STUDIES ON CULTURAL REQUIREMENTS AND CULTIVATION TECHNOLOGY OF SOME MEDICINAL MUSHROOMS

A
Thesis
By

POOJA DHIMAN

Submitted in partial fulfillment of the requirements
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Dr C D Thapa
Senior Scientist

Department of Mycology and Plant Pathology
College of Horiculture
Dr. Y S Parmar University of Horticulture
and Forestry, Nauni-173 230, Solan (HP)

CERTIFICATE-I

This is to certify that the thesis entitled, **"Studies on cultural requirements and cultivation technology of some medicinal mushrooms"** submitted in partial fulfillment of the requirements for the award of degree of **MASTER OF SCIENCE** in **Mycology and Plant Pathology** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (HP) is a record of bonafide research work carried out by **Ms. Pooja Dhiman (H-2007-31-M)** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

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

Place: Nauni-Solan
Dated:

(Dr. C D Thapa)
Major Advisor
Advisory Committee

CERTIFICATE-II

This is to certify that the thesis entitled, **"Studies on cultural requirements and cultivation technology of some medicinal mushrooms"** submitted by **Ms. Pooja Dhiman (H-2007-31-M)** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (HP) in partial fulfillment of the requirements for the award of degree of **MASTER OF SCIENCE** in **Mycology and Plant Pathology** has been approved by the Student's Advisory Committee after an oral examination of the same in collaboration with the external examiner.

Dr CD Thapa
Senior Scientist
(Major Advisor)

Dr SR Sharma
(External Examiner)

Members, Advisory Committee

Dr. BC Suman
Senior Scientist (Dept. of Mycology and Plant Pathology)

Dr. PR Gupta
Senior Scientist
(Dept. of Entomology)

Dr. (Mrs.) Neerja Rana
Asstt. Scientist
(Dept. of Basic Science)

Dean's Nominee
Dr Devender Gupta

Professor and Head
Department of Mycology and Plant Pathology

Dean
College of Horticulture
Dr. Y S Parmar University of Horticulture & Forestry,
Nauni-Solan (HP)

CERTIFICATE-III

This is to certify that all the mistakes and errors pointed out by the external examiner have been incorporated in the thesis entitled**, "Studies on cultural requirements and cultivation technology of some medicinal mushrooms"** submitted to Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan (HP) by **Ms. Pooja Dhiman (H-2007-31-M)** in partial fulfillment of the requirements for the award of degree of **MASTER OF SCIENCE** in **Mycology and Plant Pathology.**

_____

Dr. CD Thapa
Senior Scientist
(Major Advisor)

_____

Dr. SK Sharma
Professor and Head
Department of Mycology and Plant Pathology
Dr. Y S Parmar, UHF, Nauni-173 230, Solan (HP)

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Place: Nauni, **(Pooja Dhiman)**

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ABBREVIATIONS

%	Per cent
µg	Micro gram
µl	Micro litre
A. polytricha	*Auricularia polytricha*
cm	Centimeter
F. velutipes	*Flammulina velutipes*
g	Gram
G. lucidum	*Ganaderma lucidum*
h	hour
HCl	Hydrochloric acid
HPKVV	Himachal Pradesh Krishi Vishva Vidyalaya
Kg	Kilo gram
L. edodes	*Lentinula edodes*
mg	Milligram
mm	Millimeter
N	Normality
NaOH	Sodium hydroxide
^{o}C	Degree Celsius
^{o}F	Degree Fahrenheit
P. eryngii	*Pleurotus eryngii*
P. ostreatus	*Pleurotus ostreatus*
psi	pound per square inch
UHF	University of Horticulture and Forestry
V/V	Volume by Volume
W/V	Weight by Volume

Chapter-1

INTRODUCTION

Since the beginning of the human civilization, ethno botanicals such as herbs and mushrooms have been valued for both culinary and medicinal properties. The base assumption about herbal medicine is that plants contain natural substances that can promote health and alleviate illness (Mahaday, 2001). During the last decades, the use of traditional medicines had expanded globally and over- the- counter supplements have become very popular.

There are approximately 14,000 described species of 1.5 million fungi that produce fruiting bodies large enough to be considered as mushrooms. More that 50 mushroom species have yielded potential immunoceuticals (Chang, 2006). Chang and Buswell (1996) coined the term 'nutraceuticals' for the medicinal preparations from mushrooms. This new class of compounds termed "mushroom nutraceuticals" are extractable from either the mushroom mycelium and /or spent mycelial culture fluids/or the mushroom fruiting bodies (Chang and Buswell, 2008).

The annual global production of mushrooms during the year 2007 was 34,14,392 tonnes and that of India was 48,000 tonnes only (Anonymous, 2007). World production of culinary- medicinal mushrooms has reached 16 million metric tonnes per year (Sharma *et al*., 2008).

Medicinal mushrooms have an established history of use in traditional oriental medicines. Many traditionally used mushrooms from genera: *Auricularia, Flammulina, Ganoderma, Grifola, Lentinus, Trametes, Tramella* etc. have been demonstrated to possess significant medicinal properties (Wasser *et al*., 2000). Polysaccharide- protein complexes, triterpenes and nitrogen containing compounds are three basic groups of compounds responsible for the medicinal effects of mushrooms (Willard, 1991).

Ganoderma lucidum (Layss. Fr) Karst., a basidiomycete belonging to Polyporaceae and also known as Lingzhi in China and Reishi in Japan, has been widely used as a traditional medicine in the orient for more than 2000 years. It is also regarded as 'king of herbs'. The estimated turn over of *G. lucidum* production is approximately $ 2.16 billion US

(Mishra and Singh, 2008). *Ganoderma* is unique in being consumed for its medicinal, rather than nutritional value (Chang and Buswell, 1999). The specific reported attributes of Lingzhi include lowering the risk of cancer, heart diseases and infection. These health promoting effects are believed to be mediated via the antioxidant, hypotensive, anti-inflammatory and immunomodulatory properties of the triterpenes present in the mushroom (Chen and Miles, 1996).

Lentinula edodes (Berk. & Mont.) Pegler, commonly called as shiitake is an edible mushroom that has been cultivated for thousand of years. It is the second largest cultivated mushroom in the world. Besides the ability to convert ligno- cellulosic materials into food with good flavours and texture, it has medicinal and nutritional benefits to human health that places it at the forefront of cultivated mushrooms. Shiitake is rich in polysaccharide protein complexes that enhance immune system by serving as biological response modifiers. Shiitake is considered an excellent protein source because it contains essential amino acids. Eritadenine, a protein present in shiitake, helps to reduce cholesterol level in blood, lipid components of serum and also lowers the high blood pressure (Yap and Ng, 2003). Lentinan, a β- glucan polysaccharide complex present in shiitake, is used for preventing toxicity from acid foods and has antitumour activity by inhibiting growth of sarcoma- 180 (Hobbs, 1995).

Flammulina velutipes (Curt. Ex Fr.) Singer commonly called as enokitake or winter mushroom ranks fifth in total world's mushroom production. From this winter mushroom, immunomodulatory proteins viz., flammuline and fip-fve have been isolated. They lower the blood pressure and are active against tumour cells (Tkekawa, 2001). It also prevents and cures liver diseases and gastero-enteric ulcers. In addition, enokitake contains immunomodulatory, anti-tumorous and antibiotic substances (Sharma *et al.*, 2006).

Auricularia polytricha (Mont) Sacc. commonly called as black ear's mushroom, ranks fourth among all the cultivated mushrooms in production terms (Chang, 1996). It is generally used for treatment of piles, sore throat, inflammation of eyes and anaemia.

Pleurotus spp. (Oyster mushroom) is the third largest cultivated mushroom in the world. A polycyclic aromatic compound, pleurotin has been isolated from some *Pleurotus* species which are known to possess a number of medicinal properties such as anti-tumorous, anti-inflammatory, hypocholestrolaemic, antiviral, anti microbial etc. (Grigori *et al*., 2007). Among these, *Pleurotus eryngii* (Dc.: Fr.) Quel. possess immunomodulatory, anti inflammatory and anti- tumour activities whereas *Pleurotus ostreatus* (Jacq. Ex Fr.) Kummer

has antiviral and anti- mitogenic, anti- oxidant and gene protective activities. The medicinal properties of *P. ostreatus* is attributed to the presence of a compound called p- anisaldehyde and that of *P. eryngii* to eryngin (an anti fungal- peptide) and eryngeolysin (a haemolysin).

Thus, it is crucial that along with the growing interest of the population in herbs and medicinal mushrooms, scientific research should be conducted to evaluate and investigate the benefits and possible deleterious effects of ethno botanicals. Keeping in view the obvious potentialities, increasing demand and popularity of medicinal mushrooms, the present study was carried out with following objectives:

1) To work out the nutritional requirements of test fungi.
2) Cultivation studies:

- To evaluate different substrates for spawn production.
- To select different substrates for the production of fruiting bodies of three medicinal mushrooms viz., *Ganoderma lucidum, Lentinula edodes* and *Flammulina velutipes*.

Chapter-2

REVIEW OF LITERATURE

Fungi and plants have been considered as the most abundant source of lead compounds for the development of therapeutics by the biopharmaceutical industry. Now- a - days, there is an increasing demand for powder and extracts of medicinal mushrooms. However, most of the mushrooms are still wild and need much improvement in order to become established as a common mushroom. The information available on various aspects of some important medicinal mushrooms has been reviewed under following heads:

Lentinula edodes

Selection of media

Different workers had shown best mycelial growth of *Lentinula edodes* on Potato dextrose agar, Yeast potato agar and Malt extract agar (Jodon and Royse, 1979; Campbell and Slee, 1987;Chang and Miles, 1989). Pacumbaba and Pacumbaba (1999) recorded solid media viz., YMMBSA (Yeast extract, Malt extract , multigrain oatmeal and brown sugar agar YVMBSA (Yeast extract,V-8 vegetable juice, multigrain oat meal and brown sugar agar) and broths viz., YVMBS (Yeast extract, V-8 vegetable juice, multigrain oat meal and brown sugar), YVMS (Yeast extract, V-8 vegetable juice, multigrain oat meal and sucrose), MVBS (multigrain oat meal, V-8 vegetable juice, brown sugar and sucrose) as the excellent media and broths for the growth of shiitake.

Furlan *et al*. (1997) recorded highest rate of mycelial growth of *L. edodes* on Wheat dextrose agar medium. Kaur and Lakhanpal (1999) reported wood extract agar medium to support maximum mycelial growth of *L. edodes* among fifteen different solid media studied and Glucose- asparagine as best liquid medium among five different liquid media tested by them.

Temperature studies

Royse (1989) while working with two isolates of *Lentinula leteritia* reported that optimum temperature for maximum vegetative growth of shiitake is in the range of 23 to 25^0C. Temperature of 25^0C is optimum for the best mycelial growth of *L. edodes* (Kaur and Lakhanpal, 1999; and Singh and Singh, 2005). Tnglet *et al.* (2006) while working with whey permeate for cultivation of *L. edodes* recorded 23.6^0C as optimum temperature for mycelial

growth of the fungus.

pH studies

Furlan *et al*. (1997) conducted experiments to select suitable pH for growth of *L. edodes*. They recorded best mycelial growth of the fungus at pH 4.0. Kaur and Lakhanpal (1999) recorded pH optima of 4.5 for best mycelial growth of *Lentinula edodes*. Singh and Singh (2005) stated that a pH of 5.5 was optimum for mycelial growth of *L. lateritia*. Best mycelial growth of *Lentinula edodes* was found at a pH 7.0 (Sharma *et al*., 2006). Tnglet (2006) proved that a pH of 5.0 is best for maximum mycelial growth of *Lentinula edodes* in whey permeate solid medium.

Carbon nutrition

Carbon is the most important constituent of the media which helps in the synthesis of proteins, protoplasm, nucleic acid, enzymes and cell wall materials. Lambert (1950) reported that it is an essential nutrient for the mushroom mycelium.

Matsuo (1950) and Khan *et al*. (1991) reported that *L. edodes* grew best with starch among different carbon sources tested. Kaur and Lakhanpal (1995) studied twelve different carbon sources and glucose was recorded as the best carbon source followed by fructose and sucrose for the vegetative growth of *L. edodes.*

Maximum mycelial growth of *L. edodes* on whey permeated medium was recorded with 40 g/l of lactose (Tnglet *et al.*, 2006). Meng *et al.* (2007) tested four different carbon sources and recorded sweet Potato starch as the best carbon source. On the effect of vitamin mixtures, Mahmud and Ohmasa (2008) reported that only ascorbic acid worked together with thiamine for increasing the growth of *L. edodes*.

Nitrogen nutrition

Nitrogen is considered important next to carbon as functional and structural element in growth of fungi. Addition of vitamin mixture supported growth only when thiamine was present. Boyle (1998) proved through his studies that nitrogen availability limits the growth rate of fungi on wood while availability of simple carbohydrates, micronutrients or vitamins increases the growth. Among twenty four nitrogen sources studied by Kaur and Lakhanpal (1999), peptone was recorded as best nitrogen source with cysteine giving similar results.

Tsivileva (2005) through his studies proved that among all the natural amino acids

studied as nitrogen sources and nine divalent cations as inorganic additives, L- asparagine and Ca^{2+} exhibited positive effects with regard to the formation of pigmented mycelial film in liquid culture. Kachlishvili (2006) recorded that hydrolytic enzymes and laccase yield increased by supplementation of medium with an additional nitrogen source.

Meng *et al.* (2007) while studying the effect of four nitrogen sources viz., soybean flour, wheat bran, peptone and Yeast extract on mycelial production of *L. edodes* found wheat bran as the best nitrogen source. The results of the studies done by Mahmud and Ohmasa (2008) cleared that the Yeast extract containing medium showed very good result for enhancing high temperature tolerance of mycelium of *L. edodes*, but methionine was not at all effective.

Trace element nutrition

Investigations done by Kaur and Lakhanpal (1995) showed that out of different trace elements viz., boron, iron, manganese, molybdenum and zinc that were tested, manganese (2 ppm) was noticed as the most effective trace element. Kang *et al.* (2001) inoculated *L. edodes* strain Xianggu Cr-02 on to potassium dihydrogen phosphate liquid medium containing potassium dihydrogen phosphate at seven different concentrations and magnesium sulphate liquid medium supplemented with seven different concentrations of magnesium sulphate. The results proved that potassium dihydrogen phosphate at different concentrations had greater effect on biomass production as compared with magnesium sulphate. The mycelial growth of *L. edodes* was highest with 0.05 per cent magnesium sulphate and 0.01 per cent potassium dihydrogen phosphate.

Vitamin nutrition

Vitamins have a catalytic function in the cell and are known to be the precursors of enzymes or constituent part of enzymes. Majority of fungi belonging to Agaricales were found to require thiamine, replaceable for most of the pyrimidine moiety (Jennison *et al.*, 1965). Haruhiko (1967) reported that *L. edodes* required thiamine for growth of mycelium. Kaur and Lakhanpal (1995) recorded thiamine (20 ppm) as the best vitamin source among eight different vitamins studied including a mixture.

Shin *et al.* (2000) proved through their studies on *L. edodes* that thiamine is required in trace amount (1.5µg-2 µg/l) for the vegetative growth of *L. edodes* and almost all thiamine

added to basal medium was used for fruit body formation with minimum requirement of 10 µg/l. Mahmud and Ohmasa (2008) showed experimentally that addition of biotin, bases (A+C) and organic acid (tartaric acid) to basal medium increased the efficiency of some high temperature tolerant *L. edodes* strains.

Growth regulator nutrition

There is evidence that cytokinins, auxin and gibberellic acid have profound influence on mycelial growth of fungi (Chandra, 1974). Growth regulators such as IAA are produced by a number of fungi *in vitro* (Cochrane, 1958).

Tan and Chang (1989) reported that when *L. edodes* was grown on a chemically defined liquid media using perlite as a supporting medium, gibberellic acid (5-100ppm) promoted maximum mycelial growth but IAA, kinetin and gibberellic acid (each at 5-300 ppm) had no effect on fructification. Studies done by Kaur and Lakhanpal (1995) revealed that gibberellic acid (20-40 ppm) was best among five different growth regulators studied for mycelial growth of *L. edodes*. However, Siwulski and Sobieralski (1998) proved that among three different concentrations of gibberellic acid used (100, 150 and 200 ppm), gibberellic acid at 150 ppm concentration resulted in maximum vegetative yield of *L. edodes.*

Substrate for spawn production

Constantin and Matruchot (1894) were the first to prepare spawn on sterilized horse manure. Sinden (1936) predicted that the speedy growth of the mycelium was accelerated and the quality improved by adding small amounts of calcium salts to the cereal substrates. Cayley (1937) produced spawn on chopped straw, hay and on a mixture of crushed oat, coarse sand and compost. The most important advancement was the use of grain spawn patent by Sinden (1932) and Klingman (1950). Terrier (1945) used wheat grains as the basal medium for spawn production. Uzonyi (1959) adjusted the pH of the manure spawn at 7.2 who further emphasized that good results were obtained from corn grains and manure spawn having the pH 6.5 and 7.4, respectively. Stoller (1962) reported that the spawn prepared on grains gave better yield than the compost spawn. A combination of four parts of gypsum to one part of calcium carbonate by weight prevented sticking of grains. Hu and Lin (1972) reported 50 and 60 per cent moisture content of the wheat grain and compost, respectively is required for better spawn production. Thapa *et al.* (1976) and Suman (1989) stated that the spawn substrate should be sterilized at 22 psi for 2 hours to eliminate latent contaminants.

Dewangan *et al.* (2007) used seven different spawn substrates namely; wheat grains, sorghum grains, paddy grains, sawdust alone, sawdust + wheat straw + paddy straw and sawdust + cotton waste in order to evaluate mycelial development for production of spawn of two strains of *L. edodes.* The results revealed that Chinese strain of *L. edodes* took less time for mycelial run (25 days) on wheat grains and sorghum grains.

Substrate for sporophore production

The scientific method to artificially inoculate natural logs with pure culture of shiitake myeclium was developed by Dr. Shozaburo Minura in Japan (Stamets, 1993).Attempts to cultivate shiitake in India were made by Bakshi and Puri (1978); Dhar (1978); Verma and Singh (1981); and Suman and Seth (1982) but no details on the cultivation aspects were described. However, Suman and Seth (1982) reported the composition of media for cultivation of *L. edodes*. Brodziak and Wazyny (1980) cultivated *L. edodes* on various combinations of rye straw bark and sawdust of oak, alder and pine. Highest production was recorded on sawdust + rye straw followed by sawdust + bark + straw with DM values of 3.3-5.6 and 2.9-3.6 g/l of culture jars, respectively containing 100 gm substrates. Among the different sawdust evaluated, oak sawdust gave the best result. Growth accelerators further gave upto 22 per cent increase in production on sawdust or bark mixture without straw.

Tan and Chang (1989) reported the best growth of *L. edodes* on sawdust enriched with used tea leaves, wheat bran and calcium carbonate. Diehle and Royse (1986) reported that the mixture of maple and birch sawdust substrate was colonized with mycelium of *L. edodes* in 40 days emphasizing it as the best combination of sawdust for cultivation.

Royse *et al.* (1990) performed experiment to determine the effect of selected saccharides on mushroom yield and fruit size of shiitake when grown on synthetic substrates. Substrate formulations of mixed hardwood sawdust, wheat bran and millet were ammended with sucrose, fructose and glucose individually. It was noted that addition of glucose (0.6-1.2 per cent DW) stimulated mushroom yield by 11-20 per cent while fructose (1-2 per cent) and glucose (0.6 per cent) addition also resulted in yield increase.

Mata (1992) suggested through his studies on two strains each of *L. edodes* and *Lentinula boryanus* that mycelial growth rate for the four strains tested were obtained on non-fermented shavings, which suggests that fermentation of the substrate negatively affects the

growth of the mycelium. In India, Shukla (1994) reported the cultivation of shiitake on oak logs.

Kaur and Lakhanpal (1995) cultivated *L. edodes* on various types of sawdust in polypropylene bags. They recorded that colonization was rapid in a mixture of Eucalyptus and Populus sawdust (40 days) and slowest in Poplus+ Acacia sawdust (70 days*)*. Eucalyptus + Poplus sawdust gave highest yield and biological efficiency followed by Poplus sawdust alone.

Wu (1993) and Oie (1996) observed five stages of mycelial growth in *L. edodes* viz., normal spawn run, mycelial coat formation, mycelial bump formation (blister or popcorn stage), pigmentation and coat- hardening stage (browning and bark forming stage). Royse (1997) used water soaking for fruiting induction after browning and bark forming stage for 2-4 h. at 12^0C while Stamets (1993) reported water soaking for 24-48 h as a shock treatment for inducing fruiting.

Palomo *et al.* (1998) inoculated spawn of *L. edodes* on bean, barley and maize residues, sugarcane bagasse, coffee hulls and sawdust of nine different tree species in plastic bags. Best results with respect to mycelial growth and production of fruiting bodies were recorded on Eucalyptus sawdust alone and in combination with rice dust (90 per cent sawdust and 10 per cent rice dust).

Mata and Gaitan (2004) made an attempt to cultivate five edible strains of *L. edodes* on wheat straw substrates. They concluded that complete mycelial spread on substrate took 60 days. The yields fluctuated from 6.2- 13.9 per cent, biological efficiency ranged from 24.8-55.6 per cent and production rates varied from 19-55 per cent.

Eira *et al. (*2005) inoculated spawn of *L. edodes* on substrates based on ground maize cob namely, maize cob (90 per cent) + rice bran (10 per cent), maize cob (45 per cent) + Eucalyptus sawdust (45 per cent) + rice bran (10 per cent), maize cob (50 per cent) + Eucalyptus sawdust (50 per cent) and maize cob (100 per cent). Good mushroom yield was observed in all the treatments with highest yield obtained in maize cob and rice bran combination.

***Pleurotus* spp.**

a) Solid media selection

Humfeld and Sugihara (1952) found that synthetic medium containing Glucose-asparagine was most suitable for good yield of mushrooms. Rangad and Jandaik (1977) stated that PDA fortified with Yeast extract supported maximum vegetative growth of different *Pleurotus* spp. studied. Use of PDA cum Yeast extract for growth and maintenance of stock cultures has been recommended by other workers (Kneebone *et al.*, 1972). Sharma and Jandaik (1984) reported that among all the solid media tried, PDA and Glucose yeast agar supported insignificantly maximum growth of Spain isolate whereas maximum growth of Kabul isolate of *P. eryngii* was recorded on Yeastal potato dextrose agar medium. Furlan *et al.* (1997) studied different growth media with regard to mycelial growth and recorded that Wheat dextrose agar medium promoted highest rate of mycelial growth.

b) Liquid media selection

Good growth of *Pleurotus* spp. on Dimmick's and Czepek's solution was recorded by many workers (Jandaik and Kapoor, 1975; Rangad and Jandaik, 1977). Rangad (1981) studied twelve different liquid media for comparative suitability for vegetative growth and found that Glucose- asparagine solution favored significantly maximum growth of all the three edible fungi studied.

Sharma and Jandaik (1984) tried twelve different liquid media and recorded maximum growth of Spain isolate of *P. eryngii* on Sabouraud's solution while synthetic mucor was recorded as best medium for Kabul isolate. Zhang *et al.* (2005) recorded that the mycelial pallets grew well on Potato soybean powder medium, Soybean maize powder medium and Potato peptone medium.

Temperature studies

Block *et al.* (1959) found that temperature range of 21-26^{0}C appeared to be the optimum for vegetative growth of *P. ostreatus.* The optimum temperature for vegetative growth of *Pleurotus* spp. was recorded as 25^{0}C (Rangad and Jandaik, 1977). Soni (1979) recorded best growth of Kabul isolate of *P. eryngii* at 20^{0}C. Zadrazil (1976) recorded 30^{0}C as optimum temperature for the mycelial growth of *P. ostreatus* and *P. florida* and 25^{0}C for *P. eryngii*. Optimum temperature range for mycelial growth of *P. ostreatus* was recorded to be

25-30^{0}C (Balazs *et al.*, 1987).

Sharma and Jandaik (1984) studied the effect of different temperature regimes on mycelial growth of two isolates of *P. eryngii* and reported that 25±1^{0}C was the optimum temperature for vegetative growth of Spain and Kabul isolates. Highest mycelial growth of two species of *Pleurotus* viz., *Pleurotus sajor- caju* and *Pleurotus flabellatus* was observed at 30^{0}C (Ram and Pant, 2001). Ali *et al.* (2004) found that maximum radial growth of *P. ostreatus* occurred at a temperature range of 25-30^{0}C.

pH studies

Maximum growth of *P. ostreatus* occurred at a pH of 5.0 (Block *et al.*, 1959). Again, an initial pH of 4.5 was found to be optimum for growth of *P. ostreatus* (Sugimori *et al.*, 1971). Hashimoto and Takahashi (1976) observed that maximum growth of *P. ostreatus* occurred at pH 5.0. The optimum pH requirement of different *Pleurotus* spp. studied by Rangad and Jandaik (1977) was reported to be 5.6.

Zadrazil (1978) recorded optimum initial pH of 5.0 and 6.0 for *P. osteratus* and *Pleurotus florida*, respectively. Soni (1979) recorded maximum vegetative growth of Kabul isolate of *P. eryngii* at pH 6.0. Solomoko and Fedorov (1989) also found highest growth of *P. ostreatus* at 6.2-6.6 pH. Similarly, Furlan *et al.* (1997) observed maximum mycelial growth of *P. ostreatus* at 5.0 pH.

Ram and Pant (2001) recorded maximum growth of two species of *Pleurotus* viz., *Pleurotus sajor- caju* and *Pleurotus. flabellatus* at 6.0 pH. Ali *et al.* (2004) showed that 6.0 was the optimum pH level for mycelial growth of *P. ostreatus* followed by a pH of 6.5 whereas the pH range between 5-7 with optimum being 6.0 was recorded for the growth of *P. eryngii*.

Carbon nutrition

Block *et al.* (1959) recorded soluble sugars as better carbon sources than starch for growth of *P. ostreatus*. Glucose and starch were found good carbon sources for the growth of variable fungi (Srivastava and Bano, 1970). Hashimoto and Takahashi (1976) reported that mycelium of *P. ostreatus* grew faster with mannose followed by starch and glucose while sugars like xylose and arabinose were not recorded best for growth of the fungus. Sakamoto *et al.* (1978) during their studies on different carbon and nitrogen sources proved that

maximum dry mycelial weight of *P. ostreatus* was obtained when basal medium containing glucose, a carbon source was supplemented with additional starch and 2-3 per cent glucose.

Kikon (1979) observed that starch was the best carbon source with respect to vegetative growth of two strains of *P. ostreatus* viz., Florida and Grey followed by fructose and glucose. Mycelial growth of Spain isolate of *P. eryngii* was maximium with glucose followed by sucrose and starch and that of Kabul isolate of *P. eryngii* on dextrose substituted medium (Soni, 1979).

Among twenty different carbon sources tested by Sharma (1984), maltose and dextrose supported insignificantly maximum growth of Spain isolate of *P. eryngii* followed by sorbitol and fructose. Dextrose and galactose were reported to support equally good growth of Kabul isolate. Huang *et al.(*2003) recorded brown sugar as best carbon source for the growth of *P. ostreatus* among fructose, lactose, glucose, sucrose, starch and maltose tested by them. Zhang *et al.* (2005) recorded glucose (50 mg/l) and peptone (1g/l) as best carbon sources for growth of *P. eryngii* in medium.

Nitrogen nutrition

Among fungi, *Pleurotus* spp. was found to utilize elemental nitrogen indicating the ability to fix atmospheric nitrogen (Block *et al.*, 1959). Mandelin (1956) found urea and amino acids as good nitrogen sources while alanine and asparagine were not utilized equally by both strains of *P. osteratus* studied.

Similarly, among the organic nitrogen sources tried, peptone and urea were recorded to give best results in case of *P. ostreatus* (Voltz, 1972; Hashimoto and Takahashi, 1976). Jandaik and Kapoor (1975) recorded best growth of *Pleurotus sajor- caju* on asparagine and urea followed by potassium nitrate and sodium nitrate. Among different sources of nitrogen tested, asparagine yielded significantly higher mycelial dry weight in case of both Florida and Grey strains of *P. ostreatus* while alanine supported poor growth (Kikon, 1979).

Significantly better growth of Kabul isolate of *P. eryngii* was reported on urea and asparagine (Soni, 1979). Rangad (1981) observed that asparagine supported maximum average mycelial growth of *P. ostreatus* followed by iso-leucine, leucine and glutamic acid in decreasing order. Kabul isolate showed significant stimulation of growth with peptone and asparagine.

Zhang *et al.* (2005) proved through his studies that among different nitrogen sources studied, peptone and ammonium molybdate were best for the good vegetative growth of *P. eryngii*. Huang *et al.* (2006) reported that wheat bran, Yeast powder and peptone were optimum nitrogen sources for growth of *P. ostreatus*.

Trace element nutrition

The trace element requirements for mycelial growth of the fungus on dry weight basis are not easily shown because the requirements are so minute. Jandaik (1976) recorded increased growth of *Pleurotus sajor- caju* with addition of iron as micronutrient source into the basal medium. A mixture of ferrous sulphate and zinc sulphate also resulted in good mycelial growth of *Pleurotus sajor- caju*.

Soni (1979) recorded iron to support maximum growth of Kabul isolate of *P. eryngii*. Magnesium suphate and potassium dihydrogen phosphate were found to support increased growth of *P. ostreatus* (Hong, 1978). Kurtzman and Zadrazil (1982) found stimulation in growth of *P. ostreatus* with magnesium sulphate.

Studies on trace element nutrition done by Sharma (1984) revealed that zinc and iron supported increased growth of Spain isolate of *P. eryngii* whereas manganese supported significantly maximum growth of Kabul isolate. Huang *et al.* (2003) recorded magnesium sulphate as the most suitable inorganic salt source for vegetative growth of *P. ostreatus*.

Vitamin nutrition

Fungi like any other living entity require minute amount of specific organic compounds for their metabolism. The growth factors are required in very small quantities for supporting their variety of reactions but not for energy production (Snell, 1951). Majority of higher fungi have completely or partly lost their ability to synthesize one of the most important growth factor, thiamine (Bohus, 1959).

Block *et al.* (1959) observed definite requirement of thiamine for the mycelial growth of *P. ostreatus* but no response was observed with the addition of biotin. The suitable concentration of thiamine for the growth of *P. ostreatus* was 100 µg/l and addition of other vitamins did not influence the growth (Hashimoto and Takahashi, 1976).

Similarly, Soni (1979) observed maximum mycelial yield of *P. eryngii* when thiamine at 50 µg/l concentration was added to the basal medium. Among different vitamins tested, biotin supported best growth of both Kabul and Spain isolates of *P. eryngii* (Sharma, 1984).

Reddy *et al.* (2002) recorded that pyridoxine HCl at 50ppm favoured vigorous growth of *P. ostreatus*. Kundu (2003) evaluated four vitamins viz., thiamine, biotin, pyridoxine and inositol (for which fungi are most frequently deficient) for their comparative effect on vegetative growth of *Pleurotus* spp. He observed the growth promoting effect in decreasing order by biotin, inositol, pyridoxine and thiamine.

Growth regulator nutrition

Briggs and Ray (1956) reported that cell free enzyme system of several Basidiomycetes inactivates the auxin. But in several cases IAA increased the growth of fungi. Hong (1978) found 0.1 ppm IAA to support good growth of *P. ostreatus* followed by 10 ppm GA and 0.1 and 0.01 ppm kinetin.

Kikon (1979) through his investigations found that IAA at all concentrations completely inhibited the growth of Florida and Grey strains of *P. ostreatus*. Similarly, Vinklarkova and Sladky (1978) recorded maximum linear growth of *P. ostreatus* with 200 ppm of IAA followed by same concentration of kinetin. Rawal and Singh (1980) observed that increased mycelial growth of *P. eryngii* occured with addition of gibbrellic acid and for other species of *Pleurotus* (*P. florida, P. ostreatus, P. sajor- caju and P. sapidus*) with α-NAA slightly but they were not significantly superior in supporting the vegetative growth.

Again, Sharma (1984) recorded that maximum growth of Spain isolate of *P. eryngii* occurred on different concentrations of 2, 4- D followed by higher concentrations (1-5 ppm) of miraculan. However, none of the growth regulators tried stimulated growth of Kabul isolates over control indicating that the concentrations tried exerted inhibitory effect on vegetative growth of the fungus. Reddy *et al.* (2002) found most favorable growth of *P. ostreatus* with the addition of IAA at 5 ppm.

Substrate for spawn production

Zadrazil (1976) and Kalberer (1976) reported that substrate spawn was equally good to the grain spawn which was costly. Spawn of *Pleurotus* spp. could be produced on various

cereal grains, millets and other farm wastes such as straw and bajra husk with success (Jandaik and Kapoor, 1974). Rangad and Jandaik (1977) recorded maximum yield of different *Pleurotus* spp. with spawn prepared from jowar grains.

Sharma (1984) proved grains of wheat, barley, jowar and bajra as equally good for vegetative growth of *P. eryngii*. Pandey and Singh (2001) recorded highest yield of *P. ostreatus* when spawn prepared on jowar grains was used followed by bajra grains. However, spawn prepared on wheat straw alone gave lower yield. Hussain and Hussain (2004) inoculated mycelial bits of *P. ostreatus* on wheat, sorghum and maize grains and recorded highest radial growth on maize grains.

Substrate for sporophore production

Falck (1917, 1919) first reported cultivaton of *Pleurotus* spp. on logs. Successful cultivation of *P. ostreatus* could be done on crushed corncobs under sterile conditions (Toth, 1969). Peng (1996) used sawdust from broad leaved trees mixed with rice bran (20 per cent v/v) for cultivation of *P. ostreatus* and recorded that the substrate was suitable for replacement of rice or wheat straw. The first fruit body flush took about 60 days from inoculation.

Dubey (1999) during his cultivation trials on six locally available substrates (paddy straw, wheat straw, maize stalks, ragi straw, sugar cane leaves and groundnut shells) recorded paddy straw to produce highest number of sporophores with maximum biological efficiency followed by wheat straw. Ohga (2000) used various sawdust based substrates for cultivation of *P. eryngii* and recorded good mycelium growth on sawdust from *Crytomeria japonica*, while sawdust from *Larix kaempferi* was found unsuitable for cultivation of this mushroom.

Recycling of the waste bed logs for the cultivation of *Lentinula edodes* as a resource for the cultivation of *Pleurotus ostreatus* and *Flammulina velutipes* was investigated by Makoto *et al.* (1999). It was recorded that there was no significant difference in the yield of fruiting bodies using sawdust medium from waste bed logs and that from softwood. However, the number of days required for initiation and harvest of fruiting bodies on sawdust medium from waste bed logs was shorter as compared from that with sawdust medium from softwood.

Pleurotus spp. could colonize and produce fruiting bodies on pre- treated conifer (*Pinus* spp.) wood chips but they did not readily colonize non- pretreated conifer wood, due

to presence of inhibitory components (Croan, 2004). Hussain and Hussain (2004) recorded highest yield of *P. ostreatus* on wheat straw (50 per cent) and maize straw (50 per cent). Ohga and Royse (2004) evaluated *Cyperus alternifolius* (umbrella plant) supplemented with wheat bran and *Crytomeria japonica* alone as substrates for cultivation of oyster mushroom. They recorded 31- 46 per cent greater mycelial growth on *Cyperus alternifolius* substrate as compared with *Crytomeria japonica* and the yield was also 20- 23 per cent higher on the former. Shah *et al.* (2004) recommended sawdust as the best substrate for oyster mushroom cultivation as it gave highest yield, biological efficiency and the number of fruiting bodies.

Iqbal *et al.* (2005) during their cultivation trials on *P. ostreatus* recorded maximum fruit body production from chick pea straw substrate followed by wheat straw whereas, spawn run, formation of pinheads and maturation of fruit bodies occurred earlier with sugarcane waste, sunflower and chick pea straw as growing substrate.

Among the different wood substrates used for the cultivation of *P. ostreatus*, Tisdale *et al.* (2006) recorded highest yield and biological efficiency on sawdust from nitrogen fixing trees (*Casuarina equisetifolia, Trema orientalis and Falcataria molucama*). Substrate combinations of 25 per cent coconut residue + 75 per cent sawdust gave maximum sporophore yield of *P. ostreatus* and this was significantly different from that recorded with 100 per cent coconut residue and sawdust.

Das and Mukherjee (2007) cultivated *P. ostreatus* on different dry weed plants viz., *Sida acuta, Ageratum conyzoides, Cassia sophera, Parthenium argenotatum, Tephrosia purpurea, Leanotis* spp. and *Lantana camera*. They recorded that *Leanotis* spp. mixed with rice straw was the best substrate for fruit body production of *P. ostreatus.*

Estrada and Royse (2007) reported that *P. eryngii* fruiting yields were significantly higher in substrates containing manganese (50µg/l) and soybean than in the basal cotton seed hull/ sawdust substrate. Sevda and Mehmet (2008) cultivated *P. eryngii* on different agricultural wastes and recorded lowest yield on mixture of wheat straw and cotton straw (1:1) but highest yield was recorded when 20 per cent rice bran was added to the mixture.

Auricularia polytricha

Media selection

Thakur and Bhandal (1993) recorded best mycelial growth of *A. polytricha* on PDA.

Khan *et al.* (1991) reported MEA as the best medium for vegetative growth of *A. polytricha* among MEA, PDA, and Wheat extract agar tried.

Temperature studies

A temperature of 25^0C was recorded optimum for the mycelial growth of *A. polytricha* (Khan *et al.*, 1991; Thakur and Bhandal, 1993; and Xu and Yun, 2003). Maximum biomass production of *A. polytricha* occurred at 25^0C (Jonathan *et al.*, 2009).

pH studies

Khan *et al.* (1991) recorded pH 7.0 as optimum for mycelial growth of *A. polytricha* but Thakur and Bhandal (1993) found a pH of 5.5- 6.0 to be optimum for growth of *A. polytricha*. Xu and Yun (2003) recorded an initial pH of 5.0 for maximum biomass and exo-polymer production. Jonathan *et al.* (2009) recorded good biomass production of the fungus at pH 6.5.

Carbon nutrition

Khan *et al.* (1991) while evaluating different carbon sources for vegetative growth of *A. polytricha* recorded starch as best carbon source. Sucrose was recorded as most suitable carbon source for mycelial growth of *A. polytricha* (Xu and Yun, 2003). However, Jonathan *et al.* (2009) recorded glucose followed by fructose as best carbon sources for maximum biomass yield of the fungus.

Nitrogen nutrition

Peptone was recorded as most suitable nitrogen source for vegetative growth of *A. polytricha* among different nitrogen sources tested (Khan *et al.*, 1991). Xu and Yun (2003) recorded Yeast extract as best nitrogen source for mycelial growth of *A. polytricha*. Again, peptone followed by tryptophan were found to be the best nitrogen sources for *A. polytricha* (Jonathan *et al.*, 2009).

Trace element nutrition

Di- potassium hydrogen phosphate was found as the most suitable mineral element for the growth of *A. polytricha* (Xu and Yun, 2003). Meng *et al.* (2004) proved ferrous sulphate as suitable trace element for growth and development of *A. polytricha*.

Substrate for spawn production

Khan *et al.* (1991) evaluated three types of sawdust viz., poplar, shisham and kikar for spawn production and recorded fastest growth on sawdust of kikar. Mehta and Bhandal (1991) used seven different substrates for cultivation of *A. polytricha* and recorded fastest spawn run on sawdust followed by wheat straw and rice husk while highest number of fructifications was observed on corncobs. Among nine growing substrates studied by Thakur and Bhandal (1993), good spawn run was observed on wheat straw, sugarcane baggasse, corncobs and sawdust.

Ganoderma lucidum

Media selection

Fresh isolation of *Ganoderma lucidum* from different host tree species was done on Malt extract agar medium by tissue culture method (Booth, 1971). Bilay *et al.* (2000) used different Nutrient agar media and recorded maximum growth rate of *G. lucidum* on MEA medium. Kim *et al.* (2002) recorded highest mycelial biomass yield and exo- polymer production of the fungus on Potato Malt peptone medium. Song *et al.* (2007) recorded good vegetative growth of *G. lucidum* on barley permeated medium. Nasreen *et al.* (2005) studied growth of *G. lucidum* on different culture media and recorded maximum radial growth on PDA after fifteen days of incubation.

Temperature studies

Yang and Liau (1998) recorded optimum mycelial growth of *G. lucidum* at a temperature range between 30-35^{0}C on Glucose ammonium chloride medium while optimum temperature for best mycelial growth of *G. lucidum* has been recorded at 28.3^{0}C (Lee *et al.*, 2002). Nasreen *et al.* (2005) stated that a temperature of 25^{0}C was required for optimum vegetative growth of *G. lucidum*. Similarly, Babitskaya *et al.* (2007) during their studies on the effect of conditions on polysaccharide production in submerged culture of *G. lucidum* reported a temperature range of 25-30^{0}C as suitable for mycelial growth.

pH studies

Yang and Liau (1998) recorded an initial pH of 4.0 as optimum for growth of the fungus on glucose ammonium chloride medium and an initial pH of 6.5 was found to give maximum biomass, ganodermic acid and total fruit bodies (Yang and Zhong, 2002). Lee *et al.* (2002) found a pH of 4.2 as optimum for vegetative growth of *G. lucidum.*

Nasreen *et al.* (2005) recorded that mycelium of *G. lucidum* grew best on PDA at pH 5.0. Babitskaya *et al.* (2007) recorded a pH range of 3.5- 6.0 as good for mycelial growth of *G. lucidum.* Highest level of laccase activity of *G. lucidum* was found at pH 5.0 (Mirjana *et al.*, 2007) whereas, Song *et al.* (2007) utilizing whey permeate for cultivation of *G. lucidum* reported a pH range of 3.5-5.5 suitable for maximum mycelial growth.

Substrate for spawn production

Hirt and Schnitzler (1994) assessed different cereal grains as spawn substrate for *G. lucidum* and obtained best results in terms of spawn run with millet, wheat and rye grains. Mishra and Singh (2006) reported that wheat grain spawn resulted in maximum yield (89 g/500 g dry weight of substrate) among spawn prepared on wheat, mandua and jhangora grains.

Substrate for sporophore production

Tong and Chen (1990) used sawdust mixed with 10 per cent rice bran and 3 per cent lime as substrate for cultivation of *G. lucidum* and obtained three harvests. Triratana *et al.* (1991) during his investigations regarding suitability of sawdust and available agricultural wastes as substrate for cultivation of *Ganoderma lucidum* recorded that sawdust from *Hevea brasiliensis* gave optimum mycelial growth among various other tree sawdusts viz., *Dipetrocarpus alatus, Pentacme suavis* and T*ectona grandis* tried. Siwulski and Sobieralski (2001) while evaluating mycelial growth and yield of two strains of *G. lucidum* on sawdust of oak, beech and birch reported that mycelium grew faster on birch sawdust substrate while yield recorded was highest on oak and beech sawdusts as compared with that on birch sawdust.

In India, Rai (2003) successfully cultivated reishi (*G. lucidum)* on sawdust. Dadwal and Jamaluddin (2004) reported that sawdust was the best substrate for cultivation of *G. lucidum*. Tiwari *et al.* (2004) used different hosts viz., *Boswellia serrata, Delonix regia, Poplus deltoides, Leucaena leucocephala and Lannea coromandelica* for artificial cultivation in control condition. The better performance in terms of production was observed on *Delonix regia.*

Mishra and Singh (2008) evaluated yield and biological efficacy of three isolates of *G. lucidum* on different agro wastes supplemented with wheat bran (2.5 per cent) and rice bran (5 per cent) each on wet weight basis. They recorded significantly higher yield of all the

three isolates of *G. lucidum* on wheat straw supplemented with 5 per cent rice bran.

Flammulina velutipes

Media selection

a) Solid media selection

Rangad (1981) recorded fastest mycelial run on PDA+ Yeast extract followed by PDA alone among seven different solid media studied. Sharma *et al*. (2005) recorded maximum radial growth of *F. velutipes* on Glucose- asparagine agar medium followed by Malt extract agar medium and Rice extract agar medium.

b) Liquid medium selection

Among twelve different liquid media studied, Rangad (1981) recorded maximum dry mycelial weight of *F. velutipes* on Glucose- asparagine solution followed by Elliott's solution and then on Dimmick's solution. Sharma *et al.* (2005) also recorded that among nine liquid media tried, maximum growth was recorded on Glucose- asparagine solution.

Temperature studies

Rangad (1981) found that *F. velutipes* produced maximum mycelial growth at 20^0C. Aschan (1954) has reported *F. velutipes* to grow at a temperature range of 3-4^0C to 33-34^0C with optimum being 22-26^0C. Optimum temperature for mycelial growth of *F. velutipes* was found to be 25^0C (Su *et al*., 2001; and Sharma *et al*., 2005).

pH studies

Rangad (1981) studied the interaction of different pH ranges with fungi and revealed that a pH of 5.6 supported significantly maximum growth of *F. velutipes* whearas, Su and Li (2001) recorded that a pH of 6.0 was optimum for mycelial growth of *F. velutipes.* Sharma *et al.* (2005) during their cultural studies on *F. velutipes* recorded a pH of 8.0 as optimum for maximum vegetative growth of *F. velutipes.*

Carbon nutrition

Rangad (1981) tested thirteen different carbon sources for their comparative response on the vegetative growth of *F. velutipes* and recorded sugar alcohol, mannitol as best carbon source followed by sorbitol while, least growth was recorded on raffinose and lactose. Wang (2000) during his experimental studies recorded that soluble starch, glucose, sucrose and

mannose were better carbon sources for mycelial growth of *F. velutipes.* Among thirteen different carbon sources tested for mycelial growth of *F. velutipes*, best growth was supported with addition of sucrose (Sharma *et al*., 2005).

Nitrogen nutrition

Rangad (1981) tested twenty different nitrogen sources (six inorganic and fourteen organic) to sort out the best nitrogen source for mycelial growth of *F. velutipes.* Results revealed that *F. velutipes* exhibited maximum growth with the addition of iso- leucine followed by asparagine, leucine and ammonium chloride. Reduced growth was recorded with sodium nitrite.

Sharma *et al.* (2005) tested nine different nitrogen sources and recorded asparagine as best nitrogen source. Wang (2000) recorded better growth of *F. velutipes* with soybean powder, peptone, bean cream and yeast powder as nitrogen sources.

Trace element nutrition

Fungal requirement of molybdenum is reported to be extremely low ranging from 1-10 ppb (Bilgrami and Verma, 1978). Rangad (1981) reported that maximum mycelial growth of *F. velutipes* was recorded when a mixture of trace elements was added to the basal medium followed by ferrous sulphate and zinc sulphate. Addition of other trace elements like manganese sulphate, boric acid and ammonium molybdate individually to the basal medium did not increase the growth.

Kachroo (1991) recorded effect of micronutrients on fructification of *F. velutipes* and an increase in yield by 2- 7 times over control. Primordial formation occurred earlier in copper sulphate and calcium sulphate and application of ferrous iron gave highest number of mushrooms. Cho and New (2006) recorded that only the basic salt medium with magnesium, manganese, iron and zinc as trace elements were needed for the vegetative growth of *F. velutipes*.

Vitamin nutrition

Rangad (1981) reported that among various vitamin sources tried, the average growth was found to be maximum with the addition of thiamine. Yun (1996) during cultivation studies also proved thiamine hypochlorite (0.05-1 per cent) as best vitamin for growth of *F. velutipes.* Cho and New (2006) recorded that thiamine is needed by *F. velutipes* as growth

factor.

Growth regulator nutrition

There is evidence that cytokinins, auxins and gibberellins have profound influence on the mycelial growth (Szabo *et al.*, 1972) as well as production of basidiocarps of higher fungi (Alakaldrev, 1964). Rangad (1981) recorded gibberellic acid (5 ppm) as best growth regulator for *F. velutipes* followed by succinic acid 2, 2- di methyl hydrazide (SADH) and morphactin (5 ppm).

Ding *et al*. (1990) recorded good growth of *F. velutipes* with respect to time of appearance of fruiting bodies and increased number, yield and quality of fruiting bodies on mixture of alkylal 30+ gibberellic acid. Sharma *et al.* (2005) studied the effect of four growth regulators viz., gibberellic acid, NAA, IBA and kinetin on vegetative growth of *F. velutipes* and gibberellic acid (10 ppm) was reported to support maximum mycelial growth.

Substrate for spawn production

Rangad (1981) recorded fast and maximum growth of *F. velutipes* on jowar and bajra grains closely followed by wheat and barley grains, when tried individually.

Substrate for sporophore production

Rangad (1981) successfully cultivated *F. velutipes* on various agricultural wastes viz., oak dust+ rice bran, composted wheat straw and paddy straw, poplar leaves +rice bran, wheat straw and paddy straw + crushed corncobs. Chopped paddy straw was found most suitable substrate for fruiting body production as it supported maximum yield. Supplementation of rice straw with crushed corncobs and rice husk also favoured good fruit body production.

Li (1989) during his cultivation trial recorded that mycelial run took longer time on maize cob substrate as compared with cotton seed hulls but yield was 2.9-9.6 times higher on maize cob substrate. Gavrilova and Lysenkova (1988) cultivated *F. velutipes* strain LLKH-1177 on different substrates viz., Poplus sawdust+ wheat straw, sawdust + wheat straw + spent malt and sawdust + spent malt and the highest yield was recorded on the third combination i.e. sawdust+ spent malt.

Pawlak and Siwulski (2001) cultivated *F. velutipes* on pine sawdust; beech sawdust and their mixture. Best results were recorded on mixture of pine and beech sawdust. Sharma

et al. (2005) used eight different substrates and recorded fastest mycelial growth of *F. velutipes* on pasteurized grass and highest biological efficiency on sawdust of broad leaved trees.

Sharma *et al.* (2006) recorded the effects of different supplementations on the mycelial growth, enzyme production and yield of *F. velutipes.* Wheat bran (10 per cent) supported the fastest mycelial growth followed by addition of wheat bran at 5 per cent concentration. Other supplements (soybean meal, cotton seed cake and deoiled soybean) resulted in reduced linear growth and no fruit body induction was recorded.

Environmental parameters

a) Temperature

Callieux and Diop (1976) found that *Pleurotus eryngii* fruits until 25^0C but 20-25^0C was the optimum temperature. Cayrol (1978) cultivated *P. ostreatus* and *P. pulmonarius* at 25^0C and 20-26^0C, respectively. *Pleurotus eryngii* was reported to fruit well below 18^0C (Zadrazil, 1982). Sharma (1984) reported maximum yield of *P. eryngii* in a temperature range of 16-21^0C. Dubey (1999) successfully cultivated four species of *Pleurotus* viz., *P. flabellatus, P. ostreatus, sajor- caju* and *P. cystidiosus* at room temperature of 21-28^0C.

Stamets and Chilton (1983) recorded that for spawn run of *L. edodes* the substrate temperature of 77^0F (25^0C) with pH optima of 5-6 was needed and for pinhead formation and fruiting an air temperature of 59-68^0F (15-20^0C)was required. Campbell and Slee (1987) observed that vegetative growth of shiitake required a temperature of 24-28^0C and a temperature range of 12-20^0C was found to be optimum for fruiting of shiitake. The temperature range for maximum vegetative growth of *L. edodes* was found to be 23-25^0C and optimum temperature for fruiting varied between 5-20^0C (Royse, 1989). Kaur and Lakhanpal (1995) reported that shiitake required a temperature of 18^0C for fruiting. To obtain a homogeneous production of *Lentinula edodes*, it was necessary to undertake a thermal shock, which consists of lowering the temperature of substrate to at least 10^0C. Placing the bags in a refrigerator for 24 hours, at a temperature between 4-8^0C was sufficient to accomplish the thermal shock. During the fructification process, it was necessary to maintain temperature of 10-23^0C (Lopez *et al.*, 2004).

Samajpati amd Bandyopadhyay (1981) reported that for cultivation of *G. lucidum* a

temperature of 25±5^{0}C temperature was required. Growing rooms should be maintained at 28-30^{0}C during fruiting of *G. lucidum* (Tong and Chen, 1990). Dadwal and Jamalluddin (2004) emphasized to maintain a temperature of 28-30^{0}C in the cropping rooms for fruiting of *G. lucidum.* Tiwari *et al.* (2004) successfully got the fruiting of *G. lucidum* when polypropylene bags containing the substrate were kept at 28-30^{0}C. Primordial initiation of *G. lucidum* was very fast at 30±2^{0}C and took only a week whereas at 24±2^{0}C, it was delayed by another week (Verma and Pandey, 2006).

Successful cultivation of *Flammulina velutipes* requires a temperature of 15-16^{0}C (Gardezi and Ahmad, 1997). Sharma *et al.* (2008) reported that *F. velutipes* required an incubation temperature of 25-28^{0}C during spawn run and 15-16^{0}C during fruiting. The temperature requirements in case of *F. velutipes* for spawn run was recorded as 72- 77^{0}F and for primordial formation and induction of fruiting it was 70-75^{0}F (Stamets and Chilton, 1983)

Relative humidity

Different levels of relative humidity were observed for different species of *Pleurotus* by various workers. Callieux and Diop (1976) reported 85-95 per cent relative humidity for better growth of *Pleurotus eryngii*. Zadrazil (1978) stated that good growth of *Pleurotus* spp. occurs at a relative humidity of 69-80 per cent. Very high humidity resulted in abnormal fruit bodies with very long stipe (Jandaik and Kapoor, 1974; and Block *et al.*, 1959). Samajpati and Bandyopadhyay (1981) reported that optimum relative humidity for cultivation of *P. ostreatus* was 75-80 per cent. Sharma (1984) has recorded maximum yield of *P. eryngii* at relative humidity of 85 per cent. Dubey (1999) while evaluating different locally available substrates and organic amendments recorded 71-85 per cent relative humidity required for fruiting of *Pleurotus* spp.

Stamets and Chilton (1983) stated that for spawn run *L. edodes* needed 60-75 per cent humidity for logs and 90 per cent for sawdust. Stamets (2000) emphasized that shiitake needs a relative humidity of 95-100 per cent for spawn run and inducing primordia and 60-80 per cent for its fruiting.

Tong and Chen (1990) successfully cultivated *G. lucidum* at a relative humidity of 85-90 per cent. Watering in the form of mist spray was carried out at least twice a day. Dadwal and Jamalludin (2004) insisted to maintain a humidity of 70-90 per cent for fruiting of *G. lucidum*. Tiwari *et al.* (2004) reported that 70-90 per cent humidity was required for fruiting

of *G. lucidum*. Verma and Pandey (2006) reported primordial initiation in *G. lucidum* required relative humidity of 90-95 per cent whereas delayed initiation was recorded at 80-85 per cent humidity. Mishra and Singh (2008) maintained relative humidity of 85-90 per cent by sprinkling water for pinning of *G. lucidum*. After pin head formation, the humidity was reduced to 80 per cent and after cap thickening, it was again lowered to 70 per cent.

Stamets and Chilton (1983) reported that during spawn run and fruiting of *F. velutipes*, relative humidity of 90-100 per cent and 85 per cent, respectively is needed.

Aeration

It is generally believed that *Pleurotus* spp. required less oxygen than many other microorganisms (Ginterova, 1973). Zadrazil (1976) stated that *Pleurotus* spp. can tolerate higher concentration of carbon dioxide which also acts as shield against many competitive microorganisms. Stimulatory effect of carbon dioxide on mycelial growth has also been observed by Zadrazil (1975) but higher concentrations resulted in abnormal elongation and branching of stipes (Kurtzman, 1979; Zadrazil, 1978). Insufficient ventilation and low light exposure resulted in regeneration of sporophores of *Pleurotus* spp. (Eger, 1965, 70; Zadrazil and Schneidereit, 1972).

Stamets (2000) recorded that during spawn run, induction of primordia and fruiting, ventilation of 0-1 h, 4-7 h and 4-8 h, respectively were required for *L. edodes*. Stamets and Chilton (1983) stated that for spawn run of *F. velutipes*, no aeration was required while, for primodial and fruit body formation fresh air exchange of 4/h was required.

Light

Positive phototropism in *Pleurotus* spp. has been demonstrated by Block *et al.*, (1959) and Gyurko (1972). The need for light to promote fruit body development in different *Pleurotus* spp. has been demonstrated by several workers (Kaufert, 1935; Eger, 1979; Jandaik and Kapoor, 1976). Zadrazil (1976, 78) recorded that *Pleurotus* spp. needed light for 15 minutes every day during cropping and in the absence of light sporophores with long and thin stipes and reduced pilei were produced. Kalberer and Vogel (1974) recorded that decreased light intensity resulted in longer and heavier stipe of *P. ostreatus*. Vollandnail (1981) on the contrary reported that light was not needed by *Pleurotus* spp. for fruiting but sporophores with thin, elongated stipes and white pilei were produced in dark. Sharma (1984) recorded a

light exposure for 3-5 minutes/ 24 h to stimulate the primordial initiation and yield in *P. eryngii*. Furthermore, in absolute darkness poorly developed pilei with long and thin stipe were produced.

During spawn run, pining and fruiting of *L. edodes*, no light was required (Stamets and Chilton, 1983). Miles and Chang (1989) reported that for spawn run of *L. edodes* no light was required however, in the day/ night cycle towards the end of the spawn run, light was conducive for the induction of primordia. Light exposure during the vegetative phase was necessary for sporocarp formation of *L. edodes* (Royse, 1984). Eight hours of light was necessary for cropping of *L. edodes* (Royse, 1985). Lopez *et al*. (2004) insisted on total darkness of cropping rooms for fructification of shiitake. Singh and Mishra (2008) stated that light exposure of 50-200 lux was required for the development of fruiting bodies in case of shiitake.

Bags of *Ganoderma lucidum* should be kept in dark room for fruit body induction (Tiwari *et al.*, 2004). Yun (1996) cultivated *Flammulina velutipes* with success when light of 60 lux was provided to the cropping room while, Gardezi and Ahmad (1997) reported that *F. velutipes* required complete darkness for fruiting. No light was required for spawn run and pining of *F. velutipes* while some light was needed for fruiting (Stamets and Chilton, 1983).

Incubation Period

Diehle and Royse (1986) recorded that it took 40 days for complete substrate colonization of mixture of maple and birch sawdust in case of *L. edodes*. Whereas, colonization of *L. edodes* on sawdust enriched with tea leaves, wheat bran and calcium carbonate was recorded within 35 days of incubation (Tan and Chang, 1989). Miles and Chang (1989) reported that for one strain, 60 days were sufficient for maturation of mycelial mat of *L. edodes*, depending on the strain. Similarly, Kaur and Lakhanpal (1995) observed that substrate containing mixture of Poplus and Eucalyptus sawdust was completely colonized with the mycelium of *L. edodes* in 40 days. Depending on the strain, *L. edodes* required a spawn run period of 1- 2 months, 5-7 days for inducing primordia and 5-8 days for fruiting (Stamets, 2000). During cultivation of *L. edodes* on coffee waste, Lopez *et al.* (2004) reported that after 50-70 days, the mycelium colonized the entire substrate.

The inoculated bags of *Ganoderma lucidum* took 45 days for complete mycelial

growth at 30^0C in BOD incubator (Tiwari *et al.*, 2004). Dadwal and Jamalludin (2004) obtained fruit body of *G. lucidum* after 40 days of incubation. Mishra and Singh (2006) recorded that it took nearly 30 days for substrate colonization of *G. lucidum.*

Chapter-3

MATERIALS AND METHODS

The research work on "**Studies on cultural requirements and cultivation technology of some medicinal mushrooms"** was carried out in the laboratory of the Department of Mycology and Plant Pathology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan (HP). The present investigations were carried out during the year 2007-08 and 2008-09. The cultures of the medicinal mushrooms viz., *Ganoderma lucidum, Auricularia polytricha, Flammulina velutipes, Lentinula edodes. Pleurotus ostreatus* and *Pleurotus eryngii* were procured from Directorate of Mushroom Research (DMR), Chambaghat, Solan. The details of material and methods for carrying out the present studies are given below under following heads:

3.1 GLASSWARE AND CHEMICALS

Corning glassware and BDH chemicals were used throughout the experiments. The glasswares were cleaned with acid dichromate cleaning solution (potassium or sodium

dichromate 200 g, sterilized distilled water 800 ml, concentrated sulphuric acid 1200 ml), followed by several washings in running tap water and finally rinsed with double glass distilled water. Ninety mm diameter Petri dishes were used for solid media studies. In case of cultural studies, 'Erlenmeyer's conical flasks of 100 ml capacity were employed. For spawn production and cultivation trials, polypropylene bags of one-kilogram capacity were used.

3.2 MAINTENANCE OF PURE CULTURES

All the cultures were maintained on Malt extract agar medium and kept in a refrigerator at 4^0C for further use in cultural and cultivation studies. The cultures were periodically subcultured on 2.0 per cent Malt extract agar medium.

3.3 CULTURAL STUDIES OF SELECTED MUSHROOM SPECIES

The cultural studies were conducted to understand in detail the nutritional requirements and environmental parameter needs of the selected medicinal mushroom species.

3.3.1 Selection of basal medium

Twelve different media (synthetic, semi- synthetic and natural) were tested out of which six were solid and rest six were the liquid media. The best medium was used for further maintenance, multiplication and selection of suitable medium for physiological studies. The composition and method of preparation of media were the same (Appendix-I) as described by Tuite (1969). In case of solid medium, 2 per cent agar was incorporated for solidification whereas, in case of liquid media, agar was excluded.

3.3.1.1 Preparation of Inoculum

In case of solid media studies, twenty ml of sterilized agar media were poured in each pre-sterilized (at 180^0C in hot air oven) Petri plates under aseptic conditions. Each Petri plate was then inoculated aseptically with the bits of test culture. Inoculum used in different experiments was obtained from the periphery of the actively growing colonies of different cultures. For cultural studies, 4mm diameter discs of mycelial mat were cut with the help of a pre- sterilized cork borer and sufficient care was exercised to avoid the use of thick agar block with mycelial discs.

For liquid medium, 25 ml of the medium was poured into 100 ml flasks and after sterilization at 15 psi for 20 minutes, these flasks after cooling were inoculated in the same

way as in case of solid medium above.

3.3.1.2 Sterilization

Different media were sterilized at 15 psi for 20 minutes except where specifically mentioned. In case of heat sensitive solutions, fractional sterilization was accomplished for 30 minutes for three consecutive days at no pressure (to avoid effect of hydrolysis). Sterilization of different media, both solid and liquid, was done in an autoclave at 15 psi pressure for 20 minutes and all the glasswares were sterilized at 180^0C for 1½ hours in an electric oven. The cork borer and inoculation needle were initially dipped in ethyl alcohol, finally flame sterilized and used after complete cooling.

3.3.1.3 Hydrogen- ion concentration

The pH of the basal solid and liquid media was adjusted with the help of a Systronic type 1 expanded pH meter to fix the pH at 6.5. The pH of the solid medium was initially kept at 6.5.

3.3.1.4 Incubation

Petri plates containing basal (solid) medium and inoculums of different cultures under study were incubated in BOD incubator at 25 ± 1^0C till the Petri plates containing solid media were fully colonized with the mycelial mat in any one of the treatments used whereas the flasks containing liquid medium were incubated for 21 days.

3.3.1.5 Recording of vegetative growth

In case of solid media, the growth was recorded at different intervals depending on the rate of growth of the fungi. The growth was measured by taking average of linear growth of the colony in two directions at right angle and the morphological characters were also recorded. In liquid media studies, 21 days old mycelial mats of different cultures were filtered through Whatman No. 1 filter paper disc of 11cm diameter which were dried to constant weight (recorded) in an electric oven at 70 ± 5^0C, cooled in a dessiccator (having fused calcium chloride at its bottom) and weighed in an electric balance containing small flecks of calcium chloride so as to avoid absorption of moisture (ambient) during weighing. Dry weight of the mycelium was obtained by subtracting the initial weight of the oven dried Whatman No. 1 filter paper from the final weight and expressed in milligrams. The best liquid and solid media were used for further cultural studies.

3.3.2 Effect of temperature

To study the effect of different temperature regimes, flasks (100 ml) containing basal medium along with inoculums of different cultures were incubated at different temperatures viz., 5, 10, 15, 20, 25, 30, and 35 in different incubators for 21 days. Each treatment was replicated thrice and data analyzed.

3.3.3 Effect of hydrogen- ion concentrations

In this experiment, basal medium was adjusted at different pH levels viz., 4.0, 4.5, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 with the help of a Systronic type 1 expanded pH meter using N/10 HCl or N/10 NaOH and finally inoculated with 4 mm mycelial bits of the cultures and incubated at 25±1 ^{0}C for 21 days in BOD incubator. Each treatment was replicated thrice. Type of growth was also recorded and data obtained was subjected to statistical analysis. statistically.

3.3.4 Effect of different carbon sources

To study the effect of different carbon sources on the mycelial growth of test cultures, the carbon source present in the liquid basal medium for each culture was substituted by different carbon sources. Treatment without any carbon source acted as control. The carbon source in the basal medium was replaced by different carbon compounds so as to provide or supply equal amount of carbon per litre as obtained from the carbon source originally standardized for particular medium. The quantity of each carbon source per litre of medium is shown in Appendix-II. All the treatments were replicated thrice and sterilized at 15 psi pressure for 20 minutes and inoculated with 4 mm mycelial bits of different cultures after cooling. These were further incubated at 25±1^{0}C in BOD incubator. Observations on dry mycelial weight were recorded after 21 days of incubation along with the type of growth in various treatments and data analyzed statistically.

3.3.5 Effect of different nitrogen sources

The study in respect of nitrogen was also carried out with selected basal liquid medium. Treatment without any nitrogen source served as control. The quantity of each nitrogen source required for one litre of medium is given in Appendix-III. Each treatment was replicated thrice, sterilized at 15 psi for 20 minutes and inoculated with 4 mm mycelial culture bits of different cultures aseptically which were then incubated at 25±1^{0}C temperature

in BOD incubator. The data on dry mycelial weight was recorded after 21 days of inoculation.

3.3.6 Effect of different trace elements

The different trace elements and their concentrations studied were as under:

Trace element	Concentrations (ppm)		
Iron (ferrous sulphate)	0.025	0.05	1
Boron (boric acid)	0.025	0.05	1
Manganese (manganese sulphate)	0.025	0.05	1
Zinc (zinc sulphate)	0.025	0.05	1
Molybdenum (ammonium molybdate)	0.025	0.05	1

The stock solutions were prepared in double glass distilled water. The medium was purified by adding calcium carbonate at the rate of one gram per litre (Steinberg, 1950). Two controls without any trace element were also kept. Among the two, one treatment was purified using Whatman filter paper No. 1 after adding calcium carbonate and the other was kept unpurified. Again, three replications under each treatment were kept. The incubation period, temperature and statistical analysis were the same as for nitrogen and carbon sources.

3.3.7 Effect of different vitamin sources

Following concentrations of vitamins were tried to study their effect on mycelia growth:

Vitamin	Concentrations (ppm)
Niacin	2, 5, 10
Folic acid	2, 5, 10
Riboflavin	2, 5, 10
Ascorbic acid	2, 5, 10
Pantothenic acid	2, 5, 10
Thiamine hypo chlorite	2, 5, 10

The stock solutions of all vitamins except biotin were prepared in double glass distilled water and stored at 2-5^{0}C in a refrigerator. One control treatment with no vitamin

was also maintained. The stock solution of biotin was prepared in 5 ml of 50 per cent ethanol and volume made up with double glass distilled water. The basal medium (liquid) for vitamin study was purified by adding 5 g charcoal per litre of basal medium and then filtered through Whatman No. 1 filter paper as suggested by Mathur *et al.* (1950).

3.3.7 Effect of different growth regulators

The stock solutions of all except gibberellic acid (GA) were prepared in double glass distilled water and stored at 4^0C in a refrigerator. GA was first dissolved in 10 ml acetone and then required dilutions were prepared. Following concentrations of different growth regulators were used:

Growth regulator	Concentrations (ppm)		
Gibberellic acid (GA)	10	25	50
Indole acetic acid (IAA)	10	25	50
Indole-3- butyric acid (IBA)	10	25	50
Kinetin	10	25	50
Nephtha- acetic acid (NAA)	10	25	50
Benzyl adenine (BA)	10	25	50

3.4 EVALUATION OF SUBSTRATES FOR SPAWN PRODUCTION

3.4.1 Preparation of Mother culture

For preparing the mother culture, wheat grains were used as substrate. The wheat grains were cleaned and then soaked in water for 2 hours. Rotten grains or those floating on water were removed. These grains were boiled in tap water for at least 10- 15 minutes. Care was taken not to overboil the grains so that they may not rupture. Excess water was drained off by keeping on a wire mesh for 8- 10 h. Lime (calcium carbonate) and gypsum (calcium sulphate) in the ratio of 1:3 were added to the grains to prevent sticking of grains and for maintaining proper pH i.e. 6.5-6.8.

Properly mixed grains were filled in bottles or polypropylene bags up to $2/3^{rd}$ of the capacity. These were then tightly plugged with non- absorbent cotton with the help of a plastic ring and autoclaved at 22 psi pressure for 2 h. The sterilized bags or bottles were taken out from autoclave while still hot and shaken vigorously to avoid clumping of grains. After cooling, these bottles or bags were immediately transferred to inoculation chamber and kept under UV light for minimum thirty minutes for surface sterilization.

Actively growing cultures of each fungus were used for inoculation of these bottles/ bags. Each bag was inoculated aseptically with two mycelial agar bits. The inoculated bags were then incubated at 24 ± 1^0C till the grains were fully impregnated with the mycelium of inoculated fungus culture (15-20 days) and were ready as master culture for furthur multiplication. These bags were stored at 4^0C and used for further multiplication of spawn.

3.4.2 Selection of suitable substrate for spawn production

Following substrates were evaluated for their suitability to support fastest mycelial spread of each of the mushroom studied:

Wheat grains

Bajra grains

Wheat grains + Bajra grains (1:1)

Sawdust

Sawdust+ Bajra grains (1:1)

Sawdust+ Wheat grains (1:1)

Wheat straw

Wheat grains+ gur (5 per cent)

Wheat straw was cut into small pieces of upto 1.0 cm length. Sawdust and wheat straw were soaked in cold water for different periods depending upon their capacity to retain water. Moisture content of 68-70 per cent was adjusted in each case and were filled in polypropylene bags which were then sterilized at 22 psi for 2 hours. The grains were used after boiling in the same way as in case of commercial spawn production of *Agaricus brunnescens* (Munjal, 1973).Three replications of each treatment were kept and fresh master culture prepared in each case was used as an inoculum. The inoculated bags were incubated at 25±1 ^{0}C. Downward linear growth of the mycelium was recorded by taking the average of maximum and minimum growth after fixed intervals depending on the growth rate of the fungi. Type of growth was also recorded.

3.5 EVALUATION OF SUBSTRATES FOR FRUIT BODY PRODUCTION

Different agricultural and forestry waste materials that were tried to see the ability of different cultures to colonize and form fruiting bodies are as follows:

Crushed corncobs

Pine needles

Pine needles + Robinia wood chips (2:1)

Pine needles + sawdust + Robinia wood chips (2:1:1)

Pine needles + wheat straw (1:1)

Wheat straw

Wheat straw + sawdust (1:1)

Wheat straw + corncobs (1:1)

Pasteurized compost

Sawdust

Sawdust + Alnus wood chips (1:2) + 5 per cent rice bran

Sawdust + Robinia wood chips (1:2) + 5 per cent rice bran
Sawdust + Eucalyptus wood chips (1:2) + 5 per cent rice bran

In all the treatments sawdust of *Toona ciliata* was used. The substrates were soaked in water for different periods depending on their ability to retain water. Moisture content of 60–70 per cent with 6.5 pH was maintained in each case. Gypsum at the rate of 5 per cent was added in each of the wet substrate. The ingredients were mixed thoroughly with hands which were then filled in polypropylene bags (one kilogram/ bag) and tightly plugged with non-absorbent cotton. Sterilization was carried out at 22 psi for two hours. Ten replicates of each treatment were kept throughout the cultivation studies.

3.5.1 Spawning and spawn- run

The pasteurized substrate was inoculated aseptically on the surface with freshly prepared spawn of each culture at the rate of 4 per cent. The spawn run period varied for each of the mushroom. Temperature as well as relative humidity was maintained around 25 ± 1^0C and 85 per cent, respectively. Little or no fresh air was supplied during the incubation period. Water was sprayed on the walls and flour of the incubation room for maintaining the humidity. The bags were also sprayed daily with tap water (to maintain the humidity) on the surface only taking care that free water did not enter into the substrate. Time taken for complete mycelial run in each substrate was recorded.

3.5.2 Incubation period

The bags were opened after complete spawn run by making vertical slits in four places with a sterilize blade to expose the upper surface and the substrate blocks were subjected to optimum cropping conditions as above for fruiting body formation.

In case of *Ganoderma lucidum* and *Flammulina velutipes*, the colonized bags were kept for one week to ensure maturation of mycelium. For *L. edodes* also, the bags were not transferred immediately to the cropping room but the mycelium was allowed to undergo a number of changes after complete substrate colonization. Firstly, it formed a thick sheet on the outer surface of the substrate termed as ‘established mycelial stage’, then bumps (which appeared like primordia) began to form that created air spaces between the mycelial coat and the plastic bag which enhanced brown pigmentation, the next stage. When the mycelial coat on the substrate completely turned brown like bark of the tree, the bags were given shock treatment (dipped in water at 12^0C for 4-6 h). Finally, the bags were shifted to cropping

room. After first harvest, the substrate bags were again given shock treatment by dipping them in water for 4 h at 12^0C.

3.5.3 Crop management

After complete mycelial run, the bags were shifted to cropping room. The temperature and relative humidity were maintained according to the requirements of each mushroom for fruiting. For *Lentinula edodes, Pleurotus ostreatus, Pleurotus eryngii* and *Flammulina velutipes,* temperature was lowered to 15-20^0C whereas, for *G. lucidum* it was maintained at 28±2^0C. The bags were sprayed with tap water once or twice a day as and when required for maintaining a relative humidity of 85-90 per cent. Ample ventilation was provided in cropping room for development of pinheads into fruit bodies. Here also, time taken for pinhead formation and fruit body induction was recorded.

3.5.4 Picking

Mushrooms were picked, trimmed off at the substrate level, counted and weighed separately for each replication at every harvest. Fully mature mushrooms were picked by slightly and carefully twisting them so that the young developing pinheads in the vicinity of the mature sporophores did not become vulnerable to any sort of damage. It is noteworthy that fruiting bodies of G*anoderma. lucidum* became ready to harvest when later turned red leaving no whitish margin around the edge. The harvesting was done by cutting the base of the mushroom stalk with a knife. Yield was calculated as weight (kg) of mushrooms produced/ kilogram of substrate.

3.5.6 Sanitation

Before spawning, cropping bags were thoroughly sterilized/disinfected with 2 per cent formalin solution to avoid any surface contaminantion. Equipments as well as other articles were cleaned/disinfected with 2 per cent formalin solution before taking them in to the cropping room. Use of clean water for spraying purposes was a daily practice.

3.6 STATISTICAL ANALYSIS

The data recorded in each experiment was subjected to statistical analysis wherever required. The differences exhibited by treatments were tested for their significance by employing the Completely Randomized Design (CRD) as given by Gomez and Gomez (1976).

Chapter-4

EXPERIMENTAL RESULTS

Results of the present investigations are described below under following headings:

4.1 CULTURAL STUDIES

4.1.1 Selection of basal medium

a) Solid medium

b) Liquid medium

4.1.2 Effect of different temperature regimes

4.1.3 Effect of different hydrogen- ion concentrations

4.1.4 Effect of different carbon sources

4.1.5 Effect of different nitrogen sources

4.1.6 Effect of different trace elements

4.1.7 Effect of different vitamin sources

4.1.8 Effect of different growth regulators

4.2 SELECTION OF SUBSTRATES FOR SPAWN PRODUCTION

4.3 SELECTION OF SUBSTRATES FOR SPOROPHORE PRODUCTION

4.1 Cultural studies

4.1.1 Selection of basal medium

All the selected medicinal fungi were grown on six solid and six liquid media. Linear growth of test fungus and average dry mycelial weight were recorded after fixed intervals and the data are presented in Table- 1 and Table-2, respectively.

Liquid media selection

Six different synthetic and semi- synthetic media were selectively tried for the growth of the test fungi. Average dry mycelial weight was recorded after 21 days of incubation and the data obtained are presented in Table 1.1

Table 1.1: Effect of different liquid media on the mycelial growth of medicinal fungi

Liquid Media	*Pleurotus ostreatus*		*Flammulina velutipes*		*Lentinula edodes*		*Pleurotus eryngii*		*Auricularia polytricha*		*Ganoderma lucidum*	
	A.G.* (mg)	Type of growth	A.G. (mg)	Type of growth	A.G. (mg)	Type of growth	A.G. (mg)	Type of growth	A.G. (mg)	Type of growth	A.G. (mg)	Type of growth
Richard's	86.56	Aerial, strandy	73.85	Thin, strandy	84.25	Dense silky strandy	72.50	Fluffy	12.33	Appressed, strandy	84.25	Thick, dense mat
Sabouraud's	75.19	Fluffy, strandy	63.33	Fluffy, slightly strandy	70.33	Fluffy, slightly silky	88.65	Fluffy. strandy	25.0	Fluffy , strandy	70.33	Fluffy, dense mat
Glucose-asparagine	77.67	Aerial, dense strandy	86.47	Dense, cottony	93.74	Slightly dense silky	101.3	Fluffy,slightly strandy	34.0	Appressed,, slightly dense	93.74	Slightly thick, strandy
Thornton's solution	51.95	Thin, strandy	33.48	Thin, sparse, strandy	47.10	Dense silky strandy	59.17	Fluffy,slightly strandy	8.67	Thin transparent	47.10	Thin, strandy
Asthana and Hawker's	70.86	Transparent strandy	50.81	Appressed	34.60	Thin silky, strandy	80.41	Fluffy,slightly strandy	18.67	Thin transparent	34.60	Slightly dense mat
Elliott's	64.49	Thin, strandy	43.88	Thin, strandy	61.44	Transparent strandy	68.55	Fluffy,slightly strandy	39.33	Appressed	61.44	Slightly dense
Mean	71.12		58.84		65.24		78.43		71.12		85.86	
$CD_{0.05}$	1.92		2.05		2.11		2.06		1.51		2.26	
SE	0.91		0.94		0.97		0.95		0.72		1.04	

*** Average mycelial weight (mg) after 21 days of incubation**

The data presented in Table 1.1 indicates that Richard's solution is significantly the best medium for the mycelial growth (86.56 mg) of *Pleurotus ostreatus* followed by Glucose- asparagine solution (77. 67 mg). Moderate to good growth was recorded in Sabouraud's solution (75.19 mg). Significantly minimum growth was observed in Thornton's solution (51.95 mg) followed by Elliott's solution (64.49 mg). In all the treatments, aerial and strandy growth was recorded. However, in Sabouraud's solution fluffy growth was observed.

Significantly maximum growth (86.47mg) of *F. velutipes* was recorded in case of Glucose- asparagine solution followed by Richard's solution (73.85 mg). Moderate to good growth was observed in Sabourauds'solution (63.33 mg) whereas, minimum growth (33.48 mg) was observed in Thornton's medium followed by Asthana and Hawker's solution (50.81 mg) as is shown in Table 1.1. The type of growth was dense cottony on Glucose- asparagine solution, strandy in Richard's, Thornton's and Elliott's solution and appressed in Asthatna and Hawker's solution.

Glucose- asparagine solution supported significantly maximum (93.74 mg) mycelial growth of *L. edodes* followed by Richard's solution (84.25 mg). Significantly minimum (34.60 mg) mycelial dry weight was recorded in Asthana and Hawker's solution. Statistically, best vegetative (101.3 mg) growth of *P.eryngii* was supported by Glucose- asparagine solution followed by Sabouraud's solution (88.65 mg). Least growth was recorded on Thornton's (59.17 mg) followed by Elliott's solution (68.55 mg). The growth recorded was significantly different in all these treatments (Table 1.1). Silky and strandy type of growth was observed in all cases except, Sabouraud's where type of growth was fluffy.

Data on growth of *A. polytricha* on various media (Table 1.1) indicated that Elliot's solution supported insignificantly best average dry mycelia weight (39.33 mg), followed by Sabouraud's solution (25.0 mg). However, least growth (8.7 mg) was recorded in Thornton's solution followed by Richard's solution (12.33 mg). Similarly, significantly maximum growth (93.74 mg) of *G. lucidum*

was observed in Glucose- asparagine solution followed by Richard's solution (84.25 mg). However, Asthana and Hawker's solution was found to support significantly minimum (34.60 mg) growth followed by Thornton's (47.1 mg). Appressed type of growth was observed in all the treatments except in Sabouraud's medium where fluffy type of growth was recorded

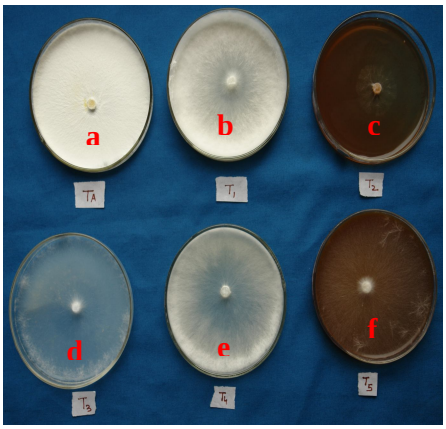
Selection of solid media

To select the most suitable solid medium for routine culturing of test fungi, linear growth on six solid media were tested and compared as shown in Table 1.2a and 1.2b.

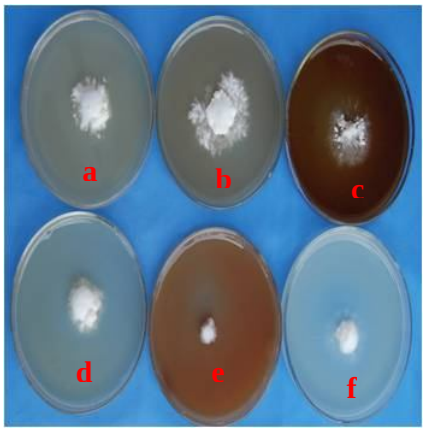
The data presented in Table 1.2a showed that though *P. ostreatus* could grow on all media tried but Potato dextrose agar supported insignificantly maximum growth (72.3 mm) followed by Malt extract agar (66.8 mm) and Compost extract agar (62.1mm) which was statistically at par with growth on Glucose- asparagine agar medium (61.0 mm). Insignificantly minimum growth (42.0 mm) was recorded with Czapek's dox agar medium.

The significance of interaction between media and days for *P. ostreatus* indicated that there was significant difference in the linear growth on various solid media at different intervals studied. Significantly maximum growth (90 mm) was recorded on eighth day of incubation on Potato dextrose agar, Malt extract agar, Compost extract agar and Glucose- asparagine agar media. Statistically minimum growth (61.8 mm) was exhibited by Czapek's dox agar on eighth day of incubation. Dense to thin strandy type growth of the fungi was observed.

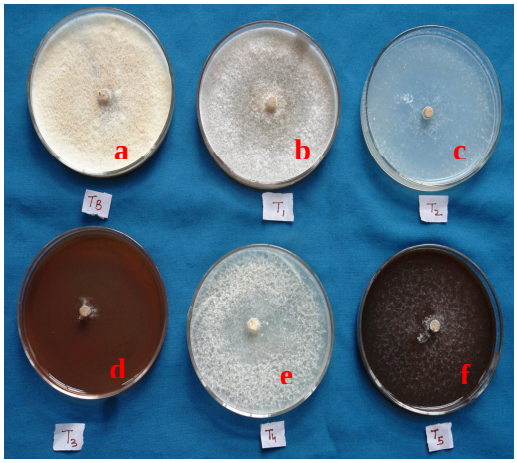
It is obvious from the data presented in Table 1.2a that irrespective of intervals, significantly maximum growth (47.4 mm) of *P. eryngii* was recorded on Malt extract agar medium followed by Potato dextrose agar (45.4 mm) and Compost extract agar (44.6 mm). Significantly minimum growth (28.3 mm) was obtained on Czapek's dox agar. Similarly, the interaction between media and intervals revealed that there was significant difference in the linear growth on various solid media at different intervals studied. Significantly maximum growth



1(a) **Growth of *Pleurotus ostreatus* on a) PDA b) MEA c) Czapek's dox d) Dimmick's agar e) Glucose-asparagine f) CEA media**



1(b) **Growth of *Pleurotus eryngii* on a) PDA b) MEA c) CEA d) Glucose -asparagine e) Czapek's dox f) Dimmick's agar media**



1(c) **Growth of *Flammulina velutipes* on a) PDA b)MEA c) Dimmick's agar d) Czapek's dox e)Glucose- asparagine f) CEA media**

(86.8 mm) was recorded after 10 days of incubation on Malt extract agar medium, followed by Potato dextrose agar (83.8 mm) and Compost extract agar (78.3 mm) media. These treatments were significantly different from one another. Statistically minimum growth (54.0 mm) was exhibited by Czapek's dox after 10 days of incubation. Irrespective of media, significantly maximum average growth (73.4 mm) was recorded on tenth day. There was five fold increases in growth after 10 days of incubation in comparison to growth recorded after 4 days. Dense to thin fluffy type of growth was observed in all the treatments.

The data recorded for growth of *Flammulina velutipes* on different solid media revealed that irrespective of intervals, significantly maximum growth (68.8 mm) was recorded on Glucose- asparagine agar medium followed by Potato dextrose agar (61.9 mm) and Malt extract agar (58.8 mm). Significantly minimum growth (38.9 mm) was recorded on Czapek's dox agar medium. Irrespective of media, significantly maximum growth (82.1 mm) was recorded after 8 days of incubation which was three times higher than the growth after 4 days of incubation (24.8 mm). Interaction between media and interval revealed that significantly maximum average growth (90 mm) of the fungus was recorded in Potato dextrose agar and Glucose- asparagine agar media after 8 days. These two treatments were statistically at par with each other whereas, significantly minimum growth (68.0 mm) was recorded on Czapek's dox agar medium after 8 days of incubation (Table 1.2a). Dense to slightly dense cottony type of growth was observed except, in CEA and Czapek's dox and Dimmick's agar where strandy growth was noticed.

Similarly, Table 1.2b cleared that maximum growth (62.2 mm) of *L. edodes* was recorded on Malt extract agar followed by Potato dextrose agar (59.2 mm). However, significantly minimum growth (9.4 mm) was recorded on Czapek's dox agar followed by Dimmick's agar (33.2 mm). Irrespective of media, significantly maximum growth (62.5mm) was recorded after 12 days of incubation which was almost four times to the growth after 4 days of incubation. Statistical interaction between media and interval indicate that Malt extract agar medium supported significantly maximum average growth (90 mm) after 12

Table 1.2a: Effect of different solid media on the mycelial growth of cultures of medicinal fungi

Medium	*Pleurotus ostreatus*					*Pleurotus eryngii*					*Flammulina velutipes*				
	Radial growth (mm)				Type of growth	Radial growth (mm)				Type of growth	Radial growth (mm)				Type of growth
	Interval					Interval					Interval				
	4th day	6th day	8th day	GM*		4th day	8th day	10th day	GM		4th day	6th day	8th day	GM	
PDA	44.0	83.0	90	72.3	Dense, strandy	15.3	37.3	83.8	45.4	Dense Fluffy	31.0	64.8	90	61.9	Dense cottony
MEA	34.8	77.8	90	66.8	Dense, strandy	15.3	40.8	86.8	47.4	Dense fluffy	27.3	61.0	88.0	58.8	Dense cottony
CEA	28.7	67.8	90	62.1	Thin, strandy	17.0	38.3	78.3	44.6	Dense fluffy	17.3	51.3	75.0	47.9	Strandy
Czapek's dox	22.3	42.0	61.8	42.0	Strandy	9.0	22.3	54.0	28.3	Less dense, fluffy	2.67	36.0	68.0	38.9	Thin, strandy
Dimmick's	27.8	51.3	76.0	51.7	Strandy	14.0	30.0	64.0	36.0	Fluffy	22.3	45.8	81.3	49.8	Strandy
Glucose-asparagine	28.0	65.0	90	61.0	Thin, strandy	18.3	34.8	74.0	42.3	Dense, fluffy	38.0	78.3	90	68.8	Slightly dense, cottony
GM	30.9	64.1	82.9			14.83	33.89	73.4			24.8	56.2	82.1		
Effect	$CD_{0.05}$	SE				$CD_{0.05}$	SE				$CD_{0.05}$	SE			
Treatment (T)	1.12	0.55				1.02	0.51				1.01	0.5			
Interval(I)	0.79	0.39				0.72	0.36				0.71	0.35			
Treatment* Interval (T*I)	1.94	0.96				1.76	0.88				1.75	0.86			

*** Grand mean**

days of incubation whereas Czapek's dox agar supported minimum growth (24.3 mm) after 12 days of incubation. The type of growth was strandy, in general. However, on Compost extract agar and Czapek's dox agar media the growth observed was slightly appressed.

It is obvious from Table 1.2b that significantly maximum growth (54.1 mm) of *Auricularia polytricha* was supported by Malt extract agar medium followed by Potato dextrose agar (46.6 mm). However, significantly minimum growth (11.4 mm) was recorded on Czapek's dox agar medium followed by Dimmick's agar (22.8 mm). Irrespective of medium, significantly maximum average growth (54.5 mm) was found after 3 weeks of incubation. This growth was almost four folds greater than growth recorded after first week. There was significant difference in growth at various intervals. In general, the type of growth in general was appressed. However, on Compost extract agar and Czapek's dox agar, it was slightly strandy.

Data in Table 1.2b revealed that insignificantly maximum growth (67.8 mm) of *G. lucidum* was recorded on Potato dextrose agar medium followed by Malt extract agar (65.6 mm). However, significantly minimum growth (5.9 mm) was recorded on Czapek's dox agar followed by Dimmick's (28.3 mm). Irrespective of media, significantly maximum growth (66.7 mm) was observed after 10 days of incubation. About four fold increase in growth was noticed after ten days of incubation as compared to growth recorded after four days. The significance of interaction between media and interval indicates that there was significant difference in the linear growth on various solid media at different intervals studied. Significantly maximum growth (90 mm) was recorded after 10 days of incubation on Potato dextrose agar, Malt extract agar and Compost extract agar medium which was statistically at par with one another. Thin to thick strandy growth was noticed in all the media studied.

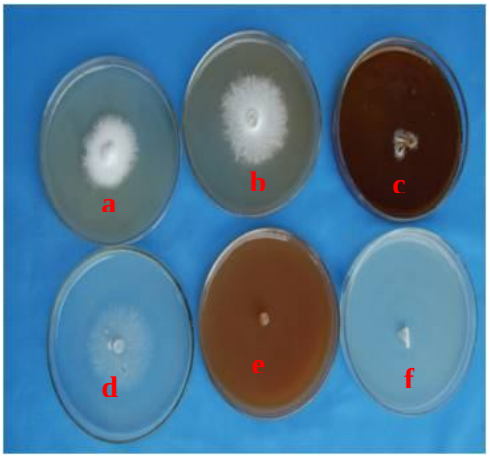
4.1.2 Effect of temperature

To study the effect of various temperature regimes, flasks containing the basal medium and inoculum of test fungi were incubated at different temperatures

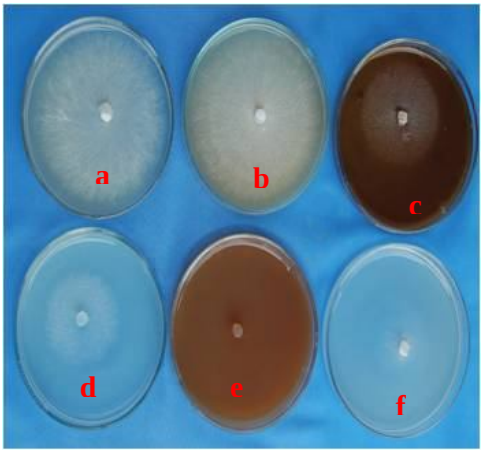
Table 1.2 b: Effect of different solid media on the mycelial growth of cultures of medicinal fungi

Medium	*Auricularia polytricha*					*Lentinula edodes*							*Ganoderma lucidum*					
	Radial growth (mm)				Type of growth	Radial growth (mm)						Type of growth	Radial growth (mm)					Type of growth
	Interval					Interval							Interval					
	1st week	2nd week	3rd week	GM*		4th day	6th day	8th day	10th day	12th day	GM		4th day	6th day	8th day	10th day	GM	
PDA	21.8	48.0	70.0	46.6	Appressed	25.0	42.0	62.3	79.0	87.8	59.2	Silky, strandy	28.3	65.0	88.0	90	67.8	Thick, strandy
MEA	25.3	60.0	77.0	54.1	Thin, Appressed	24.3	46.0	68.0	82.8	90.0	62.2	Slightly dense, strandy	27.3	62.0	83.0	90	65.6	Thick, strandy
CEA	10.8	33.8	55.3	33.2	Slightly dense strandy	18.3	25.3	34.8	45.8	56.3	36.1	Appressed	20.0	45.0	75.3	90	57.6	Less dense, strandy
Czapek's dox	0.0	7.8	26.8	11.4	Thin, strandy	0.0	0.0	7.0	15.8	24.3	9.4	Thin, appressed	0.0	0.0	7.7	16.0	5.9	Thin, strandy
Dimmick's	10.3	22.0	36.0	22.8	Appressed	14.3	21.3	31.0	44.0	55.3	33.2	Thin, strandy	13.3	21.3	33.3	45.3	28.3	Strandy
Glucose-asparagine	16.8	45.0	62.0	41.2	Thin, Appressed	19.3	26.0	35.3	49.8	61.3	38.3	Strandy	17.3	34.0	52.0	68.8	43.0	Thick, strandy
GM	14.1	36.1	54.5			16.9	26.7	39.7	52.8	62.5	26.7		17.7	37.9	56.6	66.7		
Effect	$CD_{0.05}$		SE			$CD_{0.05}$			SE				$CD_{0.05}$			SE		
Treatment(T)	0.81		4.0			0.61			0.31				0.98			0.49		
Interval(I)	0.57		0.28			0.56			0.28				0.80			0.40		
Treatment* Interval (T*I)	1.41		0.70			1.36			0.67				1.98			0.98		

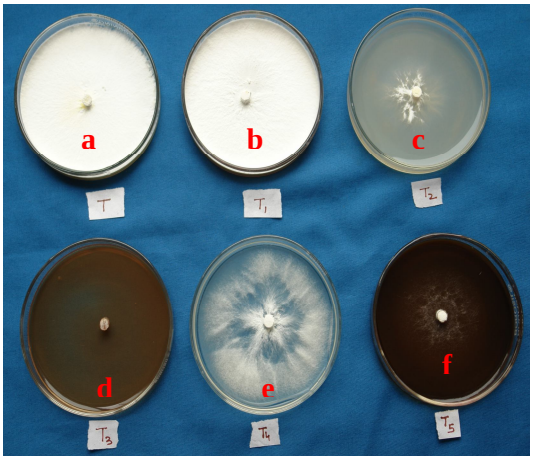
*** Grand mean**



2(a) **Mycelial growth of *Auricularia polytricha* on a) PDA b) MEA c) CEA d) Glucose-asparagine e) Czapek's dox f) Dimmik's agar media**



2(b) **Growth of *Lentinula edodes* on a) MEA b) PDA c) CEA d) Glucose-asparagine agar e) Czapek's dox f) Dimmick's agar media**



2(c) **Growth of *Ganoderma lucidum* on a) PDA b) MEA c) Dimmik's agar d) Czapek's dox e) Glucose-asparagine f) CEA media**

Plate-2

ranging between 5^0C to 35^0C for 21 days. Average mycelial dry weight of test fungi was recorded and data obtained are presented in Table 2.

It is clear from the data that all the test fungi could grow over a wide range of temperatures (5-35^0C) but for *Ganoderma lucidum* and *Auricularia polytricha* the favourable temperature range was found to be 10-35^0C. Significantly maximum average growth of *Pleurotus ostreatus* was recorded at 25^0C (82.13 mg) followed by 30^0C (73.87 mg) and 20^0C (70.26 mg) where the growth was significantly different. In case of *Pleurotus eryngii*, maximum growth occurred at 25^0C (85.5 mg) followed by 20^0C (67.9 mg) and 30^0C (61.70 mg). Insignificantly minimum growth in both cases was recorded at 5^0C followed by 35^0C where growth recorded was 5.25 mg and 38.76 mg, respectively for *P. ostreatus* and 4.06 and 30.1 mg, respectively for *P. eryngii*.

Again the statistical analysis of data presented in Table 2 revealed that significantly maximum growth of *Flammulina velutipes* and *Lentinula edodes* was observed at 25^0C supporting 85.16 mg and 98.41 mg mycelial weight, respectively. This was followed by 30^0C in case of *F. velutipes* (71.79 mg) and 20^0C in case of *L. edodes* (84.05 mg). Significantly minimum growth of both fungi occurred at 5^0C i.e. 12.73 mg in *F. velutipes* and 4.17 mg in case of *L. edodes* which was followed by 10^0C in both cases but in *L. edodes* (31.75 mg), it was statistically at par with mycelial growth at 35^0C.

As is obvious from the data (Table 2) that both *Auricularia polytricha* and *Ganoderma lucidum* failed to grow at 5^0C. However, increased mycelial growth was recorded at 10^0C and 15^0C in both the fungi. Significantly maximum growth of both these fungi was found at 25^0C supporting 45 mg and 129.1 mg growth, respectively of *Auricularia polytricha* and *Ganoderma lucidum*. This was followed by growth at 30^0C in both cases where growth was 37.7 mg and 113.6 mg, respectively.

In *P. ostreatus*, aerial and thin to dense and strandy growth was recorded, in general. The type of growth in *P. eryngii* was fluffy at all incubation temperatures. However, it was fluffy and strandy at 20, 25 and 30^0C. In *F.*

Table 2: Mycelial growth of cultures of different medicinal fungi at various temperature regimes

Temp.* (^{0}C)	*P. ostreatus*		*F. velutipes*		*L. edodes*		*P. eryngii*		*A. polytricha*		*G. lucidum*	
	A.G. (mg)**	Type of growth	A.G. (mg)	Type of growth	A.G. (mg)	Type of growth	A.G. (mg)	Type of growth	A.G. (mg)	Type of growth	A.G. (mg)	Type of growth
5	5.25	Thin, strandy	12.73	Transparent strandy	4.17	Thin, strandy	4.06	Slightly fluffy	0.0	-	0.0	-
10	22.25	Thin, strandy	28.72	Transparent strandy	31.75	Thin, strandy	21.0	Fluffy	9.0	Thin, appressed	23.10	Slightly dense strandy
15	46.41	Slightly aerial, strandy	53.30	Thin, cottony	55.02	Thin, strandy, silky	45.70	Fluffy	17.0	Thin, appressed	44.30	Slightly dense strandy
20	70.26	Slightly Aerial strandy	66.77	Slightly dense, cottony	84.05	Dense silky	67.90	Fluffy, strandy	29.0	Slightly thick, strandy	93.10	Thick strandy mat
25	82.13	Aerial, dense strandy	85.16	Slightly dense, cottony	98.41	Dense silky	85.50	Fluffy, strandy	45.0	Slightly dense, appressed	129.10	Thick strandy mat
30	73.87	Aerial dense strandy	71.79	Less dense, cottony	63.56	Less dense, silky	61.70	Fluffy, strandy	37.70	Appressed	113.60	Slightly thick, strandy
35	38.76	Aerial thin, strandy	45.92	Thin, strandy	32.66	Thin, slightly strandy	30.10	Less fluffy	22.0	Thin, appressed	52.80	Slightly strandy
Mean	48.42		52.06		52.80		45.12		48.40		65.10	
$CD_{0.05}$	1.82		1.37		1.32		1.47		1.91		2.29	
SE	0.86		0.64		0.63		0.70		0.89		1.07	

*** Temperature (^{0}C)**

****Average mycelial weight (mg) after 21 days of incubation**

velutipes, strandy growth was observed at 5, 10 and 35^0C and at other temperature regimes, it was found to be cottony.

In *G. lucidum*, thin to thick strandy mycelial mat was noticed at all the temperature regimes. The growth of *A. polytricha* in all the treatments was appressed however, in *L. edodes*, the growth recorded was silky and strandy.

4.1.3 Effect of different hydrogen - ion concentrations

Different pH range from 4.0- 9.0 were tried to see their effect on vegetative growth of all the test fungi. Average mycelial dry weight of test fungi was recorded after an incubation period of 21 days and presented in Table 3.

From the data given in Table 3, it is clear that significantly minimum growth of all the test fungi was recorded at pH 9.0 followed by pH 8.5 which differed significantly from one another. The amount of growth at pH 9.0 and 8.5 was 15.02 mg and 22.54 mg, respectively in *Pleurotus ostreatus*, 15.26 mg and 20.43 mg, respectively in case of *Pleurotus eryngii*, 25.83 mg and 32.04 mg, respectively in *Flammulina velutipes*, 21.89 mg and 28.79 mg, respectively in *Lentinula edodes*. No growth was recorded in case of *Auricularia polytricha* at pH 9.0 but a growth of 4.67 mg was recorded at 8.5 pH and in *Ganoderma lucidum*, it was 20.43 mg and 25.26 mg at 9.0 and 8.5 pH, respectively.

Significantly maximum growth (90.55 mg) of *P. ostreatus* was observed at pH 6.0 followed by pH 5.5 (83.22 mg) as is apparent from Table 3. The table also signified that maximum growth of *P. eryngii* occurred at 5.5 pH (88.76 mg) followed by pH 6.0 (76.45 mg) and pH 5.0 (72.35 mg). The growth at pH 4.0 (30.61 mg) and 7.5 (29.05 mg) was statistically at par with one another. Strandy growth of *P. ostreatus* was observed at all the pH ranges studied and it was fluffy in case of *P. eryngii*.

Similarly, maximum growth (86.91 mg) of *F. vlutipes* was recorded at pH 5.5 followed by pH 5.0 (80.77 mg) and 6.0 pH (72.61 mg). The Table 3 also revealed that insignificantly maximum growth of *L. edodes* was recorded at pH

Table 3: Mycelial growth of cultures of different medicinal fungi at various hydrogen - ion concentrations

pH	*P. ostreatus*		*F. velutipes*		*L. edodes*		*P. eryngii*		*A. polytricha*		*G. lucidum*	
	A.G. (mg)*	Type of growth	A.G. (mg)	Type of growth	A.G. (mg)	Type of growth	A.G. (mg)	Type of growth	A.G. (mg)	Type of growth	A.G. (mg)	Type of growth
4.0	**36.84**	**Thin, strandy**	**35.59**	**Thin, slightly, strandy**	**83.74**	**Thin, slightly strandy**	**30.61**	**Slightly fluffy**	**7.0**	**Transparent, appressed**	**82.74**	**Thin, strandy**
4.5	**45.20**	**Thin, strandy**	**58.75**	**Thin, cottony**	**113.1**	**Thin, slightly strandy**	**46.57**	**Slightly fluffy**	**12.33**	**Transparent, appressed**	**107.56**	**Slightly thick, strandy**
5.0	**73.53**	**Slightly dense, strandy**	**80.77**	**Dense, cottony**	**108.7**	**Silky, strandy**	**72.35**	**Fluffy, slightly strandy**	**25.67**	**Appressed, strandy**	**136.74**	**Slightly dense,strandy**
5.5	**83.22**	**Silky,dense strandy**	**86.91**	**Dense, cottony**	**68.31**	**Silky, strandy**	**88.76**	**Fluffy, slightly strandy**	**32.67**	**Appressed, strandy**	**125.27**	**Dense, thick strandy**
6.0	**90.55**	**Slightly dense, strandy**	**72.61**	**Slightly dense, cottony**	**56.70**	**Silky, dense, strandy**	**76.45**	**Fluffy , slightly strandy**	**42.33**	**Appressed, strandy**	**94.99**	**Dense, thick strandy**
6.5	**60.84**	**Strandy**	**67.40**	**Slightly appressed, cottony**	**51.36**	**Less dense, silky**	**54.74**	**Fluffy, less dense**	**51.0**	**Appressed**	**77.42**	**Thick, strandy**
7.0	**51.18**	**Aerial, strandy**	**51.06**	**Thin, cottony**	**47.35**	**Less dense, silky**	**49.10**	**Fluffy**	**36.67**	**Slightly appressed**	**59.12**	**Thin, strandy**
7.5	**29.97**	**Aerial, strandy**	**44.96**	**Thin, cottony**	**39.09**	**Thin, silky**	**29.05**	**Fluffy**	**20.67**	**Thin, appressed**	**44.14**	**Slightly strandy**
8.0	**25.05**	**Strandy**	**40.15**	**Thin, cottony**	**34.90**	**Thin ,silky**	**23.76**	**Thin, fluffy**	**14.67**	**Thin, appressed**	**32.48**	**Thin slightly, strandy**
8.5	**22.54**	**Slightly strandy**	**32.04**	**Thin, strandy**	**28.79**	**Slightly appressed**	**20.43**	**Thin, fluffy**	**4.67**	**Thin, slightly strandy**	**25.26**	**Thin, slightly strandy**
9.0	**15.02**	**Slightly strandy**	**25.83**	**Thin, strandy**	**21.89**	**Slightly appressed**	**15.26**	**Thin, slightly fluffy**	**0.0**	**-**	**20.43**	**Thin, slightly strandy**
Mean	**48.54**		**54.28**		**59.44**		**46.10**		**48.54**		**73.3**	
$CD_{0.05}$	**1.28**		**1.69**		**1.56**		**1.69**		**1.32**		**1.60**	
SE	**0.62**		**0.81**		**0.75**		**0.82**		**0.64**		**0.78**	

***Average mycelial weight (mg) after 21 days of incubation**

4.5 (113.10 mg) followed by pH 5.0 (108.70 mg) and pH 4.0 (83.74 mg). Cottony growth on *F. velutipes* was noticed in general, however at 4.0 and 9.0, the growth was strandy and it was slightly appressed at 6.5. In case of *L. edodes*, the growth was silky and strandy in general, but at pH 8.5 and 9.0, it was slightly appressed.

It is obvious that a pH of 6.5 favoured comparatively higher mycelial growth of *A. polytricha* (51.0 mg) followed by growth at pH 6.0 (42.33 mg) and 7.0 (36.67 mg). Data in the same table revealed that maximum growth of *G. lucidum* occurred at pH 5.0 (136.74 mg), which was significantly different from other treatments. This was followed by the growth at pH 5.5 (125.27 mg) and 4.5 (107.56 mg). In *A. polytricha*, the growth recorded was thick to transparent and appressed, however in *G. lucidum*, the growth was dense to thin strandy.

4.1.4 Effect of different carbon sources

Carbon is the most important factor for vegetative growth of the fungi. Different fungi respond to a carbon source differently and donot prefer the same carbon source for their maximum growth. Ten different carbon sources belonging to the groups of monosaccharides, disaccharides, sugar alcohol and polysaccharides were tested for determining their comparative response on the vegetative growth of the test fungi In case of *Pleurotus ostreatus*, starch supported insignificantly maximum growth (98.60 mg) followed by glucose (90.5 mg) and sucrose (84.3 mg).Treatment without any carbon source was the least preferred one and minimum growth was recorded with mannitol (48.63 mg) followed by fructose (55.8 mg) and Maltose (60.2 mg). Dextrose, xylose and galactose supported moderately good growth (66-78 mg) and the difference in growth was statistically insignificant. The type of growth noted was aerial and strandy but in case of control and treatment containing starch, the growth was slightly fluffy.

Among the carbon sources tested, glucose supported significantly maximum growth of *P. eryngii* (113.5 mg) followed by sucrose (110.5 mg) and dextrose (103.5 mg) that were statistically different from one another. Although, significantly minimum growth was recorded in control (18.9 mg) yet among carbon sources evaluated, least growth was observed with galactose (52.3 mg)

followed by mannitol (59.3 mg). Moderate to good growth was recorded with Maltose (94.5 mg), xylose (88.7 mg) and starch (81.2 mg) as is evident in Table 4. In general, fluffy type of growth was recorded in all the treatments however, in some treatments slightly strandy growth was also observed.

Best mycelial growth of *F. velutipes* was favoured by the addition of sucrose (107.33 mg) to the basal medium followed by dextrose (94.23 mg). However, minimum vegetative growth was observed in the medium without any carbon source i.e. control (22.76 mg) followed by sugar alcohol i.e. mannitol (51.95 mg). Starch (87.9 mg) and glucose (84.13 mg) supported moderately good growth and were significantly different from each other (Table 4). In almost all the treatments, fluffy growth of the fungus was noticed.

The monosaccharide, glucose supported significantly maximum growth of *L .edodes* (116.60 mg) followed by fructose (98.09 mg) and sucrose (94.28 mg) that were significantly different from one another. Significantly minimum growth was recorded with control (27.98 mg) followed by sugar alcohol, mannitol (42.15 mg). Starch and dextrose were observed as moderately good carbon sources supporting 81.8 mg and 78.31 mg growth, respectively (Table 4). Thin to dense silky and strandy type of growth was noted in all the treatments.

In case of *A. polytricha*, the polysaccharide, starch favoured the best vegetative growth (65.3 mg) followed by sucrose (53.3 mg) and fructose (45.3 mg), whereas significantly poor growth was observed in control (4.7 mg) and among carbon sources, mannitol supported the minimum growth (7.67 mg). Galactose (14.0 mg) and xylose (19.0 mg) also supported poor mycelial growth. Moderately good growth (40 mg) was observed when dextrose was added. Among the carbon sources tested for the mycelial growth of *G. lucidum*, simple sugar, glucose supported maximum growth (126.3 mg) and was significantly different from all other treatments. Starch was recorded as the second best carbon source (118.5 mg) followed by dextrose (106.1 mg). Again, sugar alcohol, mannitol supported insignificantly minimum growth (44.8 mg) followed by galactose (61.7 mg) among the carbon sources tested although, poorest growth

Table 4: Mycelial growth of different medicinal fungi on various carbon sources

Carbon source	*Pleurotus ostreatus*		*Flammulina velutipes*		*Lentinula edodes*		*Pleurotus eryngii*		*A. polytricha*		*G. lucidum*	
	A.G. (mg)*	Type of growth	A.G. (mg)	Type of growth	A.G. (mg)	Type of growth	A.G. (mg)	Type of growth	A.G. (mg)	Type of growth	A.G. (mg)	Type of growth
Sucrose	84.30	Aerial, dense strandy	107.33	Slightly dense, cottony	94.28	Silky, strandy	110.50	Fluffy , strandy	53.30	Appressed, dense, strandy	96.70	Dense, thick mat
Fructose	55.80	Thin, strandy	66.94	Thin, cottony	98.09	Thin silky strandy	76.10	Fluffy	45.30	Thin, strandy	90.40	Appressed, slightly dense
Glucose	90.50	Aerial thin, strandy	84.13	Cottony	116.60	Silky, strandy	113.50	Fluffy dense strandy	33.0	Appresed , slightly strandy	126.30	Dense, strandy mat
Maltose	60.20	Aerial, slightly strandy	72.96	Slightly dense cottony	56.43	Silky, strandy	94.50	Fluffy slightly strandy	29.0	Appressed, slightly strandy	68.40	Dense slightly strandy mat
Dextrose	78.29	Strandy	94.23	Slightly strandy	78.31	Slightly dense strandy	103.50	Fluffy, dense strandy	40.0	Appressed, dense strandy	106.10	Dense, strandy
Lactose	63.03	Thin, strandy	58.58	Thin, cottony	70.68	Thin, strandy	68.60	Slightly strandy	24.0	Slightly dense, strandy	75.80	Dense, thick
Starch	98.60	Slightly fluffy, strandy	87.90	Thick, cottony	81.82	Slightly dense, strandy	81.20	Strandy slightly fluffy	65.3	Dense, strandy	118.50	Slightly strandy
Mannitol	48.63	Thin, strandy	51.95	Thin, cottony	42.15	Transparent, strandy	59.30	Fluffy	7.67	Appressed, slightly strandy	44.80	Thin, strandy
Galactose	66.69	Thin, strandy	78.95	Thin slightly cottony	63.22	Thin, strandy	52.30	Fluffy	14.0	Thin, appressed	61.70	Thin, slightly dense
Xylose	70.68	Strandy	63.39	Thin, cottony	68.77	Thin, silky, strandy	88.70	Fluffy	19.0	Thin, strandy	81.90	Thin, strandy
Control	33.18	Strandy, slightly fluffy	22.76	Cottony	27.98	Silky, strandy	18.90	Fluffy	4.70	Thin, strandy	37.50	Thick, strandy
Mean	68.30		71.74		72.58		78.8		68.3		85.52	
$CD_{0.05}$	1.52		2.01		1.70		1.82		1.59		1.52	
SE	0.74		0.97		0.82		0.88		0.77		0.74	

***Average mycelial weight (mg) after 21 days of incubation**

among all treatments was observed in control (37.5 mg). Moderately good growth was observed with the addition of sucrose (96.7 mg) and fructose (90.4 mg) as carbon sources into the basal medium. The type of growth, in general was dense to thin appressed.

4.1.5 Effect of different nitrogen sources

Nitrogen has been considered as an essential source for the growth of various fungi. Innumerable studies on the nitrogen requirements of different fungi in the past have shown the differential response of different fungi to inorganic as well as organic sources of nitrogen.

In the present study, fifteen different organic, inorganic and complex sources of nitrogen were tried to see their comparative effect on the mycelial growth of test fungi against the control with no nitrogen source. Mycelial dry weight was recorded after 21 days of incubation as per data given in Table 5. It is apparent that among the various nitrogen sources tested, ammonium nitrate supported insignificantly maximum growth (101.3 mg) of *P. ostreatus* followed by leucine (90.6 mg) and potassium nitrate (87.1 mg), whereas ammonium sulphate (81.3 mg) and ammonium chloride (77.90 mg) supported moderately good growth. Sodium nitrite (18.30 mg) followed by valine (39.50 mg) gave less encouraging results among all the treatments although the growth was higher than that observed in nitrogen free medium (25.5 mg). Moderate growth was recorded with addition of histidine (56.0 mg) and proline (55.5 mg) which were statistically at par with each other. The type of growth recorded was aerial varying from slightly to dense strandy but in case of alanine, ammonium phosphate and sodium nitrite, slightly appressed growth was recorded.

Again Table 5 highlighted that leucine supported insignificantly maximum growth (137.09 mg) of *P. eryngii* followed by aspartic acid (128.53 mg), ammonium chloride (111.73 mg) and alanine (97.24 mg). Poor results with respect to mycelial growth were recorded with ammonium phosphate and methionine (51.23 mg and 58.03 mg, respectively), though the growth was higher than that observed on nitrogen free medium (30.95 mg). Interestingly, sodium nitrite resulted in significantly minimum growth (10.32 mg) as compared to

growth in nitrogen free medium (30.95 mg). The growth in treatments containing ammonium nitrate (62.91 mg) and sodium nitrate (64.16 mg) was statistically at par with one another. Dense to thin fluffy growth was recorded in all the treatments.

The vegetative growth of *F. velutipes* was best favoured by the addition of leucine (103.60 mg) as nitrogen source followed by ammonium sulphate (91.90 mg) and ammonium chloride (87.50 mg). Significantly minimum (22.30 mg) average mycelial dry weight was recorded in sodium nitrite followed by control (28.10 mg). The growth with treatments namely, alanine (74.20 mg) and urea (72.60 mg); ammonium nitrate (58.20 mg) and potassium nitrate (57.0 mg); and aspartic acid (49.90 mg) and ammonium phosphate (49.30 mg) were statistically at par with each other (Table 5). Dense to thin cottony type of growth was recorded in all treatments, in general. However, in sodium nitrate and potassium nitrate the growth was slightly strandy.

Statistical analysis of data presented in Table 5 also revealed that ammonium sulphate supported significantly maximum (128.4 mg) vegetative growth of *L. edodes* followed by ammonium chloride (119.4 mg) and sodium nitrate (109.5 mg). Poorest growth (13.0 mg) was recorded with sodium nitrite containing treatment followed by control (24.1 mg) and valine (39.6 mg) that were significantly different from one another. Silky and strandy type of growth was recorded in all the cases except, in sodium nitrite where slightly appressed and in leucine where slightly fluffy growth were recorded.

For *A. polytricha*, significantly maximum average dry mycelial weight was recorded on ammonium chloride (73.0 mg) followed by ammonium sulphate (65.0 mg) and potassium nitrate (61.30 mg). The treatments namely, aspartic acid (48.30 mg) and ammonium nitrate (48.17 mg); alanine (38.67 mg) and urea (38.67 mg); and histidine (31.33 mg) and sodium nitrate (29.0 mg) were statistically at par with each other and supported moderately good growth. No growth was recorded in the treatment containing sodium nitrite whereas; significantly minimum growth (11.0 mg) was recorded in control and valine (12.33 mg) that were statistically at par with each other. Dense to thin appressed

Table 5: Mycelial growth of different medicinal fungi on various nitrogen sources

Nitrogen Source	*P.ostreatus*		*F. velutipes*		*L. edodes*		*P. eryngii*		*A. polytricha*		*G. lucidum*	
	A.G. (mg)*	Type of growth	A.G. (mg)	Type of growth	A.G. (mg)	Type of growth	A.G. (mg)	Type of growth	A.G. (mg)	Type of growth	A.G. (mg)	Type of growth
L- Aspartic acid	**53.30**	**Aerial, strandy**	**49.90**	**Slightly dense, cottony**	**61.50**	**Silky, thin, strandy**	**128.53**	**Slightly fluffy**	**48.30**	**Thin, appressed**	**133.89**	**Thick strandy**
DL-Methionine	**47.10**	**Aerial, strandy**	**39.40**	**Thin, cottony**	**56.40**	**Silky, thin, strandy**	**58.03**	**Fluffy**	**15.33**	**Thin, appressed**	**57.07**	**Thin, strandy**
Ammonium nitrate	**101.30**	**Aerial, slightly strandy**	**58.20**	**Slightly strandy, cottony**	**78.10**	**Slightly thick, strandy**	**62.91**	**Thick, fluffy**	**48.17**	**Slightly appressed, strandy**	**64.92**	**Thick appressed, strandy**
Ammonium sulphate	**81.30**	**Aerial, slightly strandy**	**91.90**	**Silky, thick cottony**	**128.40**	**Thin, silky, strandy**	**81.29**	**Thick, fluffy**	**65.0**	**Thin, slightly appressed**	**151.40**	**Slightly thick, strandy**
Ammonium chloride	**77.90**	**Aerial, slightly strandy**	**87.50**	**Slightly dense, cottony**	**119.40**	**Slightly strandy**	**111.73**	**Fluffy, slightly strandy**	**73.0**	**Thin , slightly appressed**	**168.57**	**Less dense, strandy**
Ammonium phosphate	**69.60**	**Aerial, appressed strandy**	**49.30**	**Silky, thick cottony**	**50.70**	**Thin, strandy**	**51.23**	**Fluffy**	**18.0**	**Appressed**	**47.13**	**Thin, strandy**
Sodium nitrate	**63.20**	**Thin, silky strandy,**	**84.60**	**Thin, strandy**	**109.50**	**Slightly strandy**	**64.16**	**Thin, fluffy**	**29.0**	**Thin, appressed**	**106.33**	**Thin, strandy**
Sodium nitrite	**18.30**	**Slightly appressed**	**22.30**	**Thin, cottony**	**13.0**	**Thin appressed, strandy**	**10.32**	**Thin, fluffy**	**0**	**-**	**12.99**	**Thin, strandy**
L(-) Histidine	**56.0**	**Aerial, strandy**	**78.70**	**Thin, slightly cottony**	**98.50**	**Silky, strandy**	**94.32**	**Fluffy**	**31.33**	**Slightly strandy**	**80.34**	**Thin, strandy**
L(+) Valine	**39.50**	**Aerial, slightly strandy**	**34.20**	**Thin, cottony**	**39.60**	**Transparent strandy**	**75.67**	**Fluffy**	**12.33**	**Thin, appressed**	**42.13**	**Slightly appressed**
L(-) Proline	**55.50**	**Silky, strandy**	**69.50**	**Thin, cottony**	**88.90**	**Transparent thin, strandy**	**69.08**	**Slightly strandy**	**23.0**	**Appressed**	**68.15**	**Dense ,strandy**
DL- L-Alanine	**65.90**	**Slightly appressed, strandy**	**74.2**	**Slightly fluffy, cottony**	**82.0**	**Thin, slightly strandy**	**97.24**	**Thin, strandy**	**38.67**	**Less dense, appressed**	**92.52**	**Strandy**
Urea	**73.20**	**Silky, thick strandy**	**72.60**	**Fluffy, cottony**	**86.60**	**Slightly thick, strandy**	**84.41**	**Fluffy, slightly strandy**	**38.67**	**Thin, appressed, strandy**	**86.53**	**Thick ,appressed**
Potassium nitrate	**87.10**	**Aerial, strandy**	**57.0**	**Slightly thick, strandy**	**95.20**	**Silky, thin, strandy**	**73.39**	**Fluffy**	**61.30**	**Appressed**	**61.30**	**Thin, strandy**
Leucine	**90.60**	**Aerial, strandy**	**103.6**	**Slightly thick, cottony**	**106.6**	**Slightly fluffy, strandy**	**137.09**	**Dense, fluffy**	**53.67**	**Dense, appressed**	**128.26**	**Thick, strandy mat**
Control	**25.50**	**Aerial, strandy**	**28.1**	**Thin, slightly cottony**	**24.10**	**Thin, silky, strandy**	**30.95**	**Fluffy**	**11.0**	**Appressed**	**11.0**	**Thick, strandy mat**
Mean	**62.80**		**62.60**		**77.41**		**76.9**		**62.83**		**85.2**	
$CD_{0.05}$	**1.63**		**1.91**		**1.81**		**1.95**		**2.35**		**1.69**	
SE	**0.80**		**0.94**		**0.89**		**0.96**		**1.30**		**0.83**	

***Average myclial weight (mg) after 21 days of incubation**

type of growth was recorded, in general. However slightly strandy growth was observed in treatment containing urea, ammonium nitrate and histidine.

The data given in Table 5 indicates that ammonium chloride supported significantly maximum (168.57 mg) mycelial growth of *G. lucidum* followed by ammonium sulphate (151.40 mg) and aspartic acid (133.89 mg). Though, the growth was least on control (11.0 mg) containing no nitrogen source but amongst all the sources, sodium nitrite supported the minimum growth (12.99 mg). In all the treatments thick to thin strandy growth was recorded. However, in urea, valine and ammonium nitrate slightly appressed growth was observed.

4.1.6 Effect of different trace elements

Five trace elements were tried for their effect on the growth of test fungi. The details of purification of medium, concentrations of trace elements, sterilization, inoculation and incubation have already been described under material and methods. The average dry mycelial weight recorded is presented in Table 6a and 6b.

From the perusal of data presented in Table 6a, it is clear that significantly maximum average growth of *Ganoderma lucidum* was recorded with 2 ppm manganese sulphate (162.4 mg) followed by 2 ppm ferrous sulphate (150.3 mg). Significantly minimum growth was recorded with ammonium molybdate (111.4 mg), zinc sulphate (111.4 mg) followed by manganese sulphate (112.2 mg) all at 10 ppm concentrations that were statistically at par with one another. In comparison to purified control that supported 122.3 mg of growth, addition of 5 and 10 ppm boric acid; ammonium molybdate (2 ppm & 10 ppm); zinc sulphate, ferrous sulphate and manganese sulphate all at 10 ppm concentration; and control with unpurified medium resulted in decreased growth. The type of growth varied from thick to thin strandy but with addition of ammonium molybdate and zinc sulphate appressed growth was observed.

The statistical analysis of vegetative growth of *Pleurotus ostreatus* with varying concentrations of different trace elements given in the Table 6a revealed

that significantly maximum growth (114.3 mg) was recorded with the addition of 2 ppm ferrous sulphate followed by 2 ppm manganese sulphate (110.2 mg) and 5 ppm zinc sulphate (101.3 mg) while significantly minimum mycelial growth was observed with unpurified control (75.63 mg) followed by 10 ppm boric acid (82.6 mg). Treatments containing boric acid at 2 ppm (86.5 mg) and 10 ppm (82.6 mg) concentrations; and 10 ppm ammonium molybdate (85.4 mg) were also recorded to reduce the mycelial growth in comparison to control (87.71 mg). Aerial and strandy type growth was noticed in all the treatments.

Insignificantly maximum growth of *Pleurotus eryngii* was recorded with 2 ppm ferrous sulphate (99.4 mg) followed by 2 ppm zinc sulphate (93.6 mg) and 5 ppm ferrous sulphate (90.8 mg). Statistically, minimum growth (73.6 mg) was recorded with the addition of 5ppm boric acid that was significantly at par with 10 ppm ammonium molybdate (74.3 mg). In comparison to control (82.4 mg), decreased growth was recorded with the addition of boric acid at 2 ppm (81.1 mg), 5 ppm (73.6 mg) and 10 ppm (76.3 mg) concentrations; ammonium molybdate at 5 ppm (76.9 mg) and 10 ppm (74.3 mg); ferrous sulphate (79.6 mg), zinc sulphate (81.8 mg); and manganese sulphate (81.4 mg) all at 10 ppm concentration. Fluffy type of growth was noticed in all the treatments.

It is apparent from Table 6b that in *Auricularia polytricha*, 5 ppm ammonium molybdate supported significantly maximum growth (58 mg) followed by 2 ppm ferrous sulphate (52.3 mg), whereas minimum growth (30 mg) was recorded with the addition of 2 ppm boric acid in the basal medium followed by 10 ppm manganese sulphate (33.3 mg) and 10 ppm boric acid (34.3 mg) which were statistically at par with unpurified control (33.3 mg). Treatment with ferrous sulphate (38.0 mg), zinc sulphate (35.3 mg) both at 10 ppm concentration and 5ppm manganese sulphate (37.7 mg) also resulted in decreased growth (Table 6b). Dense to thin appressed type of growth was observed in all the treatments.

It was also found that addition of 2 ppm ferrous sulphate resulted in significantly maximum growth (117.3 mg) of *Flammulina velutipes* followed by

Table 6a: Mycelial growth of different cultures of medicinal fungi on various trace elements

Trace element	*Pleurotus ostreatus*				*Pleurotus eryngii*				*Ganoderma lucidum*			
	Av. Growth (mg)****			Type of growth	Av. growth (mg)			Type of growth	Av. growth (mg)			Type of growth
	Conc. (ppm)*				Conc. (ppm)				Conc. (ppm)			
	2	5	10		2	5	10		2	5	10	
Boric acid	86.5	97.7	82.6	Aerial ,thin, strandy	81.1	73.6	76.3	Fluffy	124.4	118.6	114.5	Thick, strandy
Ammonium molybdate	88.6	90.5	85.4	Aerial, slightly dense, strandy	84.1	76.9	74.3	Fluffy, slightly appressed	117.5	127.2	111.4	Thick, appressed
Ferrous sulphate	114.3	99.4	91.0	Aerial, dense, strandy	99.4	90.8	79.6	Fluffy, dense strandy	150.3	131.2	116.5	Thick, strandy
Zinc sulphate	92.1	101.3	93.0	Aerial, dense, strandy	93.6	86.7	81.8	Dense, fluffy	127.2	138.5	111.4	Slightly appressed
Manganese sulphate	110.2	94.5	90.0	Aerial, strandy	88.4	84.9	81.4	Fluffy	162.4	138.1	112.2	Thin, strandy
Control (p)**	87.71			Aerial, dense strandy	82.4			Fluffy	122.3			Thick, strandy
Control (UP)***	75.63			Dense, strandy	76.1			Thin, fluffy	117.5			Thick, strandy
Mean	92.79				83.03				125.97			
$CD_{0.05}$	1.59				1.64				1.54			
SE	0.78				0.80				0.76			

***Concentration (ppm) ** Control with purified medium *** Control without purified medium ****Average mycelial weight (mg) after 21 days of incubation**

Table 6b: Mycelial growth of different cultures of medicinal fungi on various trace elements

Trace element	*Auricularia polytricha*				*Flammulina velutipes*				*Lentinula edodes*			
	Av. growth (mg)**** Conc. (ppm)*			Type of growth	Av. growth (mg) Conc. (ppm)			Type of growth	Av. growth (mg) Conc. (ppm)			Type of growth
	2	5	10		2	5	10		2	5	10	
Boric acid	30.0	40.7	34.3	Slightly appressed	56.2	88.6	73.0	Slightly dense, cottony	95.9	91.3	86.6	Silky, strandy
Ammonium molybdate	47.0	58.0	41.3	Thin, appressed	89.0	97.5	109.1	Slightly dense, cottony	90.5	87.5	85.4	Thin, silky, strandy
Ferrous sulphate	52.3	45.0	38.0	Appressed	117.3	89.9	87.57	Dense, strandy	118.9	97.7	88.6	Slightly dense, strandy
Zinc sulphate	48.0	41.0	35.3	Dense, appressed	97.8	104.0	94.0	Thin, cottony	101.6	93.1	84.7	Silky, strandy
Manganese sulphate	43.0	37.7	33.3	Thin, appressed	112.6	107.0	91.1	Slightly dense, cottony	130.0	109.7	99.0	Silky, strandy
Control (p) **	40.7			Appressed	88.8			Slightly dense, cottony	91.0			Thick, strandy
Control (up)***	33.3			Thin, appressed	71.7			Thin, cottony	79.9			Strandy
Mean	41.47				93.2				95.9			
$CD_{0.05}$	1.59				1.40				1.66			
SE	0.78				2.84				0.82			

***Concentration (ppm) ** Control with purified medium *** Control without purified medium ****Average mycelial weight (mg) after 21 days of incubation**

2 ppm manganese sulphate (112.6 mg), 10 ppm ammonium molybdate (109.1 mg) and 5 ppm manganese sulphate (107 mg). Insignificantly minimum growth was recorded with the addition of 2 ppm boric acid (56.2 mg).Treatment with unpurified control was also recorded to reduce the mycelial growth (71.7 mg) in comparison to control (88.8 mg) (Table 6b). Thin to dense cottony type growth was noticed in all the treatments.

Statistical analysis of the data given in Table 6b revealed that significantly maximum average dry mycelial weight (130.0 mg) of *Lentinula edodes* was recorded with 2 ppm manganese sulphate followed by 2 ppm ferrous sulphate (118.9 mg) and 5 ppm manganese sulphate (109.7 mg). Significantly minimum vegetative growth was recorded in unpurified control treatment (79.9 mg) followed by 10 ppm ammonium molybdate (85.4 mg) and 10 ppm boric acid (86.6 mg) that were statistically at par with one another. The type of growth varied from dense to thin strandy and silky.

4.1.7 Effect of different vitamin sources

Six vitamins were tried for their effect on the vegetative growth of test fungi. Average dry weight of the mycelium produced by each test fungus was recorded and presented in Table 7a and Table 7b, respectively.

As seen from the data given in the Table 7a, 0.05 ppm thiamine supported insignificantly maximum (74.5 mg) average dry mycelial weight of *A. polytricha* followed by 0.025 ppm concentration of same vitamin (67.5 mg). The data indicated that thiamine at all three tested concentrations supported good growth of mycelium of the fungus. Although, biotin at 0.025 ppm concentration supported third best vegetative growth (65.5 mg) amongst all the treatments followed by 0.05 ppm concentration of same vitamin (59.5 mg). However, 1 ppm niacin supported significantly minimum growth (31.9 mg) followed by 1 ppm pentothenic acid (34.0 mg). Comparing with the growth in control (42.5 mg), niacin at 0.025 ppm and 1 ppm; pentothenic acid at all the concentrations tried and riboflavin at 0.025 ppm and 1 ppm resulted in decreased growth, indicating that these vitamin concentrations exerted inhibitory effect on the growth of the

fungus. The type of growth recorded varied from dense to thin appressed. However, in case of treatment containing folic acid, the growth was slightly strandy.

Among all the vitamins tried, 0.025 ppm thiamine supported significantly maximum growth (120.2 mg) of *L. edodes* (Table 7a) followed by 0.05 ppm biotin (114.1 mg), 0.025 ppm niacin (105.6 mg) and 0.05 ppm thiamine (105.4 mg) that were statistically at par with one another. Significantly reduced growth was recorded with the addition of higher concentrations of folic acid (0.05 and 1 ppm) and pentothenic acid (1 ppm). All these treatments were statistically at par with one another. The Table clearly indicates that in comparison to control, folic acid at all three tested concentrations resulted in reduced growth. Silky and strandy growth was noticed in all treatments.

Again, Table 7a shows that significantly maximum growth (108.5 mg) of *Flammulina velutipes* was observed with 0.025 ppm thiamine followed by 0.025 ppm biotin (100 mg) and again 0.05 ppm thiamine (98.2 mg). One ppm concentration of pentothenic acid supported significantly minimum growth (79.4 mg). As compared to control (90.4 mg), decreased growth was recorded with 0.025 ppm pentothenic acid (84.9 mg), 1ppm concentration each of biotin (85.3 mg), folic acid (84.4 mg) and riboflavin (83.7 mg). Cottony type growth of the fungus was observed in all the treatments except in folic acid where slightly appressed growth was noticed.

It is evident from the data presented in Table 7b that the vegetative growth of *Pleurotus ostreatus* was best favoured by the addition of 0.05 ppm thiamine (114.3 mg) followed by 0.025 ppm biotin (105.8 mg) and 0.05 ppm niacin (103.8 mg). Addition of pentothenic acid at all concentrations resulted in decreased growth with significantly minimum growth (67.6 mg) at 1ppm. Similarly, addition of 0.05 and 1ppm of folic acid and 0.05 and 1ppm of riboflavin also resulted in reduced growth in comparison to control (80.9 mg). Strandy and slightly dense type growth of fungi was observed in these treatments.

Table 7a: Effect of vitamins on the mycelial growth of different test fungi

Vitamin source	*Auricularia polytricha*				*Flammulina velutipes*				*Lentinula edodes*			
	Av. growth (mg)* Conc. (ppm)**			Type of growth	Av. growth (mg) Conc. (ppm)			Type of growth	Av. growth (mg) Conc. (ppm)			Type of growth
	0.025	0.05	1		0.025	0.05	1		0.025	0.05	1	
Niacin	35.5	47.5	31.9	Thin, appressed	91.0	96.6	88.3	Thin, cottony	105.6	93.8	91.4	Strandy
Thiamine	67.5	74.5	57.0	Dense, appressed	108.5	98.2	87.0	Dense, cottony	120.2	105.4	97.1	Dense, silky, strandy
Pentothenic acid	41.5	36.5	34.0	Appressed	84.9	91.3	79.4	Less dense, cottony	94.5	88.7	85.9	Less dense, strandy
Biotin	65.5	59.5	52.5	Appressed	100.0	91.4	85.3	Dense, cottony	103.6	114.1	93.7	Silky, strandy
Folic acid	56.5	51.0	45.5	Appressed, slightly strand	90.6	88.2	84.4	Thin, slightly appressed	89.8	86.5	85.0	Silky, strandy
Riboflavin	37.5	52.5	41.0	Dense ,appressed	88.4	92.8	83.7	Thin, cottony	98.4	93.6	88.5	Strandy, slightly dense
Control	42.5			Appressed	90.4			Dense, cottony	96.3			Silky, strandy
Mean	48.77				90.37				96.29			
$CD_{0.05}$	1.44				1.45				1.43			
SE	0.71				0.72				0.70			

***Average dry mycelial weight (mg) after 21 days of incubation **Concentration (ppm)**

Table 7b: Effect of vitamins on the mycelial growth of different test fungi

Vitamin source	*Pleurotus ostreatus*				*Pleurotus eryngii*				*Ganoderma lucidum*			
	Av. growth (mg)* Conc. (ppm)**			Type of growth	Av. growth (mg) Conc. (ppm)			Type of growth	Av. growth (mg) Conc. (ppm)			Type of growth
	0.025	0.05	1		0.025	0.05	1		0.025	0.05	1	
Niacin	90.8	103.8	88.1	Aerial, Thin, strandy	90.0	100.1	82.8	Fluffy	124.3	118.6	114.5	Dense, slightly appressed
Thiamine	101.5	114.3	89.3	Aerial, dense strandy	95.5	126.4	91.2	Dense, fluffy	157.6	135.7	128.3	Thick, strandy
Pentothenic acid	79.3	72.0	67.6	Thin, strandy	83.0	79.1	76.9	Thin, fluffy	132.9	127.7	118.4	Less dense, strandy
Biotin	105.8	93.4	79.4	Strandy	109.2	94.2	88.6	Fluffy	137.0	144.8	129.7	Less dense, appressed
Folic acid	82.5	75.8	70.8	Dense, strandy	78.6	85.5	76.5	Fluffy	131.4	125.1	119.9	Thick, strandy
Riboflavin	84.5	77.9	72.8	Strandy	93.5	87.1	81.9	Thin, fluffy	141.4	135.0	128.3	Thick ,strandy
Control	80.9			Aerial, strandy	85.7			Fluffy	125.0			Thick, strandy
Mean	85.8				89.8				130.3			
$CD_{0.05}$	1.56				1.38				1.70			
SE	0.77				0.68				0.84			

***Average dry mycelial weight (mg) after 21 days of incubation** ****Concentration (ppm)**

The same Table also revealed that maximum average dry mycelial weight (126.4 mg) of *Pleurotus eryngii* was recorded with 0.05 ppm thiamine followed by 0.025 ppm biotin (109.2 mg) and 0.05 ppm niacin (100.1 mg). Insignificantly minimum growth was recorded with 1ppm folic acid (76.5 mg) followed by 1ppm pentothenic acid (76.9 mg) that were significantly at par with each other. It is evident that pentothenic acid at all three tested concentrations i.e. 0.025, 0.05 and 1 ppm supporting 83.0, 79.1, 76.9 mg of growth, respectively resulted in reduced growth when compared with control (85.7 mg). However, decreased growth was also recorded with 1ppm niacin (82.8 mg), 0.025 ppm folic acid (78.6 mg) and 1ppm riboflavin (81.9 mg). Dense to slightly dense and fluffy growth was recorded in all the treatments.

From the statistical analysis, it is obvious that highest mycelial growth (157.6 mg) of *Ganoderma lucidum* occurred with 0.025 ppm thiamine followed by 0.05 ppm biotin (144.8 mg) and 0.025 ppm riboflavin (141.4 mg). In general, the addition of all the vitamins resulted increased growth except for niacin at 0.025 ppm (124.3 mg), 0.05 ppm (118.6 mg) and 1 ppm (114.5 mg),1 ppm pentothenic acid (118.4 mg) and 1 ppm folic acid (119.9 mg) in comparison to control. In general, strandy growth was noticed but in biotin and niacin the growth was slightly appressed.

4.1.8 Effect of different growth regulators

In the present study, six different growth regulators at three varying concentrations were tried. The data obtained are presented in Table 8a and 8b.

It is obvious from the statistically analysis that highest mycelial growth (136.0 mg) of *P. ostreatus* occurred by the addition of 25 ppm gibberellic acid followed by 50 ppm kinetin (124.2 mg) and again 10 ppm gibberellic acid (119.9 3 mg). It is evident that all the growth regulators increased the vegetative growth of fungus in comparison to control (89.25 mg) except 10 ppm NAA and 50ppm IBA that had no significant effect on the growth and were statistically at par with the control. Twenty five ppm NAA and 50 ppm BA had very little positive effect on growth and were significantly at par with the growth in control (Table 8a).

Aerial and dense to thin strandy mycelial growth was recorded in all the treatments.

Again, 50 ppm gibberellic acid resulted in significantly maximum mycelial growth (144.4 mg) of *P. eryngii* which was statistically different from all other treatments. This was followed by 25 ppm kinetin (136.3 mg) as is evident from Table 8a. It is noteworthy here that gibberellic acid and kinetin at all the three concentrations tried resulted in good mycelial yield. However, significantly minimum growth (82.02 mg) was recorded with 50 ppm BA followed by 50 ppm NAA (86.49 mg). Inhibitory effect of higher concentrations of BA, IAA and NAA was recorded when data obtained was compared with the growth in control. In all the treatments dense to slightly dense fluffy growth was noticed.

It is apparent from Table 8a that all the growth regulators had pronounced influence on the growth of *G. lucidum* except, 50 ppm IAA in comparison to growth in control. Significantly maximum growth (178 mg) was obtained with the addition of 50 ppm gibberellic acid followed by 25 ppm kinetin (168.0 mg). It is obvious from the table that gibberellic acid and kinetin at all the three tested concentrations resulted in significantly very good growth in comparison to control. The growth in all these treatments was significantly different from one another. Significantly minimum growth (129.6 mg) was recorded with 50 ppm IAA. Poor growth was also recorded with 50 ppm NAA (133.3 mg), 25 ppm IAA (134.5 mg), 50 ppm IBA (135.1 mg) which were statistically at par with control (132.7 mg). In general, thick to thin strandy growth was noticed in all the treatments.

Gibberellic acid at 50 ppm concentration resulted in significantly maximum growth (68.0 mg) of *A. polytricha* followed by 50 ppm kinetin (62.0 mg) and again 25 ppm gibberellic acid (60.0 mg) where growth was statistically different from one another. Significantly minimum growth (34.3 mg) was recorded with the treatment containing 50 ppm NAA followed by 50 ppm IAA (37.3 mg). Growth with the addition of 10 ppm IAA (41.0 mg) and 25 ppm NAA

(40.7 mg) was statistically at par with that in the control (40.3 mg). The results in the Table 8b clearly indicated that all the growth regulators were effective in increasing the growth of *A. polytricha* except higher concentrations of IAA and NAA (Table 8b). Dense to thin, appressed growth was supported by all the treatments.

Table 8b indicated that for *F. velutipes*, addition of 50 ppm gibberellic acid resulted in significantly maximum average dry mycelial growth (133.8 mg) followed by 50 ppm kinetin (120 mg) and 50 ppm IBA (118.5 mg). When compared with the growth in control (88.63 mg), 10 ppm each of IAA (85.3 mg) and NAA (88.1 mg) were found to exert slightly inhibitory effect on the growth, though these treatments were found to be statistically at par with the control. Growth with the addition of 10 and 25 ppm BA, and 25 ppm IAA (90.9 mg) was also statistically at par with control. Dense to thin, cottony mycelial growth was recorded, in general but NAA supported slightly strandy growth.

Again, the vegetative growth of *L. edodes* was found to be best favoured with the addition of 50 ppm gibberellic acid (137.2 mg) followed by 25 ppm kinetin (127.8 mg) and again 25 ppm gibberellic acid (125.1 mg) that were statistically different from one another. Insignificantly minimum growth (82.1 mg) was observed with the addition of 50 ppm IAA followed by 50 ppm BA (84.4 mg) and control (89.3 mg) that was statistically at par with the growth in 25 ppm IAA (89.5 mg). The study indicated that the growth regulators play a significant role in growth of the fungi (Table 8b).All the treatments supported strandy and silky type growth of the fungus

4.2 EVALUATION OF SUBSTRATES FOR SPAWN PRODUCTION

In order to cultivate any edible fungus, preparation of spawn is a must and in the present study, eight different substrates singly or in combinations were tried to see their suitability for preparation of spawn. The method of sterilization of substrates and preparation of spawn was the same as described in materials and methods.

Table 8a: Effect of growth regulators on the mycelial growth of different test fungi

Growth regulator	*Pleurotus ostreatus*				*Pleurotus eryngii*				*Ganoderma lucidum*			
	Av. growth (mg)* Conc. (ppm)**			Type of growth	Av. growth (mg) Conc. (ppm)			Type of growth	Av. growth (mg) Conc. (ppm)			Type of growth
	10	25	50		10	25	50		10	25	50	
IAA	107.47	114.20	101.0	Aerial, thin, strandy	110.30	98.14	89.06	Fluffy	139.40	134.50	129.60	Dense, strandy
IBA	104.30	98.45	88.60	Aerial, strandy	99.57	116.0	95.01	Thin, fluffy	159.20	144.0	135.10	Dense, strandy
NAA	87.41	89.42	95.10	Thin, strandy	97.69	91.71	86.49	Fluffy	149.20	141.0	133.30	Slightly appressed, strandy
GA	119.93	136.0	106.90	Dense, strandy	118.40	124.7	144.40	Dense, fluffy	155.40	164.60	178.0	Less dense, strandy
BA	101.92	95.40	89.80	Dense, strandy	92.46	88.40	82.02	Fluffy	142.40	150.10	137.60	Less dense, strandy
Kinetin	106.45	112.20	124.20	Less dense, strandy	105.90	136.30	110.60	Less dense, fluffy	154.30	168.0	148.30	Thick, strandy
Control	89.25			Aerial, dense, strandy	90.29			Fluffy	132.70			Thick, strandy
Mean	103.59				104.08				147.19			
$CD_{0.05}$	1.88				1.65				1.46			
SE	0.93				0.82				0.72			

***Average dry mycelial weight (mg) after 21 days of incubation **Concentration (ppm)**

Table 8b: Effect of growth regulators on the mycelial growth of different test fungi

Growth regulator	*Auricularia polytricha*				*Flammulina velutipes*				*Lentinula edodes*			
	Av. growth (mg)*			Type of growth	Av. growth (mg)			Type of growth	Av. growth (mg)			Type of growth
	Conc. (ppm)**				Conc. (ppm)				Conc. (ppm)			
	10	25	50		10	25	50		10	25	50	
IAA	41.0	46.0	37.3	Thin, appressed	85.3	90.9	93.6	Slightly dense, cottony	93.5	89.5	82.1	Silky, strandy
IBA	57.3	51.0	44.7	Appressed	95.5	107.0	118.5	Thin, cottony	117.8	101.8	96.5	Silky, strandy
NAA	52.2	40.7	34.3	Appressed , strandy	88.1	95.0	99.8	Cottony, slightly strandy	105.9	111.6	102.7	Thin, strandy
GA	49.0	60.0	68.0	Appressed	96.6	109.7	133.8	Dense, cottony	121.5	125.1	137.2	Thin, silky, strandy
BA	54.6	46.3	42.3	Appressed	89.7	91.6	97.6	Dense, cottony	101.3	92.2	84.4	Strandy
Kinetin	48.3	55.19	62.0	Thin, appressed	96.4	103.0	120.0	Less dense, cottony	114.8	127.8	108.3	Dense, strandy
Control	40.3			Appressed	88.63			Dense, cottony	89.3			Silky, slightly strandy
Mean	48.98				100.04				105.37			
$CD_{0.05}$	1.50				1.42				1.67			
SE	0.72				0.70				0.83			

***Average dry mycelial dry weight (mg) after 21 days of incubation **Concentration (ppm)**

It is clear from results given in Table 9a and 9b that *Pleurotus ostreatus, Pleurotus eyrngii* and *Ganoderma lucidum* took significantly less time for complete colonization of substrate therefore, readings regarding linear growth in these cases were taken at shorter intervals. Table 9a indicates that irrespective of interval, treatment containing combination of wheat grains and bajra grains supported significantly maximum (58.8 mm) linear downward mycelial growth of *L. edodes* followed by bajra grains alone (54.8 mm) and wheat grains (51.3 mm). Wheat straw favoured significantly minimum growth (28.8 mm) followed by sawdust alone (40.0 mm). However, interaction studies of substrate and interval showed that maximum growth (97.3 mm) was attained on treatment with wheat and bajra grains followed by bajra grains (91.7 mm) after 18 days of incubation.

Maximum downward linear growth (63.6 mm) of *F. velutipes* was recorded in substratum containing combination of wheat and bajra grains followed by bajra grains alone (59.7 mm). Significantly minimum growth (38.1 mm) was recorded in treatment containing wheat grains and gur, again followed by wheat straw (41.2 mm). Insignificantly maximum growth (79.3 mm) was recorded after 18 days of incubation, irrespective of the substratum. The significance of interaction between different substrates and intervals indicate that there was significant difference in the linear growth on various substrates at different intervals studied. Significantly maximum growth (94.0 mm) was recorded on treatment containing mixture of wheat and bajra grains after 18 days of incubation followed by bajra grains (90.7 mm) and wheat grains (87.3 mm) alone after the same incubation period. Statistically minimum growth (62.7 mm) was recorded on treatment with wheat grains + gur mixture after 18 days of incubation (Table 9a).

Irrespective of intervals, maximum mycelial growth (75 mm) of *P.eryngii* was observed with treatment containing mixture of wheat and bajra grains followed by bajra grains (71.9 mm) and wheat grains (69.0 mm), whereas minimum growth (35.2 mm) was recorded on mixture of wheat grains and gur (Table 9a). Similarly, irrespective of the substrate used significantly maximum

3(a) **Mycelial growth of *Lentinula edodes* on sawdust + bajra grains, sawdust + wheat grains, wheat straw, sawdust, wheat grains + gur, bajra grains, wheat grains + bajra grains and wheat grains (from left to right)**

3(b) **Mycelial growth of *Flammulina veluipes* on wheat grains + sawdust, wheat grains + gur, bajra grains, wheat grains, wheat + bajra grains, sawdust+ bajra grains, wheat straw, sawdust (from left to right)**

3(c) **Mycelial growth of *Pleurotus eryngii* on wheat grains+ bajra grains, saw dust + wheat grains, wheat straw, saw dust, bajra grains, bajra grains + saw dust, wheat grains, wheat grains + gur**

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growth (89.8 mm) was recorded after 9 days of incubation. There was significant difference in growth at various intervals studied. The interaction studies between treatments and intervals indicate that maximum growth (110 mm) was recorded after 9 days of incubation on substrates like wheat grains, bajra grains, mixture of wheat and bajra grains and the wheat straw. The difference among these treatments was insignificant.

Pleurotus ostreatus exhibited significantly maximum vegetative growth (72.0 mm) on bajra grains followed by combination of wheat and bajra grains (69.4 mm) and wheat grains (68.0 mm). There was significant difference in growth among these treatments. Irrespective of substrate tried, maximum downward linear growth (89.2 mm) occurred after 9 days of incubation. Similarly interaction studies between treatment and intervals revealed that there was significant difference in linear downward growth on different substrates after various intervals studied. On an average, maximum linear growth (110 mm) was recorded after 9 days on substrates such as wheat grains, bajra grains, wheat straw and combination of wheat and bajra grains which were statistically at par with one another (Table 9b).

It is obvious from Table 9b that *A. polytricha* could also grow and colonize different substrates with considerable variation. However, significantly maximum linear growth (43.0 mm) was recorded on bajra grains followed by combination of wheat and bajra grains (41.2 mm). Significantly minimum growth was observed on wheat grains and gur combination (22.8 mm) followed by sawdust (27.1 mm). There was significant difference between growth in all these treatments. Irrespective of the substrate used, maximum growth (56.9 mm) was recorded after 30 days of incubation. Interaction studies of substrates and intervals showed that bajra grains supported maximum growth (70.0 mm) after 30 days of incubation followed by wheat and bajra grain combination (69.3 mm) which were statistically at par with each other. Significantly minimum growth (40.0 mm) was observed on wheat grains and gur combination after 30 days of incubation.

Table 9a: Linear mycelial growth of different test fungi on various substrates for spawn production

Substrates	*Lentinula edodes*					*Flammulina velutipes*					*Pleurotus eryngii*				
	Linear growth (mm)				Type of growth	Linear growth (mm)				Type of growth	Linear growth (mm)				Type of growth
	6th day	12th day	18th day	GM		6th day	12th day	18th day	GM		3rd day	6th day	9th day	GM	
Wheat grains	15.8	50.0	88.3	51.3	Fine strandy	30.3	58.0	87.3	58.6	Strandy, less dense	26.3	70.6	110.0	69.0	Fluffy, strandy
Bajra grains	18.3	54.3	91.7	54.8	Fine, strandy	27.7	60.7	90.7	59.7	Strandy, less dense	28.7	77.0	110.0	71.9	Fluffy, strandy
Wheat +Bajra grains	20.7	58.3	97.3	58.8	Silky, strandy	33.0	63.7	94.0	63.6	Dense, strandy	31.3	83.7	110.0	75.0	Fluffy, strandy
Wheat grains + gur	14.0	40.7	73.7	42.8	Silky, strandy	16.0	35.7	62.7	38.1	Thin, strandy	12.3	33.7	59.7	35.2	Thin fluffy
Sawdust	11.0	38.0	71.0	40.0	Strandy	22.0	41.3	72.7	45.3	Thin, strandy	14.3	39.3	68.0	40.6	Less dense, fluffy
Sawdust + Wheat grains	13.3	42.7	78.7	44.9	Dense, strandy	26.0	51.0	80.7	52.6	Dense, strandy	18.0	46.0	74.0	46.0	Fluffy, slightly strandy
Sawdust + Bajra grains	20.7	40.7	76.0	47.9	Dense, strandy	24.7	47.7	77.7	50.0	Dense, strandy	20.0	47.7	76.7	48.1	Fluffy, slightly strandy
Wheat straw	9.33	27.3	49.7	28.8	Thin, strandy	16.3	38.3	69.0	41.2	Slightly dense, strandy	22.7	66.0	110.0	66.2	Dense, fluffy
GM	15.4	44.0	78.3			24.5	49.5	79.3			21.7	58.0	89.8		
Effect	$CD_{0.05}$		SE			$CD_{0.05}$		SE			$CD_{0.05}$		SE		
Treatment (T)	0.84		0.42			0.83		0.41			0.85		0.42		
Interval (I)	0.52		0.26			0.51		0.25			0.52		0.26		
T*I	1.47		0.73			1.43		0.71			1.47		0.73		

***Grand mean**

4(b) **Mycelial growth of *Ganoderma lucidum* on wheat grains+ sawdust, wheat grains + bajra grains, wheat grains, sawdust + bajra grains, wheat straw, wheat grains + gur, wheat grains + gur, bajra grains, sawdust (from left to right)**

4(c) **Mycelial growth of *Pleurotus ostreatus* on wheat grains+ bajra grains, saw dust + wheat grains, wheat straw, saw dust, bajra grains, bajra grains + saw dust, wheat grains, wheat grains + gur (from left to right)**

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Table 9b: Linear mycelial growth of different test fungi on various substrates for spawn production

Substrate	*Auricularia polytricha*					*Ganoderma lucidum*					*Pleurotus ostreatus*				
	Linear growth (mm)				Type of growth	Linear growth (mm)				Type of growth	Linear growth (mm)				Type of growth
	10th day	20th day	30th day	GM*		4th day	8th day	12th day	GM		3rd day	6th day	9th day	GM	
Wheat grains	16.7	36.7	64.0	39.1	Thin, strand	38.3	69.0	110.0	72.4	Dense, strandy	26.7	67.3	110.0	68.0	Strandy, slightly fluffy
Bajra grains	20.3	38.7	70.0	43.0	Thin, strand	35.3	66.0	110.0	70.4	Dense, strandy	29.0	77.0	110.0	72.0	Strandy, slightly fluffy
Wheat + Bajra grains	17.7	36.7	69.3	41.2	Thin, strand	36.0	68.3	110.0	71.4	Dense, strandy	25.3	73.0	110.0	69.4	Dense, strandy
Wheat grains + gur	8.0	20.0	40.0	22.8	Transparent, strand	29.0	60.0	85.0	58.0	Thin, strandy	13.0	37.3	58.3	36.2	Less dense, strandy
Sawdust	11.0	24.3	46.0	27.1	Very thin, strand	26.7	54.7	80.3	53.9	Thin, strandy	15.7	43.7	64.3	41.2	Dense, strandy
Sawdust + Wheat grains	12.7	26.0	53.7	30.8	Transparent, strand	33.7	63.3	91.7	62.9	Dense, strandy	18.0	56.7	73.0	49..2	Silky, strandy
Sawdust + Bajra grains	11.7	29.7	55.0	32.1	Transparent, strand	32.3	62.0	87.7	60.7	Dense, strandy	20.0	59.0	78.0	52.3	Silky, strandy
Wheat straw	14.0	31.7	57.0	34.2	Thin, strand	29.0	47.3	76.0	50.8	Less dense, strandy	22.7	64.7	110.0	65.8	Dense, strandy
GM	14.0	30.5	56.9			32.5	61.3	93.8			21.3	59.3	89.2		
Effect	$CD_{0.05}$		SE			$CD_{0.05}$		SE			$CD_{0.05}$		SE		
Treatment (T)	1.17		0.58			0.99		0.49			0.76		0.38		
Interval (I)	072		0.35			0.60		0.30			0.47		0.23		
T*I	2.71		1.01			1.71		0.85			1.33		0.66		

***Grand mean**

It is clear from table 9b that growth of *G. lucidum* was best favoured by wheat grains (72.4 mm) followed by mixture of wheat and bajra grains (70.4 mm). There was significant difference in growth on these treatments. Significantly minimum growth (50.8 mm) was recorded on wheat straw followed by sawdust (53.9 mm). Significantly maximum (93.8 mm) vegetative growth was recorded after 12 days of incubation, irrespective of the substrates used. The significance of interaction between substrate and interval revealed that significantly maximum (110 mm) linear growth was observed on wheat grains, bajra grains and combination of wheat and bajra grains after 12 days of incubation. Growth on these treatments was statistically at par with one another. However, minimum growth (76.0 mm) was observed on wheat straw after twelve days of incubation which was significantly different from all other treatments.

It can be inferred from the Table 9a and 9b that type of growth of all the test fungi on all different type of substrates varied from dense to thin strandy. However, in case of *P. eryngii*, the growth was fluffy to slightly strandy. In *A. polytricha* though the growth was strandy but it was thin and transparent in almost all the treatments.

It can also be inferred from the study that sawdust and mixture of wheat grains and gur did not support significant growth in case of all the edible fungi studied. However, wheat grains, bajra grains and mixture of wheat and bajra grains were found to support significantly best growth among all the substrates used. Therefore, later substrates can be successfully used for spawn production of the edible fungi.

4.3 SELECTION OF SUBSTRATES FOR FRUIT BODY PRODUCTION

To find out the most suitable and economical substrate for cultivation of different edible mushrooms under study, different forest and agricultural wastes were screened for the yield potential, with or without supplementation as mentioned in materials and methods. The data regarding time taken for spawn run, initiation of pinning of the test fungi on various substrates and yield data of

fruit bodies are presented in Table 10a.

The perusal of data (Table 10a) indicate that significantly minimum time (15days) for substrate colonization by mycelium of *Pleurotus eryngii* was recorded on wheat straw and wheat straw + corncobs followed by corncobs and pine needles + wheat straw which were statistically at par with one another. Among these, maximum time for complete colonization was taken on sawdust (25 days) followed by pasteurized compost (24 days) and pine needles + Robinia woodchips + sawdust (23.33 days). The difference in these treatments was statistically insignificant. After the complete colonization of the substrate, blocks were kept for fruiting. It is obvious from the table that minimum time (15 days) for initiation of pinning was taken by wheat straw + corncobs followed by corncobs (16 days) and this difference in time taken was statistically at par .On the contrary maximum time (29 days) for initiation of pinning was observed on pine needles + Robinia woodchips + sawdust that was statistically at par with sawdust alone. Similarly, yield data in Table 10a revealed that wheat straw alone supported maximum yield (571.33 g) and number of sporophore (61) of *P. eryngii* followed by corncobs (501.0 g) and wheat straw + corncobs (490.67 g).There was significant difference in yield on these substrates. Pine needles supported significantly lower yield (77.33 g) followed by mixture of pine needles and wood chips (156.0 g).

The data regarding average spawn run of *Pleurotus ostreatus* on various substrates (Table 10 a) showed that wheat straw when used individually supported fast growth and colonized the whole substrate within 10 days of inoculation followed by mixture of wheat straw + sawdust (11.33 days); wheat straw + corncobs (12 days); mixture of pine needles + Robinia wood chips + sawdust (12.33 days); and corncobs alone (13 days) where growth was significantly at par with one another. However, the fungus showed minimum growth on pasteurized compost (20 days) followed by mixture of sawdust + Eucalyptus wood chips supplemented with 5 per cent rice bran (18.33 days); and mixture of pine needles + wheat straw (18.33 days)that were statistically at par with one another.

Similarly, data on time taken for pinning (Table 10a) of *P.ostreatus* revealed that significantly minimum time for initiation of pinning (9 days) was observed on wheat straw followed by corncobs (10 days) and mixture of sawdust and Alnus wood chips supplemented with 5 per cent rice bran (12 days). Maximum time for initiation of pinning (19.7 days) was taken on substrate containing only the sawdust followed by pine needles (19 days) which were statistically at par with each other.

It is clear from the data given regarding yield (Table 10a) of *P. ostreatus* on different substrates that treatment containing mixture of sawdust and Robinia wood chips supplemented with 5 per cent rice bran supported maximum yield (511 g) and number of sporophores (60) followed by sawdust + Eucalyptus wood chips+ 5 per cent rice bran yielding 501.33 g with 55 number of sporophores and sawdust + Alnus wood chips + 5 per cent rice bran yielding 491.33 g of sporophores. Significantly much lower yield was recorded on pine needles (87.33 g) followed by pasteurized compost (108.67 g). Moderately good yields were obtained with wheat straw + corncobs (462 g) followed by wheat straw (478.67 g) and corncobs (421.33 g) when used alone.

It is obvious from the perusal of data in Table 10a that mycelium of *F. velutipes* could permeate all the substrates with considerable variation. However, mixture of pine needles + Robinia wood chips + sawdust was found to be the most suitable substrate as far as the substrate colonization was concerned. The fungus took 21 days for spawn run followed by wheat straw alone (24 days); mixture of sawdust, Alnus wood chips and 5 per cent rice bran (24 days); sawdust + Robinia wood chips + 5 per cent rice bran (24.33 days); and wheat straw + sawdust (24.67 days). However, significantly maximum time for spawn run (31 days) was found in case of pine needles alone and wheat straw + corncobs (31 days) that were statistically at par with each other. Statistical data on initiation of pinning revealed that pasteurized compost failed to produce fruiting bodies. Significantly minimum time (17 days) for primordial formation was noticed

5(a) Primordial formation of *Pleurotus ostreatus* on fully colonized substrate

5(b) Immature fruiting bodies of *Pleurotus ostreatus*

5(c) Mature fruiting bodies of *Pleurotus ostreatus*

5(d) Primordial formation of *Pleurotus eryngii* on fully colonized substrate bags

5(e) Immature fruiting bodies of *Pleurotus eryngii*

5(f) Mature fruiting bodies of *Pleurotus eryngii*

Plate -5

Table 10a: Effect of various substrates on the productivity of different edible fungi

Substrate	*Pleurotus eryngii*				*Pleurotus ostreatus*				*Flammulina velutipes*			
	T. S. (days)	T.P. (days)	No. of fruiting bodies	Yield (g)	T.S. (days)	T.P. (days)	No. of fruiting bodies	Yield (g)	**T. S. (days)**	**T.P. (days)**	**No. of flushes**	**Yield (g)**
Wheat straw	14.67	20.0	60.67	571.33	9.67	8.67	46.67	478.67	23.67	17.0	2	32.67
Corncobs	16.67	15.67	58.0	501	13	10.3	40.67	421.33	28.33	32.0	2	39.49
Pine needles	22.0	23.0	12.0	77.33	17.67	19.0	14.0	87.33	31.0	26.0	2	19.89
P. compost	24.0	25	18.0	103	19.67	17.3	18.0	108.67	29.0	0	0	0
Sawdust	25.0	28.7	30.0	333.33	16.67	19.7	29.0	293.67	25.67	24.0	1	18.0
Sd+rb+Rwc	21.67	26.0	41.0	457.33	15.67	13.0	59.67	511.0	24.33	21.67	2	50.68
Sd+rb+Awc	19.0	27.3	45.67	466.33	17.69	11.7	51.33	491.33	24.0	21.0	2	49.0
Sd+rb+Ewc	21.0	22.0	40.33	449.33	18.33	12.3	55.0	501.33	26.0	22.33	2	54.0
Pine needles+ Robinia Woodchips	21.0	27.0	22.0	156.0	16.33	16.7	23.0	174.0	29.0	33.0	2	32.3
Pn +Rwc+ Sd	23.33	29.3	27.0	176.0	12.33	13.0	25.67	261.67	21.33	26.33	1	34.14
Pine needles + Wheat straw	17.0	23.7	30.0	189.67	18.33	15.7	32.67	362.0	27.33	25.33	1	24.49
Wheat straw + Sawdust	20.0	27.0	37.0	441	11.33	13.7	35.67	381.0	24.67	20.0	1	24.84
Wheat straw + corncobs	15.0	14.67	52.67	490.67	12.0	12.3	45.67	462.0	31.0	28.33	2	36.49
$CD_{0.05}$	1.50	1.47	3.20	2.86	1.66	1.63	1.90	1.72	1.70	1.78		3.38
SE	0.73	0.72	1.56	1.41	0.81	0.80	0.93	0.84	0.83	0.87		1.65

*T.S.: **Time taken for complete spawn run (days), T.P.: Time taken for initiation of pinning (days), P. Compost: Pasteurized Compost, Sd+rb+Rwc: Sawdust + rice bran + Robinia wood chips, Sd+rb+Awc: Sawdust + rice bran + Alnus wood chips, Sd+rb+Ewc: Sawdust + rice bran + Eucalyptus wood chips, Pn +Rwc+ Sd: Pine needles + Robinia wood chips + sawdust**

on wheat straw and maximum time (32 days) was taken on corncobs alone and pine needles + Robinia wood chips (33 days) which were statistically at par with each other. Number of flushes varied from 1 to 2, in general.

Sawdust + Eucalyptus wood chips + 5 per cent rice bran was found to be the most suitable substrate for fruit body production of *F. velutipes* as it yielded maximum fruit bodies (54 g) on this substrate. The same mixture except for Eucalyptus wood chips replaced by Robinia wood chips and in other with Alnus wood chips supported the next best yield. The yield in both the cases was statistically at par with each other. Pine needles and sawdust alone supported least production of fruiting bodies where production was again statistically at par with each other. Moderately good fruit body production was noted on corncobs (39.49 g), wheat straw + corncobs (36.49 g), and pine needles + Robinia wood chips + sawdust (34.14 g) in decreasing order.

Results given in Table 10b indicates that significantly minimum time for colonization in case of *G. lucidum* was taken by pine needles+ Robinia wood chips + sawdust (19 days) followed by sawdust + rice bran + Alnus wood chips (20 days) that were statistically at par with each other. Statistically maximum time for substrate colonization was observed in case of corncobs (35 days). Data regarding primordial formation indicated that sawdust + Eucalyptus wood chips supplemented with 5 per cent rice bran took minimum time for primordial formation (8 days) after complete substrate colonization followed by sawdust + Alnus wood chips supplemented with 5 per cent rice bran (11 days) and sawdust + Robinia wood chips+ 5 per cent rice bran (12 days). This indicated that supplementation of sawdust reduced the time taken for sporophore development. Significantly maximum time for initiation of primordia was observed on mixture of pine needles and wheat straw (25 days).

When time taken from initiation of fruit body production to maturity of sporophores was noted, it was observed that statistically minimum time was taken by mixture of wheat straw and corncobs (26 days), corncobs alone (27 days) and sawdust + Robinia wood chips + 5 per cent rice bran (28 days), all of


6(a) Immature fruiting bodies of *Flammulina velutipes*


6(b) Mature fruiting bodies of *Flammulina velutipes*

Plate-6

which were statistically at par with one another. However, maximum time for maturity of sporophores was observed in case of sawdust (33 days) and mixture of wheat straw + sawdust (34 days) that were statistically at par with each other. Significantly maximum number of fruit bodies were obtained on sawdust + Robinia wood chips (8 in number) and sawdust + Alnus wood chips (8 in number) both supplemented with 5 per cent rice bran and they were found to be statistically at par with each other.

The results of cultivation of *Ganoderma lucidum* revealed that all the substrate, either alone or in combination, resulted in the development of fruit bodies and all the substrates varied significantly from one another in terms of yield except pasteurized Compost and pine needles (Table 10b) where no fruiting was observed. Mushroom Compost got contaminated by mould competitors after the substrate bags were opened for induction of fruiting. It was observed that supplementation of sawdust with 5 per cent rice bran gave comparatively higher yield. Among these treatments sawdust + Alnus wood chips gave significantly higher yield (308.37 g) followed by sawdust + Eucalyptus wood chips (291.89 g) and sawdust + Robinia wood chips (265.41 g). However, sawdust alone yielded 224.75 g of sporophores that was otherwise higher than the yield from all other substrates tried except for mentioned as above. Similarly, pine needles alone yielded no fruit body but in combination with Robinia wood chips and sawdust, it resulted in moderately good yield (185.85 g). Combination of pine needles and wheat straw as substrate resulted in moderately good results in terms of yield (118.02 g). In wheat straw, sporophore yield was much lower (73.67 g) but in combination with sawdust moderately good yield (139.95 g) was obtained.

In general, 2- 3 flushes were recorded, but in case of wheat straw and mixture of pine needles and wheat straw only one flush was obtained. The yield in successive flushes was comparatively low in all the cases.

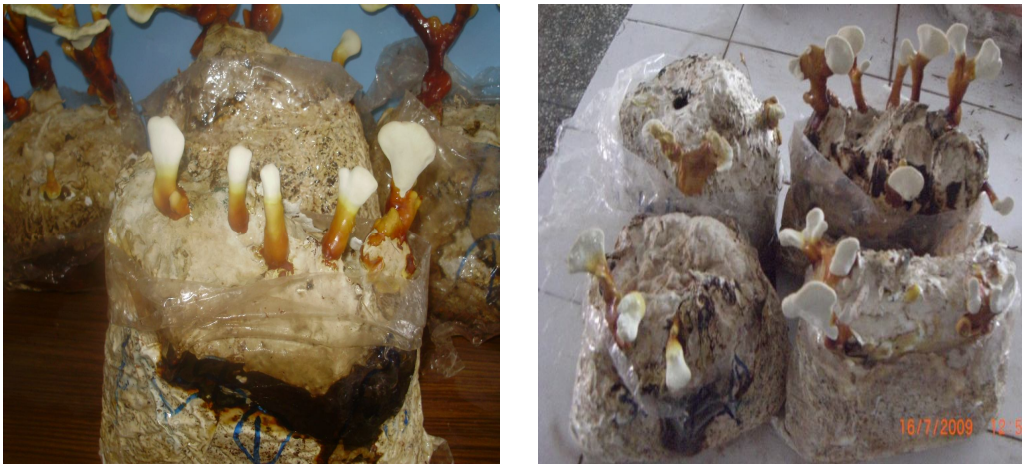
It can be inferred from Table 10b that colonization of different substrates with the mycelium of *Lentinula edodes* was achieved at the durations varying from 42- 68 days. The minimum time taken for colonization (42 days) was

recorded in case of the substrate containing mixture of sawdust, Robinia wood chips supplemented with 5 per cent rice bran followed by pine needles + wood chips + sawdust (45 days) that were statistically at par with each other. In sawdust alone, the complete mycelial colonization took 62 days that was statistically at par with mixture of wheat straw and sawdust and wheat straw + corncobs. Corncobs when used individually were colonized in a much longer time (68 days) which was statistically different from all other treatments.

When days from mycelial colonization to complete browning were counted. It was recorded that in the mixture of sawdust + Robinia wood chips + 5 per cent rice bran (13 days), and sawdust + Eucalyptus wood chips + 5 rice bran minimum time (13 days each) was taken for complete browning followed by sawdust + Alnus wood chips +5 per cent rice bran (15 day) and all these treatments were statistically at par with one another. However, browning was not observed in case of pine needles although the substrate was completely colonized by the mycelium of the fungus. Maximum time for complete browning of the substrate was recorded on mixture of pine needles + Robinia wood chips + sawdust (34 days). It is noteworthy that pasteurized compost was not permeated completely with the mycelium of the fungus, the growth stopped after few days and got contaminated after some time..

In the colonized substrate, mycelium underwent a number of changes between colonization and primordial formation. Initially it formed a thick sheet on the outer surface of the substrate. When the bags were completely permeated, bumps of different sizes and shapes appeared on it and the colour of the colonized substrate started changing into brown colour.

Among all the treatments, only sawdust and its combination with wood chips and rice bran supported the initiation of pinning. Maximum time (31 days) for primordial formation was taken by sawdust when used alone followed by its mixture with 5 per cent rice bran and Eucalyptus wood chips (30 days). Minimum time for initiation of pinning was observed on sawdust + 5 per cent rice bran + Robinia wood chips (25 days) followed by same combination but for



7(a) Primordial formation of *Ganoderma lucidum* on fully colonized substrate bags

7(b) Immature fruiting bodies of *Ganoderma lucidum*

7(c) Mature fruiting bodies of *Ganoderma lucidum*

Plate -7

Table10b: Effect of various substrates on the productivity of different edible fungi

Substrate	*Ganoderma lucidum*						*Lentinula edodes*					
	T.S. (days)	**T.P. (days)**	**T.M. (days)**	**No. of fruiting bodies**	**No. of flushes**	**Yield (g)**	T.S. (days)	T.B. (days)	T.P. (days)	No. of fruiting bodies	No. of flushes	Yield (g)
Wheat straw	30.33	20.33	29.67	3.67	1	73.67	57.33	29.0	0	0	0	0
Corncobs	35.33	21.67	27.0	4.0	2	55.59	67.67	24.33	0	0	0	0
Pine needles	29.33	0	0	0	0	0	59.0	0	0	0	0	0
P. compost	33.0	0	0	0	0	0	0	0	0	0	0	0
Sawdust	29.67	15.67	33.0	5.33	2	224.75	61.67	21.67	31	1.67	1	24.67
Sd+rb+Rwc	24.33	12.33	27.67	7.67	3	265.41	42.0	13.33	25	5.33	1	230.94
Sd+rb+Awc	20.33	10.67	28.67	7.67	3	308.37	48.0	14.67	28	6.67	1	251.08
Sd+rb+Ewc	22.0	8.33	29.0	6.33	3	291.89	46.0	13.33	30	4.33	1	218.94
Pine needles+ Robinia wood chips	26.33	21.67	31.0	4.0	3	135.37	56.0	19.33	0	0	0	0
Pn +Rwc+ Sd	19.0	17.33	31.33	5.33	2	185.85	45.33	33.67	0	0	0	0
Pine needles + Wheat straw	23.0	25.0	30.67	3.67	1	118.02	50.0	37.0	0	0	0	0
Wheat straw + Sawdust	26.67	15.67	33.67	4.33	2	139.95	61.67	25.67	0	0	0	0
Wheat straw + corncobs	32.33	19.0	26.33	4.33	1	54.25	62.0	22.33	0	0	0	0
$CD_{0.05}$	1.97	1.80	1.70	1.14		6.34	6.34	1.29	0.93	0.54		3.55
SE	0.96	0.88	0.83	0.83		3.09	3.09	0.63	0.45	0.26		1.73

* T. M.: **Time taken for maturity of fruit bodies (days), T.B.: Time taken for complete browning of the substrate (days), Sd+rb+Rwc: Sawdust + rice bran + Robinia wood chips, Sd+rb+Awc: Sawdust + rice bran + Alnus wood chips, Sd+rb+Ewc: Sawdust + rice bran + Eucalyptus wood chips, Pn + Rwc+ Sd: Pine needles + Robinia wood chips + sawdust**

wood chips of Robinia replaced by Alnus wood chips (28 days). The primordial developed into mature fruit bodies in 5-9 days. Maximum number of fruit bodies and yield (251.08 g) were recorded on mixture of sawdust + Alnus wood chips + 5 per cent rice bran followed by sawdust + Robinia wood chips + 5 per cent rice bran (230.94 g). Minimum number of sporophores and yield (24.67 g) was observed in sawdust alone. Under present study, only one flush was obtained in all the treatments even when the substrates were dipped in water at 12^0C for 10-12 h after the first harvest.

8(a) Fully colonized bags of *Lentinula edodes*

8(b) Substrate bags showing initiation of browning of the mycelium of *Lentinula edodes*

8(c) Substrate bags showing formation of bumps or popcorn like blisters on the surface

8 (d) Substrate bags showing complete browning of the mycelium of *Lentinula edodes*

8(e) Immature fruting bodies of *Lentinula edodes*

8(f) Mature fruiting bodies of *Lentinula edodes*

Plate-8

Chapter-5

DISCUSSION

Six different medicinal mushrooms were studied with respect to their cultural requirements and cultivation aspect. Many of these medicinal mushrooms are not cultivated commercially in the world, but they offer best prospects with respect to the nutritive and medicinal values.

Physiological studies in mushrooms are important not only to the scientists but for mushroom growers as well because one should know about their temperature, pH, moisture, light and nutritional requirements for crop management. However, utilization of various nutrients can be more accurately studied in relation to mycelial growth under *in vitro* conditions. Different workers have reported difference in growth at different concentrations of the same chemical. It is also known that chemicals which are stimulatory at one concentration may be toxic at another. For successful introduction, it is necessary to understand its physiological requirements and substrate suitable for spawn production and cultivation. The results obtained from the present work on these aspects are discussed below.

Physiological studies not only form the basis for mushroom crop management but are also a pre- requisite for effective planning and preparations for successful crop production. It also provides vital informations having direct bearing on yield, quality, timing and other characteristics of fruiting bodies.

Various solid media in natural, semi- synthetic or synthetic form were used for getting desirable and readily available mycelial growth which is necessary for maintenance of culture as well as to serve as a source of inoculum. Out of six agar media, significantly fastest growth of *P. ostreatus* was recorded on PDA medium followed by Malt extract agar. However, several workers have supplemented PDA with yeast to obtain better mycelial growth (Lockard and Kneebone, 1962; Kneebone *et al.*, 1972; Rangad and Jandaik, 1977; and Sharma, 1984). Nevertheless, PDA is still acknowledged as the most popular universal medium for routine culture multiplication. Fastest growth of *P. eryngii* was recorded on MEA medium followed by

PDA. However, Sharma and Jandaik (1984) reported PDA and Glucose Yeast agar media to support significantly maximum growth of Spain isolate whereas maximum growth of Kabul isolate was recorded on Yeastal potato dextrose agar medium. Fastest growth of *F. velutipes* was observed on Glucose- asparagine agar medium followed by Potato dextrose agar. The results are in agreement with the findings of Sharma *et al.* (2005). However, Rangad (1981) recorded fastest mycelial run on PDA fortified with yeast extract.

For *L. edodes*, MEA followed by PDA were found to be the best media as compared to other media tried. PDA, Yeast potato agar and MEA were found to support good growth of *L. edodes* (Jodon and Royse, 1979; Campbell and Slee, 1987; Chang and Miles, 1989). Significantly maximum growth of *G. lucidum* was recorded on PDA followed by MEA. Nasreen *et al.* (2005) also recorded best and fastest growth of the fungus on PDA. On the contrary, Bilay *et al.* (2000) recorded maximum growth of the fungus on MEA.

MEA medium followed by PDA were found to be the preferred media for mycelial growth of *A. polytricha*. These results are in agreement with the findings of Thakur and Bhandal (1993). However, Khan *et al.* (1991) reported slightly different results where MEA was found as the best medium for vegetative growth of *A. polytricha* among MEA, PDA and Wheat extract agar. The variation in above studies may be due to the differences in strains of the test fungi and the nutritional status of different ingredients used during above studies.

For selecting the basal medium, six different solutions were tried. Maximum mycelial growth of *P. ostreatus* was recorded on Richard's followed by Glucose- asparagine solution. In case of *P. eryngii*, significantly maximum growth was recorded on Glucose- asparagine solution followed by Sabouraud's solution. The results are contradictory with the findings of workers like Jandaik and Kapoor (1975), and Rangad and Jandaik (1977) who recorded good growth of *Pleurotus* spp. on Dimmick's and Czapek's dox solution. The possible reason behind this difference might be the presence of fairly high quantity of glucose in Glucose- asparagine solution and in Richard's solution which were selected as the basal media for *P. eryngii* and *P. ostreatus*, respectively as compared to Dimmick's solution in which only 10g dextrose is present as carbon source while fungi are well known to have higher carbon requirements for their growth and development. However, Glucose- asparagine was also found as the most suitable basal

medium by Rangad (1981). Sharma and Jandaik (1984) noticed significantly maximum growth of Spain isolate of *P. eryngii* on Sabouraud's solution while synthetic mucor solution favoured the growth of Kabul isolate .

Significantly maximum growth of *F. velutipes* was recorded in Glucose- asparagine solution followed by Richard's solution. These results are in accordance with the findings of Rangad (1981) who recorded Glucose- asparagine followed by Richard's solution as best basal media for mycelial growth of the fungus. In *L. edodes*, maximum mycelial growth was recorded on Glucose- asparagine solution followed by Richard's solution. These results are in agreement with the findings of Kaur and Lakhanpal (1999) on different liquid media studies for selection of basal medium.

Among all the liquid media tested in the present investigations, Elliott's solution was found to support maximum mycelial growth of *A. polytricha* followed by Sabouraud's solution. Similarly, in case of *G. lucidum*, Glucose- asparagine solution followed by Richard's solution were found to support good mycelial growth. No information regarding this aspect for both the above fungi could be found in literature for comparison. Moderately good growth of all the test fungi was recorded on Sabouraud's solution which could be attributed to the presence of peptone which is a complex nitrogen source.

In the present investigations, all the six test fungi grew well at different temperatures ranging from 10- 35^{0}C, in general but *P. ostreatus*, *P. eryngii*, *F. velutipes* and *L. edodes* were found to grow even at 5^{0}C. On the contrary Balazs and Szabo (1979) recorded no growth of *Pleurotus* spp. below 10^{0}C. However, these results indicate that both the *Pleurotus* spp. can survive under diverse climatic conditions, which is a preferentially desirable attribute for cultivation of any mushroom under study. Significantly maximum growth of all the six edible fungi was recorded at 25^{0}C. More or less similar results were recorded by Zadrazil (1976) and Sharma and Jandaik (1984) in *P. eryngii*. Again, Rangad and Jandaik (1976) got the highest mycelial yield of *P. ostreatus* at 25^{0}C which supports the results of the present study. However, for some other species of *Pleurotus*, slightly higher temperature (30^{0}C) has been recorded to be optimum for mycelial growth by Zadrazil (1976). A temperature range between 21- 26^{0}C has been optimized for the growth of *P. ostreatus* by Block *et al.* (1959).

Kaur and Lakhanpal (1999), Singh and Singh (2005) and Royse (1989) in their studies have optimized the temperature for mycelial growth of *L. edodes* at 25^0C. Again, in case of *A. polytricha,* as described earlier, a temperature of 25^0C was recorded to be the best followed by 30^0C for the vegetative growth of the fungus in the present investigations. These results are in agreement with the results obtained by Khan *et al.* (1991), Thakur and Bhandal (1993), Xu and Yun (2003), and Jonathan *et al.* (2009). Similar results were recorded by Nasreeen *et al.* (2005) for *G. lucidum*. However, for some other species, slightly higher temperature (26-35^0C) has been recorded to be optimum for vegetative growth of *G. lucidum* by different workers (Yang and Liau, 1998; Lee *et al.*, 2002; Babitskaya *et al.*, 2007). The optimum temperature recorded by these workers varied between 26-35^0C. Su *et al.* (2001) and Sharma *et al.* (2005) found maximum mycelial growth of *F. velutipes* at 29^0C which clearly justifies the results of the present study. Whereas, Rangad (1981) recorded maximum growth of *F. velutipes* at 20^0C indicating that there could be difference in strain of the fungus evaluated.

In the present study, when different levels of pH were adjusted, good growth enough to make a comparative study was recorded. A pH value of 4.5 gave significantly maximum growth of *L. edodes* followed by pH 5.0 and 6.0. These findings are in accordance with the reports given by Kaur and Lakhanpal (1999). However, 4.0, 5.0 and 7.0 pH have been recorded optimum for the vegetative growth of *L. edodes* by Furlan *et al.* (1997), Tnglet *et al.* (2006) and Sharma *et al.* (2006), respectively.

Generally, a pH value between 5.0 and 6.5 have been found optimum for the growth of different *Pleurotus* species by various workers (Hashimoto and Takahashi, 1976; Zadrazil, 1976, 1978; Rangad and Jandaik, 1977; Soni, 1979; Sharma, 1984; Furlan *et al*., 1997). In the present study also maximum mycelial growth of *P. ostreatus* was recorded at pH 6.0 followed by pH 5.5 and that of *P. eryngii* with 5.5 pH followed by a pH of 6.0. Similar results were obtained by Ali *et al.* (2004) for *P. ostreatus* who recorded a pH range of 5-7, with optimum being 6.0 as best for *P. eryngii*.

Comparatively highest mycelial growth of *A. polytricha* was recorded with pH 6.5 followed by pH 6.0. Different workers have reported a pH range of 5.0-6.0 as optimum for the growth and exo-polymer production of the fungus (Thakur and Bhandal, 1993; Xu and Yun,

2003). Khan *et al.* (1991) have recorded a neutral pH of 7.0 favourable for mycelial growth of fungus. The results of the present studies totally match the results of Janathan *et al.* (2009) who recorded maximum biomass production of *A. polytricha* at 6.5 pH.

Generally, a pH value between 3.5-6.5 has been reported optimum for growth of different *Ganoderma* species (Yang and Liau, 1998, Yang and Zhong, 2002 ; Lee *et al.*, 2002 ; Nasreen *et al.*, 2005 ; Babitskaya *et al.*, 2007 ; Mirjana *et al.*, 2007 ; Song *et al.*, 2007). In the present study, maximum growth of the fungus was recorded at pH 5.0 followed by pH 6.5 which is in the range of pH optimized by various workers mentioned above. Maximum mycelial growth of *F. velutipes* was recorded with pH 5.5 followed by pH 5.0. The results are strongly contrary to those obtained by Sharma *et al.* (2005) who have reported alkaline pH of 8.0 for maximum growth of the fungus. However, the results are in accordance to the findings of Rangad (1981). Slight variation in results were found when results were compared with those obtained by Su *et al.* (2001), who got maximum mycelial yield of *F. velutipes* at pH 6.5. The results regarding growth of test fungi at various pH levels in present study indicated that all these Basidiomycetous fungi showed poor response with respect to the growth at alkaline pH.

Among ten carbon sources studied to see their comparative effect on growth of six test fungi, starch followed by glucose and sucrose witnessed significantly maximum vegetative growth of *P. ostreatus* while moderate growth was recorded with dextrose, xylose and galactose. These results are in agreement with results obtained by Block *et al.* (1959), and Srivastava and Bano (1970). However, these results are in partial agreement with the findings of Sakamoto *et al.* (1978) who recorded good growth of *P. ostreatus* with glucose supplemented with additional starch and Hashimoto and Takahashi (1976) who reported that mycelium of *P. ostreatus* grew faster with mannose followed by starch and glucose

In case of *P. eryngii,* glucose followed by sucrose and dextrose were found to support significantly maximum growth of *P. eryngii.* However, moderate to good results were recorded with the addition of Maltose, xylose and starch. These results agree with the findings of Soni (1979); and Zhang *et al.* (2005) who recorded maximum mycelial growth of the fungus with glucose followed by sucrose. However, the results do not agree with those recorded by Sharma (1984) who reported significantly maximum growth of Spain isolate with sorbitol and fructose and in case of Kabul isolate, with addition of dextrose and galactose. This difference in the

growth might be due to the variation in isolates.

In case of *F. velutipes*, maximum mycelial growth was recorded with the addition of sucrose followed by dextrose. These results are supported by the findings of Sharma *et al.* (2005) who also recorded sucrose as the best carbon source for *F. velutipes*. Significantly good mycelial growth of *F. velutipes* was recorded by Wang (2000) on starch, glucose, sucrose and mannose. However, the results of the present study proved mannitol to be a poor source for vegetative growth of *F. velutipes* and these are in strong contradiction with the results of Rangad (1981) who reported mannitol as best carbon source for growth of *F. velutipes*.

Again, in case of *L. edodes* glucose, a monosaccharide supported significantly maximum growth of the fungus followed by fructose and sucrose. Moderately good growth was recorded with starch and dextrose. Starch was recorded as best carbon source for *L. edodes* by Matsuo (1950); Khan *et al.* (1991); and Meng *et al.* (2007). However, the results of the present study are in agreement with those obtained by Kaur and Lakhanpal (1995). They also recorded good growth of the fungus with glucose followed by fructose and sucrose.

Best growth of *A. polytricha* was recorded with starch in the present study followed by sucrose and fructose. Similar results were obtained by Khan *et al.* (1991) but slightly different results were recorded by Xu and Yun (2003) and Jonathan *et al.* (2009) who have reported sucrose and glucose, respectively as best carbon sources for the fungus. The reason behind these variations might be difference in the strains tested and growing media used.

In the present study, simple sugar, glucose was found to support maximum mycelial growth of *G. lucidum* followed by starch and dextrose. However, due to insufficient literature regarding cultural requirements of *G. lucidum*, there is no evidence to support the results of the study. It is noteworthy that significantly minimum growth in all the six test fungi, was supported by the sugar alcohol, mannitol in comparison to control. The importance of sugar alcohols (sorbitol) for increasing vegetative growth of *P. ostreatus* has been emphasized by Hong (1978) and Oso (1977) and that of mannitol by Voltz (1972). These differences in the ability to utilize different sources of carbon is probably an expression of physiological differences in the species or strains studied by different workers. However, better utilization of starch by almost all the six

test fungi is attributed to the capability of these fungi to utilize glucose and also in breaking down the complex polysaccharide, starch into readily assimiliable glucose molecules.

Among fifteen different nitrogen sources belonging to organic and inorganic groups of nitrogen evaluated for determining their comparative response on vegetative growth of different test fungi, maximum growth of *P. ostreatus* was recorded with the addition of ammonium nitrate followed by leucine whereas in case of *P. ergngii*, it was recorded with the addition of leucine followed by aspartic acid. Mandelin (1956) has reported amino acids to be the good nitrogen sources for the growth of *P. ostreatus*. The studies done by Voltz (1972); Hashimoto and Takahashi (1972); Kikon (1979); Rangad (1981); and Huang *et al.* (2006) contradict the results of the present investigations obtained in case of *P. ostreatus*. According to these workers, peptone, asparagine and urea were recorded efficient nitrogen sources for the fungus. In our study, fairly good results were obtained with urea while peptone and asparagine were not tried. Again, in case of *P. eryngii*, urea, peptone and asparagine have been reported good nitrogen sources for the growth of fungus by different workers (Soni, 1979; and Zhang *et al*., 2005). However, in present study moderately good results were recorded with urea and the difference might be due to variation in strain used.

Iso-leucine followed by asparagine, leucine and ammonium chloride were recorded best nitrogen sources for the growth of *F. velutipes* by Rangad (1981) and asparagine was also reported as best nitrogen source by Sharma *et al.* (2005). The results of the present study were found to be in accordance to the findings of Rangad (1981) to some extent because leucine followed by ammonium sulphate and ammonium chloride were found as best nitrogen sources for the mycelial growth of *F. velutipes* by the workers. Again, for *G. lucidum* ammonium chloride followed by ammonium sulphate and asparatic acid were recorded as best nitrogen sources. No work has been done on this aspect of the fungus for comparison.

Peptone was recorded as most suitable nitrogen source for growth of *A. polytricha* by Khan *et al.* (1991) and Jonathan *et al.* (2009). In the present study, peptone was not evaluated but significantly maximum growth of the fungus was recorded with ammonium chloride followed by ammonium sulphate and potassium nitrate. Among amino acids, leucine was found to give best result however, Jonathan *et al.* (2009) reported tryptophan as best nitrogen source among amino

acids. This difference in nitrogen utilization might be due to difference in the strain used. Among the nitrogen sources used in the present study, ammonium chloride followed by ammonium sulphate and aspartic acid were found to support significantly maximum mycelial growth of *L. edodes*. On the contrary, Kaur and Lakhanpal (1999) reported peptone and Tsivileva *et al.* (2005) found natural amino acids and L- asparagine to be the best nitrogen sources for this fungus. However, peptone and asparagine were not tried in the present study.

It is noteworthy that reduced growth in all the six fungi with sodium nitrite (no growth in case of *A. polytricha*) might be due to toxicity of nitrite at acidic pH at which the test fungi were grown as also reported by Cochrane and Conn (1950). The preferential utilization of nitrogen from ammonium or nitrate salts may be due to difference in the inherent capacity of different edible fungi or their species or strains to use these salts as nitrogen source.

Among the five micro-nutrients tried at three varying concentrations to see their comparative effect on growth, it was recorded that the growth of *P. ostreatus* was best favoured by addition of 2 ppm ferrous sulphate followed by 2 ppm manganese sulphate, while 2 and 10 ppm boric acid and 10 ppm ammonium molybdate reduced the growth when added individually to the basal medium. Slightly different results were recorded by Kurtzman and Zadrazil (1982); Hong (1978) and Huang *et al.* (2003) who recorded good results with addition of magnesium in the form of magnesium sulphate at low concentrations. However, iron in the form of ferric chloride has also been reported to increase growth of different *Pleurotus* spp. by several workers (Jandaik and Kapoor, 1976; Soni, 1979; Sharma, 1984). Again, for *P. ergngii*, significantly maximum growth was recorded with addition of 2 ppm ferrous sulphate followed by 2 ppm zinc sulphate and 5 ppm ferrous sulphate. Significantly minimum growth was recorded with the addition of 5 ppm boric acid and 10 ppm ammonium molybdate. These results are in accordance with the findings of Soni (1979); and Sharma (1984) on Spain and Kabul isolates of *P. ergngii*. Reduction in growth with the addition of boron and molybdenum may possibly be attributed to concentrations used in the present study, which were on the higher side as other concentrations have not been studied.

Investigations done by Kaur and Lakhanpal (1995) showed manganese at 2 ppm concentration to be the effective trace element for *L. edodes*. The results of the present study also

agree with these findings because manganese sulphate (2 ppm) followed by 2 ppm ferrous sulphate were recorded best micronutrient sources for the fungus. On the contrary, Kang *et al.* (2001) have reported 0.05 per cent magnesium sulphate to support maximum mycelial weight of *L. edodes*.

In case of *A. polytricha*, 5 ppm ammonium molyldate was found to support significantly maximum mycelial growth followed by 2ppm ferrous sulphate whereas minimum growth was recorded with the addition of 2ppm boric acid into the basal medium. The results are slightly in accordance with the findings of Meng *et al.* (2004) who proved ferrous sulphate as suitable trace element source. On the contrary, di-potassium hydrogen was identified as most suitable mineral element by Xu and Yun (2003).

Rangad (1981) reported maximum growth of *F. velutipes* when mixture of trace elements was added to basal medium followed by ferrous sulphate and zinc sulphate. No increase in growth was recorded with addition of manganese sulphate, boric acid and ammonium molybdate. Only basic salt medium containing magnesium, manganese, iron and zinc as trace elements were recorded to be needed for vegetative growth of *F. velutipes* (Cho and New, 2006). The results of the present study are also in accordance with the results of above mentioned workers. Ferrous sulphate (2 ppm) followed by 2 ppm manganese sulphate were found to support significantly maximum growth of *F. velutipes* whereas minimum growth was supported with 2 ppm boric acid.

In the present study addition of 2 ppm manganese sulphate followed by 2 ppm ferrous sulphate were recorded to support significantly maximum growth of *G. lucidum*. However, significantly minimum growth was recorded with 10 ppm ammonium molybdate, 10 ppm zinc sulphate and 10 ppm manganese sulphate. It may be due to higher concentrations of the trace elements used that may reduce the growth of the fungus at that particular concentration. It is known that magnesium, zinc and iron are generally required in small quantities by various organisms (Zadrazil and Kurtzman, 1982). However, no evidence could be obtained to support or contradict these findings due to scanty literature.

Fairly good mycelial growth of different test fungi on medium that is totally deficient in vitamins showed that they can be at least considered partially deficient for vitamin requirement.Various authors have obtained stimulated growth of *P. ostreatus* at different concentrations (0.1 to 0.5ppm) of vitamins tested (Sugimori, *et al.*, 1971; Hashimoto and Takahashi, 1972; Jandaik and Kapoor, 1976; Hong, 1978; and Bukhalo and Solomoko, 1979). Among different vitamins tested in the present investigations, thiamine at 0.05 ppm concentration followed by 0.025 ppm biotin and 0.05 ppm niacin were recorded to support maximum mycelial growth of *P. ostreatus*. Thiamine in general, has been reported to give best growth of different *Pleurotus* spp. by various workers (Bukhalo and Solomoko, 1979; Hong, 1978; Hashimoto and Takahashi, 1976; Jandaik and Kapoor, 1976) when used at lower concentrations. However, there seems to be some difference in the relative effect of some of the vitamins with regard to *Pleurotus* species, which may possibly be due to different species as well as wide diversity of basal medium used by different workers.

In the present study, growth of *Pleurotus. eryngii* was best favoured by the addition of 0.05 ppm thiamine followed by 0.025 ppm biotin and 0.05 ppm niacin. Significantly, minimum growth was recorded with 1ppm folic acid followed by 1ppm pentothenic acid. Soni (1979) also recorded similar growth results for *P. eryngii*. Whereas, Kundu (2003) found growth promoting effect (in decreasing order) of biotin, inositol, pyridoxine and thiamine on vegetative growth of *Pleurotus* spp. The variation might be due to the difference in species. However, various workers have reported decreasing effect of some vitamins on the growth of certain fungi, not deficient for those particular vitamins (Elliott, 1949; Lilly and Barnett, 1951; Mathur *et al.*, 1950; Wirth and Nord, 1942). In certain cases, vitamins may have an inhibitory effect on growth, particularly when present in excessive dosages. The inhibitory effect of vitamins may be due to the inter-related effects of temperature and amount of particular vitamin (Barnett and Lilly, 1948). The exogenous supply of thiamine at 0.025 ppm followed by 0.025 ppm biotin significantly supported maximum growth *of F. velutipes.* However, decreased growth was recorded with 0.025 ppm pentothenic acid, 1ppm concentration each of biotin, folic acid and riboflavin. The results obtained in the present investigations are in agreement with findings of Rangad (1981); Yun (1996); and Cho and New (2006) who also recorded good growth with thiamine.

Maximum utilization of thiamine by *L. edodes* added to the basal medium was reported

by several workers (Jennison *et al.*, 1965; Haruhiko, 1967; Kaur and Lakhanpal, 1995; Shin *et al.*, 2000). In the present study, 0.025 ppm thiamine was found to support significantly maximum growth followed by 0.05 ppm biotin. Folic acid at all the tested concentrations resulted in decreased growth. Mahmud and Ohmasa (2008) proved that addition of biotin increased the efficiency of high temperature tolerant *L. edodes* strains. The results of the study cleared that folic acid has inhibitory effect on the growth of fungus and this effect may be strain specific.

In *A. polytricha*, addition of 0.05 ppm thiamine followed by 0.025ppm concentration of same vitamin and 0.025 ppm biotin were recorded to support maximum mycelial growth of *A. polytricha* and addition of 0.025 and 1 ppm of niacin, pentothenic acid at all three tested concentrations i.e. 0.025, 0.05 and 1 ppm and 1 ppm riboflavin were recorded to reduce growth probably due to their inhibitory effect on the growth of fungi at given concentrations. Similarly, 0.025 ppm thiamine followed by 0.05 ppm biotin and 0.025 ppm riboflavin were recorded to support maximum mycelial growth of *G. lucidum*. However, all the tested concentrations of niacin, 1 ppm pentothenic acid and 1ppm folic acid were recorded to inhibit the growth of the fungus probably due to their toxicity at given concentration on the given fungus strain. There is again no evidence from literature to support the results of both these fungi.

The microorganisms found growing on a straw substrate are expected to synthesize their own vitamin requirement, but it can not be true in case of growth regulators. There is evidence that cytokinins, auxins and gibberellins have profound influence on mycelial growth (Szabo *et al.*, 1972) as well as on the production of basidiocarps of higher fungi (Aleksandrov, 1964). In *P. ostreatus*, maximum mycelial growth was recorded with addition of 25 ppm gibberellic acid followed by 50 ppm kinetin. Whereas, in comparison to control, addition of 10 ppm NAA and 50 ppm IBA had no significant effect on growth. On the contrary, several workers (Hong, 1978; Vinklarkova and Sladky, 1978; and Reddy *et al.*, 2002) recorded increased growth of *P. ostreatus* with the addition of IAA. However, they recorded gibberellic acid and kinetin equally good for the growth of the fungus. Kikon (1979) found that IAA at all concentrations completely inhibited the growth of Florida and Grey strains of *P. ostreatus*.

Gibberellic acid (50 ppm) resulted in significantly maximum mycelial growth of *P. eryngii* followed by 25 ppm kinetin and significantly minimum growth was recorded with 25

ppm benzyl adenine followed by 50ppm IAA. Inhibitory effects of higher concentrations of NAA were also recorded on the growth of fungus. Rawal and Singh (1980) have reported gibberellic acid as the best preferred growth regulator for *P. eryngii*. They also showed NAA to support significantly little growth of *Pleurotus* spp. Contradicting results with regard to mycelial growth of *P. eryngii* were recorded by Sharma (1984) who recorded maximum growth of Spain isolate of the fungus with different concentrations of 2, 4-D followed by higher concentration of miraculan.

Growth regulators such as IAA have been produced by a number of fungi *in vitro* (Cochrane, 1958). In general, lower concentrations of various growth regulators studied by different workers have been reported to stimulate growth whereas,higher concentrations have been found to inhibit the growth as compared to control (Hong, 1978, and Vinklarkova and Sladky, 1978). In *L. edodes*, the vegetative growth was best favoured with addition of 50 ppm gibberellic acid followed by 25 ppm kinetin. Significantly minimum growth was observed with 50 ppm IAA followed by 50 ppm benzyl adenine. Similarly, Tan and Chang (1989); Kaur and Lakhanpal (1995); and Siwulski and Sobieralski (1998) have also recorded maximum growth of *L. edodes* on gibberellic acid. However, IAA was recorded to support moderately good growth (Tan and Chang, 1989) which is contrary to the results of present study. This controversy could probably be due to variation in concentrations of different growth substances tried by the workers as it has long been considered that substances which are stimulatory at one concentration may be toxic at another.

For *A. polytricha*, maximum growth was recorded with addition of 50 ppm gibberellic acid followed by 50 ppm kinetin. However, significantly minimum growth was recorded with addition of higher concentration of IAA and NAA. Gibberellic acid (50 ppm) supported maximum growth of *G. lucidum* followed by 25 ppm kinetin. However, minimum growth was recorded with 50 ppm IAA followed by 50 ppm NAA, 25 ppm IAA and 50 ppm IBA. Again, there is no report in the literature to support the results for both the fungi.

Addition of 50 ppm gibberellic acid followed by 50 ppm kinetin and 50 ppm IBA were found to support significantly maximum growth of *F. velutipes*. However, significantly minimum of growth was observed with 10 ppm each of IAA and NAA. Rangad (1981) and Sharma *et al.* (2005) have reported gibberellic acid to support maximum growth of fungus.

However, Ding *et al.* (1990) recorded best growth of *F. velutipes* with respect to time and appearance of fruiting bodies and increased number, yield and quality of fruiting bodies with application of mixture of alkylal 30 per cent+ gibberellic acid.

Cereal grains, especially wheat and rye are the universally accepted substrate for spawn production. However, various workers have tried other substrates or agricultural wastes also because of the easy availability, accessibility or low expenses. In the present study, for the selection of cheap and readily available substrates for spawn production, eight substrates (including cereal grains, straw, sawdust alone and in combination) were tried and the mycelial impregnation by different test fungi at periodic intervals was studied. Significantly minimum time for substrate colonization by mycelium of *P. ostreatus* was taken on bajra grains after 9 days of incubation followed by mixture of wheat and bajra grains and wheat grains alone. The longest period for colonization was taken on the mixture of wheat grains + gur and sawdust alone. Significantly maximum time for substrate colonization was taken on sawdust alone but after supplementation with bajra and wheat grains significantly good results were recorded. Poor results on sawdust alone could be due to poor nutrition or lack of aeration and space for the mycelial spread and colonization. Spawn of *Pleurotus* spp. has been produced on various cereal grains, millets and other farm wastes such as straw and bajra husk with success (Jandaik and Kapoor, 1974). Rangad and Jandaik (1977) recorded maximum yield of different *Pleurotus* spp. with spawn prepared from jowar grains. Similar results were recorded by Hussain and Hussain (2004) for *P. ostreatus* on maize grains.

In case of *P. eryngii*, maximum mycelial growth was observed on mixture of wheat and bajra grains followed by bajra grains alone. These results are fovoured by the studies done by Sharma (1984) who proved wheat, barley, jowar and bajra grains as equally good for vegetative growth of the fungus. Moderately good results in case of both the fungi were recorded on wheat straw. However, findings of Pandey and Singh (2001) have cleared that spawn prepared on wheat straw alone gave lower yield. On the contrary, use of spawn prepared on paddy and wheat straw has been reported by different workers (Huhnke, 1961; Lelley, 1974; Zadrazil, 1976; Kalberer, 1976; and Bano *et al.*, 1979) to give equally good yields as grain spawn. The good results obtained with the use of these spawn could be due to increased inoculum level or contact point per unit surface area as compared with equal weight of grains making faster colonization

than the grain substrate. Dewangan *et al.* (2007) recorded fastest mycelial run on grain substrate i.e. wheat and sorghum grains. In the present study also, good results with respect to mycelialrun on *P. eryngii* were obtained with mixture of wheat and bajra grains followed by bajra grains alone. However, significantly minimum growth was recorded with wheat straw followed by sawdust alone.

Quick and fastest growth of *F. velutipes* was recorded on cereal grains such as jowar, bajra and millets by several workers (Hashimoto and Takahashi, 1976; Rangad, 1981).The results of present investigations are in accordance with the findings of these workers. Maximum linear growth of mycelium of *F. velutipes* was recorded on mixture of wheat and bajra grains followed by bajra grains alone. However, significantly minimum growth was recorded with mixture of wheat grains and gur even after 18 days of incubation which may be due to the detrimental effect of gur when used with wheat grains.

Hirt and Schnitzler (1994) obtained good spawn run of *G. lucidum* on cereal grains such as millet, wheat and rye. Again, wheat grains were found to support maximum yield of *G. lucidum* when spawn prepared on wheat, mandhua and jhangora grains were tested. Amongst all the substrates used in present investigations, wheat grains were found to favour fastest mycelial run of the fungus followed by mixture of wheat and bajra grains. These results are in accordance with the findings of above mentioned workers. However, maximum time for spawn run was recorded with wheat straw.

In case of *A. polytricha*, fastest spawn run was observed on bajra grains followed by mixture of wheat and bajra grains. Significantly minimum growth was observed on mixture of wheat grains and gur followed by sawdust alone. These results are contradictory to those observed by Thakur and Bhandal (1993). They obtained fastest spawn run on sawdust alone followed by wheat straw. This may be due to difference in the strain of the fungus used and the type of sawdust used. However, the grains impregnated with the mycelium offer the advantage that the spawn can readily be mixed evenly throughout the substrate forming many centres of inoculation from which the mycelium can grow. The superiority of jowar grains in spawn production has been proved by various workers in different species of mushrooms (Munjal, 1973; Kumar *et al.*, 1975; Chauhan and Pant, 1988; Iqbal *et al.*, 1988).

For evaluating the substrate best suited for cultivation of *P. ostreatus* and *P. eryngii*, different forest and agricultural wastes were tried for yield potential with or without supplementation with 5 per cent rice bran. In *P. ostreatus* it was recorded that wheat straw was colonized in minimum time of incubation that is 10 days followed by mixture of wheat straw + sawdust (*Toona ciliata*). Slowest spawn run was noticed with pasteurized compost followed by mixture of sawdust + Eucalyptus wood chips supplemented with 5 per cent rice bran and wheat straw + pine needles. Again, minimum time for initiation of fruiting bodies was observed on wheat straw followed by corncobs whereas maximum time was taken on sawdust alone followed by pine needles which were statistically at par with each other. From the yield data, it was noticed that maximum number of sporophores and yield was obtained from treatment containing sawdust + Robinia wood chips + 5 per cent rice bran followed by sawdust + Eucalyptus wood chips + 5 per cent rice bran and the treatment containing wood chips of Alnus replacing Eucalyptus wood chips. Lowest yield was recorded with pine needles followed by the pasteurized compost. It may be due to the absence of wooden chips or cellulosic material in the pasteurized compost and presence of inhibitory compounds like resins in the pine needles.

Kurtzman and Zadrazil (1982) have also recommended supplementation of cultivation substrates with various protein rich materials for higher yields of different *Pleurotus* species. Sawdust alone was found poor source of nutrition for *P. ostreatus*. More or less similar waste materials like sawdust or wood shavings (Block *et al.*, 1959; Omori, 1974; and Zadrazil, 1980), paddy straw and wheat straw (Park *et al.*, 1975; Jandaik and Kapoor, 1976; Kandaswamy and Ramaswamy, 1976; Zadrazil, 1976; Singh and Rajarathnam, 1977; Bhaskaran *et al.*, 1978; Cayrol, 1978; Balazs and Szabo, 1979; Bano *et al.*, 1979; Sivaprakasam and Kandaswamy, 1980; Stanek and Bisko, 1982; Ginterova *et al.*, 1982; and Delmas and Mamoun, 1983) and crushed corncobs (Toth, 1969; Gyurko, 1969; Kostadinov and Stefanov, 1977; and Sharma, 1984) have been used with success for the cultivation of various *Pleurotus* species. It also appears that plant waste can be utilized by several *Pleurotus* spp. but their nutritional availability to these species varies (Zadrazil, 1980). The ability of different *Pleurotus* spp. to utilize such diverse waste materials may be due to their high saprophytic ability to decompose and utilize lignin containing materials (Nair, 1980). Paddy straw followed by wheat straw was recorded to give highest sporophore yield of *P. ostreatus* by Dubey (1999); and Hussain and Hussain (2004).

Pleurotus spp. could colonize and produce fruiting on pre- treated conifer (*Pinus* spp.) wood chips but they did not readily colonize non- pretreated conifer wood due to presence of inhibitory component (Croan, 2004). Good yield on sawdust was observed by Shah *et al.* (2004). Sawdust from nitrogen fixing trees mixed with 25 per cent coconut residue was recorded to give efficient results in comparison to the use of sawdust alone (Iqbal *et al.*, 2005). Das and Mukherjee (2007) successfully cultivated *P. ostreatus* on different dry weed plants among which *Leonotis* spp. mixed with rice straw yielded maximum fruit body production. Sawdust substrate supplemented with manganese and soybean was reported to give better yield than the use of sawdust alone (Estrada and Royse, 2007). During the cultivation trials of *P. eryngii* in the present study, minimum time for substrate colonization was recorded on wheat straw and mixture of wheat straw + corncobs. Maximum time for complete colonization was taken on sawdust followed by pasteurized compost. Minimum time for initiation of pinning was taken on mixture of wheat straw + corncobs. On the contrary, maximum time for initiation of pinning was recorded on mixture of pine needles + Robinia wood chips + sawdust. Wheat straw was found to support maximum sporophore yield of *P. eryngii* followed by corncobs and wheat straw + corncobs. Pine needles supported significantly minimum sporophore yield. From the present investigations, it may be concluded that both the species of *Pleurotus* can be cultivated on commercial scale using substrates like wheat straw and corncobs.

In the present investigations, significantly maximum yield of *F. velutipes* was obtained in the present study from mixture of sawdust + Eucalyptus wood chips+ 5 per cent rice bran followed by sawdust + Robinia wood chips + 5 per cent rice bran. The pine needles and sawdust were found to support least production of sporophores. However, no fruiting was observed on pasteurized compost. The colonized bags got contaminated after few days of substrate colonization. Minimum yield on pine needles may be attributed to some inhibitory compounds present in pine needles. Moderately good yield was recorded on corncobs and mixture of wheat straw and corncobs. However, minimum time for substrate colonization was taken on mixture of pine needles + sawdust + Robinia wood chips and maximum time for spawn run was taken on pine needles + Robinia wood chips combination. In general, 1 to 2 flushes were obtained during the cultivation trial. Chopped paddy straw and rice bran supplemented with crushed corncobs and rice husk were found to favour maximum sporophore production (Rangad, 1981). Use of

sawdust for maximum sporophore production has been recommended by many workers (Pawlak and Siwulski, 2001; Gavrilova and Lysenkova, 1988; Sharma *et al.*, 2005). Li (1989) successfully cultivated *F. velutipes* on maize cobs. However, it was found that spawn run on maize cobs took longer time as compared with seed hulls. Sharma *et al.* (2006) recorded the effects of supplementation on the yield of *F. velutipes*. They noticed that supplementation with 10 per cent rice bran supported fastest mycelial growth in comparison to other supplements such as soybean meal, cotton seed cake and deoiled soybean.

In general, the complete colonization of substrate by *L. edodes* was achieved in 42-68 days. Minimum time for substrate colonization was recorded in the mixture of sawdust + Robinia wood chips + 5 per cent rice bran followed by pine needles + Robinia wood chips + sawdust. Maximum time for complete colonization was observed on corncobs. Whereas, minimum time for complete browning of substrate was observed on mixture of sawdust + Robinia wood chips + 5 per cent rice bran and sawdust + Eucalyptus wood chips + 5 per cent rice bran. Maximum time for browning was taken on mixture of pine needles + Robinia wood chips+ sawdust followed by combination of sawdust + Eucalyptus wood chips + 5 per cent rice bran. Maximum time for complete browning was taken on mixture of pine needles + Robinia wood chips + sawdust. However, browning was not observed on pine needles whereas slow spawn run was observed on pasteurized compost where the mycelial run stopped after few days and the bags got contaminated. Pinning was only observed on sawdust and its combination with wood chips and 5 per cent rice bran. Among these, maximum time for initiation of pinning was noticed on sawdust alone whereas minimum time was observed on mixture of sawdust + 5 per cent rice bran + Robinia wood chips. Maximum number of fruiting bodies and yield were recorded on mixture of sawdust + Alnus wood chips + 5 per cent rice bran and minimum on sawdust alone. During the course of experiment, only one flush was obtained. Contrary to the results, Brodziak and Wazyny (1980) have reported the successful cultivation of *L. edodes* on pine bark and sawdust. However, in present study pine needles alone were not recorded as suitable substrate for the growth of *L. edodes* probably due to presence of some inhibitory compounds which were inhibitory to the growth of the fungus. Besides, Brodziak and Wazyny (1980) also reported the cultivation of *L. edodes* on various substrates and their combinations such as rye straw, bark and sawdust of oak and alder. Use of sawdust alone and its supplementation with various

supplements have also been reported by Tan and Chang (1989) and Diehle and Royse (1986). Successful cultivation of *L. edodes* has also been reported on non -fermented wood shavings (Mata, 1992), oak log (Shukla, 1994), wheat straw (Mata and Gaitan, 2004) and crushed maize cobs alone and in combination with sawdust and rice bran (Eira *et al.*, 2005). Kaur and Lakhanpal (1995) found rapid mycelial colonization and good yield of the fungus on mixture of sawdusts of Eucalyptus and Poplus. Contrary to the results of present study, they got 3 flushes in all. However, number of fruiting bodies were less at third flush in comparison to other two flushes. In the present study, five stages of mycelial growth were observed viz., mycelial growth, mycelial coat formation, mycelial bump formation, pigmentation and coat-hardening stage which were also recorded by Wu (1993) and Oie (1996).

Suitability of sawdust and available agricultural wastes as substrate for cultivation of *G. lucidum* has been reported by Triratana *et al.* (1991); Siwulski and Sobieralski (2001); Sharma and Rai (2002); Dadwal and Jamaluddin (2004); and Tiwari *et al.* (2004). Mishra and Singh (2008) have reported high yield and biological efficiency of *G. lucidum* on wheat straw supplemented with 5 per cent rice bran.

In the present study, all the substrates tried except, pine needles and pasteurized compost, resulted in fruiting body production, though spawn run was also noticed on pine needles and pasteurized compost. Highest yield was recorded with mixture of sawdust + Alnus wood chips + 5 per cent rice bran followed by sawdust + Eucalyptus wood chips + 5 per cent rice bran. Moderately good yield was recorded on sawdust and poor results with respect to yield were recorded on wheat straw alone. In all two to three flushes were obtained, except for wheat straw and mixture of pine needles and wheat straw where only one flush was obtained. However, minimum time for substrate colonization was observed on mixture of pine needles + Robinia wood chips + sawdust followed by sawdust + 5 per cent rice bran + Alnus wood chips. Maximum time for substrate colonization was observed on corncobs. Minimum time for primordial formation was noticed on sawdust + Eucalyptus wood chips + 5 per cent rice bran and maximum on mixture of pine needles and wheat straw. Maturity of sporophores took minimum time on mixture of wheat straw and corncobs and mixture of sawdust + 5 per cent rice bran and Robinia wood chips and maximum time on sawdust alone. However, maximum number of fruiting bodies were obtained on sawdust + Robinia wood chips + 5 per cent rice bran substrate

and sawdust + Alnus wood chips + 5 per cent rice bran. Better performance of *G. lucidum* was observed in *Delonix regia* (Tiwari *et al.*, 2004). Therefore, readily available sawdust can be exploited successfully in combination with other cheap, easily available and economical sources as supplements for commercial cultivation of specialty mushrooms.

Chapter-6

SUMMARY

The present investigations entitled **"Studies on cultural requirements and cultivation technology of some medicinal mushrooms"** were carried out during 2007-09. Six medicinal mushrooms namely, *Pleurotus ostreatus, Pleurotus eryngii, Auricularia polyrticha, Lentinula edodes, Flammulina velutipes* and *Ganoderma lucidum* were evaluated with respect to their cultural requirements and the cultivation aspect. Cultural studies of all the six test fungi were carried out under *in vitro* conditions. It was observed that *G. lucidum* and *P. ostreatus* in general showed strandy type of growth whereas mycelial growth of *L. edodes* was silky and strandy, cottony and strandy in *F. velutipes*, fluffy growth in *P. eryngii* and appressed type in case of *A. polytricha*.

Among six different solid media tested, PDA was adjudged to be the best medium for maximum radial growth of *P. ostreatus* and *G. lucidum*; MEA in case of *A. polytricha, L. edodes* and *P. eryngii* and Glucose- asparagine agar medium for *F. velutipes.* However, Czapek's dox agar medium supported poor mycelial growth of all the test fungi. Evaluation of different liquid media revealed that good mycelial growth of *P. ostreatus* occurred on Richard's solution, *A. polytricha* on Elliott's solution and that of *F. velutipes, L. edodes, G. lucidum* and *P. eryngii* on Glucose- asparagine solution. Comparatively, poor growth of *G. lucidum* and *L. edodes* was recorded on Asthana and Hawker's solution, and that of *P. ostreatus. P. eryngii, A. polytricha* and *F.velutipes* on Thornton's solution.

All the test fungi were found to grow over wide range of temperature (5-35^{0}C) with the exception of *A. polytricha* and *G. lucidum* where this growth range was between 10- 35^{0}C. However, the optimum temperature for the vegetative growth of all the test fungi was recorded to be 25^{0}C. The study revealed that extremely low (below 15^{0}C) and high temperature (35^{0}C and above) were deleterious for the growth of fungi. The best pH for mycelial growth of *F. velutipes* and *P. eryngii* was recorded as 5.5 whereas it was 5.0 for *G. lucidum*, 6.0 for *P. ostreatus*, 6.5 for *A. polytricha* and 4.5 for *L. edodes.* All the six test fungi showed poor growth at alkaline pH and among these, *A. polytricha* was recorded to show no growth at extreme pH of 9.0.

Out of ten carbon sources evaluated, sucrose followed by dextrose were found as the best carbon sources for vegetative growth of *F. velutipes* as against starch for *P. ostreatus* and *A. polytricha* and glucose for *G. lucidum, P. eryngii* and *L. edodes*. Sugar alcohol, mannitol was found to be the least preferred carbon source by all the test fungi. Amongst fifteen different nitrogen sources, maximum mycelial growth of *A. polytricha* and *G. lucidum* was recorded with the addition of ammonium chloride that of *P. eryngii* and *F. velutipes* with leucine, *P. ostreatus* with ammonium nitrate and *L. edodes* with the addition of ammonium sulphate as nitrogen source. However, significantly minimum growth was recorded with addition of sodium nitrite whereas, mycelium of *A. polytricha* failed to grow when transferred to basal medium containing sodium nitrite as nitrogen source.

Of the different trace elements tested, 2 ppm ferrous sulphate supported significantly maximum mycelial growth of *P. ostreatus* and *P. eryngii* whereas, addition of 2 ppm manganese sulphate was found to support maximum growth of *G. lucidum, L. edodes* and *F. velutipes*. In case of *A. polytricha*, addition of 5 ppm ammonium molybdate was found to support good mycelial growth. Amongst different vitamin sources evaluated, thiamine at 0.025 ppm concentration proved to be the best vitamin supporting maximum mycelial growth of *G. lucidum, L. edodes* and *F. velutipes* whereas, same vitamin at 0.05 ppm concentration was found to be best source for mycelial growth of *A. polytricha, P. ostreatus* and *P. eryngii* followed by biotin in all the cases.

Screening of different growth regulators revealed that 50 ppm gibberellic acid supported significantly maximum growth of all the test fungi except *P. ostreatus* where 25 ppm gibberellic acid was found to support maximum growth of the fungus in comparison to control containing no growth regulator followed by 50 ppm kinetin (*F. velutipes*, *A. polytricha* and *P. ostreatus*) and 25 ppm kinetin (*G. lucidum, P. eryngii* and *L. edodes*).

Out of all the substrates tested for spawn production, only grain substrates showed their suitability with regards to rate of mycelial growth on the grain substrates, mixture of wheat grains+ bajra grains supported good growth of *L. edodes, F. velutipes. P. eryngii* and *G. lucidum*. However, bajra grains were recorded to support maximum growth of *A. polytricha* and *P. ostreatus*. Treatments containing wheat grains + gur was in general least preferred substrate.

However, *L. edodes* and *G. lucidum* showed poor mycelial run on wheat straw.

Evaluation of substrates for fruit body production revealed that maximum mycelial growth, yield and sporophore number of *P. eryngii* occurred on wheat straw and on mixture of wheat straw + corncobs whereas, pine needles were found to support significantly minimum yield. Fastest mycelial growth of *P. ostreatus* was observed on wheat straw whereas, mixture of sawdust + 5 per cent rice bran + Robinia wood chips was recorded to give highest sporophore yield. Minimum yield was again recorded on pine needles.

Fastest mycelial colonization of *F. velutipes* and *G. lucidum* was found on the mixture of pine needles + Robinia wood chips + sawdust. However, maximum yield of *F. velutipes* was recorded on sawdust + Eucalyptus wood chips + 5 per cent rice bran and 1-2 flushes of sporophores were observed. Maximum sporophore yield of *G. lucidum* was recorded on sawdust + Alnus wood chips supplemented with 5 per cent rice bran whereas, on pine needles and pasteurized compost no fruiting was observed. Two to three flushes were obtained, in general.

In case of *L. edodes*, fastest mycelial run was observed on substrate containing mixture of sawdust + Robinia wood chips + 5 per cent rice bran closely followed by mixture of pine needles + Robinia wood chips + sawdust. However, fruit body production was recorded only on sawdust and its supplementation with 5 per cent rice bran. Among these treatments, mixture of sawdust + 5 per cent rice bran + Alnus wood chips was recorded to yield maximum sporophore. Only one flush of fruit bodies was recorded.

Chapter-7

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DR. YS PARMAR UNIVERSITY OF HORTICULTURE AND FORESTRY
NAUNI, SOLAN (H.P.) 173 230
DEPARTMENT OF MYCOLOGY AND PLANT PATHOLOGY

Title of thesis	**:**	**Studies on cultural requirements and cultivation technology of some medicinal mushrooms**
Name of student	**:**	Pooja Dhiman
Admission Number	**:**	H-2007-31-M
Name of Major Advisor	**:**	Dr C D Thapa
Major field	**:**	Plant Pathology
Minor field(s)	**:**	(i) Biochemistry (ii) Entomology
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ABSTRACT

The present investigations entitled "Studies on cultural requirements and cultivation technology of some medicinal mushrooms" was undertaken to understand the cultural requirements, spawn production technique of some medicinal mushrooms viz., *Auricularia polytricha, Pleurotus ostreatus, Pleurotus eryngii, Flammulina velutipes, Lentinula edodes* and *Ganoderma lucidum* and above all their cultivation technique so as to commercialize the cultivation of later five mushrooms. Different solid media were tried for maintaining the pure cultures and PDA and MEA were found to support maximum radial growth. In case of various liquid media, Glucose- asparagine solution was found to support maximum mycelial growth of *F. velutipes, L. edodes, P. eryngii* and *G. lucidum* while Elliott's and Richard's solution supported good growth in case of *A. polytricha* and *P. ostreatus*, respectively. An optimum temperature of 25^0C and pH in acidic (4.5) to slightly acidic (6.5) range were found to be preferred by these fungi. Glucose (a monosaccharide), sucrose (a disaccharide) and starch (a polysaccharide) were recorded as good carbon sources. In case of nitrogen sources, ammonical nitrogen and among amino acids, leucine, alanine and aspartic acid supported good growth. Trace elements viz., molybdenum, iron and manganese, vitamins viz., thiamine and biotin, and growth regulators viz., gibberellic acid supported best mycelial growth of the test fungi. For spawn production, grain spawn was evaluated as good substrate. While for cultivation, good yield was recorded using supplemented (5 per cent and wood chips) sawdust in all the test fungi while wheat straw was recorded to give good yield in case of *P. ostreatus* and *P. eryngii* that could be recommended for commercial cultivation as they showed superior yield than other substrates.

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

Countersigned

Professor and Head
Department of Mycology and Plant Pathology
Dr. YSP, UHF, Nauni, Solan (H P)

APPENDIX-I

Composition of different media used in the present study

1. Solid media

Constituent	Quantity (g)
A. Potato dextrose agar	
Peeled potato	200
Dextrose	20
Agar	20
Distilled water	1000ml
B. Glucose-asparagine agar	
Glucose	30
Asparagine	1
Agar	20
Magnesium sulphate	0.5
Potassium-di-hydrogen phosphate	1.5
Distilled water	1000ml
C. Compost extract agar	
Synthetic compost	250
Agar	20
Distilled water	1000ml
D. Czapek's-dox agar	
Sodium carbonate	2
Di-potassium hydrogen phosphate	1
Magnesium sulphate	0.5
Potassium chloride	0.5
Sucrose	30
Ferrous sulphate	0.01
Agar	20
Distilled water	1000ml
E. Dimmick's agar	
Glucose	10
Di-potassium hydrogen phosphate	0.5
Sodium chloride	0.5
Magnesium sulphate	0.2
Sodium nitrate	2
Agar	20
Distilled water	1000ml
F. Malt extract agar	
Malt extract	25
Agar	20
Distilled water	1000ml

2. Liquid media

Constituent	Quantity (g)
A. Asthana and Hawker's	
Potassium nitrate	3.5
Potassium di-hydrogen phosphate	1.75
Magnesium sulphate	0.75
Glucose	5
Distilled water	1000ml
B. Glucose- asparagine solution	
Glucose	30
Asparagine	1
Magnesium sulphate	0.5
Potassium di-hydrogen phosphate	1.5
Distilled water	1000ml
C. Thornton solution	
Di-potassium hydrogen phosphate	1
Magnesium sulphate	0.2
Calcium chloride	0.1
Sodium chloride	0.1
Ferric chloride	0.002
Asparagine	0.5
Potassium nitrate	0.5
Mannitol	1
Distilled water	1000ml
D. Sabouraud's solution	
Glucose	40
Peptone	10
Distilled water	1000ml
E. Richard's solution	
Potassium nitrate	10
Potassium di-hydrogen phosphate	5
Magnesium sulphate	2.5
Ferrous chloride	0.02
Sucrose	50
Distilled water	1000ml
F. Elliott's solution	
Potassium di-hydrogen phosphate	1.36
Sodium carbonate	1.06
Magnesium sulphate	0.5
Dextrose	5.0
Asparagine	1
Distilled water	1000ml

APPENDIX-II

Amount of different carbon sources added to the basal medium individually in order to replace the carbon source of the medium

Carbon Source	Molecular weight	Quantity (g*) per litre of basal medium
A. Richard's medium		
Lactose (C_{12} $H_{22}O_{11}$)	360.31	52.60
Glucose (C_6 $H_{12}O_6$)	180.15	52.62
Xylose ($C_5H_{10}O_5$)	150.13	52.63
Sucrose (C_{12} $H_{22}O_{11}$)	342.30	49.99
Fructose (C_6 $H_{12}O_6$)	180.15	52.63
Galactose (C_6 $H_{12}O_6$)	180.15	52.63
Starch (C_6 $H_{12}O_6$)	182.17	53.22
Mannitol ($C_6H_{14}O_6$)	182.18	53.26
Maltose (C_{12} $H_{22}O_{11}$)	342.12	49.97
Dextrose (C_6 $H_{12}O_6$)	180.15	52.64
* To replace 50g sucrose in Richard's medium		
B. Elliott's medium		
Lactose (C_{12} $H_{22}O_{11}$)	360.31	5.0
Glucose (C_6 $H_{12}O_6$)	180.15	5.0
Xylose ($C_5H_{10}O_5$)	150.13	5.0
Sucrose (C_{12} $H_{22}O_{11}$)	342.30	4.75
Fructose (C_6 $H_{12}O_6$)	180.15	5.0
Galactose (C_6 $H_{12}O_6$)	180.15	5.0
Starch (C_6 $H_{12}O_6$)	182.17	5.06
Mannitol ($C_6H_{14}O_6$)	182.18	5.06
Maltose (C_{12} $H_{22}O_{11}$)	342.12	4.75
Dextrose (C_6 $H_{12}O_6$)	180.15	5.0
* To replace 5g dextrose in Elliott's medium		
C. Glucose-asparagine medium		
Lactose (C_{12} $H_{22}O_{11}$)	360.31	30
Glucose (C_6 $H_{12}O_6$)	180.15	29.99
Xylose ($C_5H_{10}O_5$)	150.13	30
Sucrose (C_{12} $H_{22}O_{11}$)	342.30	28.5
Fructose (C_6 $H_{12}O_6$)	180.15	29.99
Galactose (C_6 $H_{12}O_6$)	180.15	29.99
Starch (C_6 $H_{12}O_6$)	182.17	30.33
Mannitol ($C_6H_{14}O_6$)	182.18	30.33
Maltose (C_{12} $H_{22}O_{11}$)	342.12	30.0
Dextrose (C_6 $H_{12}O_6$)	180.15	30.01
* To replace 30g glucose in Glucose-asparagine medium		

APPENDIX- III

Amount of different nitrogen sources added to the basal medium individually in order to replace the nitrogen source of the medium

Nitrogen Source	Molecular weight	Quantity (g)* per litre of basal medium
A. Richard's medium		
Ammonium nitrate (NH_4NO_3)	80.04	7.88
Ammonium phosphate (NH_6PO_4)	115.03	11.33
Ammonium sulphate ($H_8N_2SO_4$)	132.14	6.51
Potassium nitrate (KNO_3)	101.10	9.96
Urea (CH_4N_2O)	60.06	2.96
Ammonium chloride (NH_4Cl)	53.49	5.27
L (-) Proline ($C_5H_9NO_2$)	115.13	11.34
Alanine ($C_3H_7NO_2$)	89.09	8.78
L- Aspartic acid ($C_4H_7NO_4$)	133.10	13.11
Valine ($C_5H_{11}NO_2$)	117.15	11.54
Sodium nitrate ($NaNO_3$)	83.99	8.25
L-Histidine ($C_6H_9N_3O_2$)	155.19	5.09
L (-) Leucine ($C_6H_{13}NO_2$)	131.18	12.93
D L- Methionine ($C_5H_{11}NO_2S$)	149.21	14.70
Sodium nitrite ($NaNO_2$)	69.90	6.80
*To replace 10g potassium nitrate in Richard's medium		
B. Glucose-asparagine and Elliott's media		
Ammonium nitrate (NH_4NO_3)	80.04	1.09
Ammonium phosphate (NH_6PO_4)	115.03	1.56
Ammonium sulphate ($H_8N_2SO_4$)	132.14	0.89
Potassium nitrate (KNO_3)	101.10	1.37
Urea (CH_4N_2O)	60.06	0.41
Ammonium chloride (NH_4Cl)	53.49	0.73
L (-) Proline ($C_5H_9NO_2$)	115.13	1.56
Alanine ($C_3H_7NO_2$)	89.09	1.21
L- Aspartic acid ($C_4H_7NO_4$)	133.10	1.80
Valine ($C_5H_{11}NO_2$)	117.15	1.59
Sodium nitrate ($NaNO_3$)	83.99	1.39
L-Histidine ($C_6H_9N_3O_2$)	155.19	0.70
L (-) Leucine ($C_6H_{13}NO_2$)	131.18	1.78
D L- Methionine ($C_5H_{11}NO_2S$)	149.21	2.02
Sodium nitrite ($NaNO_2$)	69.90	0.94
*To replace 1g asparagine in Glucose-asparagine and Elliott's media		

APPENDIX – IV

ANOVA 1: Analysis of variance for evaluation of different liquid media for mycelial growth of *Pleurotus ostreatus* (Table 1.1)

Source of variance	DF	SS	MS	F
Treatment	5	2127.99	425.59	366.65
Error	12	13.93	1.16	
Total	17	2141.92	125.99	

ANOVA 2: Analysis of variance for evaluation of different liquid media for mycelial growth of *Lentinula edodes* (Table 1.1)

Source of variance	DF	SS	MS	F
Treatment	5	7446.66	1489.33	1058.82
Error	12	16.88	1.41	
Total	17	7463.53	439.03	

ANOVA 3: Analysis of variance for evaluation of different liquid media for mycelial growth of *Flammulina velutipes* (Table 1.1)

Source of variance	DF	SS	MS	F
Treatment	5	5668.05	1133.61	856.99
Error	12	15.85	1.32	
Total	17	5683.92	334.35	

ANOVA 4: Analysis of variance for evaluation of different liquid media for mycelial growth of *Pleurotus eryngii* (Table 1.1)

Source of variance	DF	SS	MS	F
Treatment	5	3408.14	681.63	501.17
Error	12	16.13	1.34	
Total	17	3424.26	201.43	

ANOVA 5: Analysis of variance for evaluation of different liquid media for mycelial growth of *Auricularia polytricha* (Table 1.1)

Source of variance	DF	SS	MS	F
Treatment	5	2189.33	437.87	606.28
Error	12	8.67	0.72	
Total	17	2198.0	129.29	

ANOVA 6: Analysis of variance for evaluation of different liquid media for mycelial growth of *Ganoderma lucidum* (Table 1.2)

Source of variance	DF	SS	MS	F
Treatments	5	2189.33	437.87	606.28
Error	12	8.67	0.72	
Total	17	2198.0	129.29	

ANOVA 7: Analysis of variance for evaluation of different solid media for mycelial growth of *Pleurotus ostreatus* (Table 1.2 a)

Source of variance	DF	SS	MS	F
Treatments	5	5347.2	1069.4	780.40
Interval	2	25009	12505	9124.96
Treatments*Interval	10	1070	107	78.08
Treatments * Interval * Replications	36	49.33	1.37	s
Total	53	31476		

ANOVA 8: Analysis of variance for evaluation of different solid media for mycelial growth of *Pleurotus eryngii* (Table 1.2 a)

Source of variance	DF	SS	MS	F
Treatments	5	2333.9	466.79	406.56
Interval	2	32178	16089	14013.05
Treatments*Interval	10	817.44	81.54	71.02
Treatments * Interval * Replications	36	41.33	1.15	
Total	53	35369		

ANOVA 9: Analysis of variance for evaluation of different solid media for mycelial growth of *Flammulina velutipes* (Table 1.2 a)

Source of variance	DF	SS	MS	F
Treatments	5	5276.7	1055.3	949.80
Interval	2	29617	14809	13327.85
Treatments*Interval	10	617.89	61.79	55.61
Treatments * Interval * Replications	36	40.0	1.11	
Total	53	35552		

ANOVA 10: Analysis of variance for evaluation of different solid media for mycelial growth of *Auricularia polytricha* (Table 1.2 a)

Source of variance	DF	SS	MS	F
Treatments	5	11203	22407	3102.46
Interval	2	14718	73591	10189.46
Treatments*Interval	10	1217.9	121.79	168.63
Treatments * Interval * Replications	36	26.0	0.72	
Total	53	27165		

ANOVA 11: Analysis of variance for evaluation of different solid media for mycelial growth of *Lentinula edodes* (Table 1.2 a)

Source of variance	DF	SS	MS	F
Treatments	5	27928.53	5585.71	8378.56
Interval	2	24807.49	6201.87	9302.81
Treatments*Interval	10	2991.58	149.58	224.37
Treatments * Interval * Replications	36	40.0	0.67	
Total	53	52618		

ANOVA 12: Analysis of variance for evaluation of different solid media for mycelial growth of *Ganoderma lucidum* (Table 1.2 a)

Source of variance	DF	SS	MS	F
Treatments	5	34946	6989.2	4885.63
Interval	2	25151	8383.7	5860.44
Treatments*Interval	10	4911.4	327.4	228.88
Treatments * Interval * Replications	36	68.67	1.43	
Total	53	65077		

ANOVA 13: Analysis of variance for mycelial growth of *Pleurotus ostreatus* at different temperature regimes (Table 2)

Source of variance	DF	SS	MS	F
Treatments	6	14720.63	2453.22	2261.57
Error	14	15.19	1.08	
Total	20	14735.82	736.79	

ANOVA 14: Analysis of variance for mycelial growth of *Flammulina velutipes* at different temperature regimes (Table 2)

Source of variance	DF	SS	MS	F
Treatments	6	11497.95	1916.32	3147.31
Error	14	8.52	0.61	
Total	20	11506.47	575.32s	

ANOVA 15: Analysis of variance for mycelial growth of *Lentinula edodes* at different temperature regimes (Table 2)

Source of variance	DF	SS	MS	F
Treatments	6	19171.11	3195.18	5614.17
Error	14	7.97	0.57	
Total	20	19179.08	958.95	

ANOVA 16: Analysis of variance for mycelial growth of *Pleurotus eryngii* at different temperature regimes (Table 2)

Source of variance	DF	SS	MS	F
Treatments	6	14753.35	2458.89	3499.63
Error	14	9.84	0.70	
Total	20	14763.18	738.16	

ANOVA 17: Analysis of variance for mycelial growth of *Auricularia polytricha* at different temperature regimes (Table 2)

Source of variance	DF	SS	MS	F
Treatments	6	4490.57	748.43	628.68
Error	14	16.67	1.19	
Total	20	4507.24	225.36	

ANOVA 18: Analysis of variance for mycelial growth of *Ganoderma lucidum* at different temperature regimes (Table 2)

Source of variance	DF	SS	MS	F
Treatments	6	41423.93	6903.99	4030.32
Error	14	23.98	1.71	
Total	20	41447.92	2072.39	

ANOVA 19: Analysis of variance for mycelial growth of *Pleurotus ostreatus* at different hydrogen - ion concentrations (Table 3)

Source of variance	DF	SS	MS	F
Treatments	10	19784.23	1978.42	3475.0
Error	22	12.53	0.57	
Total	32	19796.75	618.65	

ANOVA 20: Analysis of variance for mycelial growth of *Flammulina velutipes* at different hydrogen - ion concentrations (Table 3)

Source of variance	DF	SS	MS	F
Treatments	10	12625.16	1262.52	1275.79
Error	22	21.77	0.98	
Total	32	12646.94	395.22	

ANOVA 21: Analysis of variance for mycelial growth of *Lentinula edodes* at different hydrogen - ion concentrations (Table 3)

Source of variance	DF	SS	MS	F
Treatments	10	28668.37	2866.84	3387.93
Error	22	18.62	0.85	
Total	32	28686.99	896.67	

ANOVA 22: Analysis of variance for mycelial growth of *Pleurotus eryngii* at different hydrogen - ion concentrations (Table 3)

Source of variance	DF	SS	MS	F
Treatments	10	18462.28	1846.23	1859.62
Error	22	21.84	0.99	
Total	32	18484.13	577.63	

ANOVA 23: Analysis of variance for mycelial growth of *Auricularia polytricha* at different hydrogen - ion concentrations (Table 3)

Source of variance	DF	SS	MS	F
Treatments	10	8256.91	825.69	1362.39
Error	22	13.33	0.61	
Total	32	8270.24	258.45	

ANOVA 24: Analysis of variance for mycelial growth of *Ganoderma lucidum* at different hydrogen - ion concentrations (Table 3)

Source of variance	DF	SS	MS	F
Treatments	10	48878.24	4887.82	5484.08
Error	22	19.61	0.89	
Total	32	48897.85	1528.06	

ANOVA 25: Analysis of variance for mycelial growth of *Pleurotus ostreatus* on various carbon sources (Table 4)

Source of variance	DF	SS	MS	F
Treatments	10	10892.78	1089.28	1344.88
Error	22	17.82	0.81	
Total	32	10910.60	340.96	

ANOVA 26: Analysis of variance for mycelial growth of *Flammulina velutipes* on various carbon sources (Table 4)

Source of variance	DF	SS	MS	F
Treatments	10	15891.38	1589.44	1128.24
Error	22	30.99	1.41	
Total	32	15922.37	497.57	

ANOVA 27: Analysis of variance for mycelial growth of *Lentinula edodes* on various carbon sources (Table 4)

Source of variance	DF	SS	MS	F
Treatments	10	19380.0	1890.02	1918.0
Error	22	22.22	1.01	
Total	32	19402.23	606.32	

ANOVA 28: Analysis of variance for mycelial growth of *Pleurotus eryngii* on various carbon sources (Table 4)

Source of variance	DF	SS	MS	F
Treatments	10	23872.44	2387.24	2067.43
Error	22	25.40	1.15	
Total	32	23897.84	746.81	

ANOVA 29: Analysis of variance for mycelial growth of *Auricularia polytricha* on various carbon sources (Table 4)

Source of variance	DF	SS	MS	F
Treatments	10	11066.91	1106.69	1259.34
Error	22	19.33	0.88	
Total	32	11086.24	346.45	

ANOVA 30: Analysis of variance for mycelial growth of *Ganoderma lucidum* on various carbon sources (Table 4)

Source of variance	DF	SS	MS	F
Treatments	10	24477.67	2447.77	3056.02
Error	22	17.62	0.80	
Total	32	24495.24	765.48	

ANOVA 31: Analysis of variance for mycelial growth of *Pleurotus ostreatus* on various nitrogen sources (Table 5)

Source of variance	DF	SS	MS	F
Treatments	15	23805.31	1587.02	1647.94
Error	32	30.82	0.96	
Total	47	23836.12	507.12	

ANOVA 32: Analysis of variance for mycelial growth of *Flammulina velutipes* on various nitrogen sources (Table 5)

Source of variance	DF	SS	MS	F
Treatments	15	26210.36	1747.36	1323.89
Error	32	42.24	1.32	
Total	47	26252.59	558.57	

ANOVA 33: Analysis of variance for mycelial growth of *Lentinula edodes* on various nitrogen sources (Table 5)

Source of variance	DF	SS	MS	F
Treatments	15	51190.49	3412.69	2879.95
Error	32	37.92	1.18	
Total	47	51228.42	1089.97	

ANOVA 34: Analysis of variance for mycelial growth of *Pleurotus eryngii* on various nitrogen sources (Table 5)

Source of variance	DF	SS	MS	F
Treatments	15	48860.02	3257.34	2370.97
Error	32	43.96	1.37	
Total	47	48903.98	1040.51	

ANOVA 35: Analysis of variance for mycelial growth of *Auricularia polytricha* on various nitrogen sources (Table 5)

Source of variance	DF	SS	MS	F
Treatments	15	20822.33	1388.16	546.16
Error	32	81.33	2.54	
Total	47	20903.66	444.76	

ANOVA 36: Analysis of variance for mycelial growth of *Ganoderma lucidum* on various nitrogen sources (Table 5)

Source of variance	DF	SS	MS	F
Treatments	15	90643.06	6042.87	5879.22
Error	32	32.89	1.03	
Total	47	90675.95	1929.28	

ANOVA 37: Analysis of variance for mycelial growth of *Pleurotus ostreatus* on various trace elements (Table 5)

Source of variance	DF	SS	MS	F
Treatments	16	4377.85	273.62	297.38
Error	34	31.28	0.92	
Total	50	4409.13	88.18	

ANOVA 38: Analysis of variance for mycelial growth of *Pleurotus eryngii* on various trace elements (Table 6a)

Source of variance	DF	SS	MS	F
Treatments	16	2411.15	150.69	155.36
Error	34	32.98	0.97	
Total	50	2444.13	48.88	

ANOVA 39: Analysis of variance for mycelial growth of *Ganoderma lucidum* on various trace elements (Table 6a)

Source of variance	DF	SS	MS	F
Treatments	16	9891.15	618.20	716.57
Error	34	29.33	0.86	
Total	50	9920.48	198.41	

ANOVA 40: Analysis of variance for mycelial growth of *Auricularia polytricha* on various trace elements (Table 6a)

Source of variance	DF	SS	MS	F
Treatments	16	2275.54	142.22	154.74
Error	34	31.25	0.92	
Total	50	2306.79	46.14	

ANOVA 41: Analysis of variance for mycelial growth of *Flammulina velutipes* on various trace elements (Table 6b)

Source of variance	DF	SS	MS	F
Treatments	16	12993.41	812.09	276.72
Error	34	49.77	2.94	
Total	50	13093.19	261.86	

ANOVA 42: Analysis of variance for mycelial growth of *Lentinula edodes* on various trace elements (Table 6b)

Source of variance	DF	SS	MS	F
Treatments	16	8126.03	507.88	508.12
Error	34	33.98	1.0	
Total	50	1860.02	163.20	

ANOVA 43: Analysis of variance for effect of vitamins on the mycelial growth of *Pleurotus ostreatus* (Table 7a)

Source of variance	DF	SS	MS	F
Treatments	18	9174.49	509.69	394.77
Error	38	49.06	1.29	
Total	56	9223.55	164.71	

ANOVA 44: Analysis of variance for effect of vitamins on the mycelial growth of *Pleurotus eryngii* (Table7a)

Source of variance	DF	SS	MS	F
Treatments	18	8133.3	451.85	477.05
Error	38	35.99	0.95	
Total	56	8169.29	145.88	

ANOVA 45: Analysis of variance for effect of vitamins on the mycelial growth of *Ganoderma lucidum* (Table 7a)

Source of variance	DF	SS	MS	F
Treatments	18	9602.17	533.45	689.90
Error	38	29.77	0.78	
Total	56	9631.95	171.99	

ANOVA 46: Analysis of variance for effect of vitamins on the mycelial growth of *Auricularia polytricha* (Table 7b)

Source of variance	DF	SS	MS	F
Treatments	18	4332.18	240.68	303.66
Error	38	29.32	0.78	
Total	56	4362.60	77.89	

ANOVA 47: Analysis of variance for effect of vitamins on the mycelial growth of *Flammulina velutipes*(Table 7b)

Source of variance	DF	SS	MS	F
Treatments	18	8699.95	483.33	652.32
Error	38	28.16	0.74	
Total	56	8728.10	155.86	

ANOVA 48: Analysis of variance for effect of vitamins on the mycelial growth of *Lentinula edodes* (Table 7b)

Source of variance	DF	SS	MS	F
Treatments	18	13183.95	732.44	715.32
Error	38	38.91	1.02	
Total	56	13222.86	236.12	

ANOVA 49: Analysis of variance for effect of growth regulators on the mycelial growth of *Pleurotus ostreatus* (Table 8a)

Source of variance	DF	SS	MS	F
Treatments	18	9241.66	513.43	575.46
Error	38	33.90	0.89	
Total	56	9275.56	165.64	

ANOVA 50: Analysis of variance for effect of growth regulators on the mycelial growth of *Pleurotus eryngii* (Table 8a)

Source of variance	DF	SS	MS	F
Treatments	18	8105.71	450.32	647.88
Error	38	26.41	0.69	
Total	56	8132.12	145.22	

ANOVA 51: Analysis of variance for effect of growth regulators on the mycelial growth of *Ganoderma lucidum* (Table 8a)

Source of variance	DF	SS	MS	F
Treatments	18	5774.25	320.79	304.64
Error	38	40.02	1.05	
Total	56	5814.26	103.83	

ANOVA 52: Analysis of variance for effect of growth regulators on the mycelial growth of *Auricularia polytricha* (Table 8b)

Source of variance	DF	SS	MS	F
Treatments	18	8107.96	450.44	592.70
Error	38	28.88	0.76	
Total	56	8136.84	145.30	

ANOVA 53: Analysis of variance for effect of growth regulators on the mycelial growth of *Flammulina velutipes* (Table 8b)

Source of variance	DF	SS	MS	F
Treatments	18	2522.6	140.14	181.79
Error	38	29.29	0.77	
Total	56	2551.89	45.57	

ANOVA 54: Analysis of variance for effect of growth regulators on the mycelial growth of *Lentinula edodes* (Table 8b)

Source of variance	DF	SS	MS	F
Treatments	18	5006.70	278.15	371.26
Error	38	28.47	0.75	
Total	56	5035.17	89.91	

ANOVA 55: Analysis of variance for linear mycelial growth of *Lentinula edodes* on various substrates for spawn production (Table 9a)

Source of variance	DF	SS	MS	F
Treatments	7	5547.32	792.47	967.09
Interval	2	47568.78	23784.39	29025.02
Treatments*Interval	14	1493.89	106.71	130.22
Treatments * Interval * Replications	48	39.33	0.82	
Total	71	54731		

ANOVA 56: Analysis of variance for linear mycelial growth of *Flammulina velutipes* on various substrates for spawn production (Table 9a)

Source of variance	DF	SS	MS	F
Treatments	7	5282.8	754.68	987.95
Interval	2	36171	18085	23675.29
Treatments*Interval	14	389.86	27.85	36.45
Treatments * Interval * Replications	48	36.67	0.8	
Total	71	41880		

ANOVA 57: Analysis of variance for linear mycelial growth of *Pleurotus eryngii* on various substrates for spawn production (Table 9a)

Source of variance	DF	SS	MS	F
Treatments	7	15457	2208.1	2741.12
Interval	2	55705	27853	34575.57
Treatments*Interval	14	3129.4	223.53	277.48
Treatments * Interval * Replications	48	38.67	0.81	
Total	71	74330		

ANOVA 58: Analysis of variance for linear mycelial growth of *Auricularia polytricha* on various substrates for spawn production (Table 9b)

Source of variance	DF	SS	MS	F
Treatments	7	3116.99	445.28	291.46
Interval	2	22502.08	11251.04	7364.32
Treatments*Interval	14	513.47	36.68	24.01
Treatments * Interval * Replications	48	73.33	1.53	
Total	71	26205.88		

ANOVA 59: Analysis of variance for linear mycelial growth of *Ganoderma lucidum* on various substrates for spawn production (Table 9b)

Source of variance	DF	SS	MS	F
Treatments	7	4295.7	613.66	566.46
Interval	2	45135	22568	20831.55
Treatments*Interval	14	1391.0	99.36	91.71

Treatments * Interval * Replications	48	52.0	1.08	
Total	71	50874		

ANOVA 60: Analysis of variance for linear mycelial growth of *Pleurotus ostreatus* on various substrates for spawn production (Table 9b)

Source of variance	DF	SS	MS	F
Treatments	7	12064	1723.4	2640.15
Interval	2	55688	27844	42654.79
Treatments*Interval	14	3640.9	260.07	398.40
Treatments * Interval * Replications	48	31.33	0.65	
Total	71	71424		

ANOVA 61: Analysis of variance for effect of various substrates on time taken for complete spawn run of *Pleurotus eryngii* (Table 10a)

Source of variance	DF	SS	MS	F
Treatments	12	406.31	33.86	42.59
Error	26	20.67	0.79	
Total	38	426.97	11.24	

ANOVA 62: Analysis of variance for effect of various substrates on time taken for initiation of pinning in *Pleurotus eryngii* (Table 10a)

Source of variance	DF	SS	MS	F
Treatments	12	784.36	65.36	84.97s
Error	26	20.0	0.77	
Total	38	804.36	21.17	

ANOVA 63: Analysis of variance for effect of various substrates on the number of fruiting bodies of *Pleurotus eryngii* produced (Table 10a)

Source of variance	DF	SS	MS	F
Treatments	12	8263.07	688.59	189.12
Error	26	94.67	3.64	
Total	38	8357.74	219.94	

ANOVA 64: Analysis of variance for effect of various substrates on the yield of *Pleurotus eryngii* (Table 10a)

Source of variance	DF	SS	MS	F
Treatments	12	1087720.10	90643.34	31283.99
Error	26	75.33	2.89	
Total	38	1087795.44	28626.2	

ANOVA 65: Analysis of variance for effect of various substrates on time taken for complete spawn run of *Pleurotus ostreatus* (Table 10a)

Source of variance	DF	SS	MS	F
Treatments	12	372.56	31.05	31.86
Error	26	25.33	0.97	
Total	38	397.9	10.47	

ANOVA 66: Analysis of variance for effect of various substrates on time taken for initiation of pinning in *Pleurotus ostreatus* (Table 10a)

Source of variance	DF	SS	MS	F
Treatments	12	398.92	33.24	28.91
Error	26	24.67	0.95	
Total	38	423.59	11.15	

ANOVA 67: Analysis of variance for effect of various substrates on the number of fruiting bodies of *Pleurotus ostreatus* produced (Table 10a)

Source of variance	DF	SS	MS	F
Treatments	12	7568.97	630.75	599.98
Error	26	27.33	1.05	
Total	38	7596.31	199.91	

ANOVA 68: Analysis of variance for effect of various substrates on the yield of *Pleurotus ostreatus* (Table 10a)

Source of variance	DF	SS	MS	F
Treatments	12	819775.59	68314.63	53285.41
Error	26	33.33	1.28	
Total	38	819808.92	21573.92	

ANOVA 69: Analysis of variance for effect of various substrates on the time taken for complete spawn run of *Flammulina velutipes* (Table 10a)

Source of variance	DF	SS	MS	F
Treatments	12	320.92	26.74	26.08s
Error	26	26.67	1.03	
Total	38	347.59	9.15	

ANOVA 70: Analysis of variance for effect of various substrates on the time taken for initiation of pinning in *Flammulina velutipes* (Table 10a)

Source of variance	DF	SS	MS	F
Treatments	12	2447.74	203.98	180.80
Error	26	29.33	1.13	
Total	38	2477.08	65.19	

ANOVA 68: Analysis of variance for effect of various substrates on the yield of *Flammulina velutipes* (Table 10a)

Source of variance	DF	SS	MS	F
Treatments	12	8041.8	670.15	165.13
Error	26	105.52	4.08	
Total	38	8147.31	214.40	

ANOVA 69: Analysis of variance for effect of various substrates on the time taken for complete spawn run of *Ganoderma lucidum* (Table 10b)

Source of variance	DF	SS	MS	F
Treatments	12	943.9	78.66	56.81
Error	26	36.0	1.38	
Total	38	979.89	25.79	

ANOVA 70: Analysis of variance for effect of various substrates on the time taken for initiation of pinning in *Ganoderma lucidum* (Table 10b)

Source of variance	DF	SS	MS	F
Treatments	12	2267.59	188.97	163.77
Error	26	30.0	1.15	
Total	38	2297.59	60.46	

ANOVA 71: Analysis of variance for evaluation of various substrates for time taken for maturity of fruiting bodies of *Ganoderma lucidum* (Table 10b)

Source of variance	DF	SS	MS	F
Treatments	12	4682.26	390.19	380.43
Error	26	26.67	1.03	
Total	38	4708.92	123.92	

ANOVA 72: Analysis of variance for effect of various substrates on the number of fruiting bodies of *Ganoderma lucidum* produced (Table 10b)

Source of variance	DF	SS	MS	F
Treatments	12	200.67	16.72	36.23
Error	26	12.0	0.46	
Total	38	212.67	5.6	

ANOVA 73: Analysis of variance for effect of various substrates on the yield of *Ganoderma lucidum* (Table 10b)

Source of variance	DF	SS	MS	F
Treatments	12	404789.42	33732.45	2362.46
Error	26	371.24	14.28	
Total	38	405160.67	10662.12	

ANOVA 74: Analysis of variance for effect of various substrates on time taken for complete spawn run of *Lentinula edodes* (Table 10b)

Source of variance	DF	SS	MS	F
Treatments	12	10504.41	875.37	32.45
Error	26	701.33	26.97	
Total	38	11205.74	294.89	

ANOVA 75: Analysis of variance for effect of various substrates on time taken for complete browning of mycelial coat of *Lentinula edodes* (Table 10b)

Source of variance	DF	SS	MS	F
Treatments	12	4893.89	407.83	691.53
Error	26	15.33	0.59	
Total	38	4909.23	129.19	

ANOVA 76: Analysis of variance for effect of various substrates on the time taken for initiation of pinning in *Lentinula edodes* (Table 10b)

Source of variance	DF	SS	MS	F
Treatments	12	6810.92	567.58	1844.63
Error	26	8.0	0.31	
Total	38s	6818.92	179.45	

ANOVA 77: Analysis of variance for effect of various substrates on number of fruiting bodies of *Lentinula edodes* produced (Table 10b)

Source of variance	DF	SS	MS	F
Treatments	12	208.56	17.38	169.46
Error	26	2.67	0.10	
Total	38	211.23	5.56	

ANOVA 78: Analysis of variance for effect of various substrates on the yield of *Lentinula edodes* (Table 10b)

Source of variance	DF	SS	MS	F
Treatments	12	373238.23	31103.19	6969.58
Error	26	116.03	4.46	
Total	38	373354.26	9825.11	

Curriculum vitae

Name **:** **Pooja Dhiman**

Father's Name **:** Sh. K D Dhiman

Date of Birth **:** 14 January, 1986

Sex **:** Female

Marital status **:** Unmarried

Nationality **:** Indian

Educational qualifications:

Certificate/Degree	Class/Grade	Board/University	Year
Matriculation	First	HP Board, Dharamshala	2001
10+2	First	HP Board, Dharamshala	2003
B.Sc. (Horticulture)	First	UHF, Nauni, Solan (H.P)	2007

Whether sponsored by some state/ Central Govt./Univ./SAARC **:** No

Scholarship/Stipend/Fellowship, any other financial assistance received during the study period **:** University merit scholarship

(Pooja Dhiman)