the cultivation of *A. bisporus* (Straatsma 2004). A few compost definitions in light of various agri-residues have been produced and prescribed by various specialists (Tewari and Sohi 1976, Shandilya 1979, Khanna and Kapoor 2016).

Compost is the result of fermentation achieved by the variety of organisms including bacteria, actinomycetes and fungi. These organisms convert and degrade the straw to shape lignin humus complex and furthermore change over the solvent type of nitrogen into microbial cell substances (Waksman and Cordon 1993, Waksman *et al* 1996). This decomposed straw alongside microbial biomass both turn into a organic and inorganic nutrition for the mushroom mycelium (Wood and Fermor 1998). The process of composting is administered by a carefully ordered changing population of organisms (Chang and Hudson 2001). Further, this microflora additionally assumes a key part towards selectivity and conditioning of the compost and makes the growth of competitor microorganisms more difficult (Stanek 2002, Ross and Harris 2003). The experimental results show that the presence of microbial population in both compost and casing soil plays vital role in *A. bisporus* cultivation. The microbial biomass exhibit in compost influences the mycelial spread during spawn run phase, while in casing soil it triggers the induction of reproductive phase of the *A. bisporus* life cycle (Ahlawat and Verma 2001).

In India, mainly four agricultural crops (maize, wheat, rice, and sugarcane) are responsible for producing maximum lignocellulosic biomass in agriculture sector. In the world, rice straw represents one of the most prominent lignocellulosic waste material that includes the leftover residue (stems, leaf sheaths and blades, and panicle remains after threshing). The annual global production of rice straw is 731 million tons and Asia alone produces 667.6 million tons of rice straw annually (Saini *et al* 2015). The agricultural residues have mainly cellulose, hemicellulose and lignin however, rice straw is having more silica content while wheat straw possess large amount of pectin and proteins (Sarkar *et al* 2012). Cocoa shells likewise contain cellulose and lignin which have been found to support good growth of the mycelium of the oyster mushroom and fruit body production. Developing countries may profit essentially from mushroom cultivation by utilizing the abundant lignocellulosic waste (Senyah 1998).

In Southern India, Tewari and Sohi (1976) used paddy straw and maize stalks (1:1, w/w) as a substitute of wheat straw for cultivation of *Agaricus bisporus*. They found that synthetic compost prepared out of maize stalks and paddy straw when mixed in equal

proportions along with other ingredients viz; ammonium sulphate, super sulphate, urea, chalk, gypsum and rice bran was considered as a good medium for mushroom cultivation. Such type of compost produced a yield of 145.5 kg/ton of dry matter, which was quite comparable to wheat straw compost. Mantel *et al* (1972) firstly standardized the method of composting. Shandilya (1989) discovered two formulations of paddy straw compost with horse manure (5:1, w/w) and chicken manure (2.5:1, w/w) as high yielding. Singh and Solomon (1995) announced the development of *A. bisporus* on sugarcane lignocellulosic deposits. Mohan (1997) utilized sugarcane waste in a blend with wheat straw and paddy straw for making compost and recorded most elevated yield of 16.8 kg/100 kg compost on WS + sugarcane junk (3:1, w/w) trailed by PS + sugarcane waste (3:1, w/w). Singh (1997) used diverse extents of wheat straw and paddy straw with sugarcane bagasse during composting.

Two formulation of compost with WS+PS (1:1, w/w) and WS + PS (1:2, w/w) have been prescribed by Punjab Agricultural University for the growers of Punjab (Garcha 1997, Khanna and Kapoor 2016). Rana (1998) additionally demonstrated that paddy straw however, does not give great physical structure to compost but rather gave a decent outcome when blended with WS in a equal amounts. Kaur and Khanna (2001) assessed two synthetic compost preparations; WS+PS (1:1) and WS+PS (1:2) and produced yield in the range of 17.9-23.7 kg/100kg compost. Wheat straw, horse manure or chicken manure give cellulose, hemicelluloses, and lignin which are used by the mushroom mycelium as the carbon source (Uddin *et al* 2012, Straatsma *et al* 2000). Simsek *et al* (2008) reported that wheat straw based compost gave the most astounding mushroom yield (23.01%) that was acquired on wheat straw and pigeon excrement based compost utilizing a blend of peat of Agacbasi (PA) with peat of Caykara (PC) (50+50; v/v) as packaging material. For waste tea leaves based compost, the most elevated mushroom yield (24.90%) were recorded on wheat straw and pigeon manure based compost utilizing a blend of PC with sand (80+20; v/v) as packaging material. Fiore *et al* (1998) studied significant effect of compost, casing and their interactions on the yield of *Agaricus bisporus*. The best outcomes were obtained with compost blend A4 (Hay + Poultry manure + sugarcane torrent + Molasses + Rice flour) joined with packaging blend (B4 9Black soil + yellow soil + sand).

Effective production of button mushroom in synthetic compost from agricultural waste was derived (Todd *et al* 2003). The most broadly utilized agro-waste for the production of eatable mushrooms are wheat straw, rice dust, saw dust or wood chip, sugarcane bagasse, cotton waste, cottonseed body, corn cob, rice or wheat grain, poultry

litter, cotton stalks, soybean straw (Pabhabraom *et al* 2007), coffee mash (Martinez and Carrera 1989) have also been utilized for growing edible mushrooms in a few countries. In Taiwan, the most mainstream agro-waste for mushroom cultivation are rice straw, cotton waste, saw dust or wood chip, rice or wheat grain. The cultivation of exotic mushrooms on agricultural wastes not only reduce disposal problems and residue accumulation but also provide an economical alternative for the production of high quality food and fodder through production of proteins which might contribute significantly to the farmers income (Philippoussis *et al* 2000). Kumar *et al* (2006) studied the suitability of seven different substrates (paddy straw, wheat straw, maize straw, sugarcane bagasse, gram straw, pea straw and rye straw) for the cultivation of *C. indica*.

Compost made from straw, horse or chicken excrement, calcium sulfate (gypsum), water and some nourishing supplements was regarded as a neglected substrate for *A. bisporus* and some other saprophytic basidiomycetes. Manure in the compost serves as N source, straw as C source. It must be pointed out that after the initial medium preparation stage, little control can be exerted over the composition of the solid substrate medium. However in composted substrates, the supplemention of the initial medium involves in mushrooms both an effective composting process along with contagious colonization and fruiting (Wood and Smith 1987).

Yilmaz et al (2007) observed early pin-head formation with a mixture of peat of Agacbasi (PA) and piece of mosaic (PM) on waste tea leaves and poultry litter based compost as compared to unsupplemented compost. Shandilya (2002) studied paddy straw compost formulations for growing button mushroom and also makes its comparison with traditional hade compost based on wheat straw and chicken manure. Filho *et al* (2008) obtained a higher yield of A. bisporus using soyabean meal (9.65%) at spawning and champ food (9.31%) before casing.

Barman *et al* (2017) used different compost formula and packaging materials for development and improvement of sporophore of *Agaricus bisporus* on paddy straw and dried tea leaf based compost. Locally accessible vermicompost (VSMS), plant soil (GS), tea waste (TW) and Spent mushroom compost of *Calocybe indica* (SMC) and their mix, for example, GS+ VSMS, TW+ VSMS, and SMC+ VSMS were utilized as packaging materials for the development of *A. bisporus*. They obtained maximum number of fruit body number in paddy straw based compost encased in vermicompost. Because of low water potential garden soil required more time for pinning. Lowest fruit body was seen in tea leaf based compost encased with tea wastage. Blend of vermi + SMC as packaging matter gave better outcome contrasted with SMC alone. The number of fruit

body was significantly increased in addition of vermicompost with the other casing materials.

Grape stalks and vine shoots as base materials for growing *A. bisporus* were financially feasible and naturally favorable (Pardo *et al* 2007). Municipal source separated food wastage (MSSFW) were likewise considered as a decent substrate for growing *A. bisporus* (Stoknes *et al* 2008) to get a yield of 27.9%. Animal beddings and residues, which are normally high in supplements, have been utilized for mushroom development (Noble *et al* 2002). Kumari and Achal (2008) reported that the main function of rice straw is to provide a reservoir of cellulose, hemicellulose and lignin which is used during growth and fructification.

Ozores (2001) showed that compost maturity is one of several issues, the composting industry is facing. The attempts were made to provide a high quality product to the agricultural community. Immature compost may be prepared for use as mulch for control of weeds in vegetable crop row-alleys. Mulches control weeds due to their physical presence on the soil as surface cover, or through the activity of phytotoxic substances. They also reduce soil erosion, decrease soil compaction, increase water holding capacity, slow nutrient release and increase microbial activity, soil temperature moderation and biological control of plant diseases.

Several studies on the cultivation of mushrooms have proposed that changes in extracellular activities of cellulases are specifically connected with growth and fruit body formation (Kaviyarasan and Natarajan 1997). Comparative examinations were additionally directed with various *Pleurotus* spp. utilizing paddy straw and these examinations have demonstrated a broadened action of cellulases after fruiting. The ability of basidiomycetous fungi to use the wide era of lignocellulosic substrates to form the protein-rich biomass is because of the nearness of a plenty of hydrolytic and oxidative chemicals. In this way, there is a need to complete research for the details of paddy straw based compost and to think about different physicochemical parameters alongside enzyme profile during growth to limit degradation losses and to upgrade composting process that would bring about better substrate arrangement and ideal use by *A. bisporus*. It is, hence, imperative to decide precisely the compositional changes during composting and mycelial development and to relate these progressions with mushroom yield and with design/level of protein creation for the diverse mushroom assortments.

In India, numerous workers have given data about the proximate arrangement of mushrooms. In any case, the outcomes revealed by most specialists vary impressively. These distinctions have been credited to variety in the species/strain utilized or due to the

method of composting, composition of the compost , age or development of fruit bodies and so forth (Garcha *et al* 1993). *A. bisporus* contains around 23.9-34.8 percent of rough protein, 51.3-62.5 percent sugars and 1.7-8.0 percent of unrefined fat (Sharma 1997). Hadwan *et al* (1993) likewise detailed 36.4 and 41.3, 45.8 and 53.4, 1.2 and 1.6, 9.0 and 11.3 percent protein, carbohydrates, fat and ash respectively, in two distinct strains of *A. bisporus*. In some prior examinations, it has been observed that mineral substance, carbohydrates and protein content was higher with wheat straw as a substrate and declined with expanding the substitution level of paddy straw (Garcha *et al* 1993). Likewise, an examination of macro elements in the fruit bodies revealed considerable variations in the three selected strains to be specific P-1, U-3, and MS-39 (Singh *et al* 2004). Gregori *et al* (2007) successfully utilized different technique and substrates for mushroom cultivation and biomass production with emphasis on the production of fruiting bodies and the production of mycelium.

Rao *et al* (1995) concluded that composting is a controlled bio-oxidative process that involves a heterogeneous organic substrate in the solid state; evolved by passing through a thermophilic phase and temporary release of phytotoxins leading to the production of carbon dioxide, water, minerals and stabilized organic matter. The main factors that play important role in composting process include: (i) environmental parameters (temperature, moisture content, pH, and aeration) and (ii) substrate parameters (C/N ratio, particle size, and nutrient content).

2.2 Physiological factors responsible for composting

2.2.1 pH, moisture, temperature

The pH can be a determinant of microbial population structure and general action. Microscopic organisms and particularly, actinomycetes are sensitive to pH (Alexander 1977), and microbial sulfate reduction is strongly reliant on pH, happening generally at pH 6-9 (Connell and Patrick 1968). Ammonification tends to raise pH, while under anaerobic conditions, fermentation (digestion without an outside electron acceptor) will prompt the creation of natural acids, which bring down pH (Miller *et al* 1991). Building operators (biochar) generally allude to the buffering specialist that raises the pH during the composting process (Jain *et al* 2018). The pH rise can be credited to expanding ash formation and mineralization of organic nitrogen because of microbial exercises (Bang-Andreasen *et al*, 2017). Finally prepared compost ought to have pH of around 7.5 (Levanon 1987, Chowdary 2010). Royse *et al* (2008) uncovered that satisfactory scope of pH during the season of spawning ought to be from 6.5-8.2. He likewise reasoned that compost containing generally high pH and no ammonia support good mycelia growth.

Kaur and Khanna (2002) demonstrated the pH scope 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 profile of all the four compost showed a set pattern during composting. It was low toward the begin, steadily increment in the middle of the turning and afterward settles toward end of composting. The pH of wheat straw compost was most reduced (6.9) toward the end of composting and pH of paddy straw compost was most astounding (8.3). *A.bisporus* mycelium develops best at a pH range of 7.2 - 7.8 . However , at low pH the growth of *A.bisporus* will be moderate and white plaster mould (*Scopulariopsis fimicola*, *S.brevicaulis*) may attack such compost. Mushroom fungi in general produce organic acid that diminish the pH of the substrate (Zadrazil and Schaeidereit 1972), which supports the spread of mycelia only. The pH of casing media anyway diminishes with the progress of the crop because of accumulation of different salts (Shandilya and Hayes 1987).

Moisture content of the composting is a vital ecological variable as it gives a medium to the vehicle of disintegrated supplements required for the metabolic and physiological exercises of microorganisms (Stentiford 1996, Cartney and Tingley 1998). Very low moisture content qualities would cause early dehydration during composting, which will capture the biological process, hence giving physically steady yet biologically unstable compost (Bertoldi *et al* 1983). Then again, high dampness (moisture) may deliver anaerobic conditions due to water logging, which will forestall and end the ongoing composting activities (Schulze 1962, Tiquia *et al* 1996). Optimum moisture content for the natural compost is around 65-67% while for synthetic compost it is 68-72%. If it is more than 72% at spawning there may not be proper air circulation, as free space will be possessed by water. Under such conditions, the anaerobic condition may win bringing about the killing of *A.bisporus* mycelium. Further, moulds like dark-coloured plaster *(P. byssina*) and white plaster (*S. fimicola*) may show up in the compost. The composting process is emphatically influenced by environment conditions (moisture content, pH, and air circulation) as it plays a substantial role to maintain suitable composting conditions. Besides, moisture content aggravates microbial movement and additionally the physical structure and hence impacts the biological decomposition of organic waste materials (Han *et al* 2014). Druilhe *et al* 2008, Poulsen and Moldrup 2007, Richard *et al* 2004 had officially seen an expansion in the air permeability when moisture content increased. They clarified that when moisture content expands, water is dragged into the little pores of the network, making larger aggrevates which thus prompt larger inter-aggregate pores and result in an expanded air permeability.

During composting, extensive amount of water can evaporate in order to control the excessive temperature, and as water content lessens the rate of decomposition diminishes, 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% of the water from the substrate and 17-46% from the casing soil which causes diminish in moisture content of the substrate and the casing soil during the growth of solid flush (Kalberer 1991). Diminishing the water potential of casing soil by adding sodium chloride lessened harvest yield, hindered fruit body development and increase the dry matter content of mushroom (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.

Temperature is the key parameter for estimating the activity of composting process. Toward the start of composting, the temperature in the compost is same as ambient air temperature. As the organisms dissimilate organics and blend new cells, heat is released and temperature in the compost pile rises. At around 40ºC, mesophilic microorganisms vanish and are replaced by thermophilic microorganisms. As the temperature increments to as high as 70ºC microbial movement achieves fast deterioration of organics. Once the waste substrate has completely disintegrated, the temperature of the heap come back to ambient levels. The succession of spread during the compost bed while framing the windrows heap ought to be planned to optimize thermophilic activity. Temperature is an essential parameter in composting.

Studying physiochemical attributes of paddy straw based synthetic compost, Subrahmanyam (1998) reported that achieving 61°C and 58°C in compost and air temperature respectively during warming up was considered as adequate activity in the compost. Average temperature of 48°C (between compost and air) during conditioning produced good compost. The maximum temperature recorded was 80°C at the middle of the stack while minimum of 67°C was found at the bottom. Kaur and Khanna (2001) reported maximum temperature during the course of composting to be 67°C-73°C among the four composts evaluated namely, wheat straw, wheat straw + paddy straw (1:1,w/w), wheat straw + paddy straw (1:2, w/w) and paddy straw alone. For all the composts, the temperature in the middle zone was found to be higher than the upper and the lowest

zones. The maximum temperature (73°C) was found in the middle zone in case of wheat straw+ paddy straw (1:2.w/w) compost. In wheat straw compost the maximum temperature recorded was 69°C. In other two composts, the maximum temperature recorded was about 67°C.

Miller (1992) presumed that temperature increments inside the composting materials as an element of initial temperature, metabolic heat evolution and heat conservation contributes considerably too high rates of decay accomplished during composting. The accomplishment of least temperature levels is essential to an effective composting process (Finstein and Morris 1975). Without a doubt, temperatures of composting material underneath 20ºC lead to significantly slow or even stop the composting process (Mosher and Anderson 1977). Temperatures more than 60ºC have additionally been appeared to diminish the movement of the microbial community or above this temperature, microbial action decays as the thermophilic optimum of microorganisms is surpassed. If the temperatures achieve 82ºC, the microbial community is seriously blocked (Fermor *et al* 1989). Kinley *et al* (1985) revealed that little variations in temperature can influence microbial activity and biomass in composting significantly more drastically than little changes in moisture content, pH, organic matter or C/N proportion.

Lacey (1973) observed that during composting *Streptomyces* was overwhelming at mild heating stage (45-50°C) and more thermophilic genera like *Thermoactinomyces*, *Micropolyspora*, and *Thermomonospora* prevailed in the wetter and more sweltering roughages, while distinctive mesophilic genera of microscopic organisms related were *Cellulomonas folia*, *Chondrococcus exiguius*, *Myxococcus virescens*, *M. fulvus*, *Thiobacillus thioxidans*, *T. denitrificans* and *Bacillus stearothermophilus* as thermophilic microscopic organisms.

2.2.2 C: N

Microorganisms infer their energy and carbon prerequisite from the deterioration of organic remains. For every 10 parts of carbon, one part of nitrogen is required for building up the cell's protoplasm. Fungi are highly efficient in carbon assimilation as compared to bacteria and actinomycetes. At few phases of decomposition, the population of microorganisms can even cross 8 billion for every gram of organic material. In this way, one can imagine the magnitude of the beneficial role played by these invisible organisms in cleaning nature and changing over waste into riches (Harikrishna 2013).

C/N ratio is one of the major factors that can affect composting (Onwosi *et al* 2017). Low C/N ratio can lead to a high NH_3 emission, while high C/N ratio might

make this process very slow due to the excessive amount of degradable OM (Bernal *et al* 2009). The optimum C/N ratio for composting is agreed to be in the range of 15–30, especially 20–25 (Guo *et al* 2012). The C/N ratio of pig manure is about 12–14 (Jiang *et al* 2015). Bulking agent, which was found to have a relative high C/N ratio, could be added to balance the C/N ratio of pig manure during composting (Chowdhury *et al* 2014). In addition, bulking agent plays an important role in the conditioning of the starting composting mixtures, which are usually used to create inter-particle voids, providing air space, regulating the moisture content of waste, and also providing carbon source for micro-organisms (Santos *et al* 2016). Corn stalk is a common and economical bulking agent (Guo *et al* 2012). Previous study has found that using corn stalk as a bulking agent not only improves the maturity of compost but also reduces gaseous emissions during composting and increases the amounts of reusable nutrients that are present in the compost (Yuan *et al* 2016).

The proportion of carbon to nitrogen (C:N) balance in mushroom mycelium is extremely important (Gea *et al* 2009). Demirer *et al* (2005) announced that so as 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. Impact of bioaugmentation and nitrogen supplementation on composting of paddy straw has been examined (Pandey *et al* 2009) and indicating the compost prepared from bioaugmented paddy straw with poultry manure are used for nitrogen supplement accomplished alluring C: N of 21:1 in one month with rented phytotoxicity and proved to be perfect for mushroom cultivation.

Kaur and Khanna (2002) discovered C:N proportion 16.4-20.3:1 for compost prepared as mixes of wheat straw and paddy straw in the proportion of 1:1 (w/w), 1:2 (w/w) and paddy straw alone. Barr and Parandi (2006) expressed that the amount of nitrogen ought to be adequate in the compost because it not only maintain appropriate C:N proportion in compost but also act as energy suppliers to the microorganisms for proper composting. Andrade *et al* (2008) revealed ideal C:N for *A. bisporus* compost supplemented with perfect compost ought to have C:N between 15 to 21:1% and no more greater than 30:1. Blanco and Almandros (2002) examined that wheat straw with 5% dry weight horse manure acclimated to C: N equivalent to 23:1 with urea was required during progressive stages of wheat straw composting. The abundance or absence of N content in the substrate might be a restricting element to fungus growth. As indicated by Rajarathnam and Bano (1989) there is a lessening of substratum degradation when N is excessively added. There is also a great variation of N content and carbon to nitrogen

(C:N) ratios in substrate from agricultural or agro industrial by-products. Colauto *et al* (2011) recommended that substrate with soybean fiber alone give higher mycelial growth than any blend with cassava fiber; the most noteworthy mycelial development on substrate is with C: N proportion of 11:1, the intermediate growth is with C: N proportion extend from 15:1 to 50:1 and the lower growth is with C: N proportion of 100:1 or higher. Randle and Flegg (1985) revealed the C: N proportion of 33:1during fermentation, 18:1 during mycelia growth and 14:1 during fruitification has standardized to deliver quality mushroom with desired yield (Zheng *et al* 1995).

2.3 Proximate analysis

Sharma and Gupta (1993) detailed that around 80 percent of the agricultural wastage are made out of cellulose, hemicellulose, and lignin that are hard to debate. Majority of the consumable fungi has enzymatic frameworks that can break these complex substances. Rajarathnam *et al* (1997) said that plant wastage are one of the main substrates for the development of a wide range of eatable mushrooms, which are capable of utilizing lignin, cellulose, hemicellulose and produce fruiting body of mushroom with excellent nutrition and medicinal attributes. Lignocellulosic materials rich in lignin, hemicelluloses and cellulose (Damisa *et al* 2008) including wood, agricultural residues and paper wastes like rice straw, corncob, corn stover, wheat straw, rice husk and bagasse etc. are particularly attractive in this content because of their relatively low cost and plentiful supply of desired product (Brijwani 2010). Deraz and Ismail (2001) and EI-Ashry *et al* (2001) reported that fungal treatment led to decrease organic matter and crude fiber contents, while crude protein and ash content increased compared with the untreated roughages.

Different cellulolytic bacteria and lignolytic fungi for lignolytic enzyme production under solid state fermentation utilizing rice straw as a substrate were screened by Brijesh *et al* (2010). Among these, *Phanerochete chrysosporium* produced the most elevated amount of hydrolytic enzymes, for example, carboxymethyl cellulose, filter paper hydrolase, cellobiase and lignin peroxidase following 30 days of fermentation. Malek *et al* (1994) grew *Hericum erinaceus, Pleurotus djamor, Ganoderma lucidum, Auricularia auricular, Lentinus sajor caju, Coriolus versicolor, Polyporus arcularius, Coprinus cinereus* on irradiated baggase and rice straw with 3% rice bran and 65% moisture content. Sugarcane bagasse and rice straw substrates contained 39.4 and 25.9% of cellulose, 22.9 and 26.9% of hemicelluloses and 19.6 and 13.9% of lignin. NDF values decreased significantly in sugarcane bagasse fermented by G. lucidum and P. arcularius

and in rice straw fermented by all 8 strains of fungus. ADF value decreased in bagasse and rice straw fermented by all the fungi.

In plant biomass, cellulose is always associated with lignin. In this manner, lignolytic microorganisms are essential in changing over bound cellulose into free shape (Tuomela *et al* 2000). The ability to degrade cellulose complexed insoluble carbohydrate can be judged by the level of endoglucanase enzyme which facilitate the degradation of cellulose into soluble carbon compounds required as nutrient for the growth of fungal mycelium (Manning and Wood 1983). Enzymatic studies by Anandh and Prakasam (2002) revealed that cellulose activity increased upto second harvest in the cased beds and in associated with the initiation of mushroom flushes. Hemicelluloses are quickly hydrolyzed since they are significantly less impervious to catalyst activity than celluloses. Some white rot fungi that deliver lignin peroxidases and laccases realize delignification of lignocellulosic crop residues. *Phanerochaete chrysosporium* is most reviewed white rot fungi for its lignolytic enzyme system.

Activity changes in several extracellular enzymes in different growth periods of *P.citrinopileatus* cultivated on fermented cotton seed hull compost. Activity peaks of endoglucanase, filter paper activity and hemicellulase activity appeared in periods between primordial formation and fruit body maturity was determined by Klamis *et al* (2008). Narain *et al* (2008) announced that mushroom mycelial growth and primordial development relies upon the lignocellulosic materials and different factors particularly the C:N proportion. As indicated by Sharma (1995), factors that influence mushroom yield were the extent of a fibrous component i.e cellulose and lignin present in the substrate. Ajonia and Tatah (2012) expressed that the biological yield shifted essentially because of substrate composition in various flushes. Further, they announced that presence of right proportion of alpha cellulose and lignin was the probable cause of higher rate of mycelium running. *Pleurotus spp*. deliver an extensive variety of hydrolytic and oxidative catalysts that empower them to effectively colonize, degrade and change over numerous lignocellulosic substances into sugars (Bano *et al* 1993).

Agaricus bisporus was developed axenically on wheat straw compost. Examination of this culture medium during growth and fruiting demonstrated that the lignin portion of straw was debased especially during the vegetative growth phase, though cellulose was degraded after the rise of the fruit bodies. A novel strategy was produced, whereby regular or engineered radio labelled lignin was blended personally with axenic compost and the rate of mineralization to CO_2 for the duration of the life-cycle of *A. bisporus* was observed constantly without culture aggravation (Junior *et al* 2010).

Mineralization rates were maximum during the vegetative growth phase and the beginning of fruiting brought an abatement of this action. A mutant strain of *A. bisporus*, which was not able to create fruiting bodies, was appeared to mineralize radiolabelled lignin continuously announced by Durant *et al* (1991). Magingo *et al* (2004) reported that the lignin and cellulose were the highly utilized components in the saw dust where in 63.7% and 43.97% of cellulose in the substrate were used by the growing mushroom. On sisal waste, the highly utilized components were volatile solids and cellulose. On paddy straw, the highly utilized components were volatile solids and hemicelluloses.

CHAPTER III

MATERIALS AND METHODS

The present investigation was carried out at Mushroom Research Complex, Department of Microbiology, Punjab Agricultural University, Ludhiana. The materials used and methods followed have been presented under the following headings and subheadings:

3.1 CULTURE PROCUREMENT AND MAINTENANCE

3.1.1 Source of culture

3.1.2 Culture medium

3.1.3 Maintenance of culture

3.2 MUSHROOM CULTIVATION TECHNOLOGY

3.2.1 Composting

3.2.2 Preparation of Spawn and Spawning

3.2.3 Casing Soil Preparation and Casing

3.2.4 Crop Management and Harvesting

3.2.5 Yield Data

3.3 PHYSIOCHEMICAL CHARACTERISTICS OF COMPOST

3.3.1 pH

3.3.2 Moisture

3.3.3 Temperature

3.3.4 Organic Carbon/Humus Content

3.3.5 Nitrogen Content

3.3.6 C:N

3.3.7 Microbial analysis of compost

3.4 PROXIMATE ANALYSIS

3.4.1 Collection and Preparation of samples

3.4.2 Neutral Detergent Fibre (NDF)

3.4.3 Acid Detergent Fibre (ADF)

3.4.4 Determination of Cellulose and Lignin

3.4.5 Determination of Hemicellulose

3.4.6 Total Ash Content

3.4.7 Determination of Crude protein

3.1 Culture Procurement and Maintenance

3.1.1 Source of culture

Agaricus bisporus Lange (Sing.) strain U3 was procured from germplasm collection bank of Mushroom Research Complex, Department of Microbiology, Punjab Agricultural University, Ludhiana.

3.1.2 Culture Medium

Potato Dextrose agar (PDA) Medium of following composition was used:

Ingredients	g/l
Potatoes	250
Dextrose	18
Agar	20
pH	5.5-6.5

Potatoes (250 g) were washed, cut into small pieces and boiled for 15 min. The extract was collected after filtration through muslin cloth. Dextrose and agar was added into it. The mixture was heated till formation of gel. The media was then dispensed into test tube to 1/4th of their capacity and plugged with non absorbent cotton and autoclaved at 15 psi for 20 min. The medium was allowed to solidify on a support to make slants.

3.1.3 Culture Maintenance

The culture was propagated on PDA at 25 ± 2°C for 15 days and stored at 4°C for 2 months before next subculture (Plate 1). Freshly prepared culture slants were used to prepare master culture and subsequently spawn bottles.

3.2 Mushroom cultivation technology

Agaricus bisporus was cultivated in winter season (September-March). The cultivation was carried out indoor under natural climatic conditions at temperature (14-22°C) and relative humidity (70-90%) at University Mushroom Research complex using standard methodology. Five steps were followed:

1) Composting
2) Preparation of spawn and Spawning
3) Casing soil preparation and Casing
4) Crop management and harvesting
5) Yield data

3.2.1 Composting

Long method composting used for preparation of wheat straw based compost as per the method of Khanna and Kapoor (2016) was followed. The substrates i.e. paddy straw and maize stalks were chopped to 4-6" size and different compost formulations using paddy straw: maize stalks (1:1w/w and 2:1w/w) were prepared (Plate 2).

Table 3.1: Compositions of Paddy straw: Maize stalks based compost formations

Compost Ingredients	Paddy straw:Maize stalks (1:1)	Paddy straw:Maize stalks (2:1)	Paddy straw
Maize stalk	150	100	-
Paddy straw	150	200	300
Wheat bran	15	15	15
Calcium ammonium nitrate	9	9	9
Urea	3	3	3
Super-phosphate	3	3	3
Muriate of potash	3	3	3
Molasses	5	5	5
Gypsum	30	30	30
Furadon 3G	150g	150g	150g
γ BHC	60ml	60ml	60ml

Paddy straw and maize stalk mixture were spread on the cemented floor and wetted thoroughly for 48 h in the form of loose heap to achieve 70-75% moisture content. Wheat bran (dry) mixed with four chemical fertilizers was moistened with water and kept covered overnight to facilitate solubilization and absorption of chemical fertilizers onto the bran. The mixture was evenly broadcasted on wetted wheat straw, mixed thoroughly and stacked. The whole mixture was made to compact rectangular pile of 5′x 5′x 5′. Seven periodic turns were given, first three turnings on every fourth day and other four turnings on every third day. A turning schedule of 2, 0, 4, 8, 12, 15, 18, 21 and 24 day was followed. At every turn approximately 30 cm layer was separated from all the exposed surfaces of the pile, mixed well and moistened. The remaining pile was dismantled and mixed well. The material was restacked in such a way that outer portion of pile was placed in centre of the new pile. Molasses, gypsum, furadan and γ BHC were mixed at the time of first, third, fifth and seventh turning, respectively. Two days after the last turn, the compost pile was lowered.

3.2.2 Preparation of Spawn and Spawning

Master culture and then generation spawn of *Agaricus bisporus* was prepared from slant culture on wheat grains in empty bottles using the standard methodology of (Garcha 1997). Healthy, uncrumpled wheat grains were washed and boiled. (Wheat grain:H_2O;1:25, w/v) for 2h to become tender without rupturing of the seed coat. Extra water was drained off and grains were allowed to cool for 6-8 hours. The boiled grains were then mixed with gypsum (Lab grade) and chalk powder @ 2 and 4% w/v, respectively. The grains were filled

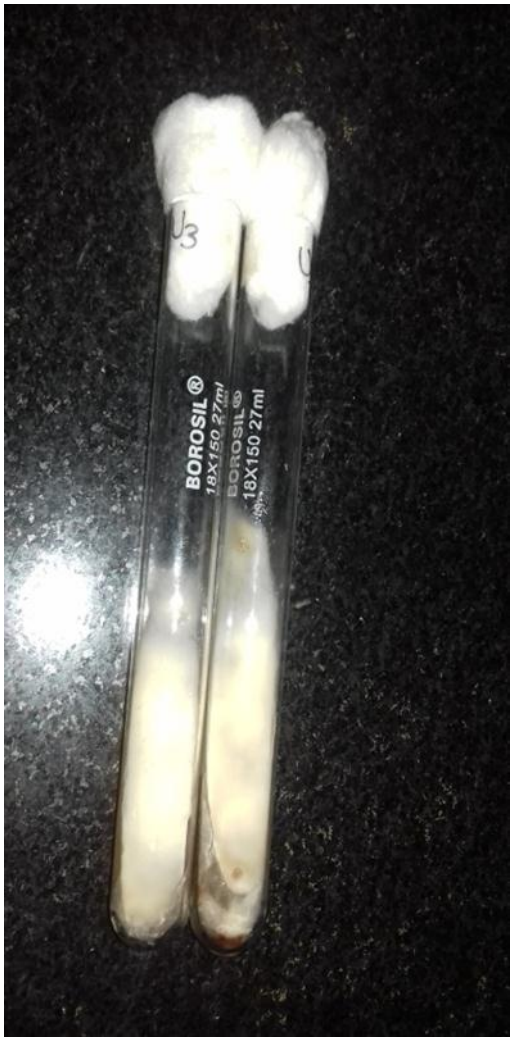

Plate 1: ***Agaricus bisporus*** **strain U3 maintained on PDA media slants**

Plate 2: Substrates a) Maize stalks b) Paddy straw used for compost preparation

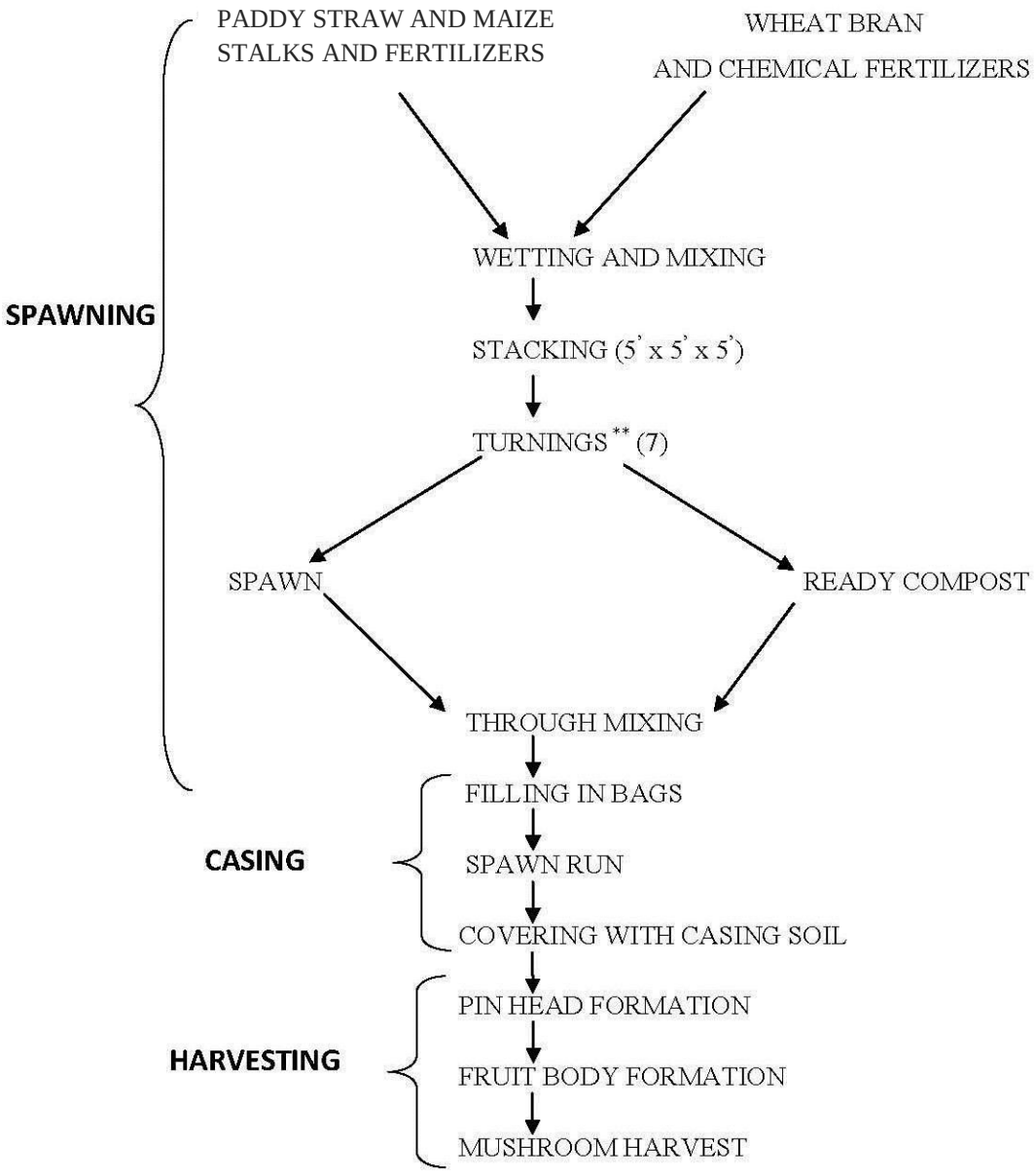

Fig.3.1: Cultivation process of *A. bisporus.*

in clean glass bottles (500 ml capacity), cotton plugged and wrapped with paper strips. The bottles were steam sterilized at 20 ± 1 lbs for 90 min.

Next day the bottles were shaken to restore transparency of glass and were inoculated with 14-15 day old culture. One agar bit measuring approximately 3x1 cm was placed inside a bottles aseptically in such a way that the mycelial surface of bit was in close contact with the grains. These were incubated at 25 ± 10°C for period of 20 days till mycelium completely covered the grains and called as Master spawn. One bottle of master spawn was used to inoculate 20 spawn bottles.

Mushroom growing was carried out in polythene bags (20"×24"). Five kg of ready compost was filled to a depth of 10-12" per bag which was spawned thoroughly at the rate of 0.6 per cent on fresh weight basis of compost. The top layer of the bag was pressed to make a smooth surface and polythene turned over it to cover the top layer of bag . Ten replicates for each of the three compost formulation were used for cultivation. The bags were arranged in rows on shelves in a room with sufficient light and cross ventilation facilities. The humidity in the room was maintained by spraying water on the floors and walls. Bags were left undisturbed until complete impregnation of compost with mycelium was achieved.

3.2.3 Casing Soil Preparation and Casing

A standard casing mixture was prepared by mixing farmyard manure (1 year old) and sandy soil (4:1 v/v). This mixture was disinfected with 4% formalin. For 100 kg casing soil, take 600 ml of formalin solution and diluted to 5 litre with water. This formalin treated casing mixture (soil) was covered with plastic sheet for 48 hrs. Before casing of bags, the casing mixture was turned frequently for 2-3 days to remove the traces of formalin. The disinfected casing mixture was used @ 2.0-2.5 kg per bag to make a uniform thick casing layer of 1.5″. Water was sprayed directly on cased bags till the end of cropping.

3.2.4 Crop Management and Harvesting

Very little or no ventilation was provided until the appearance of first pin heads. Thereafter, intermittent cross-ventilation was given for period of total 6-7 hrs per day. Mushrooms were harvested by gentle twisting and the soil end parts of fruit bodies were cut off. The yield data recorded daily for number of fruit bodies and their weight. Water was sprayed directly on cased bags till end of cropping.

3.2.5 Yield data

Yield data for total number & total weight of fruiting bodies per bag was recorded upto a maximum period of 6 weeks after appearance of pin head and percentage yield was calculated as

$$\% \text{ yield} = \frac{\text{Kg of fresh fruiting}}{\text{100 Kg of compost}}$$

3.3 Physiochemical characterization of compost

3.3.1 pH

The pH of sample was observed by the potentiometric method (Sekhon *et al* 1986) on the elico pH meter. Weigh 5g of compost sample in a 150 ml beaker, add 100 ml of distilled water and stir at least four times within a period of half an hour to attain equilibrium. The pH metre was switched on and calibrated using buffer solution to zero. With galvanometer pointer with the help of zero set knob. The sample suspension was swirled before inserting electrode into it. The selector was turned in the proper pH range and the pH was recorded.

3.3.2 Moisture

The moisture percentage was measured by drying 100g sample in an oven at 70° C to a constant weight. The difference between the final weight and initial weight was used for calculating the moisture percentage in the substrates (AOAC 2000).

$$\text{Moisture content (\%)} = \frac{\text{Fresh weight of compost (g)-Dry weight of compost(g)}}{\text{Fresh weight of compost (g)}} \times 100$$

3.3.3 Pile Temperature

The daily pile temperatures were measured by using a digital thermometer with a two meter long probe.

3.3.4 Organic Carbon /Humus Content

Organic matter in sediment consists of carbon and nutrients in the form of carbohydrates, proteins, fats and nucleic acids. Bacteria quickly eat the less resistant molecules, such as nucleic acids and many of the proteins. Total organic carbon refers to the amount of organic matter preserved within sediment.

Materials

1) Sample
2) Crucibles
3) Furnace

Procedure

Take 1g of sample in a pre weighted crucible. Place this crucible in a furnace and set at the temperature 600° C for 3-4 hours. Draw the samples from the furnace on the next day and weigh it (Nelson and Sommers 1996).

Weight of empty crucible = a

Weight of sample = b

Weight of crucible after ignition = c

% of ash = (c-a) x100/b

Organic matter/Humus content = 100 – (c-a) x 100/b = Y

% of organic carbon = Y x 0.56

3.3.5 Nitrogen content

Reagents

- Digestion mixture (K_2SO_4 : $CuSO_4$,10:1, w/w)
- Concentrated H_2SO_4
- 40%NaOH
- 0.1 N HCL
- 20 ml Boric acid
- Mixed indicator – Bromocresol green (0.5gm) and methyl red (0.1gm) dissolved in 100 ml of 95% ethanol and pH adjusted to 4.5 diluted HCL.

Estimation

0.5 gram of sample was taken in digestion flasks. To it, 5g of digestion mixture and 30 ml of concentrated sulphuric acid was added. The flasks were kept on hot plate till the contents became clear. After cooling, the volume of digested sample was made upto 50 ml. An aliquot of 25ml was distilled in Microkjedahl distillation apparatus with 50ml of 40% NaOH. The liberated ammonia was trapped in 20 ml boric acid containing 2-3 drops of mixed indicator. About 20 ml distillate was collected in 50 ml conical flask and titrated with 0.1N Hcl till the end point. A change in colour from blue to light pink appeared. A blank was also run simultaneously.

$$\% \text{ N} = \frac{\text{(x-y) x 14 x Normality of acid x V1}}{\text{V2 X S}} \times 100$$

X = Volume (ml) of 0.1 N HCL used for sample titration

Y = Volume (ml) of blank reading

V1 = Total volume (ml) made

V2 = Volume (ml) of aliquot taken

S = weight of sample in grams

3.3.6 C:N

By calculating carbon and nitrogen content of each sample individually, C:N ratio of each sample was calculated.

3.3.7 Microbial Analysis of composts

The fresh compost samples collected were analyzed for viable population of bacteria and fungi by the standard serial dilution plate count method by plating (10^{-5}, 10^{-2}) ml volume of diluted suspension (Vlassak *et al* 1992) at each turning using Media *viz.,* Nutrient Agar and Potato Dextrose Agar. The plates were incubated at 28˚C and 45˚C temperature for mesophilic and thermophilic population, respectively in incubator in triplicate. The microbial colonies appearing after the stipulated time period of incubation were counted by colony

counter. The microbial population were expressed as (CFU) g^{-1} for fungi and bacteria on dry wt. basis.

3.4 Proximate analysis

Standard methods of AOAC (2000) were followed for the determination of proximate composition of developed composts during different stages of growth i.e. Neutral detergent fibre (NDF), Acid detergent fibre (ADF), Lignin, Cellulose, Hemicellulose, Crude protein, Total ash.

3.4.1 Collection and Preparation of samples

Samples of compost for each species were taken at five different stages namely,

- Fresh chopped straw (at 1st day)
- Final grade compost (at 28th day)
- At pin head stage
- After first flush (15 days after first harvest)
- At crop termination

A compost sample of 300 g was collected from different bags at different intervals. The sample was kept in hot air oven at 60°C till samples become dry. The dried material was powdered and samples were stored in butter paper bags. Dried samples ground to pass through 40 mesh and analysed for various fractions as per AOAC (2000) method. Analysis of compost was carried out for Neutral detergent fibre (NDF), Acid detergent fibre (ADF), lignin, hemicellulose, cellulose, crude protein and total ash.

3.4.2 Determination of Neutral Detergent Fibre (NDF)

Dried sample (0.5g) was placed in a spout less beaker. 50 ml of neutral detergent solution was added to the sample. It was heated to boil on hot plate and the boiling was adjusted to an even level and was refluxed for 60 min from the onset of boiling by keeping the round bottomed flasks over the spout less beakers. The liquid content was filtered through sintered glass crucible (G-1) mounted on suction flask using an oil free vacuum pump. The washing was repeated with hot distilled water till the foam stops coming into the flask. The vacuum was removed and the sample was washed twice with acetone. The crucible was kept at 100°C in hot air oven for overnight was weighed after cooling in a desiccator.

$$NDF\ \% = W_1 - W_0 / S \times 100$$

Where,

W_1 = weight of oven dried sample and crucible

W_0 = weight of oven dried crucible

S = weight of dry sample

3.4.3 Determination of Acid Detergent Fibre (ADF)

Dried sample (0.5g) was placed in a spout less beaker. 50 ml of Acid detergent solution was added to the sample. It was heated to boil on a hot plate. The boiling was adjusted to an even level and was refluxed for 60 min from the onset of boiling by keeping the round bottomed flasks over the spout less beakers. The liquid content was filtered through sintered glass crucible (G-1) mounted on suction flask using an oil free vacuum pump. The washing was repeated with hot distilled water till the foam stops coming into the flask. The vacuum was removed and the sample was washed twice with acetone. The crucible was kept at 100°C in hot air oven for overnight and was weighed after cooling in a desiccator**.**

$$\text{ADF \%} = \frac{W_1 - W_0}{S} \times 100$$

Where,

W_1 = weight of oven dried sample and crucible

W_0 = weight of oven dried crucible

S = weight of dry sample

3.4.4 Determination of Cellulose and Lignin

Cellulose and Lignin were calculated after determining Acid Detergent Lignin (ADL).

3.4.4.1 Determination of Acid detergent lignin (ADL)

The acid detergent fiber (ADF) residue mat was covered with cold solution of 72% H_2SO_4 (w/w). Filled the crucible about half way with the acid and stirred with a rod. The lumps were broken with a glass rod and the glass rod was left in the crucible. The crucible was refilled with 72% H_2SO_4 and was stirred as the acid drains. After 3 h, suction was applied to wash the contents of the crucible with hot distilled water to neutral pH paper. The crucible was heated at 100°C in hot air oven. It was cooled in desiccator and was weighed.

3.4.4.2 Determination of Lignin

The above crucible was ignited at 550°C in muffle furnace for 3 h (or until C-free). The crucible while still hot was placed in oven at 100°C for 1 h after removing it from furnace. The crucible was cooled in a desiccator and was weighed.

$$\text{Lignin \%} = \frac{W_3 - W_2}{S} \times 100$$

Where,

W_3 = loss in weight on ignition after 72% H_2SO_4 treatment

W_2 = weight of 72% H_2SO_4 treated crucible

S = weight of dry sample

3.4.4.3 Determination of Cellulose

The cellulose content of the sample was calculated by the following formula:

$$\text{Cellulose \%} = \frac{W_1\text{-}W_2}{S} \times 100$$

Where,

W_2 = weight of 72% H_2SO_4 treated crucible

W_1 = weight of oven dried fiber and crucible

S = weight of dry sample

3.4.5 Determination of Hemi-cellulose

Hemicellulose content of compost was measured after determining Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF).

3.4.4.1 Calculation of Hemi-cellulose

Hemi-cellulose was obtained by subtracting ADF from NDF, as:

Hemi-cellulose % = NDF%-ADF%

3.4.6 Determination of Total Ash content

The crucible containing oven dried sample was ignited in a muffle furnace at 600°C for 3 h (or until carbon free i.e. no black colored straw should be visible). The crucible was placed in oven at 100°C for 1 h after removing it from furnace. The crucible containing ash was cooled and weighed. The ash content was determined according to the formula given below:

$$\text{Ash \%} = \frac{W_3\text{-}W_1}{W_2\text{-}W_1} \times 100$$

Where,

W_1 = weight of sample and crucible

W_2 = weight of oven dried sample and crucible

W_3 = weight of furnace burnt sample and crucible

3.4.7 Determination of Crude protein

The N content was estimated by Macro-Kjeldahl method. Finely ground sample 0.25g, 4-5 g of digestion mixture (Copper sulphate: Potassium sulphate, 1:9) was added in the digestion tube. After the addition of 10-12 ml of concentrated sulphuric acid, the samples were digested in automated Kel Plus digestion unit at 450ºC for 2-3 hours till the solution become clear bluish green. During digestion procedure, nitrogen present in the sample was converted to ammonium sulphate. The material after digestion was distilled in presence of 40% sodium hydroxide. The NH_4^+ liberated was received on the other end of condenser having 250 ml flask containing 25 ml of 4% boric acid-mixed indicator solution. Mixed indicator was prepared by taking bromocresol green and methyl red in 5:1 ratio in 95%

ethanol. Distillation was carried out till the distillate become almost double to its volume. The distillate was titrated with standard 0.1 N H_2SO_4 till it turns to light pink color. The reading for blank was also recorded.

Nitrogen % calculated was then by using the formula:

$$\% \text{ Nitrogen} = \frac{\text{N} \times \text{Volume of 0.1N } H_2SO_4 \text{ used} \times 14 \times \text{dilution factor}}{1000 \text{ x Wt. of sample (g)}} \times 100$$

The CP (%) content was calculated by multiplying the nitrogen by 6.25.

CHAPTER IV

RESULTS AND DISCUSSION

The present investigation entitled 'Development of compost formulation based on paddy straw and maize stalks for cultivation of *Agaricus bisporus*' was carried out. Two compost formulations were tried with paddy straw and maize stalks in different combinations (1:1; 2:1,w/w) and compared with paddy straw compost. The compost was prepared using long method of composting. The physical (moisture, pH, temperature), chemical (carbon, nitrogen, C:N) and microbiological properties (bacteria, fungi) were recorded at each turning. These compost mixtures were further evaluated for their yield potential. The results of the various experiments conducted during the course of present investigations have been presented under the following headings:

4.1 Physicochemical characteristics of different composts during composting

4.2 Proximate analysis of compost during different stages of growth of button mushroom

4.3 Yield potential of *Agaricus bisporus* of U3 strain on paddy straw and maize stalk based composts

4.1 Physicochemical characteristics of different composts during composting

The physicochemical characteristics of the compost mixtures namely paddy straw + maize stalks (1:1, w/w), paddy straw + maize stalks (2:1, w/w) and paddy straw based compost as control were carried out at different turning stages of compost formation. The data for moisture, pH, temperature, C:N ratio and microbiological counts were recorded at each turning. The data has been tabulated in the table 4.1.

The moisture content of the composts was observed to be 65.2%-67.3% in PS:MS (2:1, 1:1,w/w) respectively at the start of the composting which finally decreased to 61.3%-63.4% in PS:MS (2:1, 1:1,w/w) respectively at the time of spawning whereas for the paddy straw (control) it was 62.6% in the beginning of compost formation while at the time of spawning, it was observed to be 62.1% (Table 4.1, Fig 4.1.1). The pH profile of two composts viz., paddy straw + maize stalks (1:1, w/w), paddy straw + maize stalks (2:1, w/w) showed a similar pattern during composting. The pH was recorded as 6.4-6.7 for PS:MS (1:1, 2:1, w/w) respectively at the start of the composting which gradually increased to 7.6-7.8 for PS:MS (2:1, 1:1, w/w) respectively between the turnings and finally declined to7.2-7.3 in PS:MS (2:1, 1:1, w/w) respectively at the time of spawning while in case of paddy straw compost the pH increased from 6.5 at the start of composting and finally reached to 7.9 at the time of spawning (Table 4.1, Fig 4.1.2).

In the composts, the temperature of the middle zone of the stack was higher than the upper and the lower zone. The temperature of the compost stack was maximum at 4^{th} turning ranging from 73.4 to 76.4°C in all the composts (Table 4.1, Fig 4.1.3). The high temperature in the composting stack is an important parameter which favours the growth of thermophilic microflora

also but brings some chemical changes that are essential for the selectivity of the compost. The C:N ratio of the composts progressively narrowed down from the beginning towards the end of composting i.e. 37.7:1 to 16.6:1. The C:N ratio of the final grade compost was 18.7:1 in control (paddy straw based compost) while it was 16.6:1 and 17.2:1 in paddy straw + maize stalks compost (1:1;2:1), respectively (Table 4.1, Fig 4.1 4). The bacterial count of mesophiles was observed maximum at 2nd turning ranged from 22-24 cfu (Table 4.1, Fig 4.1.5) while that of thermophiles it was maximum in 4th turning which ranged from 130-134 cfu in all the composts (Table 4.1, Fig 4.1.6, Plate 3,4). In case of fungal count, the mesophiles showed highest count at 2nd turning ranged from 7.33-8.0 cfu (Table 4.1, Fig 4.1.7) whereas thermophiles were maximum at 4th turning which ranges from 7.0-7.33 cfu in all the composts (Table 4.1, Fig 4.1.8).

The optimum moisture content for biodegradation can vary widely for different compost mixtures and times in the composting process, ranging from near 50 to over 70% on a wet basis (Richard *et al* 2002). Shandilya (1989) showed that moisture level was upto 68% at the time of spawning for paddy straw compost. The high moisture content (>70%) especially in decomposed compost tended to reduce the yield as observed by Cormican and Staunton (1991). Kaur and Khanna (2001) reported that the moisture content of three compost namely 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%. Levanon *et al* (1983) suggested that ready compost should have pH of around 7.5. Kaur and Khanna (2001) showed the pH range of 6.9-8.3 for different compost formulations. Sharma (1991) and Savoie *et al* (1995) reported the pH to be high during the first phase of composting due to rise in ammonia and subsequently declined during the later phase due to addition of gypsum powder and dissipation of ammonia.

While studying the physiological attributes of paddy straw based synthetic compost, Subramanyam (1998) reported that achieving 61°C and 58°C in compost and air temperature respectively during warming up was considered as adequate activity in the compost. The maximum temperature recorded during this investigation was 78.8°C of the stack while minimum of 42.3°C was found. The trend of temperature in the composting pile has been also observed by Kaur and Khanna (2001). The maximum temperature achieved was 69° C for wheat straw compost, 67° C for both wheat straw + paddy straw (1:1, w/w) and paddy straw alone. The C/N ratio is simply the ratio of organic C to the weight of total N in the material (Caithness 2003). Based on physiochemical study of *A. bisporus* compost, an ideal compost should have C:N between 15-21:1 and not greater than 30:1 (Fidanza and Beyer 2009). Kaur and Khanna (2001) reported that the C:N ratio at the beginning was 32.6:1 in paddy straw compost and at the end of composting, the C:N ratio ranged from 16.3:1-19.3:1 in different composts. Lacey (1973) observed that during composting *Streptomyces* was overwhelming at mild heating stage (45-50°C) and along with thermophilic genera like *Thermoactinomyces*, *Micropolyspora*, and *Thermomonospora* were produced.

Plate 3: Mesophilic bacterial population during composting process of paddy straw + maize stalks (1:1)

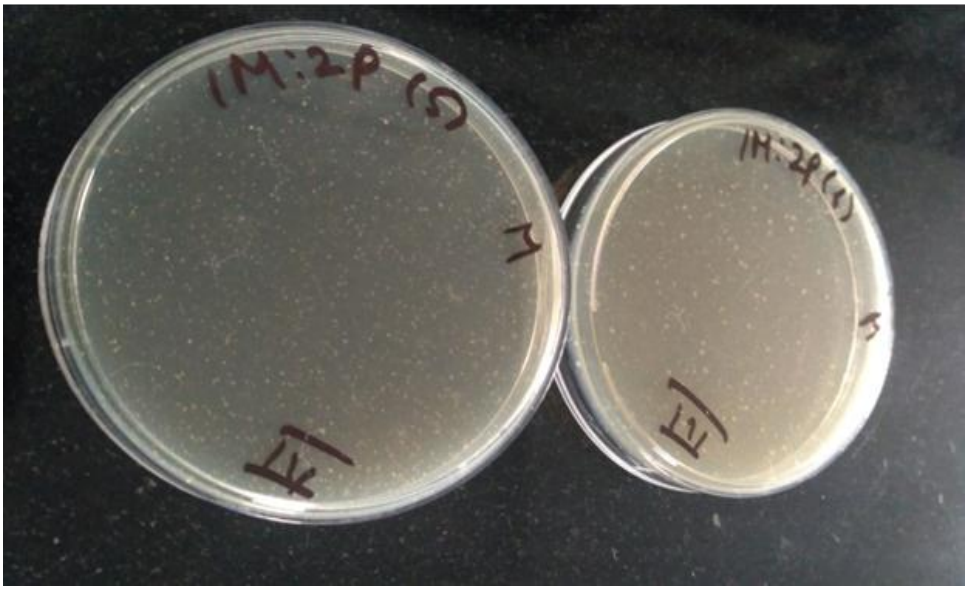

Plate 4: Mesophilic bacterial population during composting process of paddy straw + maize stalks (2:1)

Table 4.1: Physical, chemical and microbiological properties of different compost formulations.

COMPOST	TURNING (NO.)	PHYSICAL PROPERTIES			CHEMICAL PROPERTIES	MICROBIOLOGICAL COUNT			
		MOISTURE (%)	pH	TEMPERATURE (°C)	C:N	BACTERIAL($\times10^5$ cfu g^{-1} compost)		FUNGAL($\times10^2$ cfu g^{-1} compost)	
						MESOPHILIC	THERMOPHILIC	MESOPHILIC	THERMOHILIC
I	1	67.3	6.4	59.2	32.3:1	18	80	5.33	3.33
PS:MS	2	63.1	6.6	64.1	26.4:1	23	113	7.67	4.33
(1:1)	3	61.7	7.0	71.5	23.9:1	13	129	4.33	6.33
	4	60.6	7.4	73.4	21.2:1	16	134	3.67	7.00
	5	62.2	7.8	67.2	19.2:1	21	87	2.33	3.33
	6	61.8	7.5	54.3	17.1:1	20	58	1.67	2.33
	7	63.4	7.3	42.1	16.6:1	18	21	1.33	2.00
CD 5%		NS	NS	3.44	0.66	4.72	8.50	2.22	2.44
II	1	65.2	6.7	58.3	37.7:1	18	84	4.67	3.33
PS:MS	2	64.3	7.1	66.2	33.3:1	22	102	7.33	4.33
(2:1)	3	61.6	7.3	72.3	27.0:1	14	125	4.30	5.67
	4	60.1	7.6	76.4	23.6:1	18	131	3.33	7.00
	5	62.3	7.5	65.4	20.6:1	20	83	2.33	3.33
	6	63.2	7.3	53.1	18.2:1	21	61	1.67	1.67
	7	61.3	7.2	43.7	17.2:1	16	23	1.33	1.33
CD 5%		NS	NS	3.26	1.05	NS	9.17	2.12	2.56
III	1	62.6	6.5	64.4	35.2:1	18	87	5.33	3.67
CONTROL	2	60.3	6.9	67.9	30.6:1	24	110	8.00	5.00
(PS)	3	58.7	7.2	69.7	25.7:1	14	132	4.67	6.33
	4	59.4	7.5	74.4	23.5:1	9.7	130	3.67	7.33
	5	61.3	7.6	65.1	20.2:1	6.3	92	2.00	2.67
	6	63.2	7.8	55.6	19.2:1	5.7	64	1.67	1.33
	7	62.1	7.9	42.3	18.7:1	4.7	39	1.33	1.00
CD 5%		NS	NS	2.76	0.61	3.70	11.46	2.26	1.42

PS:MS- Paddy straw: Maize stalks, Control-Paddy straw, C:N- Carbon:Nitrogen

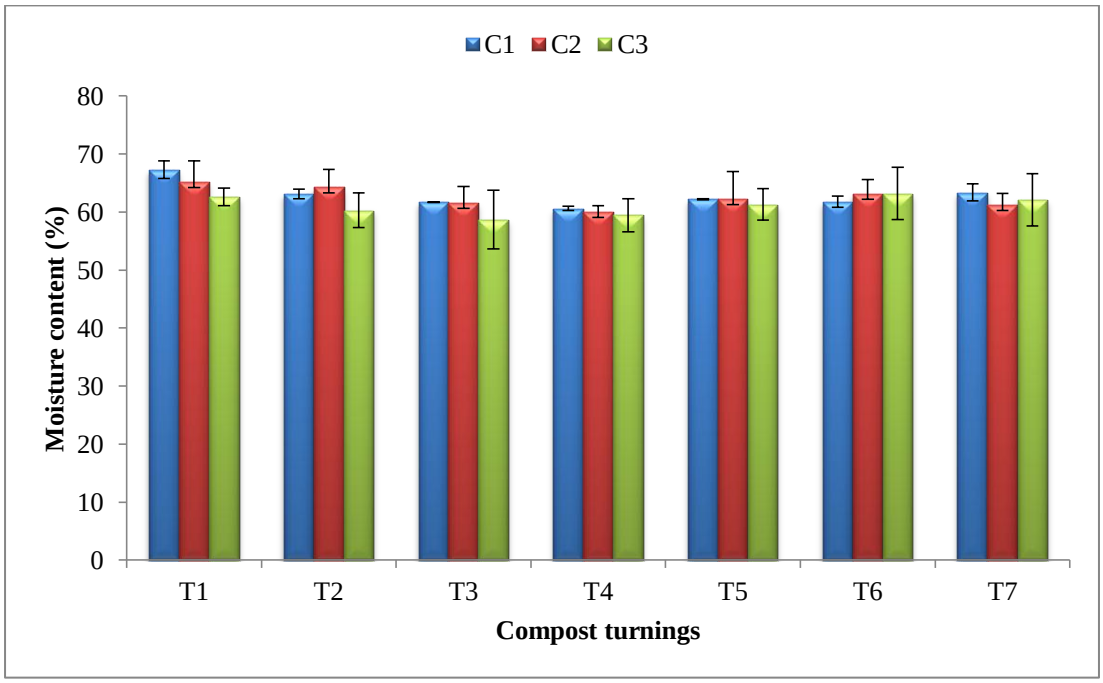

Fig.4.1.1: Moisture content (%) of compost formulations.
C1: Paddy straw: Maize stalk(1:1), C2: Paddy straw: Maize stalk(2:1), C3: Paddy straw

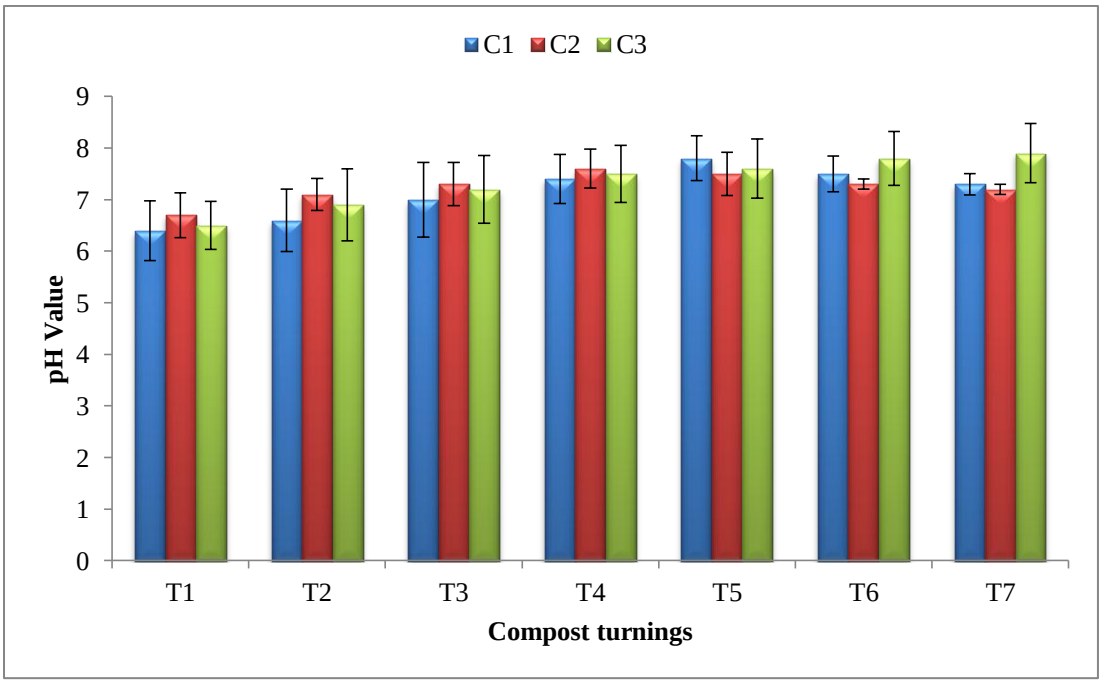

Fig 4.1.2: pH value of compost formulations.
C1: Paddy straw: Maize stalk (1:1), C2: Paddy straw: Maize stalk (2:1), C3: paddy straw

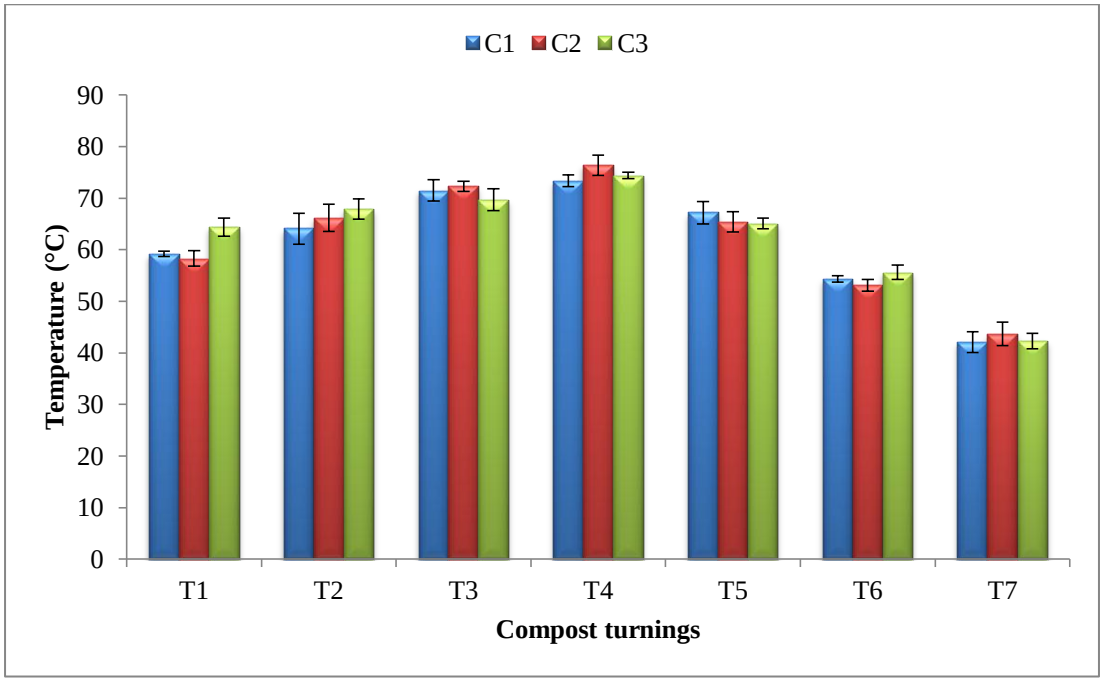

Fig.4.1.3: Temperature profile of compost formulations
C1: Paddy straw: Maize stalk(1:1), C2: Paddy straw: Maize stalk(2:1), C3:Paddy straw

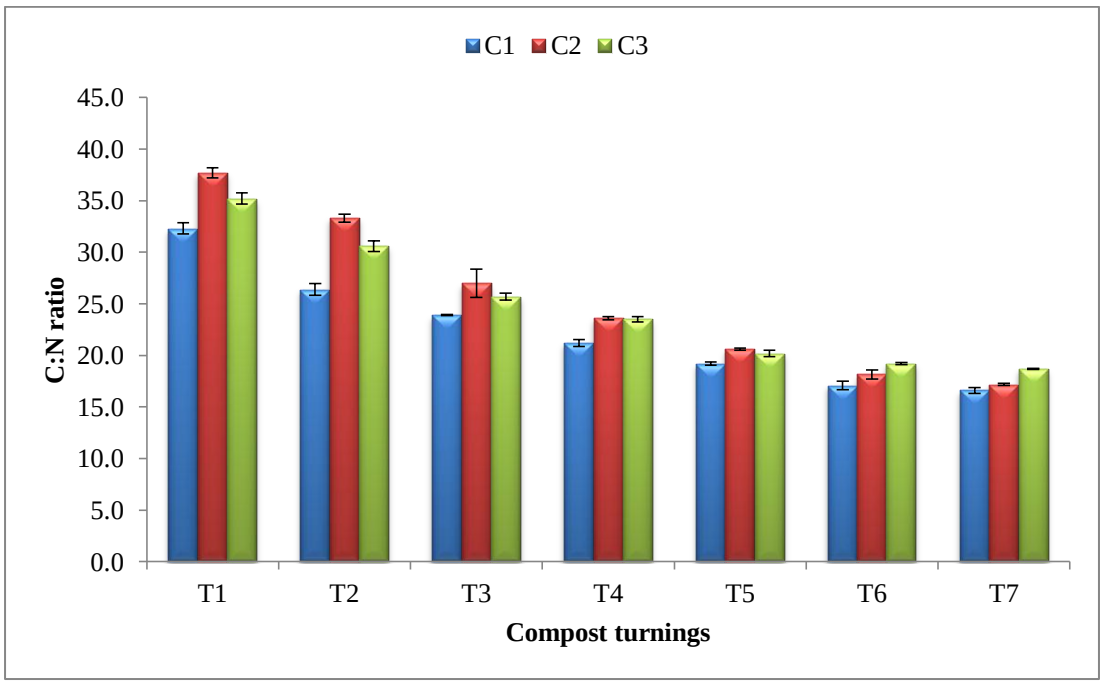

Fig. 4.1.4: C:N Ratio of compost formulations
C1: Paddy straw: Maize stalk(1:1), C2: Paddy straw: Maize stalk(2:1), C3: Paddy straw

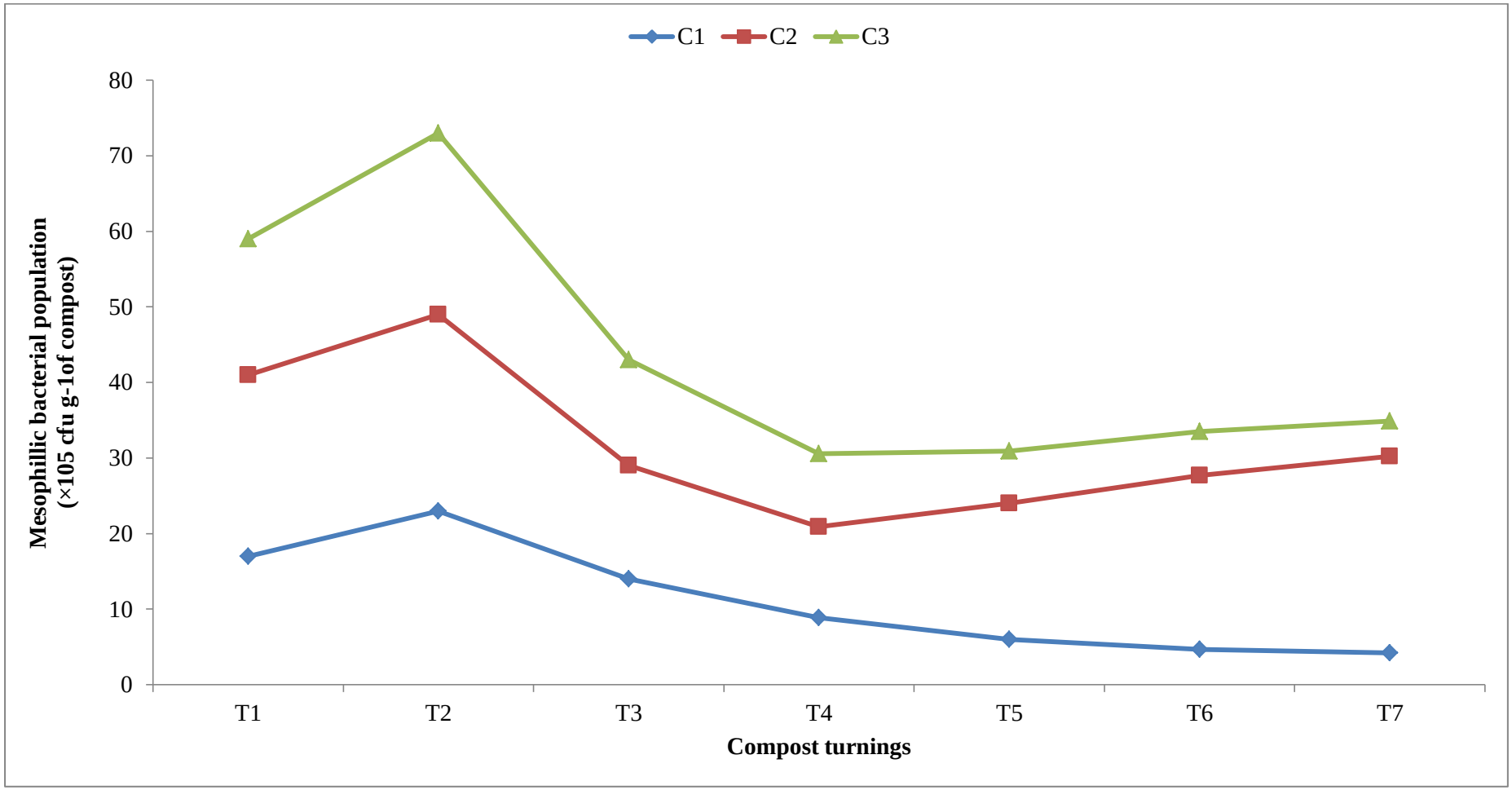

Fig.4.1.5: Mesophilic bacterial population during composting process
C1: Paddy straw: Maize stalk(1:1), C2: Paddy straw: Maize stalk(2:1), C3: Paddy straw, CFU=Colony forming unit

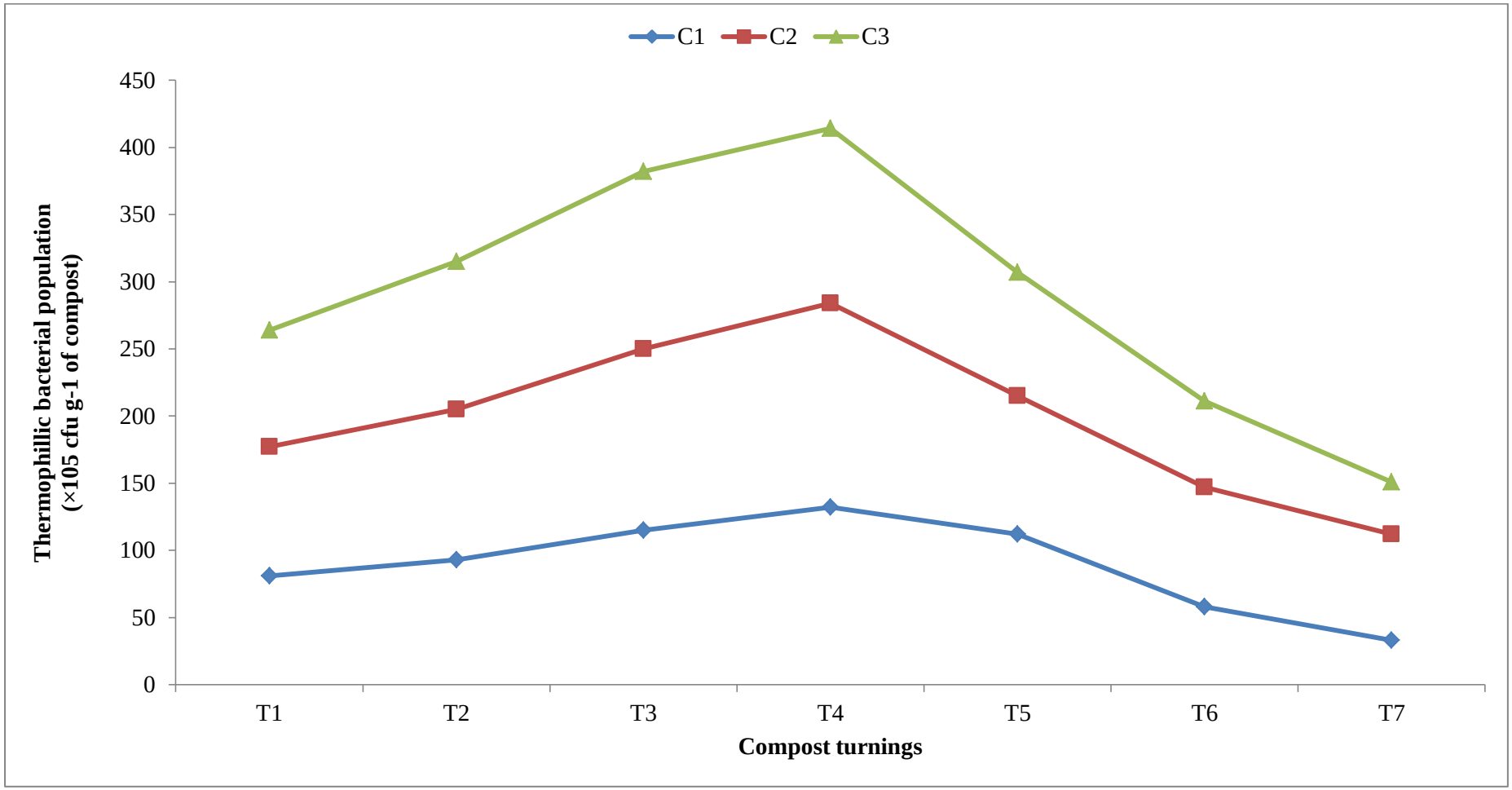

Fig 4.1.6. Thermophilic bacterial population during composting
C1: Paddy straw: Maize stalk(1:1), C2: Paddy straw: Maize stalk(2:1), C3: Paddy straw, CFU=Colony forming unit

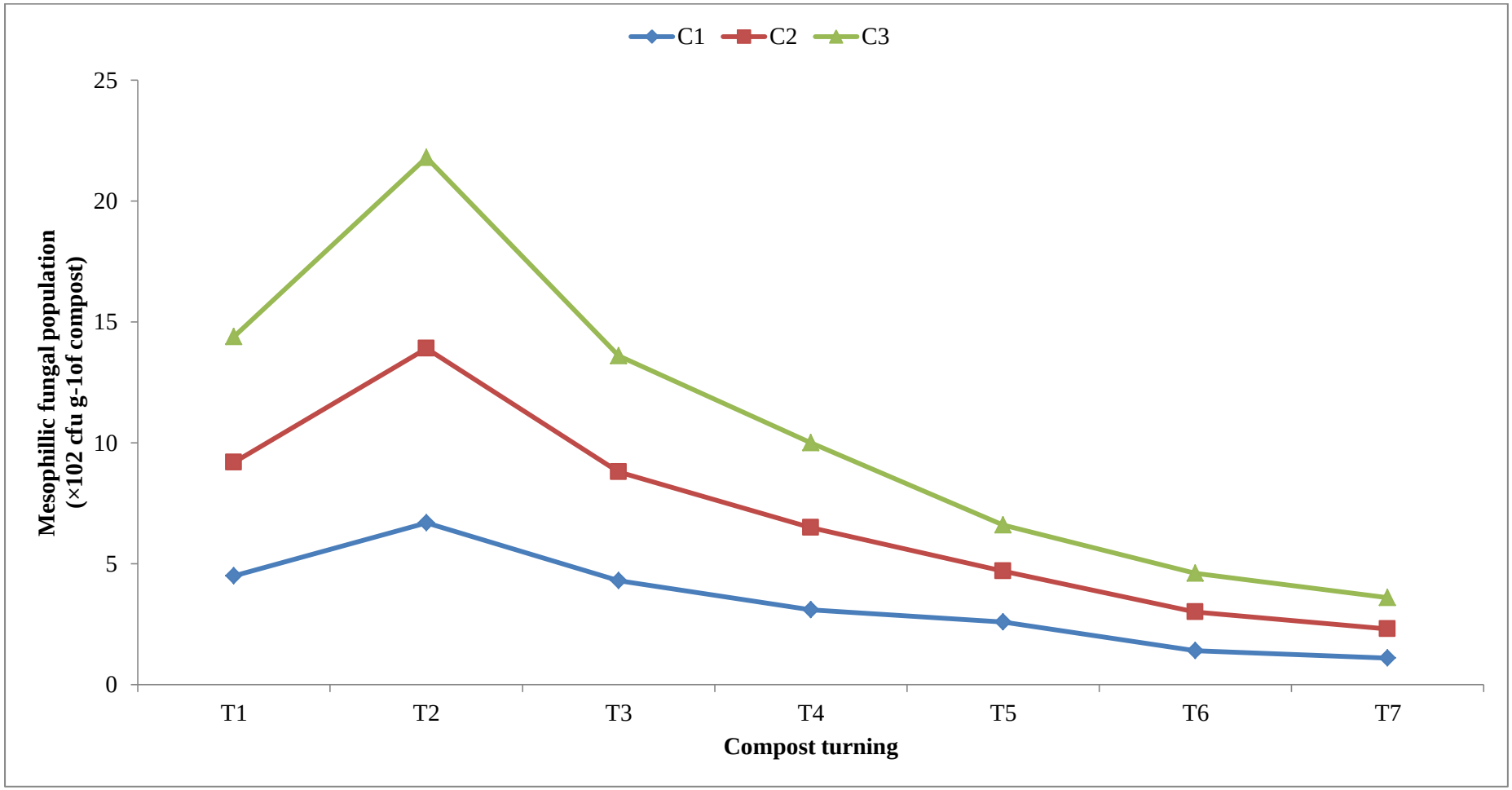

Fig.4.1.7: Mesophilic fungal population during composting
C1: Paddy straw: Maize stalk(1:1), C2: Paddy straw: Maize stalk(2:1), C3: Padddy straw, CFU=Colony forming unit

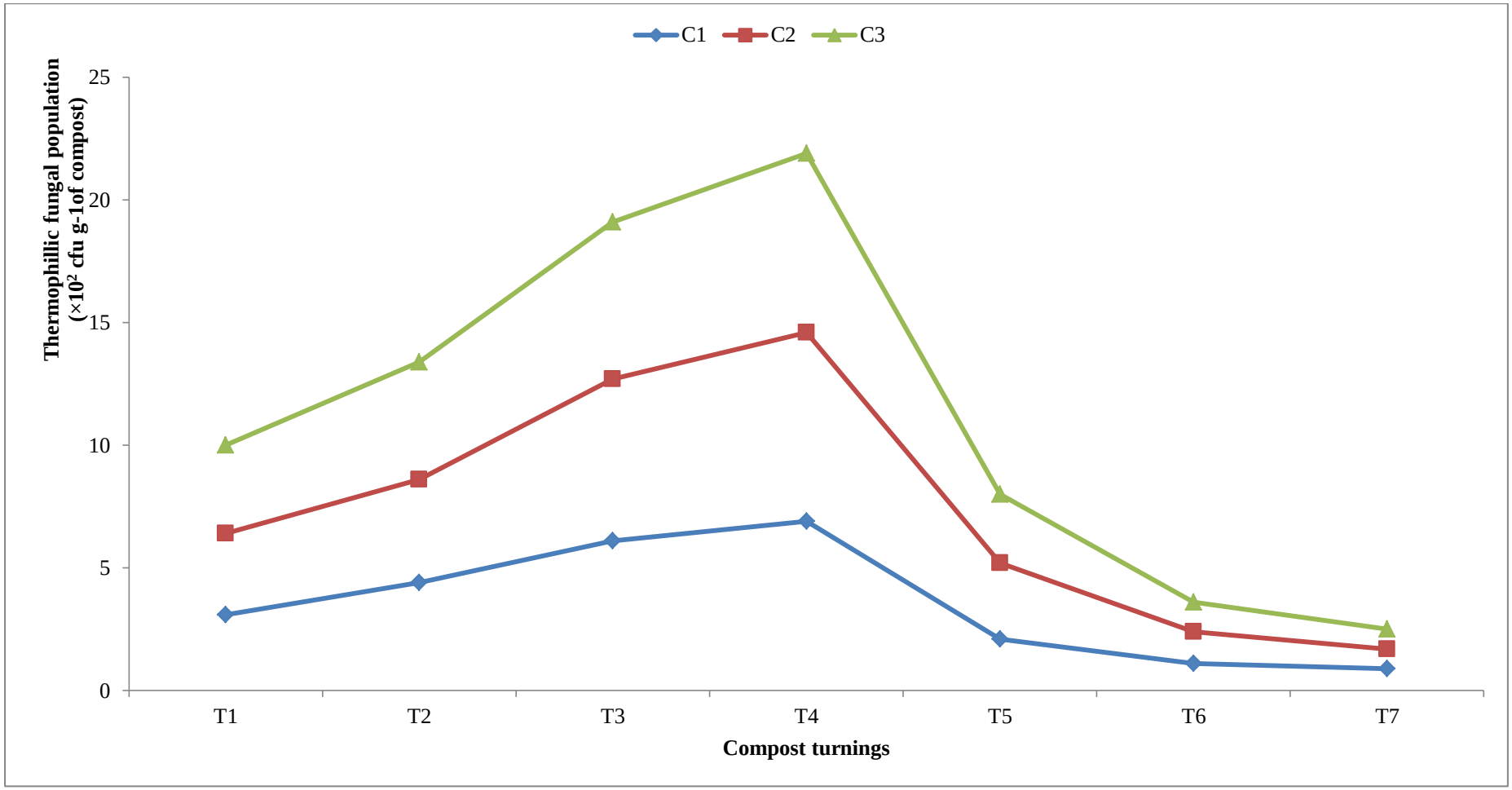

Fig.4.1.8: Thermophilic fungal population during composting
C1: Paddy straw: Maize stalk (1:1), C2: Paddy straw: Maize stalk (2:1), C3: Paddy straw, CFU=Colony forming unit

4.2 Proximate analysis of compost during different stages of growth of button mushroom

Mushroom compost prepared with paddy straw and maize stalk (1:1; 2:1, w/w) was compared with paddy straw based standard compost. The straw was analysed for proximate composition at zero day of compost, final grade compost, pin head stage, after first flush and at crop termination. The neutral detergent fibre was significantly reduced in all the compost formulation upto pin head stage (70.20%-46.20%) and there after it was statistically at par after first flush and at crop termination (Table 4.2.1, Fig 4.2.1). As the acid detergent fibre was also significantly reduced in all the compost till pin head stage (52.41%-33.72%) (Table 4.2.1, Fig 4.2.2). Total ash content in all the compost showed increment and ranged (10.90%-21.37%) (Table 4.2.1, Fig 4.2.3). There was no significant variation in crude protein content at these stages (Table 4.2.1, Fig 4.2.4). The cellulose (36.37%-20.69%) and hemi-cellulose (17.59%-8.57%) content was reduced drastically during the compost formulations leading toward pin head stage and it was stable there after (Table 4.2.2, Fig 4.2.5 and 4.2.6). The lignin content showed no significant decline during different stages of composting and crop production (Table 4.2.2, Fig 4.2.7)

The substrate composition of different composts WS + PS (1:1,1:2,w/w) and PS depicted a progressive decrease of various constituents: NDF, ADF, cellulose, hemicelluloses (Kaur 2000). Cellulose, hemicelluloses and lignin which constitute the major part of plant waste are known to have direct impact on the growth and development of mushroom fungi (Zadrazil 1975). Moorthy (1981) and Singh *et al* (1989) observed that cellulose, hemicelluloses and lignin are degraded up to an extent of 75% during the growth period. During the growth of mushroom, the cellulose content of substrates were reduced with maximum rate in fruitification stage compared to spawn run stage.

Table 4.2.1 Proximate analysis of compost during different stages of growth of button mushroom

Compost	Intervals	Neutral Detergent Fibre (%)	Acid Detergent Fibre (%)	Total Ash (%)	Crude Protein (%)
I Paddy straw:Maize stalk (1:1)	T1	70.20	52.41	10.90	15.23
	T2	52.93	41.43	13.63	12.17
	T3	48.23	36.04	16.83	10.43
	T4	45.03	29.19	18.73	9.20
	T5	43.70	26.85	21.33	8.80
CD (5%)		3.50	3.00	2.98	2.65
II Paddy straw:Maize stalk (1:2)	T1	68.23	52.03	11.20	14.80
	T2	54.73	40.57	13.63	11.80
	T3	46.20	35.32	16.77	10.23
	T4	44.17	30.34	18.73	9.03
	T5	39.50	28.24	21.37	8.44
CD (5%)		3.37	1.34	2.94	2.97
III Paddy straw (control)	T1	69.27	49.04	10.93	13.85
	T2	54.83	38.78	13.87	10.82
	T3	47.30	33.72	16.50	9.55
	T4	43.20	28.94	18.57	8.68
	T5	41.73	26.94	21.20	7.94
CD (5%)		3.00	2.60	3.62	2.14

T1 – Fresh chopped straw (first day), T2 – Final grade compost (at 28th day), T3 – Pinhead stage, T4 – After first flush, T5 – At crop termination

Table 4.2.2: Proximate analysis of compost during different stages of growth of button mushroom

Compost	Intervals	Cellulose (%)	Lignin (%)	Hemicellulose (%)
I Paddy straw:Maize stalk (1:1)	T1	31.72	10.80	15.90
	T2	23.72	9.84	14.45
	T3	20.69	9.03	12.87
	T4	17.89	8.67	10.17
	T5	16.73	8.06	9.36
CD (5%)		2.16	NS	0.79
II Paddy straw:Maize stalk (1:2)	T1	34.79	11.15	17.59
	T2	26.39	10.70	15.39
	T3	22.71	9.73	12.28
	T4	19.69	8.68	9.07
	T5	18.34	7.56	8.41
CD (5%)		2.68	NS	0.83
III Paddy straw (control)	T1	36.37	9.97	12.33
	T2	27.04	9.07	10.97
	T3	23.40	8.45	8.57
	T4	20.05	8.25	7.86
	T5	19.05	8.18	7.80
CD (5%)		3.08	NS	0.66

T1 – Fresh chopped straw (first day), T2 – Final grade compost (at 28th day), T3 – Pinhead stage, T4 – After first flush, T5 – At crop termination

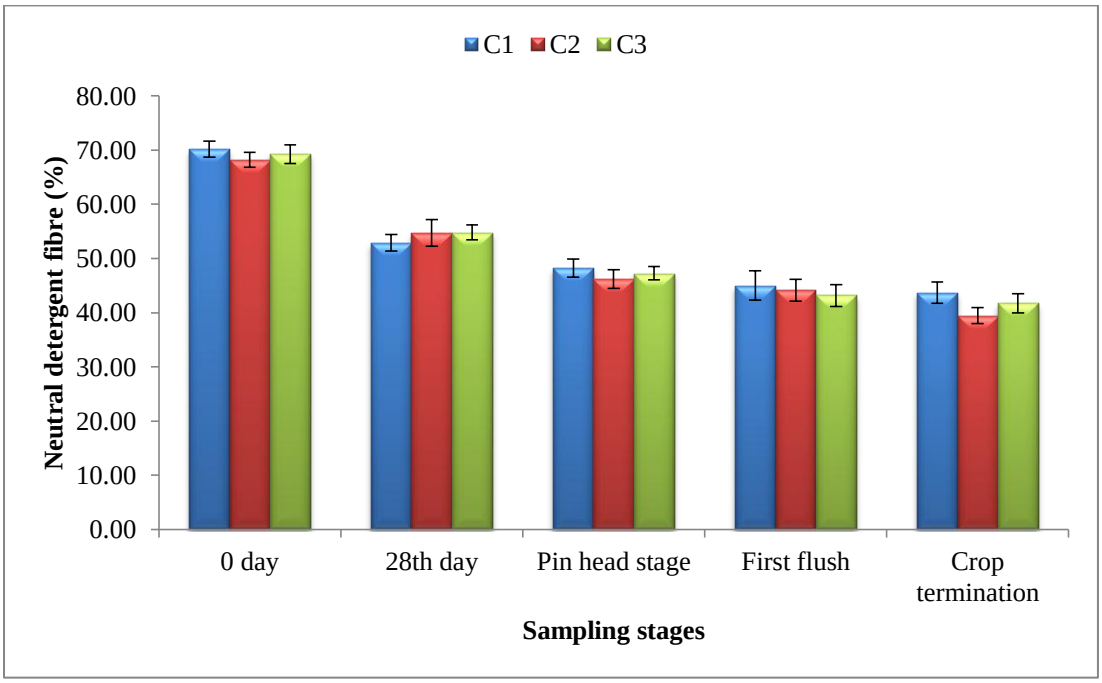

Fig.4.2.1: Neutral detergent fibre of compost formulations
C1: Paddy straw: Maize stalk(1:1), C2: Paddy straw: Maize stalk(2:1), C3: paddy straw

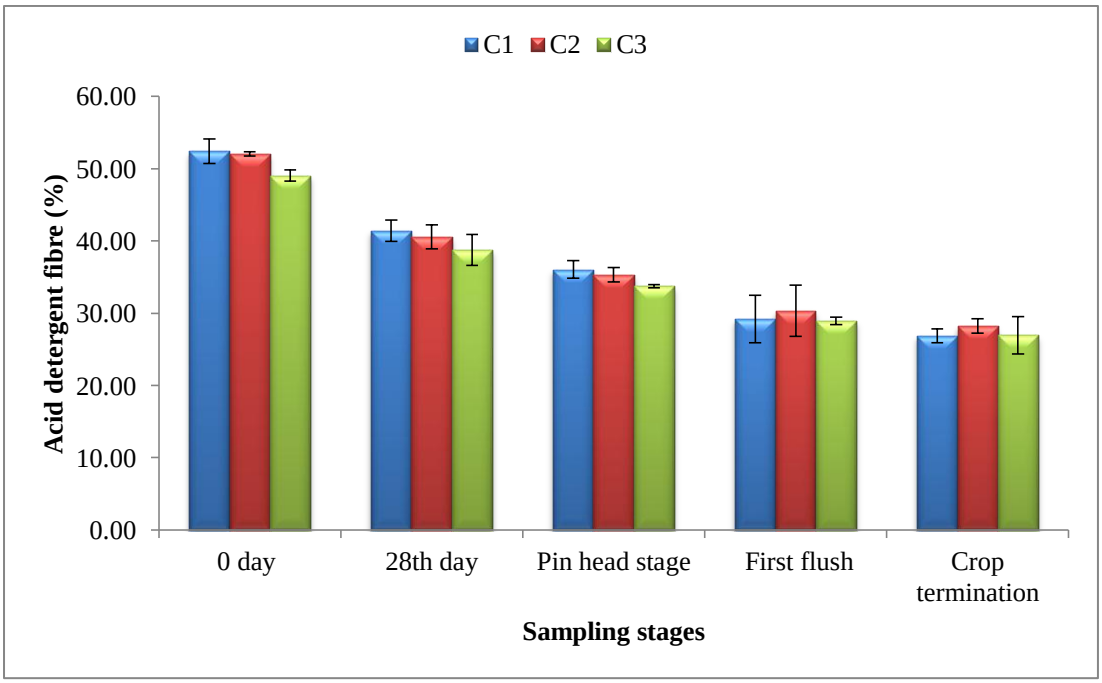

Fig. 4.2.2: Acid detergent fibre of compost formulations
C1: Paddy straw: Maize stalk(1:1), C2: Paddy straw: Maize stalk(2:1), C3: paddy straw

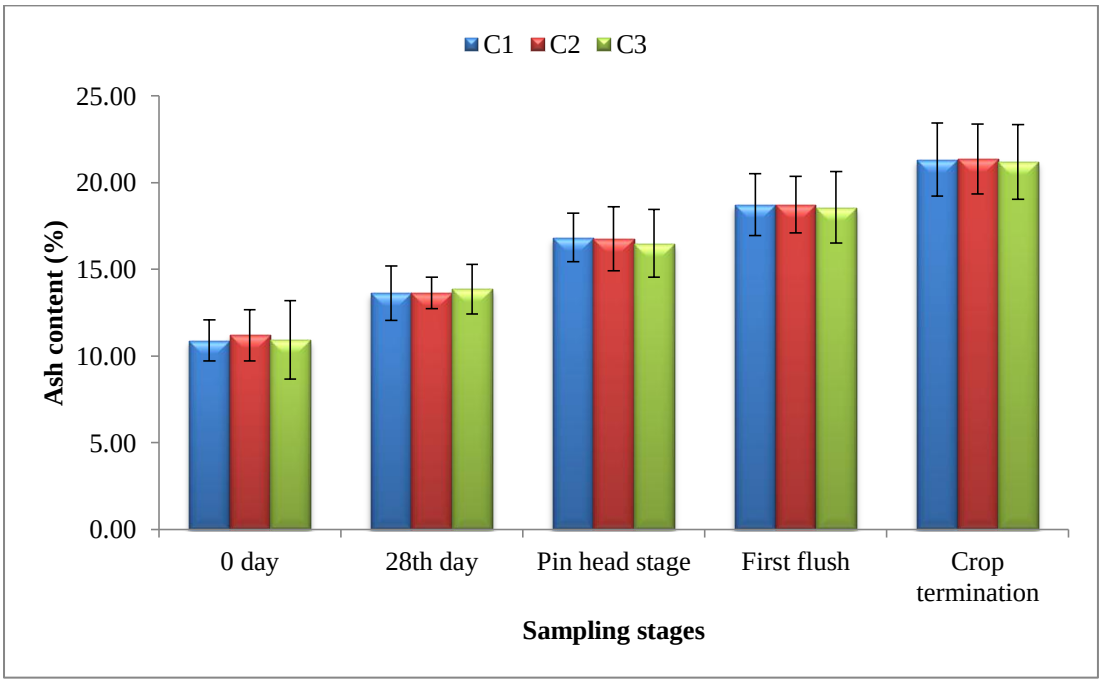

Fig.4.2.3: Ash content of compost formulations.
C1: Paddy straw: Maize stalk(1:1), C2: Paddy straw: Maize stalk(2:1), C3: paddy straw

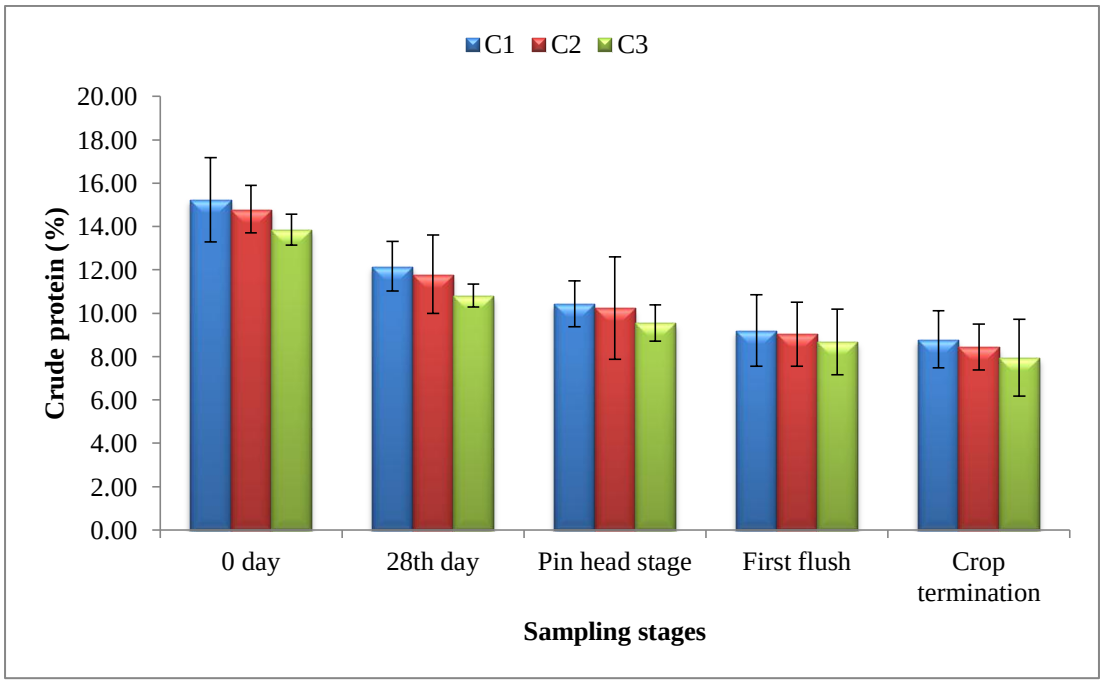

Fig.4.2.4: Crude protein of compost formulations
C1: Paddy straw: Maize stalk(1:1), C2: Paddy straw: Maize stalk(2:1), C3: paddy straw

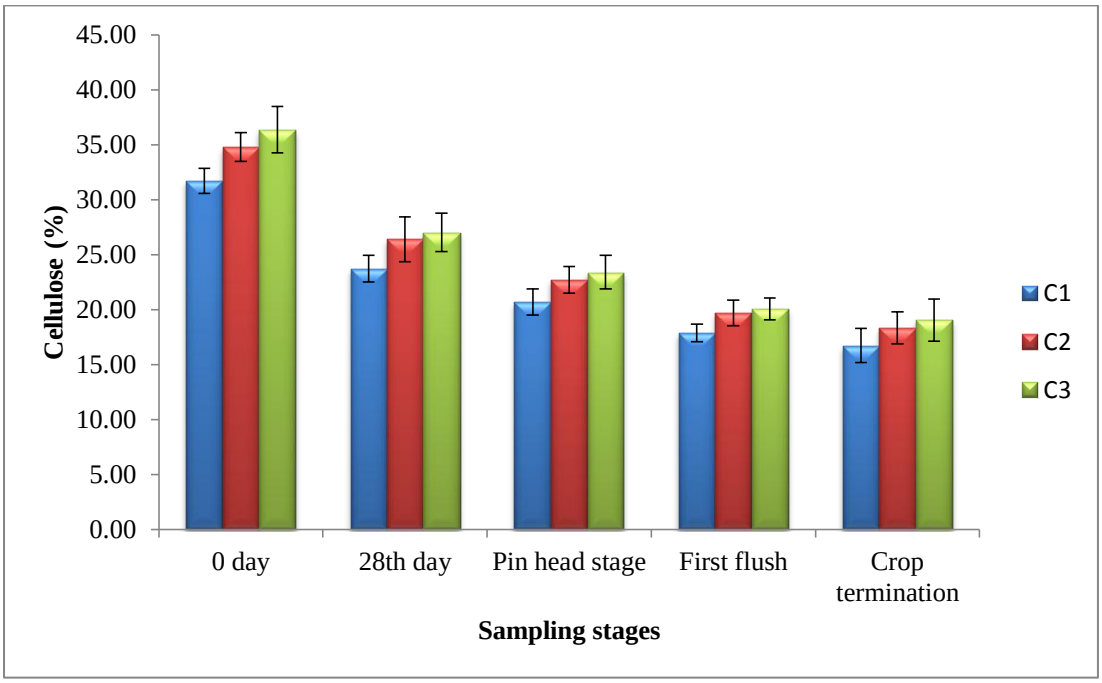

Fig.4.2.5: Cellulose content of compost formulations.
C1: Paddy straw: Maize stalk(1:1), C2: Paddy straw: Maize stalk(2:1), C3: paddy straw

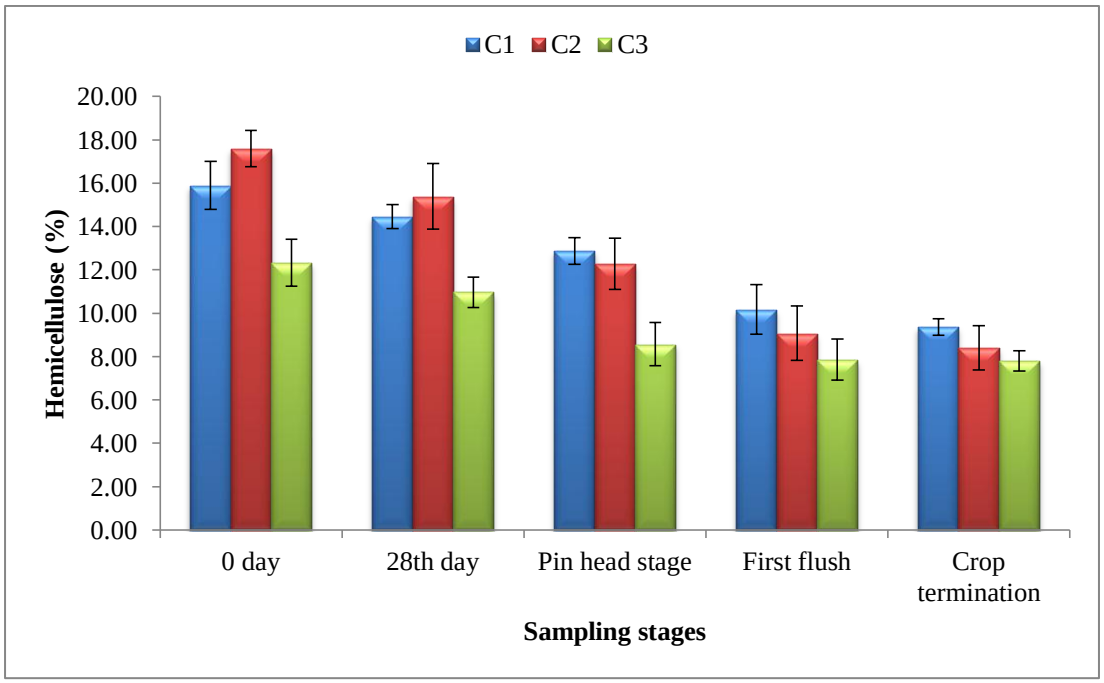

Fig.4.2.6: Hemicellulose content of compost formulations.
C1: Paddy straw: Maize stalk(1:1), C2: Paddy straw: Maize stalk(2:1), C3: paddy straw

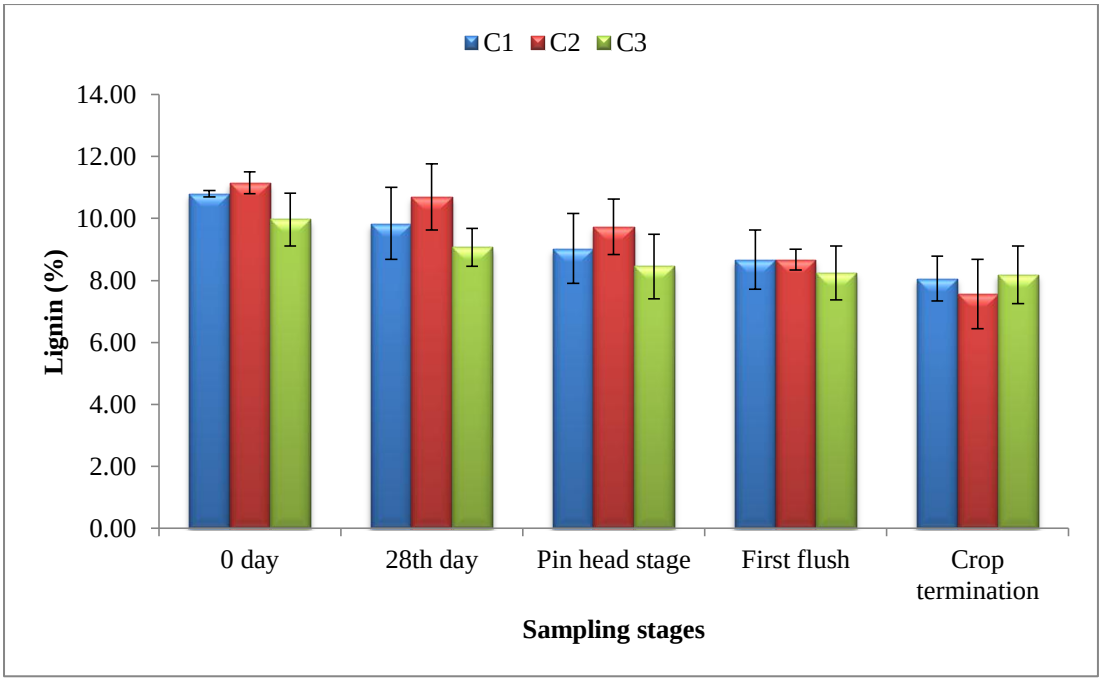

Fig.4.2.7: Lignin content of compost formulations
C1: Paddy straw: Maize stalk(1:1), C2: Paddy straw: Maize stalk(2:1), C3: paddy straw

4.3 Yield potential of *Agaricus bisporus* of U3 strain on paddy straw and maize stalk based composts

Paddy straw and maize stalk were used in 1:1, 2:1(w/w) ratio to prepare compost for the cultivation of *Agaricus bisporus* U3 strain. The observations on spawn run, case run, yield harvest and number of fruit bodies were made for 4 weeks of the harvesting. Spawn run in all compost formulations was found to complete between 21 to 28 days. The mycelial growth was visually fast on paddy straw + maize stalk based composts. Case run was completed between 25-27 days and pinning appeared between 47 to 51 days of spawning. Yield data indicated maximum yield (13.6 kg/q compost) in paddy straw + maize stalk (1:1) compost with 1563 fruit bodies/ q compost (Plate 5). Yield data obtained for paddy straw + maize stalk (2:1) was at par with that of the paddy straw based compost (Table 4.3, Fig 4.3.1, 4.3.2). Average weight of fruit bodies ranged from 8.7-12.3 g. In certain bags (5-7 bags) of paddy straw + maize stalk (2:1) showed presence of green mould with a loss of about 2% of yield (Table 4.3). It was observed that paddy straw + maize (1:1, w/w) compost was better degraded than paddy straw + maize stalk (2:1,w/w) and paddy straw composts. This showed that the compactness of paddy straw compost stack is an obstacle to the propagation of thermophilic microorganisms that are responsible for the proper degradation of the compost which was directly related to the yield. The utilization of paddy straw and maize stalks as a substitute of wheat straw delivered a yield practically identical to wheat straw was observed by Tewari and Sohi (1976). They found that synthetic compost prepared out of

Table 4.3: Yield potential of *Agaricus bisporus* of U3 strain on paddy straw, maize stalk based composts

Compost Formulation	Spawn run (d)	Case run (d)	Pinning (d)	Yield (kg/q compost)	NFB (no./q compost)	Av. Wt. of a FB (g)	Disease/ Pest
Paddy straw + Maize stalk (1:1)	21-28	27	48	13.6	1563	8.7	negative
Paddy straw + Maize stalk (2:1)	21-24	25	47	10.6	1040	10.2	positive
Paddy straw (control)	24-27	25	51	9.9	805	12.3	negative
CD (5 %)				1.57	103.3		

Bag size : 20”×24” (Polythene, 150 gauges)
No. of replicates : 3 each of 10 bags for each compost formulations (5 kg compost per bag)
Experimental design : CRD
Date of spawning : 19.12.2017
Days of spawn run : 21-28 days
Days for pinning : 20-27 after casing
Days for harvest : 4 weeks crop data
Disease by pest, loss% : Green mould; loss 2%

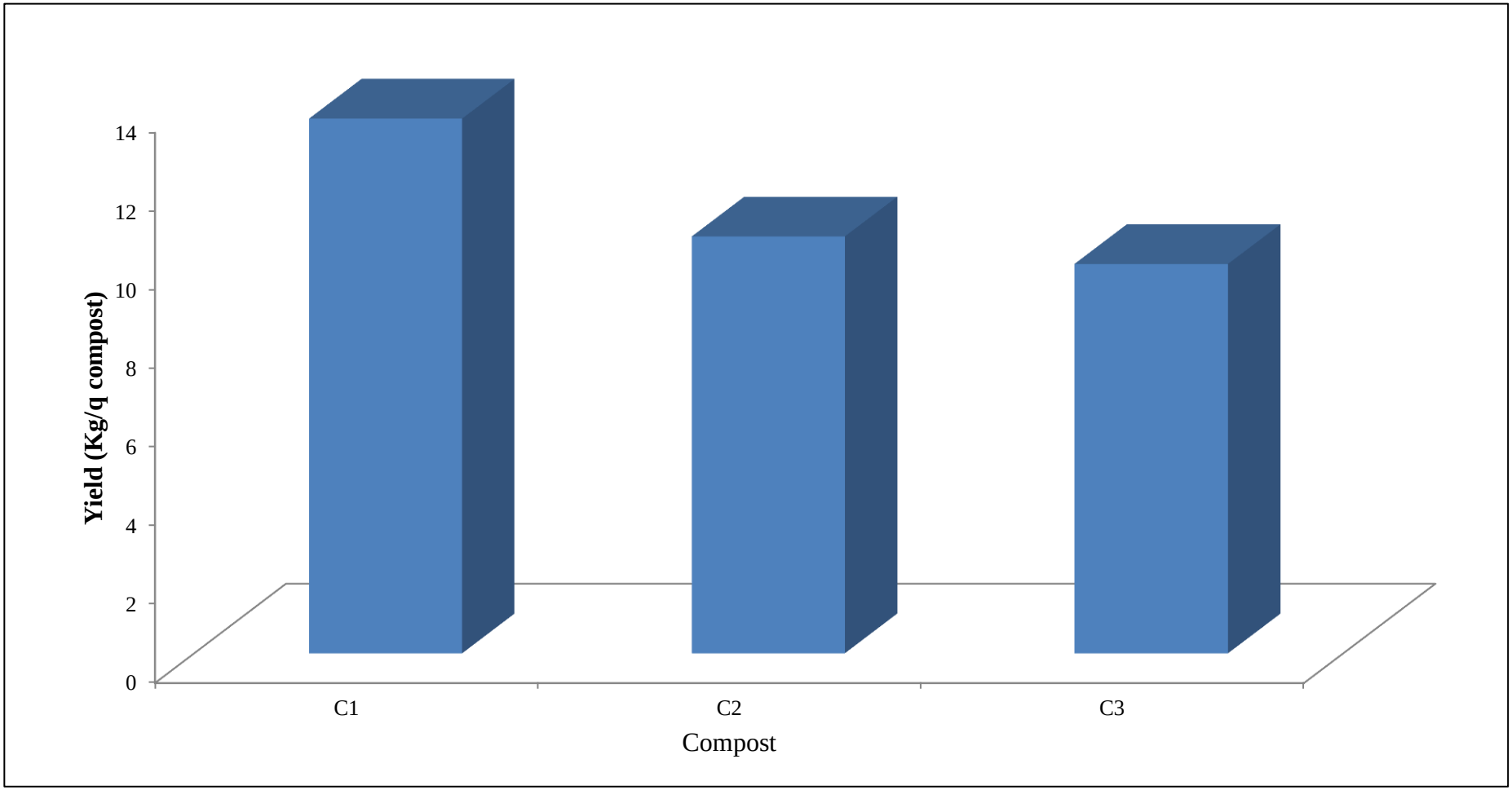

Fig.4.3.1: Yield performance of *Agaricus bisporus* with compost formulations
C1: Paddy straw: Maize stalk(1:1), C2: Paddy straw: Maize stalk(2:1), C3: Paddy straw

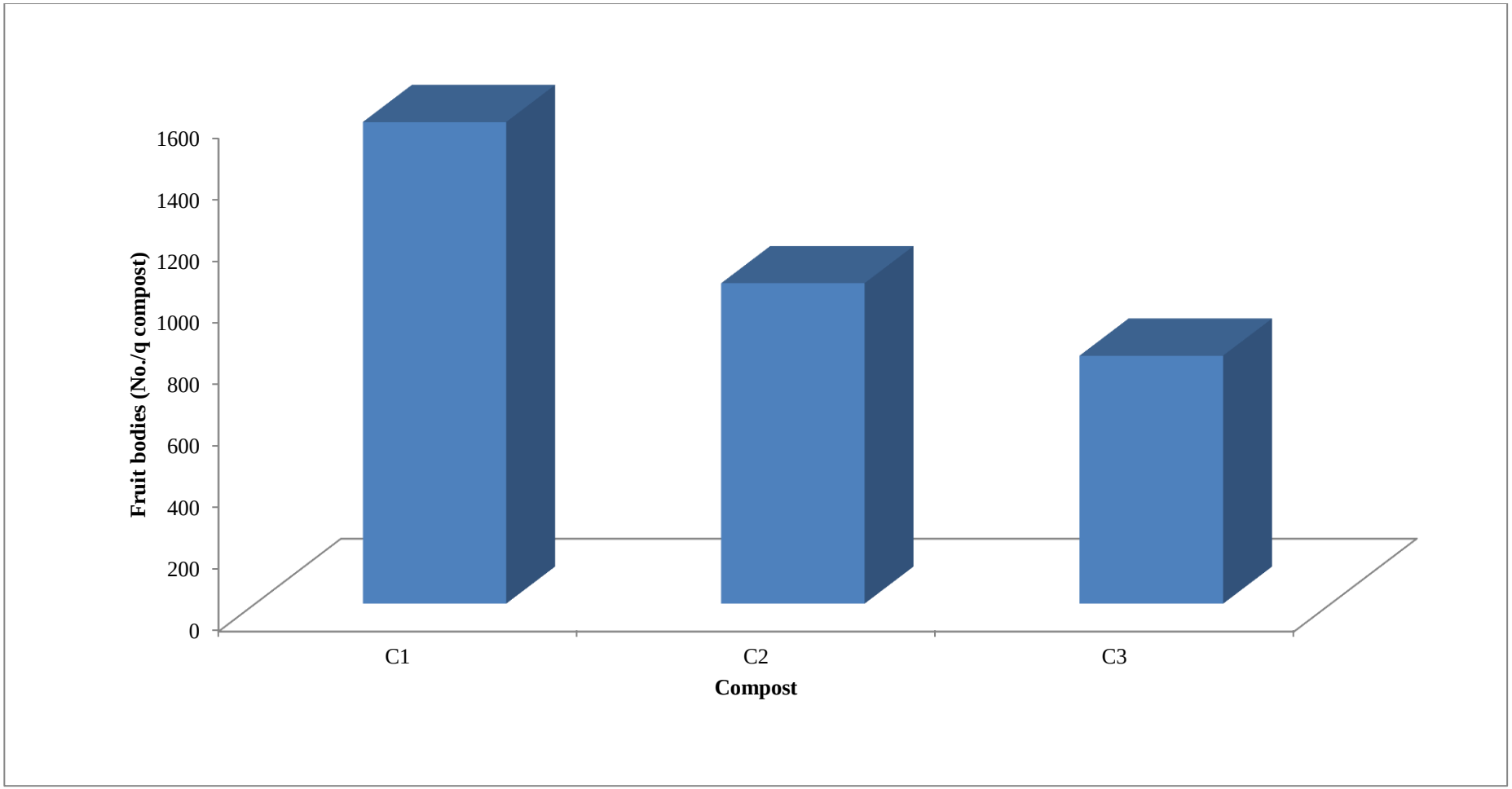

Fig.4.3.2: Number of fruit bodies of different compost formulations in *A. bisporus*
C1: Paddy straw: Maize stalk(1:1), C2: Paddy straw: Maize stalk(2:1), C3: Paddy straw

maize stalks and paddy straw when mixed in equal proportions produced a yield of 145.5 kg/ton of dry matter, which was quite comparable to wheat straw compost. Shandilya (1989) found high yield of button mushroom in two formulations of paddy straw composts with horse manure (5:1, w/w) and chicken manure (2.5:1, w/w). 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 (Garcha, 1997; Khanna and Kapoor 2016). Kaur and Khanna (2001) evaluated two synthetic compost preparations; WS+PS (1:1) and WS+PS (1:2) and obtained yields ranging from 17.9-23.7 kg/100kg compost. Singh (1997) utilized different proportions of wheat straw and paddy straw with sugarcane baggase during composting.

The growth of mushrooms fully depend upon the compost for their nutrition. The efficiency of the mushrooms to utilize the various constituents of compost depend upon the substrate used in composting which further depend upon many physiochemical factors responsible during the composting process and mushroom growth. It was concluded from this study that paddy straw + maize stalk (1:1, w/w) compost was the best formulation which could be further exploited for large scale production of mushrooms for small and marginal farmers.

Plate 5: Fruit bodies of *Agaricus bisporus* strain U3 on Paddy straw : Maize stalk (1:1)

CHAPTER V

SUMMARY

The present investigation entitled "Development of compost formulation based on paddy straw and maize stalks for cultivation of *Agaricus bisporus*" was conducted at Mushroom Research Centre, Department of Microbiology, PAU, Ludhiana. In the present study, an alternative high yielding compost formulation based on paddy straw + maize stalks as a substitute to the existing wheat straw based compost were made. Cultivation of *Agaricus bisporus* was carried out with U3 strain on paddy straw + maize stalk based composts.

The physicochemical factors-moisture, pH, temperature, C:N ratio and microbiological counts of the composts namely paddy straw + maize stalk (1:1 ; 2:1, w/w) and paddy straw compost (control) were determined at each turning during composting. The moisture content of the composts was observed to be 65.2%-67.3% in PS:MS (2:1, 1:1,w/w) respectively at the start of the composting which finally decreased to 61.3%-63.4% in PS:MS (2:1, 1:1,w/w) respectively at the time of spawning whereas for the paddy straw (control) it was 62.6% in the beginning of compost formation while at the time of spawning, it was observed to be 62.1%. The pH was recorded as 6.4-6.7 for PS:MS (1:1, 2:1, w/w) respectively at the start of the composting which gradually increased to 7.6-7.8 for PS:MS (2:1, 1:1, w/w) respectively between the turnings and finally declined to7.2-7.3 in PS:MS (2:1, 1:1, w/w) respectively at the time of spawning while in case of paddy straw compost the pH increased from 6.5 at the start of composting and finally reached to 7.9 at the time of spawning. The temperature of the compost stack was maximum at 4^{th} turning ranging from 73.4 to 76.4°C in all the composts. The C:N ratio of the composts progressively narrowed down from the beginning towards the end of composting i.e. 37.7:1 to 16.6:1. The C:N ratio of the final grade compost was 18.7 in control (paddy straw based compost) while it was 16.6 and 17.2 in paddy straw + maize stalks compost (1:1, 2:1 w/w) respectively. The bacterial and fungal count of mesophiles was observed maximum at 2^{nd} turning ranged from 24-8.0 cfu while that of thermophiles it was maximum in 4^{th} turning which ranged from 134-7.33 cfu in all the composts.

During different stages of mushroom cultivation i.e at zero day of compost, final grade compost, pin head stage, after first flush and at crop termination the straw was analysed for proximate composition by using standard methods of AOAC (2000). The neutral detergent fibre and acid detergent fibre was significantly reduced in all the compost upto pin head stage (70.20%-46.20%),(52.41%-33.72%) respectively and there after it was statistically at par after first flush and at crop termination. Total ash content in all the compost showed increment and ranged (10.90%-21.37%). There was no significant variation in crude protein content at these stages. The cellulose (36.37%-20.69%) and hemi-cellulose (17.59%-8.57%) content was reduced drastically during the compost formulations leading toward pin head stage and it was

stable thereafter. The lignin content showed no significant decline during different stages of composting and crop production.

Evaluation of these compost mixtures for mushroom production using U3 strain of *A. bisporus* was done. The observations on spawn run, case run, yield harvest and number of fruit bodies were made for 4 weeks of the harvesting. Spawn run was complete in the compost between 21 to 28 days. The mycelial growth was visually fast on paddy straw + maize stalk based composts. Case run was completed between 25-27 days and pinning appeared between 47 to 51 days of spawning. Yield data indicated maximum yield (13.6 kg/q compost) in paddy straw + maize stalk (1:1) compost with 1563 fruit bodies/ q compost. Yield data obtained for paddy straw + maize stalk (2:1) was at par with that of the paddy straw based compost. Average weight of fruit bodies ranged from 8.7-12.3 g. In certain bags (5-7 bags) of paddy straw + maize stalk (2:1) showed presence of green mould with a loss of about 2% of yield. It was observed that paddy straw + maize (1:1, w/w) compost was better degraded than paddy straw + maize stalk (2:1,w/w) and paddy straw composts. It was concluded from this study that paddy straw + maize stalk (1:1, w/w) compost was the best formulation which could be further exploited for large scale production of mushrooms for small and marginal farmers.

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APPENDIX

Experiment 4.1 Physicochemical characteristics of different composts during composting.

CD (5%) of moisture content of different compost formulations

1:1

NR = 3 NT = 7 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 67.316670 63.099990 61.666670 60.599990 62.233340
6 61.766660 63.453330

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	6	14.195310	.70	NS		
Error	14	20.286270			62.876660	7.16

2:1

NR = 3 NT = 7 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 65.200000 64.333340 61.633330 60.100000 62.333330
6 63.233330 61.300000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	6	9.5559890	1.07	NS		
Error	14	8.9520090			62.590470	4.78

---

control

NR = 3 NT = 7 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 62.633330 60.300000 58.733330 59.400000 61.333330
6 63.200000 62.133330

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	6	8.5104170	.64	NS		
Error	14	13.279580			61.104760	5.96

CD (5%) of pH of different compost formulations

1:1

NR = 3 NT = 7 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 6.4333330 6.6000000 6.9666670 7.3666670 7.8000010
6 7.5000000 7.2666660

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	6	.72666420	2.82	NS		
Error	14	.25761200			7.1333340	7.12

2:1

NR = 3 NT = 7 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 6.7000000 7.0666670 7.2666660 7.5666670 7.4666670
6 7.3000000 7.1999990

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	6	.24188230	2.13	NS		
Error	14	.11334230			7.2238100	4.66

---

control

NR = 3 NT = 7 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 6.5333330 6.9000000 7.2000000 7.5333330 7.5666670
6 7.8000000 7.8666660

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	6	.72080490	2.13	NS		
Error	14	.33904160			7.3428570	7.93

CD (5%) of temperature profile of three compost formulations

1:1

NR = 3 NT = 7 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 59.200000 64.100000 71.499990 73.400000 67.233340
6 54.300000 42.100000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	6	357.23960	92.30	3.44523		
Error	14	3.8705360			61.690470	3.19

2:1

NR = 3 NT = 7 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 58.333330 66.233330 72.266670 76.433330 65.400000
6 53.133330 43.733330

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	6	384.29690	110.57	3.26466		
Error	14	3.4754460			62.219060	3.00

---

control

NR = 3 NT = 7 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 64.400000 67.900000 69.733340 74.400000 65.100000
6 55.633330 42.333330

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	6	343.37110	138.21	2.76021		
Error	14	2.4843750			62.785710	2.51

CD (5%) of Carbon: Nitrogen ratio of three compost formulations

1:1

NR = 3 NT = 7 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 32.283340 26.366670 23.866670 21.166670 19.233330
6 17.100000 16.633330

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	6	94.227700	661.21	.661079		
Error	14	.14250840			22.378570	1.69

2:1

NR = 3 NT = 7 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 37.733330 33.333330 27.000000 23.600000 20.566670
6 18.100000 17.186670

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	6	182.47360	498.65	1.05934		
Error	14	.36593190			25.360000	2.39

---

control

NR = 3 NT = 7 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 35.233330 30.633330 25.700000 23.500000 20.166670
6 19.200000 18.733330

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	6	117.49380	941.00	.618794		
Error	14	.12486050			24.738090	1.43

CD (5%) of Mesophilic bacterial population during composting

1:1

NR = 3 NT = 7 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 18.000000 23.000000 13.000000 16.000000 21.000000
6 20.000000 18.000000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	6	32.857100	4.51	4.72682		
Error	14	7.2857140			18.428570	14.65

2:1

NR = 3 NT = 7 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 18.000000 22.000000 14.000000 18.000000 20.000000
6 21.000000 16.000000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	6	23.857100	2.49	NS		
Error	14	9.5714280			18.428570	16.79

---

control

NR = 3 NT = 7 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 18.000000 24.000000 14.000000 9.6666670 6.3333330
6 5.6666670 4.6666670

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	6	157.52380	35.19	3.70500		
Error	14	4.4762140			11.761900	17.99

CD (5%) of Thermophilic bacterial population during composting

1:1

NR = 3 NT = 7 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS

1	80.000000	113.00000	163.00000	205.00000	146.00000
6	87.000000	21.000000			

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	6	11099.850	470.90	8.50210		
Error	14	23.571430			116.42860	4.17

2:1

NR = 3 NT = 7 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS

1	84.000000	126.00000	176.00000	199.00000	106.00000
6	78.000000	23.000000			

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	6	10874.430	396.46	9.17138		
Error	14	27.428570			113.14290	4.63

---

control

NR = 3 NT = 7 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS

1	87.000000	133.00000	196.00000	224.00000	126.00000
6	64.000000	39.000000			

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	6	13731.430	320.40	11.4642		
Error	14	42.857140			124.14290	5.27

CD (5%) of Mesophilic fungal population during composting

1:1

NR = 3 NT = 7 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 5.3333330 7.6666670 4.3333330 3.6666670 2.3333330
6 1.6666670 1.3333330

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	6	15.190490	9.38	2.22824		
Error	14	1.6190450			3.7619050	33.82

2:1

NR = 3 NT = 7 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 4.6666670 7.3333330 4.3333330 3.3333330 2.3333330
6 1.6666670 1.3333330

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	6	13.079370	8.86	2.12767		
Error	14	1.4761880			3.5714290	34.02

---

control

NR = 3 NT = 7 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 5.3333330 8.0000000 4.6666670 3.6666670 2.0000000
6 1.6666670 1.3333330

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	6	17.317470	10.39	2.26077		
Error	14	1.6666630			3.8095240	33.89

CD (5%) of Thermophilic fungal population during composting

1:1

NR = 3 NT = 7 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 3.3333330 4.3333330 6.3333330 7.0000000 3.3333330
6 2.3333330 2.0000000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	6	11.079370	5.67	2.44690		
Error	14	1.9523790			4.0952380	34.12

2:1

NR = 3 NT = 7 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 3.3333330 4.3333330 5.6666670 7.0000000 3.3333330
6 1.6666670 1.3333330

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	6	12.539690	5.85	2.56348		
Error	14	2.1428550			3.8095240	38.43

---

control

NR = 3 NT = 7 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 3.6666670 5.0000000 6.3333330 7.3333330 2.6666670
6 1.3333330 1.0000000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	6	17.746040	26.62	1.42984		
Error	14	.66666520			3.9047620	20.91

Experiment 4.2 Proximate analysis of compost during different stages of growth of button mushroom

CD (5%) of Neutral detergent fibre at different stages of growth of button mushroom

1:1

NR = 3 NT = 5 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 70.200000 52.933330 48.233330 45.033340 43.700000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	347.79100	93.85	3.50058		
Error	10	3.7058590			52.020000	3.70

2:1

NR = 3 NT = 5 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 68.233340 54.733330 46.200000 44.166670 39.500000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	383.98050	111.35	3.37681		
Error	10	3.4484370			50.566660	3.67

---

control

NR = 3 NT = 5 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 69.266660 54.833330 47.300000 43.200000 41.733330

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	381.30760	140.07	3.00027		
Error	10	2.7222660			51.266670	3.22

CD (5%) of Acid detergent fibre at different stages of growth of button mushroom

1:1

NR = 3 NT = 5 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 52.410000 41.430000 36.040000 29.190000 26.850000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	316.39890	116.18	3.00092		
Error	10	2.7234380			37.184000	4.44

2:1

NR = 3 NT = 5 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 52.030000 40.570000 35.320000 30.340000 28.240000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	271.58350	494.84	1.34714		
Error	10	.54882810			37.300000	1.99

---

control

NR = 3 NT = 5 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 49.040000 38.780000 33.720000 28.940000 26.940000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	235.17290	114.95	2.60098		
Error	10	2.0458980			35.484000	4.03

CD (5%) of Total Ash content at different stages of growth of button mushroom

1:1

NR = 3 NT = 5 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS

1	10.900000	13.633330	16.833330	18.733330	21.333330

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	50.857910	18.93	2.98059		
Error	10	2.6866700			16.286660	10.06

2:1

NR = 3 NT = 5 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS

1	11.200000	13.633330	16.766670	18.733330	21.366670

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	48.692320	18.51	2.94939		
Error	10	2.6307130			16.340000	9.93

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control

NR = 3 NT = 5 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS

1	10.933330	13.866670	16.500000	18.566670	21.200000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	47.904420	12.02	3.62956		
Error	10	3.9839840			16.213330	12.31

CD (5%) of Crude protein at different stages of growth of button mushroom

1:1

NR = 3 NT = 5 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 15.233330 12.166670 10.433330 9.2000000 8.8000000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	20.658260	9.67	2.65763		
Error	10	2.1359860			11.166670	13.09

2:1

NR = 3 NT = 5 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 14.800000 11.800000 10.233330 9.0333340 8.4433340

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	19.482790	7.28	2.97467		
Error	10	2.6760010			10.862000	15.06

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control

NR = 3 NT = 5 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 13.850000 10.816670 9.5500000 8.6766670 7.9433340

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	16.149750	11.61	2.14450		
Error	10	1.3907960			10.167330	11.60

CD (5%) of Cellulose at different stages of growth of button mushroom

1:1

NR = 3 NT = 5 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 31.716670 23.716670 20.686670 17.893330 16.733330

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	107.68240	75.67	2.16926		
Error	10	1.4230960			22.149330	5.39

2:1

NR = 3 NT = 5 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 34.786670 26.386670 22.710000 19.693330 18.343330

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	130.14040	59.66	2.68564		
Error	10	2.1812500			24.384000	6.06

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control

NR = 3 NT = 5 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 36.366670 27.040000 23.400000 20.053340 19.050000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	146.72140	51.06	3.08261		
Error	10	2.8737300			25.182000	6.73

CD (5%) of Lignin at different stages of growth of button mushroom

1:1

NR = 3 NT = 5 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 10.800000 9.8400000 9.0300000 8.6700000 8.0600000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	3.4102780	2.97	NS		
Error	10	1.1464230			9.2800000	11.54

2:1

NR = 3 NT = 5 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 11.150000 10.700000 9.7300000 8.6800000 7.5600000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	6.4732360	1.85	NS		
Error	10	3.5054690			9.5639990	19.58

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control

NR = 3 NT = 5 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 9.9700000 9.0700000 8.4500010 8.2500000 8.1800000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	1.6874080	1.26	NS		
Error	10	1.3397710			8.7840000	13.18

CD (5%) of Hemicellulose at different stages of growth of button mushroom

1:1

NR = 3 NT = 5 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 15.900000 14.450000 12.870000 10.170000 9.3600000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	23.081540	120.18	.796929		
Error	10	.19206540			12.550000	3.49

2:1

NR = 3 NT = 5 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 17.590000 15.390000 12.280000 9.0700000 8.4100000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	47.092350	221.99	.837530		
Error	10	.21213380			12.548000	3.67

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control

NR = 3 NT = 5 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS
1 12.330000 10.970000 8.5700000 7.8600000 7.8000010

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	12.460510	92.65	.666886		
Error	10	.13449710			9.5060010	3.86

Experiment 4.3 Yield potential of *Agaricus bisporus* of U3 strain on paddy straw and maize stalk based composts

CD (5%) of Yield performance of *Agaricus bisporus* of different compost formulations

Yield

NR = 3 NT = 3 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS

1	13.600000	10.600000	9.9000000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	2	11.589970	18.59	1.57768		
Error	6	.62331130			11.366670	6.95

CD (5%) of Number of fruit bodies of *Agaricus bisporus* of different compost formulations

NFB

NR = 3 NT = 3 NE = 1
Analysis of CRD with equal no. of replications

TREATMENT MEANS

1	1563.0000	1055.0000	805.00000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	2	447564.00	167.33	103.348		
Error	6	2674.6670			1141.0000	4.53

VITA

Name of the student	:	Sukhmandeep Kaur
Father's name	:	S. Karnail Singh
Mother's name	:	Smt. Gurmeet Kaur
Nationality	:	Indian
Date of birth	:	29th November, 1994
Permanent home address	:	VPO Sarawan Bodla, Teh. Malout, Distt. Shri Muktsar Sahib
Email Address	:	seerutgill@gmail.com

EDUCATIONAL QUALIFICATION

Bachelor's degree	:	B.Sc. (Biotech.) Hons.
University	:	Punjab Agricultural University, Ludhiana
Year of award	:	2016
OCPA	:	7.56/10.00
Master's degree	:	M.Sc. (Microbiology)
University	:	Punjab Agricultural University, Ludhiana, Punjab- 141 004
Year of award	:	2018
OCPA	:	7.39/10.00
Title of Master's Thesis	:	Development of compost formulation based on paddy straw and maize stalks for cultivation of *Agaricus bisporus*