STUDIES ON CULTURAL, BIOCHEMICAL CHARACTERIZATION AND PRODUCTION TECHNOLOGY OF *Lentinula edodes* (Berk.) Pegler.

Thesis

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By

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CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON CULTURAL, BIOCHEMICAL CHARACTERIZATION AND PRODUCTION TECHNOLOGY OF *Lentinula edodes* (Berk.) Pegler."** submitted in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE** with major in **PLANT PATHOLOGY** of the College of Post-Graduate Studies, G.B. Pant University of Agriculture and Technology, Pantnagar, is a record of *bona fide* research carried out by **Miss Smita Puri, Id. No. 31812**, under my supervision, and no part of the thesis has been submitted for any other degree or diploma.

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

(R.R. Dwivedi)
Chairman
Advisory Committee

C E R T I F I C A T E

We, the undersigned, members of the Advisory Committee of **Miss Smita Puri, Id. No. 31812**, a candidate for the degree of **MASTER OF SCIENCE IN AGRICULTURE** with major in **PLANT PATHOLOGY**, agree that the thesis entitled **"STUDIES ON CULTURAL, BIOCHEMICAL CHARACTERIZATION AND PRODUCTION TECHNOLOGY OF *Lentinula edodes* (Berk.) Pegler."** may be submitted in partial fulfilment of the requirements for the degree.

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CONTENTS

INTRODUCTION

Mushrooms belong to a separate group of organisms called fungi. They are non-green organisms lacking chlorophyll, cannot manufacture their own food from simple inorganic materials, such as water, carbondioxide and nitrates, using energy from the sun, as in the case with the green plants. They grow saprophytically or parasitically on other organisms and derive their food from complex organic materials found in dead or living tissues of plants and animals. The term mushroom can be broadly defined as “A mushroom is a macro-fungus with a distinctive fruiting body which can be either epigeous or hypogeous and large enough to be seen with the naked eye and to be picked by hand (Chang and Miles, 1992)”. Mushrooms belong to either Basidiomycetes or Ascomycetes. Their vegetative parts mainly consist of thread-like, long, thin mycelia which under suitable conditions form fruit bodies or sporocarps.

The mushrooms comprise a large heterogenous group having various shapes, sizes, colour, all quite different in characters, appearance and edibility. Out of this large group, with more than 2000 edible species, about 300 species belonging to 70 genera are reported from India. However, only a few have been brought under cultivation or commercial scale. Out of 2000 species of prime edible mushrooms, about 80 have been grown experimentally, 20 cultivated commercially and four to five species produced on industrial scale throughout the world (Chang and Miles, 1991) and about 300 of these mushrooms possess medicinal properties (Wasser and Weis, 1999). Mushrooms are cultivated in more than hundred countries with an estimated total production of 6.16 MT (Chang, 1999). China has become an enormous producer and consumer of cultivated edible mushrooms.

Mushrooms farming in India is hardly 3 decades old and with initial lag-phase, it has started showing upward trend. From a meagre 4000 tones production during 1985, India's total mushroom production reached 70,000 tons in 2003 (AICMIP Workshop, 2004).

The role of macro-fungi (mushrooms) as food is very well known. However, many edible mushrooms are receiving additional recognition for their medicinal and tonic qualities. On the basis of properties, the mushrooms are categorized as nutraceutical, nutriceutical and pharmaceutical. The term nutraceutical has been redefined by Zeisel in 1999 as a diet supplement that delivers a concentrated form of a presumed bioactive agent from a food at dosage levels exceeding those that could be obtained from normal food. The term nutriceutical is used for a new class of compounds that have been extracted from either the mycelium or the fruiting body of a mushroom, which is consumed in the form of capsules or tablets as a dietary supplement (not in the form of a food) and which has potential therapeutic applications. Nutriceutical possess medicinal as well as nutritional attributes and when a chemically defined preparation with medicinal properties is obtained from a naturally produced mushroom, it can be referred to as a pharmaceutical (Miles and Chang, 1997).

Lentinula edodes (Berk.) Pegler. also known as Shiitake or Oak mushroom. It is a fungus native to Japan, China and Korea. The Shiitake mushroom is the second most important edible mushroom in the world from the standpoint of production just after *Agaricus bisporus*. Its share in total world production of edible mushroom was 25.4 per cent in 1997 (Table 1).

Table 1 : World production of cultivated edible mushroom in 1986 and 1997

Species	Fresh weight (× 1000 t)				Increase (%)
	1986		1997		
Agaricus bisporus	1,227	(56.2%)	1,956	(31.8%)	59.4
Lentinula edodes	314	(14.4%)	1,564	(25.4%)	398.1
Pleurotus spp.	169	(7.7%)	876	(14.2%)	418.3
Auricularia spp.	119	(5.5%)	485	(7.9%)	307.6
Volvariella volvacea	178	(8.2%)	181	(3.0%)	1.7
Flammulina velutipes	100	(4.6%)	285	(4.6%)	130.0
Tremello fuciformis	40	(1.8%)	130	(2.1%)	225.0
Hypsizygus marmoreus	–	–	74	(1.2%)	–
Pholiota nameto	25	(1.1%)	56	(0.9%)	124.0
Grifola frondosa	–	–	33	(0.5%)	–
Others	10	(0.5%)	518	(8.4%)	5,080.0
Total	2,182	(100.0%)	6,158	(100.0%)	182.2

Source : Chang (1999)

The Shiitake mushroom is a white rot wood-decay fungus that naturally inhabits the dead wood of many hard wood trees species in Asia. The name 'Shiitake' is a Japanese word, "Shii" refers to the 'Shii tree” (*Castanopsis cuspidata*) and “take” stands for “fungus of” (Mushroom). It also has medicinal properties. For a long time, this mushroom has been valued for its unique taste and flavour and as a medicinal tonic. According to a Chinese folkfare, it was capable of generating stamina, curing colds, improving the circulation and lowering blood pressure. Recent studies showed that *Lentinula* is effective in lowering serum cholesterol levels and possessing antitumor and antiviral activities (Chang and Miles, 2004). It was also believed to prevent premature aging.

Many biologically active substances, in particular polysaccharides have been isolated from *Lentinula edodes,* which are particularly effective in retarding the progress of various cancers and other diseases through immune stimulating effects (Mizuno *et al.,* 1995 and Hobbs, 1995). Other components, such as triterpenoids, organic germanium, adenosine, amino acids, vitamins, higher level of RNA which disrupts viral diseases by inducing interferon production; oleic acid, a strong inhibitor of histamine release, an immunomodulating protein which significantly reduces but does not entirely shut down antibody production, also play an important role in the immunomodulating activity of *L. edodes.* Anti HIV-activity, an inhibitory effect on HIV-replication *in vitro,* was reported from an extract of the culture medium of *L. edodes* mycelia (Tsunoda and Ishida, 1969).

A number of products prepared from *L. edodes* are sold throughout the world as dietary supplements. The market value of mushroom DS products worldwide is estimated at US $ 6 billion per year. The worldwide production of this mushroom was more than 1,564,000 MT with almost 88.8 per cent (1,397,000 MT) produced in China. It has a hearty flavour and meaty texture, a fabulous addition to almost any dish. Many brands of this mushroom product are sold in the market.

Modern researches on this mushroom's biology, biochemistry pharmacology and therapeutics have provided a firm basis for the market of *Lentinula* products. The yield and quality of the mushrooms produced, is determined by three factors : the genetic makeup of the mushroom strain, the environmental conditions in which the mushroom strain is grown and physiological and nutritional requirement of different strains. Qualities of mushrooms can be influenced by many parameters. Besides differences in strains characteristics, mushroom quality are influenced by many variations of pre and post-harvest conditions.

Many countries have developed cultivation technology of this mushroom but detailed accounts are not available in literature. The brief accounts of growing technology, available are insufficient to reproduce the results.

Keeping above in view, the present investigations were undertaken to cultivate and to know precisely active biochemical components with the following objectives :

(1) Effect of different media, pH and temperature on the radial growth.

(2) Morphological characteristics of fruiting bodies.

(3) Effect of supplementation on yield.

(4) Evaluation of different substrates for yield.

(5) Biochemical makeup of fruiting bodies : total soluble sugars, reducing sugars, non-reducing sugars and proteins.

REVIEW OF LITERATURE

Lentinus edodes (Berk.) Sing. or *Lentinula edodes* (Berk) Pegler, commonly known as the Shiitake mushroom, is a white rot wood-decay fungus that naturally inhabits the dead wood of many hardwood tree species in Asia. Cultivation of Shiitake on natural logs is an established industry, especially in Japan (San Antonio, 1981). Shiitake accounts for 25.4 per cent of total world consumption of edible mushrooms (Chang and Miles, 2004). This is a saprophytic fungus which produces edible sporophores of high value. Its commercial cultivation is being done in Japan and China on the logs. Oak is the most preferred species used as substrate (Stamets and Chilton, 1983). The cultivation of *L. edodes* (Shiitake) first began in China about AD 1100 (Nakamura, 1983; Royse *et al.*, 1985; Chang and Miles, 1987, 1989). It is believed that Shiitake cultivation techniques developed in China were introduced to the Japanese by Chinese growers (Ito, 1978).

Other common names of this mushroom are the Oakwood mushroom, Japanese forest mushroom, Black mushroom and Pasania. It is a mushroom long valued for both an unique flavour and as a medicinal tonic, for even in the Ming Dynasty (1368-1644 CE) it was claimed to be a general stimulant, curing colds, increasing blood circulation and lowering blood pressure.

Lentinula edodes is found throughout much of eastern Asia, especially China and Japan, preferring an optimal temperature of around 24°C but also extending westwards as far as Kazakhstan. It grows on the dead wood of a wide range of tree hosts, especially species of oak, beech, chestnut, hornbeam, walnut, *Elaeocarpus,* the Shii tree (*Castanopsis cuspidata*), magnolia, pines and spruce (Pegler, 2003). It is a moderately lignocellulolytic species which can produce a series of hydrolytic and oxidative enzymes used in the degradation process of the main compounds of the lignocellulosic materials (Morais *et al.,* 1999).

The available literature on *Lentinula edodes* (Berk.) Pegler in respect of taxonomic position, morphological characterization, content of different medicinally active ingredients like polysaccharides, terpenoids, proteins etc. has been reviewed as given below:

2.1 Taxonomic Position

Class	:	Basidiomycetes
Sub-class	:	Holobasidiomycetidae
Order	:	Agaricales
Family	:	Tricholomataceae
Genus	:	*Lentinula*
Species	:	*edodes*

(Alexopoulos *et al.,* 1996; Chang and Miles, 1989 and Wasser, 2002).

But some workers placed it in the order Aphyllophorales (Singer, 1986; Pegler, 1983; Hibbett and Vilgalys, 1993). In 1997, Guzman *et al.*

placed the genus *Lentinula* in order Poriales. Kirk *et al.* (2001) described the position of *Lentinula* in order Polyporales and family polyporaceae.

2.2 Morphology

According to Purkayastha and Chandra (1985) the sporophores of Shiitake mushroom usually growing on wood of deciduous trees of Fagales such as chestnut (*Castanea* sp.), oak (*Quercus* sp.), hornbeam (*Carpinus* sp.), beech (*Fagus* sp.). These sporophores were usually concentric, sometimes centrally stipitate and characterized by its whitish lamellae which do not darken with age. The pileus upto 11.0 cm in diameter, brown at first convex, then depressed sometimes umbenate when old; scales darker in the centre, lighter towards the margin, triangular or areolate, small or large consisting of filamentous interwoven hyphae. Gills crowded, edge denticulate, at first whitish, later brownish or greyish, adenate, usually get separated from stipe and becoming free. Stipe usually 3.0-4.0 × 0.8-1.3 cm long, solid, pale and reddish brown, dark brown scales present at the joints of cortina. Broad at the middle, rarely bulbous at the base, cortina colourless to light brown, ring-like in young fruiting bodies, inconspicuous with age. Flesh generally white, brownish under the surface of pileus, soft, fleshy when tender, tough and coriaceous when old. Odour mild, taste slightly acidic, not unpleasant. Hymenophoral trama regular. Basidia 4-spored. Basidiospores cylindrical to elliptical, smooth, thin walled. Hyphal system monomitic, sclerified generative hyphae present, skeletal or ligative hyphae absent, generative hyphae loosely interwoven inflating constricted at the septa with clamp connection. According to Luis (1999) Shiitake had beautiful, dark brown, fleshy basidiocarps. According to Pegler (2003) the convex to depressed, ochraceous tawny to dark vinaceous brown cap was 5-15 cm across, darker at the center, dry and smooth then breaking up into triangular scales or deeply cracking. The gills were adnate to free, crowded and whitish whilst the short, stocky stem was solid, pale reddish brown, and slightly scaly below. Gaitan and Mata (2004) cultivated Shiitake mushroom on wheat straw substrate and got sporophores with pileus diameters ranging from 5 to 20 cm.

2.3 Cultural Characteristics

Cultural characteristics of wood-decaying fungi were studied by Lyman (1907), Long and Harsch (1918), Fritz (1923) and Davidson *et al.* (1938). Davidson *et al.* (1942) using microscopic cultural characteristics, developed the first key codes for the identification of fungi that decay oak. Nobles (1948) provided an 11-character key pattern and descriptions based on cultural information to 126 basidiomycetes that decay wood. Nobles (1958) suggested the use of cultural characters in developing a taxonomy of the polyporaceae that reflects natural relationships and phylogeny.

2.3.1 Media

Fukushima *et al.* (1991) grew *Lentinula edodes* in medium containing polypeptone (2.5 g or 5.0 g/litre of water), yeast extract (2.5 or 5.0 g/litre) and glucose (50 g/litre) with salts, either under batch culture or continuous culture conditions. They found that mycelium productivity was 14 times higher in continuous culture at a dilution rate of 0.03/h than in batch culture.

Furlan *et al.* (1997) investigated mycelial growth of 7 strains of edible fungi on different growth media and under different culture conditions. They found that mycelial growth rates of *L. edodes* were higher on wheat dextrose agar (WDA) medium than on PDA at 20 to 25°C and pH 4.0. Absence of light favoured rapid mycelial development in all the strains tested.

Pacumbaba and Pacumbaba (1999) grew *Lentinula* on different culture media including YMMBSA (yeast extract, malt extract, multigrain oatmeal, brown sugar, agar), YVMBSA (yeast extract, V-8 vegetable juice, multigrain oatmeal, brown sugar, agar) and YVMSA (yeast extract, V-8 vegetable juice, multigrain oatmeal, sucrose, agar) and broths YVMBS (yeast extract, V-8 vegetable juice, multigrain oat meal, brown sugar), YVMS (yeast extract, V-8 vegetable juice, multigrain oatmeal, sucrose) and MVBS (multigrain oatmeal, V-8 Vegetable juice, brown sugar) they found that formulated culture media considerably enhanced the growth of Shiitake mycelia, production of spawn and basidiocarps in less time (2.6 to 4.1 months after inoculation) in the laboratory.

Rossi *et al.* (2000) developed *L. edodes* in different culture media, including aqueous sugarcane bagasse extracts at different concentrations (20 to 100 g/litre of bagasse), minimum medium and potato-dextrose agar medium (PDA). They found that PDA medium provided the highest speed, vigour and estimated biomass values.

Mata *et al.* (2001) cultivated 11 strains of *Lentinula edodes* on solid media : derived from malt extract (MEA); malt yeast extract (YMEA); and YMEA plus wheat straw soluble lignin derivatives (YMEA + WSLD). The results were compared with data for mycelial growth rates, of the same strains cultivated on substrates derived from wheat straw treated at different temperatures (50, 65, 75 and autoclaving at 121°C). The greatest mycelial growth rate was obtained on sterilized straw at 121°C for the majority of strains. Curvetto *et al.* (2002) evaluated the nutritive agar formulations with additions of poplar (*Populus alba*) sawdust, wheat (*Triticum durum*) bran, or milled sunflower (*Helianthus annuus*) seed hulls (SSH) for mycelium cultivation of shiitake in petri dishes. The largest mycelial growth rates were 2.75, 2.88 and 2.93 mm/day for the substrates formulated with 8 SSH : 2 wheat bran, 9SSH : 1 poplar sawdust, and 8SSH : 1 wheat bran : 1 poplar sawdust by weight, respectively. Hatvani *et al.* (2003) observed that the medium containing malt extract (15 g/l), starch (3 g/l) and oak wood chips (20 g/l) proved best for mycelial growth.

Xia and Qin (2003) investigated the effects of cotton stalk powder (CSP) on the hyphal growth and mushroom yield. They were media A (85% CSP + 15% wheat bran), B (70% CSP + 15% sawdust + 15% wheat bran), C (60% CSP + 25% sawdust + 15% wheat bran), D (45% CSP + 40% sawdust + 15% wheat bran) and E as control (80% sawdust + 20% wheat bran). The highest mushroom yield was recorded in medium C, followed by medium B. The mushroom appeared 87, 81, 79, 84 and 85 days after inoculation on the media A, B, C, D and E respectively and biological conversion efficiencies were 77, 84, 86, 78 and 81 per cent, respectively. Insignificant differences in hyphal growth speed mushroom yield and biological conversion efficiency were found among the media A, D and E, but these differences reached the significant level between the media B and E and between the media C and E. Hence, substituting sawdust with CSP was feasible for *L. edodes* production and the best medium was medium C.

2.3.2 Temperature and pH

Ding (1987) recommended a temperature of 20°C and a relative humidity of 85-90 per cent to give satisfactory colour of the fruiting bodies of *Lentinula edodes.* He also advocated that temperature changes can also cause fruiting bodies to fall.

Triratana and Tantikanjana (1989) studied the effect of temperature and light intensity on various strains of *Lentinula edodes* and they found that various strains responded differently to light intensity (150-600 lux) and temperature (11-25°C). Delpech *et al.* (1991)

found that good control of temperature is essential to prevent *Trichoderma* infection of the substrates.

Khan *et al.* (1991) observed maximum radial growth of *L. edodes* at 25°C and pH 5.0. Khan *et al.* (1995) recorded maximum growth of *L. edodes* at 25°C 12 days after inoculation on agar medium with pH 5.0. Growth was reduced markedly at temperature below 20°C and above 30°C. The most suitable pH for growth of the fungus was 5.0. Balazs *et al.* (1996) inoculated *L. edodes* into agar media containing malt extract and different concentrations of peptones at pH 5.5-7.0. The cultures were incubated at 15-30°C for 12 days and growth was then compared. They found that temperature, pH and N requirements of *Trichoderma Aspergillus, Penicillium, Chaetomium* and *Nigrospora* were similar to shiitake, indicating that modification of these factors would not help in control. It is recommended that the temperature should not exceed 25°C and the pH should be kept below 6. Increasing the initial shiitake inoculum dose is also suggested.

Furlan *et al.* (1997) observed the higher growth rates of *Lentinula edodes* at 30°C than at 20 or 25°C. They also observed high growth rates of *L. edodes* at low pH (4.0). Absence of light favoured rapid mycelium development. Maki *et al.* (2001) observed the growth of thirty four *Lentinula edodes* strains at different three pH values (5, 6 and 7) and four different temperatures (16, 25, 28 and 37°C). They found that mycelial cultivation was successful at all pH tested, while the ideal temperature for mycelial cultivation ranged between 25 and 28°C.

2.4 Evaluation of Different Substrates and Supplementation for the Yield

Mushroom cultivation is a complicated procedure, involving a number of operations, which include the preparation of a fruiting culture, spawn and compost. It is the aim of mushroom growers and researchers to try to increase the yield from a given surface area, to shorten the cropping period and to have many more flushes with an increasingly high and regular yield (Morais *et al.,* 1999).

Various species of trees have been used for the cultivation of Shiitake (San Antonio, 1981). One of the primary species used in one area of Japan in past years was the Shii-tree thus the derivation of the name Shiitake (Singer, 1961). Most production today, however, is on various species of oak (Harris, 1986; Stamets and Chilton, 1983).

In an attempt to develop a more efficient and dependable method for the production of Shiitake mushrooms researchers have focused on the cultivation of *L. edodes* on synthetic sawdust substrates. Cultivation on a supplemented sawdust substrate provides a more rapid and controlled method of cultivation than is presently possible with sectioned logs as described by Ito (1978). Synthetic substrates usually consist of sawdust mixed with nutrient supplements (Ando, 1974; Mee, 1978; Mori *et al.*, 1976; Fuzisawa *et al.* 1978a, b; Fuzisawa and Hattori, 1979; Anonymous, 1980; Han *et al.,* 1981; Fergus, 1982; Farr, 1983; Leatham, 1983; Patrick *et al.,* 1983; Royse *et al.*, 1985 and Royse, 1985).

Sawdust is one of the wood-sawmilling wastes which may reach 15 percent of the total volume. This material could be used for Shiitake's cultivation media (Djarwanto and Suprapti, 2000). Sawdust is the most popular basal ingredient used in synthetic formulations of substrate used to produce Shiitake (Miller and Jong, 1987). Other basal ingredients that may be used include straw and corn cobs or mixtures thereof.

Presently, there is a wide range of techniques used to grow Shiitake on sawdust. One common technique involves autoclaving the substrate in heat-resistant containers followed by the inoculation of cooled substrate in each container (Ando, 1974; Mee, 1978; Mori *et al.,* 1976; Fuzisawa *et al.,* 1978a, b; Fuzisawa and Hattori, 1979; Anonymous, 1980; Han *et al.,* 1981; Farr, 1983; Patrick *et al.,* 1983). It has been reported that mycelial growth, primordia formation and productivity vary with different lines on synthetic substrates (Ando, 1974; Han *et al.*, 1981).

In an effort to save time and labor, a process has been developed where the substrate can be mixed, heat treated, cooled and inoculated in the same apparatus (Royse, 1985). He used a mixture of maple and birch (60 : 40) sawdust as the main substrate ingredient and found that the proportion of sawdust (80%), millet (10%) and spring wheat bran (10%) was the best formula for nutrimental components.

Selection of the tree species for sawdust cultivation should proceed with caution. Fresh sawdust without aging by fermentation can be used for production of Shiitake only if it is from high quality tree species, such as chinkapin oak, hornbean, poplar, alder, iron wood, beech, birch and sweetgum, those graded 4, excellent by FAO (Oei, 1996).

Schunemann (1986) studied the mycelial growth of 14 strains of *Lentinula edodes* on to substrates of compressed straw, or beech (*Fagus*), coniferous or mixed (>50% mahogany) sawdust, with or without amendments of 20 per cent (w/w) soybean or maize meal, used brewer's grain or dry molasses. He observed without the amendments, the best mycelial growth after 12 days was on beech sawdust. The dry molasses amendment improved mycelial growth of nearly all the strains on all the substrates.

Chen (1988) described the cultivation of *Lentinus edodes* (including the use of gypsum in substrate), the relatively low temperature requirements of the fungus, the humidity requirements, the need for ventilation, the light requirements and the substrate pH (which is most favourable at about 5).

Triratana and Osathaphant (1988) cultivated the Shiitake on sawdust as a substitute for the usual log-wood cultivation. Some types of sawdust and agricultural residues available in tropical countries were examined for their suitability for mycelial growth in Shiitake production.

Royse *et al.* (1990) cultivated Shiitake on synthetic substrate having sawdust, wheat bran and millet, amended or non-amended with sucrose, fructose or glucose. They found that the addition of sucrose (0.6-1.2% dry weight) and fructose at 12 per cent and glucose at 0.6 per cent to the substrate stimulated mushroom yield by 11 to 20 per cent or more. They advocated that regardless of the main ingredient used, starch based supplements such as wheat bran, rice-bran, millet, rye, corn, etc. are added to the mix in a 10 to 40 per cent ratio (dry weight) to the main ingredient. These supplements serve as nutrients to provide an optimum growing medium.

Delpech *et al.* (1991) cultivated *Lentinula edodes* on available basic materials with a short production cycle (not exceeding 3 months). The substrate was wheat straw mixed with chicken feather meal. Benomyl was added to the substrate before pasteurization at 60°C for 24 h. Substrate temperature was controlled using thermodynamic sensors within the substrate blocks. Six commercial *L. edodes* strains (S 600, S 610, 4055, V084, V0122 and 072) were tested. Good control of temperature is essential to prevent *Trichoderma* infection of the substrate. A few strains only were able to give a good commercial yield on the pasteurized substrates.

Khan *et al.* (1991) cultivated the Shiitake on three types of sawdust from 'shishum' (*Dalbergia sissoom*), 'kikar' (*Acacia arabica*) and poplar (*Populus alba*) were amended with wheat bran, wheat chaff, paddy straw and cotton waste and lime and used for spawn preparation. They found that sawdust from *D. sissoo* was the most suitable for spawn preparation and all sawdust amended with cotton waste were found to give optimum results for spawn running. Moyson and Verachtert (1991) studied the effect of *Lentinus edodes* on the composition of wheat straw. They observed that Shiitake grew very well on lignocellulosic substrates breaking down a considerable amount of lignin. The initial concentration of lignin is straw was halved after 12 weeks of fungal growth, doubling the enzymatic digestibility.

Balazs and Kovacs (1993) studied that straw with additives made the best substrate of *Lentinula edodes*. The dry substrate was heat treated at 100°C, spawned and incubated. Fruiting bodies appeared 60-70 days after spawning and a yield of 20-25 kg mushrooms/100 kg heat treated wet substrate was produced.

In India, cultivation trials of Shiitake were done and mushrooms have been developed successfully (Sohi and Upadhyay, 1988; Thakur and Sharma, 1992; Shukla, 1994). Shiitake mushroom was artificially cultivated in India on wood logs, artificial medium and saw dusts and corn cobs supplemented with wheat and rice bran (Dhar, 1976; Suman and Seth, 1982; Sohi and Upadhyay, 1988; Thakur and Sharma, 1992). Shukla (1994) firstly reported the cultivation of Shiitake mushroom in India, on oak logs. According to Shukla (1995) indole-3acetic acid treated (with 5 and 10 ppm solutions) logs gave the maximum yield while maximum number of sporophores were formed in indole butyric acid. Kaur and Lakhanpal (1995) reported for the first time in India the cultivation of *L. edodes* on various types of sawdust in polypropylene bags. They found that colonization of the substrate was most rapid in a mixture of sawdust of eucalyptus and poplar (40 days) with highest yield (360 g/kg dry substrate) and with 36 per cent of biological efficiency. They also found that medium containing dextrose, peptone, thiamine, manganese and gibberellic acid supported the best mycelial growth of *L. edodes*.

Kalberer (1998) investigated the effect of substrate composition on the yield of *L. edodes*. He found that for highest yields, the substrate should contain 20 per cent corn meal, an additional N source (1% urea or 4% extracted soybean meal) and 2 per cent calcium carbonate. Aleksandrova *et al.* (1998) observed that wheat grain was best substrate for fruiting body production (upto 7 fruiting bodies per 0.5 litre of substrate).

Morais *et al.* (1999) gave a methodology for production of *Lentinus edodes* based on sterilized mixed agro-residues including sawdust, bark, bagasse, cottonseed husks, maize stover and hay/straw etc. as substrate. Zhang *et al.* (1999) raised spawn of *Lentinus edodes* for cultivation on PDA medium and the substrate was a mixture of 70 per cent deciduous tree sawdust, 20 per cent bran, 1 per cent gypsum and 1 per cent sucrose. Inoculation was done at the end of February, and mushrooms were produced in autumn. They observed that there is a lack of varieties suitable for spring cropping. Some varieties rotted before cropping. Cropping lasted 38-45 days depending on variety. Pacumbaba and Pacumbaba (1999) suggested that basidiocarp production of Shiitake mushroom on amended hardwood sawdust may have excellent economic potential commercially as it takes 1 to 2 years for basidiocarps to appear in Shiitake spawn inoculated logs.

Camargo (2000) studied the efficiency of some organic by-products available in Colombia as substrates for Shiitake cultivation. The artificial trunk technique was used, with eucalyptus sawdust and cotton seed hulls, wheat bran was added as nitrogenous supplement at 12, 20 and 28 per cent (w/w). He found that the highest total and premium quality yields were obtained with sawdust and the 20 per cent level of bran.

Djarwanto and Suprapti (2000) cultivated Shiitake on media made of sawdust of different wood species, including *Hevea brasiliensis, Eucalyptus deglupta, Dyospiros ebenum, Castanopsis argentea, Pithecelebium viringa, Swietenia mahagoni, Ecalyptus urophylla* and *Cananga odorata* enriched with rice bran and seed of *Panicum viridae* as much as 10 per cent each, and then mixed thoroughly with sufficient distilled water. They found that highest fruit body's weight and BCE were obtained from the sawdust medium of *Hevea brasiliensis* i.e. 201.64 g and 96.27, respectively.

Kovacsne and Kovacs (2000) investigated straw as a substrate for Shiitake growing. Heating of straw at 100°C for 45-60 minutes eliminated all unwanted fungal spores. During incubation (20-30 days), air temperature was kept at 18-22°C, substrate temperature at 25°C and relative humidity at approximately 80 per cent. Straw is suitable as a substrate as long as it is high quality with less than 14 per cent moisture. Both substrate nutrients and physical texture property aeration are important. Sawdust should not to be smaller than 0.85 mm (Royse and Sanchez, 2000).

Zervakis *et al.* (2001) evaluated the mycelium extension rates on seven mushroom cultivation substrates : wheat straw, cotton gin-trash, peanut shells, poplar sawdust, oak sawdust, corn cobs and olive press-cake. They found that wheat straw was the most suitable substrate for *L. edodes.* Curvetto *et al.* (2002) cultivated Shiitake with a simple substrate composition containing 37.5 per cent milled sunflower (*Helianthus annuus*) seed hulls (SSH), 0.5 per cent calcium carbonate ($CaCO_3$), 2 per cent calcium sulphate ($CaSO_4$), and 60 per cent water and got a daily production rate of 2 kg Shiitake/100 kg dry substrate for 55 days cycle production. Grodzinskaya *et al.* (2002) studied the mycelium growth (spawn) and fruit body production of Shiitake on 20 different substrates from agricultural wastes. They found that *L. edodes* prefers a mixture of rice straw and husk with maize cobs, bagasse from sugarcane, coconut fibres and coffee wastes. The cultivation of *L. edodes* began in 1979 using Quercus logs as substrate, the use of oak sawdust started in 1997 (DeLeon, 2003).

Mata and Salmones (2003) evaluated different agricultural and forest by-products as substrates for commercial cultivation of *Lentinula.* Some strains of *L. edodes* have been cultivated on pasteurized non-conventional substrates, such as coffee pulp, sugarcane bagasse, and wheat straw, showing good adaptation to this process.

Philippoussis *et al.* (2003) cultivated two strains of *Lentinula edodes* (S4080 and SIEF 0231) on oak-wood sawdust (OS), wheat straw (WS) and corn cobs (CC) substrates. They found that strain SIEF 0231 colonized OS and CC earlier than WS and heavier basidiomata were produced by WS and OS substrate. Royse and Sanchez (2003) cultivated Shiitake on synthetic substrate consisting of oak sawdust (50%), white millet (28%), winter rye (11%) and soft red wheat bran (11%) was non supplemented or supplemented with 0.2, 0.4 or 0.6 per cent (dry weight basis) precipitated calcium carbonate ($CaCO_3$). They found that supplemented substrate with 0.6 per cent $CaCO_3$ gave high yields and biological efficiencies whereas mushroom size (weight) was larger with non-supplemented substrate (16.8 g).

Silva *et al.* (2005) cultivated *Lentinula edodes* in eucalyptus residues, supplemented with 5, 10, 15 and 20 per cent (w/w) of soya, wheat or rice brans. They found that eucalyptus residues supplemented with cereal brans supported fast grown of *L. edodes,* indicating that mycelium extension is related to the bio-availability of nitrogen.

2.5 Therapeutic Potentials of *Lentinula edodes*

Chihara *et al.* (1969) isolated from the fruiting bodies of Shiitake a water soluble antitumor polysaccharide which was named '*Lentinan*' after the generic name of this mushroom. This *Lentinan* demonstrated powerful antitumor activity, preventing chemical and viral tumor development in mice and experimental models.

According to Sasaki and Takosuka (1976) the most highly researched bioactive molecule isolated from Shiitake is the pure b (la3)-D-glucan lentinan. Lentinan has an antitumour polysaccharide. *L. edodes* also contains adenine and choline, which may prevent the occurrence of cirrhosis of the liver as well as vascular sclerosis.

Tyrosinase contained in *L. edodes* tends to lower blood pressure. Two other constituents which have been isolated from *L. edodes* which tend to reduce serum cholesterol are : C6H11O4N5 and C9H11O3N5, namely [2(R), 3(R)-dihydroxy-4-(9-adenyl)-butyric acid 2(R)-hydroxy-4-(9-adenyl)-butyric acid].

According to a Chinese physician of the Ming Dynasty (1368-1644), Wu-Juei, Shiitake preserves health, improves stamina and circulation, cures cold and lowers blood cholesterol (Jong and Birmingham, 1993).

Yang *et al.* (1992), Wang *et al.* (1995) and Kobayashi *et al.* (1995) reported that a number of polysaccharides obtained from basidiomycetes, such as *Lentinus edodes* (LPS), *Ganoderma lucidum* (GPS) and *Coriolus versicolar* (CPS), have also been shown produce anti tumor effects, thus being potentially useful in cancer therapy.

2.6 Biochemical Make-up of *Lentinula edodes*

Proximate composition of *L. edodes* :

Component	Percentage by weight
Moisture	90.0-91.8
Crude protein (N × 4.38)	13.4-17.5
Crude fat	4.9-8.0
Carbohydrate	
Total	67.5-78.0
N-free	59.5-70.7
Crude fibre	7.3-8.0
Ash	3.7-7.0
Energy value	387-392

(Crisan and Sands, 1978)

Sous (1980) found that fruiting bodies of Shiitake are rich in K, P and vitamins of B complex, and have anti-viral and cytotoxic properties.

Aoyagi *et al.* (1993) cultivated 37 samples of *L. edodes* on logs and 19 samples on sawdust substrates and found that carbohydrate, Ca, Cu, Mn and Hg contents were higher in the fungi cultivated on logs and moisture, protein, ash, K, P and Zn contents were higher in fungi cultivated on sawdust substrates. He found high amounts of moisture, protein, ash, K, P and Zn in *L. edoes* cultivated on sawdust substrates.

Seidemann (1993) studied Shiitake cultivation in Japan based on various wood substrates and artificial nutrient media. He found that the fruiting body contains cyclic polysulphides which, together with other volatiles were mainly responsible for the odour, and guanosine-5-monophate for taste. Another constituent, *Lentinan*, a heavily branched (1< right arrow >3)-glucan had pharmacological properties.

Vetter (1995) observed that the amino acid concentrations in *L. edodes* were 15.24 per cent in caps and 11.35 per cent in stipes.

Longvah and Deosthale (1998) found that essential amino acid contents of *L. edodes* were 39 per cent.

Yuang *et al.* (2001) extracted a crude polysaccharide (Le) from the fruiting body of *L. edodes* with molecular weight from 14000-954000. The glucuronic acid and protein contents (%) of the 3 fractions were 24.10 and 2.01, 34.77 and 7.38, 40.05 and 25.31 in Le-1, Le-2 and Le-3.

Mattila *et al.* (2002) observed the variation in dry matter contents of mushrooms varied from 7.7 to 8.4 per cent. The dry matter of Shiitake mushrooms contained large amounts of carbohydrates i.e. 5.8

g/100 g fresh weight. *L. edodes* proved to be an especially good source of dietary fibres (3.3 g/100 g fresh weight). They also found that crude fat, ash and protein contents of the mushrooms varied from 0.31-2.09 g/100 g fresh weight.

MATERIALS AND METHODS

3.1 Cultures and their Maintenance

Two strains of *Lentinula edodes* (Berk.) Singer namely L_1 and L_2 were selected for studies. The cultures of the two strains were obtained from the Mushroom Research and Training Centre (MRTC), Pantnagar. The experiments were conducted in the above laboratory. The available background of these cultures are given in table below :

S. No.	Strains	Colour of fruiting body	Source
1.	L_1	Brown	Mushroom Research and Training Centre, Pantnagar
2.	L_2	Dark brown	Mushroom Research and Training Centre, Pantnagar

The fungus was isolated fresh, using hyphal tip method, from fully grown colony of the fungus having best mycelial growth. Each culture was multiplied in ten petriplates and 10 culture tubes containing potato dextrose agar medium. The culture tubes containing fully grown mycelium were kept in refrigerator to maintain viability of the above mushroom for long period of time.

3.2 Culture Media

3.2.1 Potato dextrose agar (PDA)

Peeled potato	:	200 g
Dextrose	:	20 g
Agar	:	20 g
Distilled water	:	1000 ml

3.2.2 Malt extract agar (MEA)

Malt extract	:	20 g
Agar	:	20 g
Distilled water	:	1000 ml

3.2.3 Yeast extract agar (YEA)

Yeast extract	:	20 g
Agar	:	20 g
Distilled water	:	1000 ml

3.2.4 Water agar (WA)

Agar	:	20 g
Distilled water	:	1000 ml

3.2.5 Teak sawdust agar (SEDA)

Teak sawdust extract	:	200 ml
Dextrose	:	20 g
Agar	:	20 g
Distilled water	:	800 ml

3.2.6 Poplar sawdust agar (PODA)

Poplar sawdust extract	:	200 ml
Dextrose	:	20 g
Agar	:	20 g
Distilled water	:	800 ml

The media were autoclaved at 20 p.s.i. for 30 minutes.

3.3 Evaluation of Radial Growth on Different Media

The experiment was carried out using six different media viz., Malt Extract Agar (MEA), Potato Dextrose Agar (PDA), Yeast Extract Agar (YEA), Water Agar (WA), Shagaun Sawdust Agar or Teak Sawdust Agar (SEDA) and Poplar Sawdust Agar (PODA) at room temperature (24-30°C). Petri plates containing 25 ml of the medium were inoculated at the center with 7 mm diameter disc of actively growing mycelium under aseptic conditions in three replications, for each strain. Observations on radial growth were recorded till the colony covered the full plate.

3.4 Effect of Different Temperatures on Radial Growth of *Lentinula edodes*

The method was same as proceeded for media evaluation but at varying temperature ranges viz., 15, 20, 25, 30 and 35°C. Each treatment was replicated three times and observations were recorded.

3.5 Effect of Different pH on Radial Growth of *Lentinula edodes*

The method was same as proceeded for temperature evaluation but at varying pH levels viz., 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 on the PDA medium. pH was adjusted using concentrated HCl and concentrated NaOH solutions. For each treatment, there were three replications and the plates were incubated at 25±1°C temperature.

3.6 Cultivation Technology

3.6.1 Spawn preparation

Grain spawn of both the strains was prepared using the standard methodology of Garcha (1994). Healthy, uncrumpled wheat grains were washed and boiled (grain : water, 1 : 25, w/v) to tender without rupturing of the seed coat. Extra water was drained off and the grains were allowed to dry on sieve overnight. The boiled grains were then mixed with commercial grade gypsum and calcium carbonate in the ratio of 4:1 (w/w) @ 15 g/kg of grains. The grains were filled in clean glass bottles and the bottles were plugged with non-absorbent cotton and sterilized at 22 lbs steam pressure (121°C) for 90 minutes. Sterilized bottles were taken out from the autoclave, while still hot and were shaken to avoid clumping of the grains.

Next day, the bottles were inoculated with bits of agar medium colonized with mycelium of pure culture (12-15 days old). Inoculated bottles were incubated at 25±1°C. About 10 days after inoculation, bottles were shaken vigorously so that mycelial threads were broken and become well mixed with the grains. Entire grains get covered with fine mycelial growth after 25 days of inoculation. This spawn is known as mother spawn or master spawn. The spawn prepared for commercial use was on sterilized grains in polypropylene bags inoculated with mother spawn.

3.6.2 Substrate preparation

The substrates used for the experiment were wheat straw, coir pith and hard-wood sawdust including shagaun or teak (*Tectona grandis*), sal (*Shorea robusta*) and poplar (*Populus alba*). They were used individually as well as in mixtures (wheat straw and poplar saw dust; 1:1, w/w), wheat straw and coir pith (1:1, w/w), wheat straw and teak sawdust (1:1, w/w), wheat straw and sal sawdust (1:1, w/w), poplar sawdust and coir pith (1:1, w/w), poplar sawdust and teak sawdust (1:1, w/w), poplar sawdust and sal sawdust (1:1, w/w), coir pith and teak sawdust (1:1, w/w), coir pith and sal sawdust (1:1, w/w), teak sawdust and sal sawdust (1:1, w/w); all enriched with 10 per cent wheat bran. All the substrates were soaked in a tank for 18 hrs. Thereafter, they were taken out from the tank and kept for 2-4 hrs to drain out excess of water. The correct water content of the mixture was determined by squeezing the mixture in the hand. All the ingredients were mixed thoroughly. For each treatment, three replications were maintained. The substrate mixture was filled only 3/4 the capacity, in 2 kg capacity polypropylene bags. The neck of the bags were plugged with non-absorbent cotton and sterilized at 22 lbs pressure (121°C) for 90 minutes. After cooling, the bags were inoculated with the two strains of the fungus.

3.6.3 Effect of supplementation on the yield of *Lentinula edodes*

The method was same as proceeded for substrate evaluation but here the substrates supplemented with wheat bran (5 and 10%, w/w) including check (no supplementation of wheat bran), were evaluated for yield (number and weight of fruiting bodies) and biological efficiency on wheat straw substrate.

3.6.4 Spawning

Spawning of the two strains was done under aseptic conditions. The grains spawn of *Lentinula edodes* were mix thoroughly @ 5 per cent in the bags prepared as above. After spawning, these bags were plugged with non-absorbent cotton and wrapped with butter paper.

3.6.5 Spawn run

The bags were kept in the crop room at relative humidity of 80-85 per cent, at 25°C temperature in the dark for 60-70 days for complete spawn run. During mycelial running, the mycelium secretes enzymes that degrade complex substances in the compost into smaller soluble molecular, which can be absorbed by the growing mycelium. After the mycelia running stage, the established mycelial stage was developed in which on substrate sheet-like thick mycelium cover develops and bumps formed, that provide increased air circulation and promote the formation of brown pigment in the mycelial coat. After the spawn run, slitting was done and relative humidity of 80-90 per cent was maintained by sprinkling water. Pinhead initiation started after 20-25 days after slitting.

3.6.5 Harvesting

Fruit bodies were harvested after maturity. Biological efficiency was calculated using the following formula :

$$\text{Biological efficiency (\%)} = \frac{\text{Fresh weight of fruit body}}{\text{Dry weight of substrate}} \times 100$$

3.7 Morphology of Fruit Bodies

Morphological differences among the fruiting bodies of different strains were studied selecting 5 fruit bodies per strain randomly per days starting from the first flush of the crop till the termination of the crop. Morphological variations among fruit bodies of different strains were studied by measuring the diameter of the pileus and stipe, thickness of the pileus and length of the stipe, length of the fruit bodies and weight per fruit body.

3.8 Biochemical Characterization

3.8.1 Estimation of total soluble sugars

The amount of total soluble sugars was estimated using the anthrone method colorimetrically (Hedge and Hofreiter, 1962). Reagents required were 2.5N HCl, Anthrone reagent (200 mg Anthrone was dissolved in 100 ml ice cold 95 per cent sulphuric acid) and standard glucose (stock- 100 mg of glucose was dissolved in 100 ml water, working standard 10 ml of stock was diluted to 100 ml with distilled water).

Sample (100 mg) was weighed into a boiling tube. It was hydrolysed by keeping it in boiling water both for 3 hr with 5 ml of 2.5 N HCl and then cooled to room temperature. The solution was neutralized with solid sodium carbonate until the effervescence ceased. The volume of solution was made to 100 ml with distilled water and centrifuged at 5000 rpm for 10 minutes. Supernatant was collected and 0.5 ml aliquot was taken for analysis. The standard was prepared by taking 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard. The volume was made to 1 ml in all the tubes including the sample tubes by adding distilled water. Then 4 ml of anthrone reagent was added. The solution was heated in a boiling water bath for 8 minutes and cooled down rapidly and the green to dark green colour was read at 630 nm. A standard graph was drawn by plotting concentration of the standard of X-axis versus absorbance on the Y-axis. From the graph, the amount of carbohydrates present in the sample tube was calculated using the following formula :

$$\text{Amount of carbohydrate present in sample (\%)} = \frac{\text{Sugar volume from graphs}}{\text{Aliquot sample used (0.5)}} \times \frac{\text{Total volume of extract}}{\text{Weight of sample}} \times 100$$

3.8.2 Estimation of reducing sugars

Reducing sugars were estimated following the Nelson-Somegyi method (Somoyi, 1945 and 1952). Reagents required were : alkaline copper tartarate arsenomolybdate reagent and standard stock glucose solution.

The sample (100 mg) was weighed and sugar was extracted with hot 80 per cent ethanol twice (5 ml each time). Supernatant was collected and evaporated on water bath. Distilled water (10 ml) was added to dissolve the sugars. Aliquots of 0.1 ml of alcohol-free extract was pipetted out to separate test tubes. The working standard solution (0.2, 0.4, 0.6, 0.8 and 1.0 ml) was pipetted out into a series of test tubes. The volume was made to 2 ml in both sample and standard tubes with distilled water. Alkaline copper tartarate reagent (1 ml) was added to each tube. The tubes were placed in boiling water for 10 minutes. Tubes were cooled down and 1 ml of arsenomolybdic acid reagent was added to all the tubes. The volume was made to 10 ml with distilled water and absorbance of blue colour at 620 nm was read after 10 minutes. From the graph, the amount of reducing sugars present in the sample was calculated using the following formula :

$$\text{Reading sugars in samples (\%)} = \frac{\text{Sugar volume from graphs}}{\text{Aliquot sample used}} \times \frac{\text{Total volume of alcohol free extract}}{\text{Weight of sample}} \times 100$$

3.8.3 Estimation of non-reducing sugars

The content of non-reducing sugars was calculated by substracting the reducing sugar from total carbohydrate content.

$$\text{Non-reducing sugars in sample (\%)} = \text{Total carbohydrate content} - \text{Amount of reducing sugars}$$

3.8.4 Estimation of proteins

The amount of proteins was calculated using the method of Lowry (Lowry *et al.,* 1951).

The reagents required were :

(i) Solution A : 2 per cent sodium carbonate in 0.1 N NaOH.

(ii) Solution B : 0.5% copper sulphate in 1% sodium potassium tartarate.

(iii) Solution C : solution A (50 ml) was mixed with 1 ml of solution B just prior to use.

(iv) Folin-ciocalteu reagent (FCR) : The commercial reagent (2N) was diluted with an equal volume of water.

(v) Stock standard protein solution : 50 mg of bovine serum albumin/50 ml of water.

(vi) Working standard solution : stock solution (10 ml) was diluted to 50 ml with distilled water to obtain 200 μg protein/ml.

The sample (0.5 g) was ground with a phosphate buffer (pH 7.0) in the mortar with the help of pestle. It was centrifuged and the supernatant was used for protein estimation. The working standard solution (0.2, 0.4, 0.6, 0.8 and 1.0 ml) was pipetted out into series of test tubes. The sample extract (0.1 ml) was also pipetted out into other test tubes. The volume was made to 1 ml with distilled water in all the tubes. A tube with 1 ml of water served as the blank. In it, 5 ml of solution C was added. It was mixed well and incubated at room temperature for 10 minutes. After it, folin ciocalteu reagent (0.5 ml) was added and mixed and the solution was incubated at room temperature in dark for 30 minutes. The absorbance at 660 nm was taken. A standard graph was drawn and the amount of protein in the sample was calculated.

3.9 Statistical Analysis

Statistical analysis of the data was done at the Computer Centre of G.B. Pant University of Agriculture and Technology, Pantnagar, using appropriate programme as per the requirement of the experiment. To compare the morphological variations among the fruit bodies of different strains simple CRD (Completely Randomized Design) was used. In order to compare the growth rates of different strains in compost, in casing soil and to compare yield variations among different strains simple CRD and two factorial CRD was used. The treatment/strain was considered first factor and different durations considered as the second factor. Critical differences (CD) calculated at 5 per cent level of significance were used for comparison of difference between the treatments means.

In the present investigation, two strains of *Lentinula edodes* (Berk.) Pegler. were evaluated culturally, quantitatively, qualitatively as well as biochemically. The data recorded in present investigation were statistically analyzed and experimental results are presented as follows.

4.1 Radial Growth of *L. edodes* strains on Different Media

The experiment was carried out to see the radial growth of two strains of *L. edodes* on different media according to the "Materials and Methods" and the data obtained are presented in Table 4.1, Fig. 4.1 and Plate 1. The results showed that among the different media tested, the fungus showed the highest growth rate (8.7 mm/day) on Potato dextrose agar (PDA) followed by Malt extract agar (MEA) (8.4 mm/day), poplar sawdust extract dextrose agar (6.8 mm/day), teak sawdust extract dextrose agar I2.5 mm/day). Least growth was observed in yeast extract agar (1.04 mm/day). No growth was obtained on water agar. Strain L_1 gave significantly higher radial growth on all the different media tested on the 8^{th} day of inoculation. These findings are in accordance with Furlan *et al.* (1997) and Rossi *et al.* (2000) who also reported that PDA medium provided the highest speed, vigour and estimated biomass values for *L. edodes*.

Table 4.1 : Radial growth of *L. edodes* (in mm) strains on different media at 25°C

Media	SEDA			MEA			PDA			PODA			YEA		
Strains / Day	L_1	L_2	Mean	L_1	L_2	Mean	L_1	L_2	Mean	L_1	L_2	Mean	L_1	L_2	Mean
2	0.93	0.5	0.72	2.7	1.3	2.0	3.3	2.3	2.8	3.3	2.0	2.7	0.0	0.0	0.0
4	3.7	2.0	2.8	19.3	16.7	18.0	18.7	19.5	19.1	18.7	16.7	17.7	0.23	0.23	0.23
6	10.3	3.5	6.9	37.7	35.3	36.5	39.0	38.9	39.0	34.0	28.6	31.3	10.2	2.7	6.5
8	22.0	18.0	20.0	69.7	68.0	68.8	69.0	69.3	69.1	58.7	50.7	54.7	12.3	4.7	8.5
Mean	9.2	6.0	7.6	32.3	30.3	31.3	32.5	32.5	32.5	28.7	24.5	26.6	5.7	1.9	3.8
Growth/day	2.8	2.3	2.5	8.5	8.2	8.4	8.6	8.7	8.7	7.3	6.3	6.8	1.5	0.6	1.04
CD at 5%															
Strains (A)	0.61			1.16			1.75			0.82			0.67		
Days (B)	0.86			1.64			2.48			1.16			0.94		
A × B	1.21			2.32			3.51			1.64			1.30		

SEDA= Shagaun Sawdust Agar, MEA = Malt Extract Agar, PDA = Potato Dextrose Agar, PODA = Poplar Sawdust Agar, YEA = Yeast Extract Agar

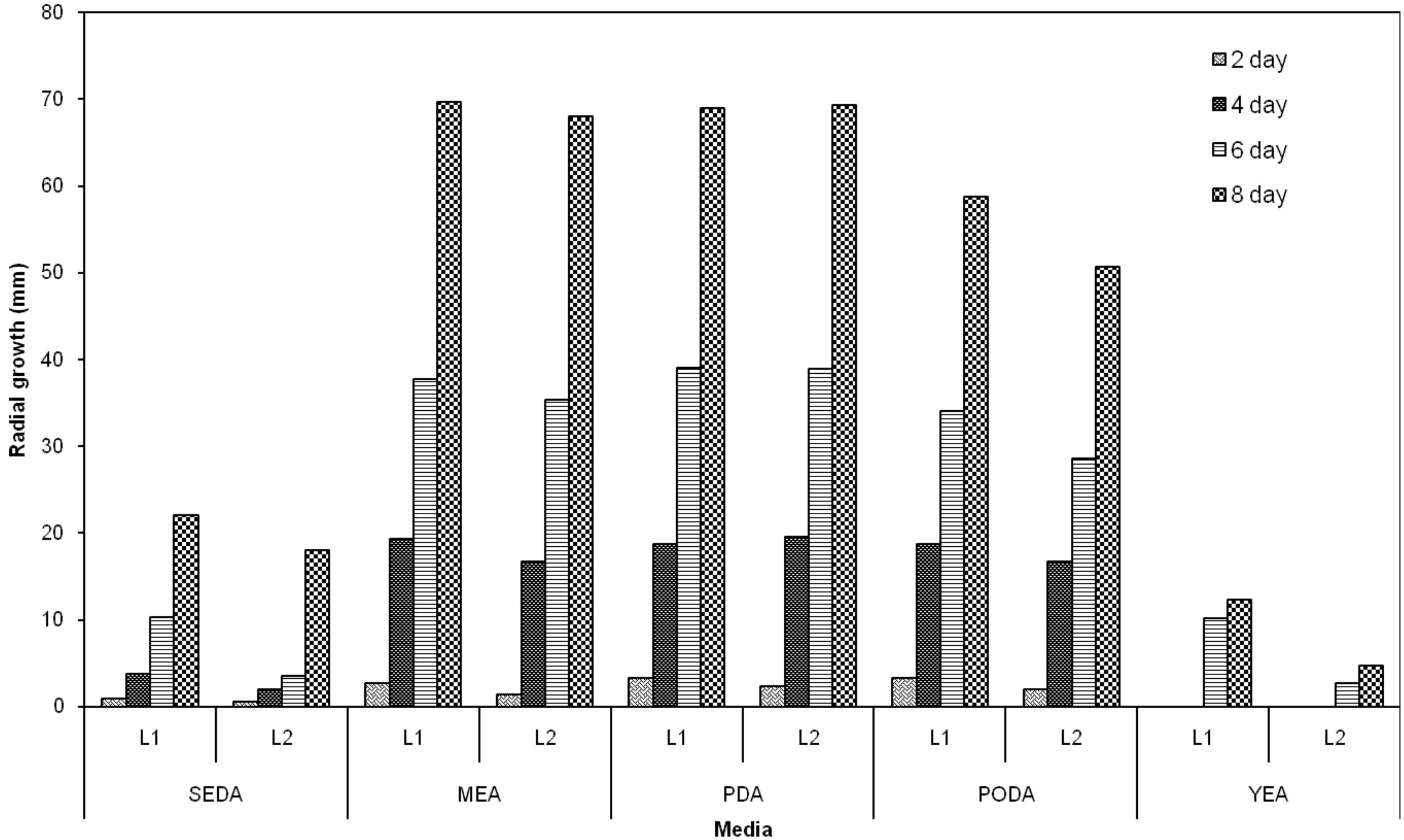
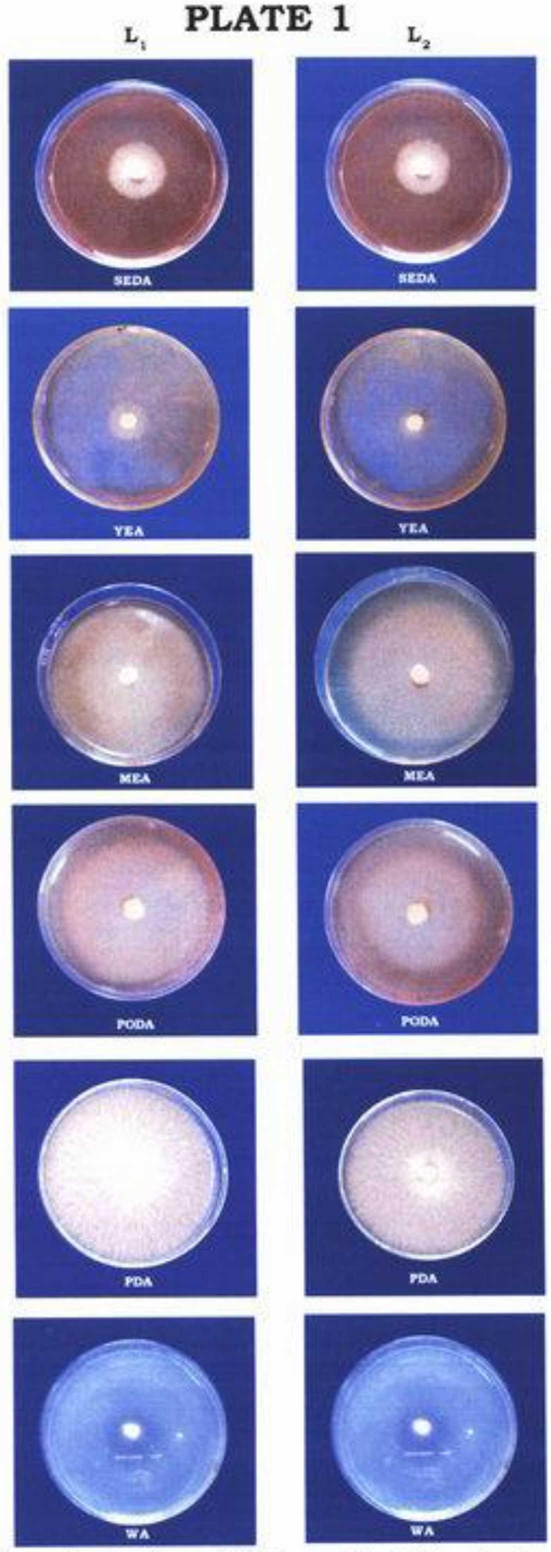


Fig. 4.1 : Radial growth of *L. edodes* strains on different media at 25°C

PLATE 1



Effect of media on the radial growth of *L. edodes* strains

4.2 Radial Growth of *L. edodes* Strains at Different Temperature

Radial growth of two strains of *L. edodes* were studied at temperature range from 15-35°C (Table 4.2, Fig. 4.2 and Plate 2) on Potato dextrose agar medium. These results revealed that among the temperature tested, the fungus showed maximum growth at 25°C. Both the strain L_1 and L_2 showed significantly higher growth rate (10.0 and 8.8 mm/day, respectively) at temperature 25°C. Decrease in average growth rate was observed the by decreasing the temperature below 15°C and increasing the temperature upto 35°C.

These results are in accordance with the findings of Khan *et al.* (1991). They also reported maximum radial growth of *L. edodes* at 25°C, whereas, Furlan *et al.* (1997) observed maximum growth rates at 30°C than at 20 or 25°C on wheat dextrose agar (WDA) medium. Maki *et al.* (2001) reported ideal temperature for mycelial cultivation ranged between 25 to 28°C.

4.3 Effect of pH on the Radial Growth of *L. edodes* strains

Radial growth of two strains of *L. edodes* was studied on PDA at different pH ranging from 4.5 to 8.0 (Table 4.3, Fig. 4.3 and Plate 3). The data revealed that the fungus grew well between 4.5 to 6.0. The fungus showed highest growth rate at pH 5.0 (9.9 mm/day). The growth rate of L_1 (10.3 mm/day) and L_2 (9.6 mm/day) were found to be significantly higher at pH 5.0. However, at pH 4.5 the growth measured

Table 4.2 : Radial growth of *L. edodes* strains (in mm) on potato dextrose agar at different temperature

Temperature	15°C			20°C			25°C			30°C			35°C		
Strains / Day	L_1	L_2	Mean	L_1	L_2	Mean	L_1	L_2	Mean	L_1	L_2	Mean	L_1	L_2	Mean
2	5.0	4.7	4.8	11.7	3.0	7.3	12.0	6.7	9.3	10.3	2.0	6.2	3.0	0.0	1.5
4	11.0	10.7	10.8	31.3	21.0	26.2	35.3	29.0	32.2	29.0	16.0	22.5	13.7	7.3	10.5
6	23.3	27.7	25.5	53.0	40.7	46.8	57.7	50.0	53.8	45.7	30.3	38.0	30.3	23.7	27.0
8	37.3	33.7	35.5	73.7	61.0	67.3	80.3	70.7	75.5	62.3	49.7	56.0	49.3	41.0	45.2
Mean	19.2	19.2	19.2	42.4	31.4	36.9	40.3	39.1	42.7	36.8	24.5	30.7	24.1	18.9	21.04
Growth/day	4.7	4.2	4.5	9.2	7.6	8.4	10.0	8.8	9.4	7.8	6.2	7.0	6.2	5.1	5.7
CD at 5%															
Strains (A)		1.7			1.8			3.2			2.1			1.2	
Days (B)		2.4			2.6			4.6			2.9			1.7	
A × B		3.4			3.7			6.5			4.2			2.4	

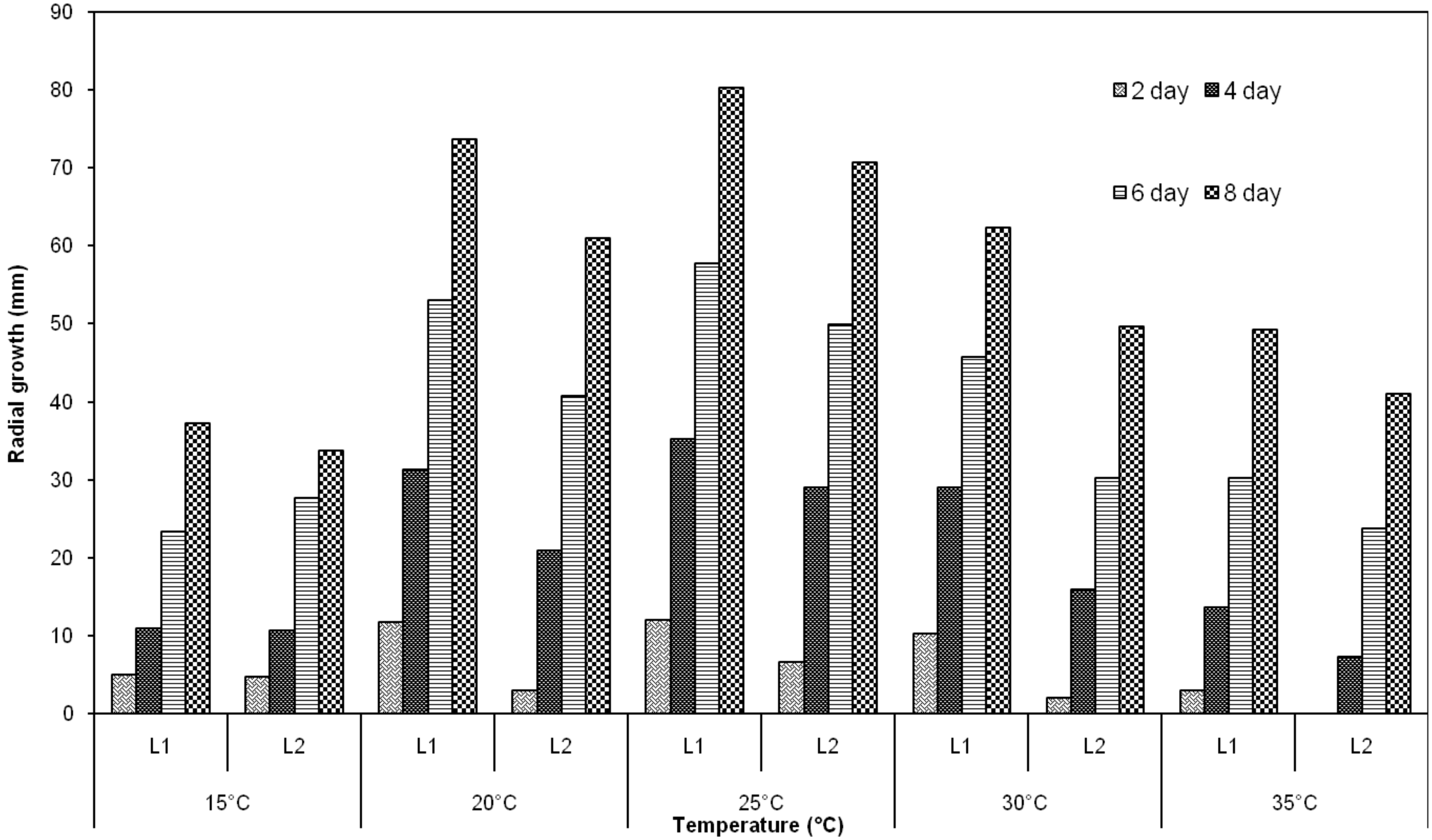


Fig. 4.2 : Radial growth of *L. edodes* strains on potato dextrose agar at different temperature

Table 4.3 : Effect of pH on radial growth (in mm) of *L. edodes* strains on Potato Dextrose Agar medium

Media	4.5			5.0			5.5			6.0			6.5			7.0			7.5			8.0		
Strains / Day	L_1	L_2	Mean	L_1	L_2	Mean	L_1	L_2	Mean	L_1	L_2	Mean	L_1	L_2	Mean	L_1	L_2	Mean	L_1	L_2	Mean	L_1	L_2	Mean
2	3.3	0.0	1.7	3.7	0.0	1.8	4.7	4.7	4.7	4.0	0.0	0.0	1.3	2.0	1.7	3.3	0.0	1.7	4.3	10.3	7.3	3.6	1.7	2.6
4	36.7	25.0	30.8	31.0	25.3	28.2	27.3	25.7	26.5	31.3	24.0	27.7	27.7	23.0	22.8	27.0	18.0	22.5	17.2	15.7	16.4	8.0	5.7	6.8
6	59.3	48.3	53.8	55.0	47.7	51.3	41.1	48.0	44.5	56.7	46.3	51.5	43.0	44.0	43.5	49.0	38.0	43.5	26.3	23.2	27.8	23.7	17.0	20.3
8	79.0	69.0	74.0	82.0	77.0	79.5	71.7	73.3	72.5	77.7	71.3	74.5	64.7	64.7	64.7	70.3	59.7	65.0	45.0	39.2	42.1	44.7	36.0	40.3
Mean	44.6	35.6	40.1	42.9	37.5	40.2	45.4	46.7	46.0	42.4	35.4	38.9	32.9	33.4	33.2	37.4	28.9	33.2	23.2	22.1	22.7	19.9	15.1	17.5
Growth/day	9.9	8.6	9.2	10.3	9.6	9.9	8.9	9.2	9.1	9.7	8.9	9.3	8.1	8.1	8.1	8.8	7.5	8.12	5.6	4.9	5.3	5.6	4.5	5.04
CD at 5%																								
Strains (A)		1.16			1.98			1.25			0.66			4.15			1.73			1.41			2.87	
Days (B)		1.64			2.80			1.77			0.93			5.87			2.45			1.99			4.07	
A × B		2.32			3.97			2.51			1.32			8.31			3.47			2.82			5.76	

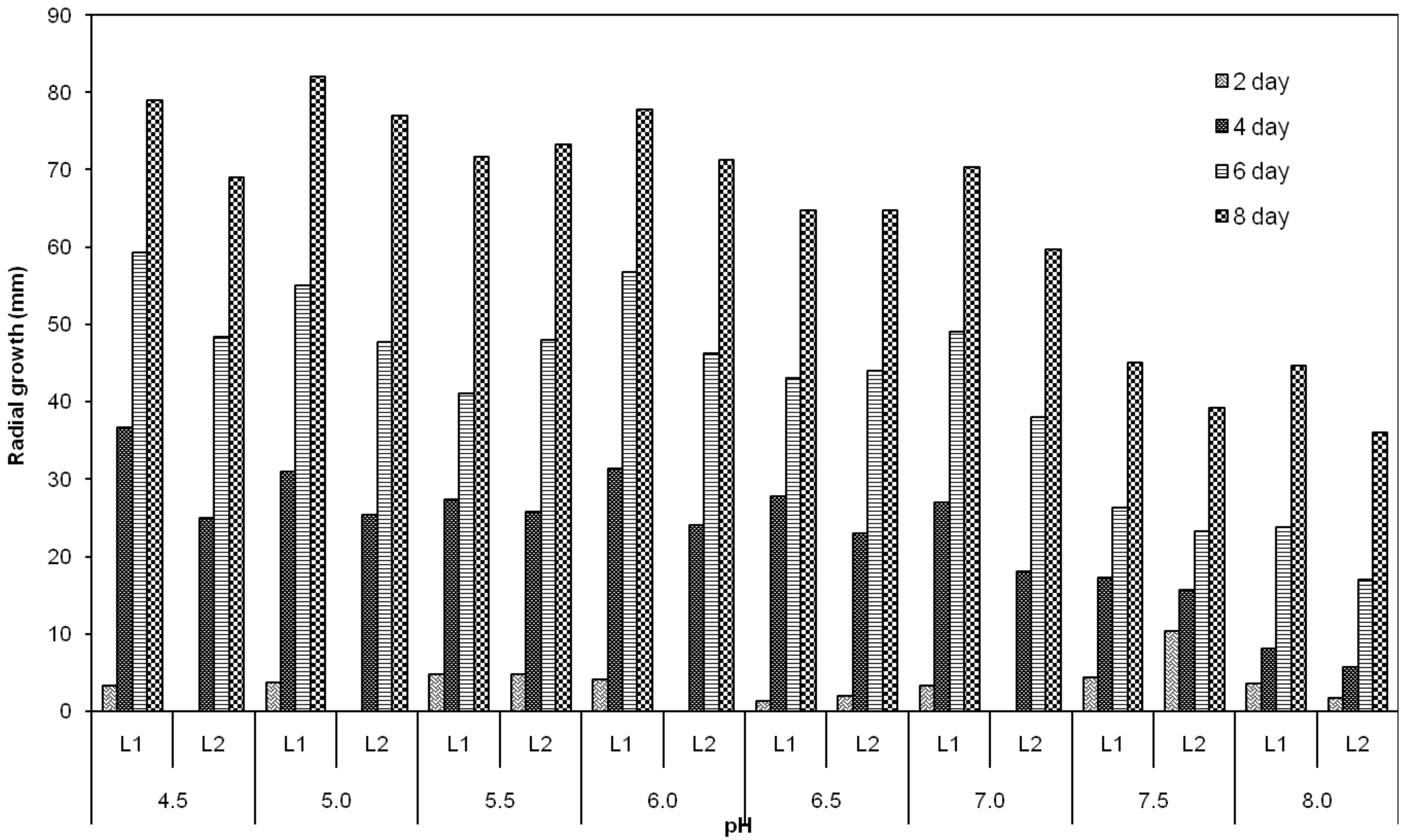
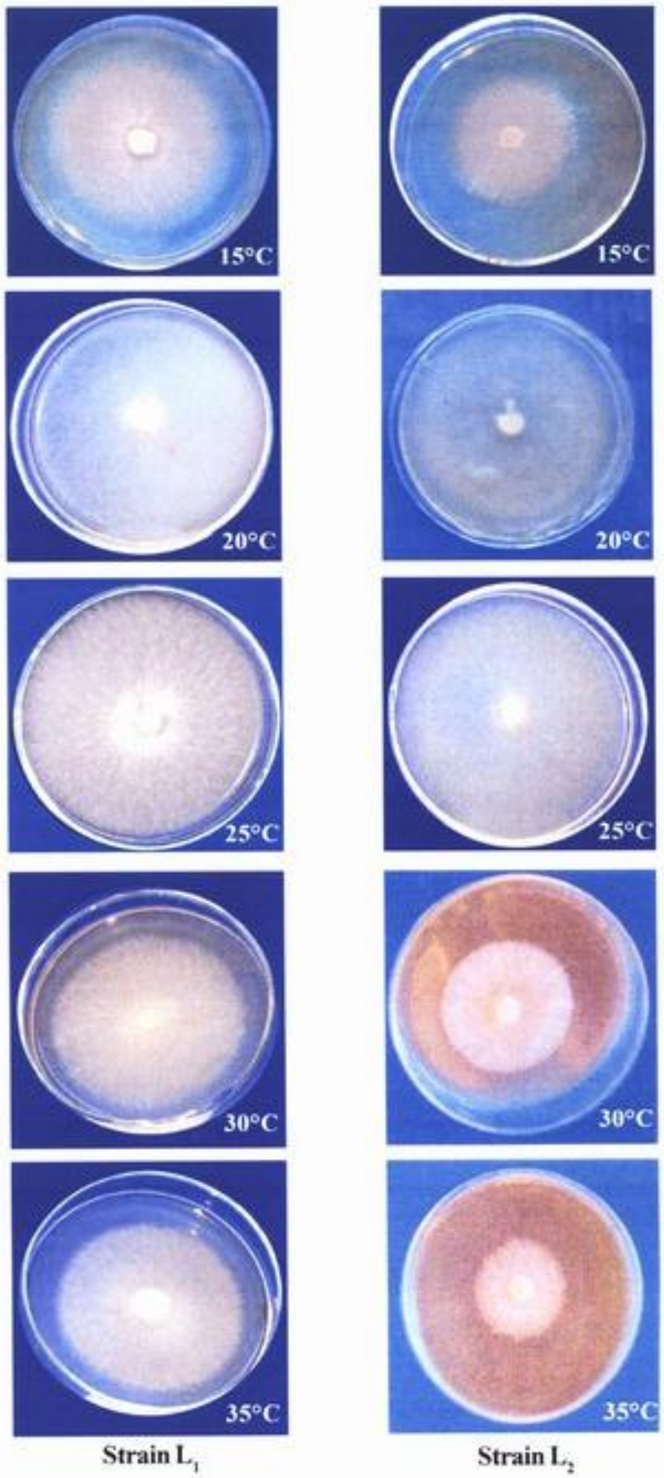


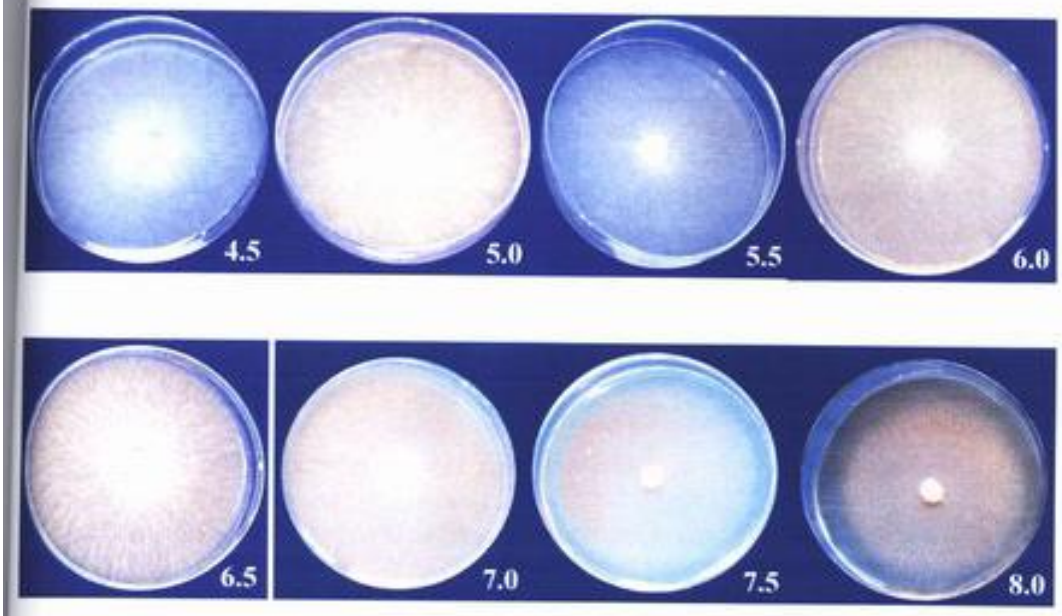
Fig. 4.3 : Effect of pH on radial growth of *L. edodes* strains on PDA medium

PLATE 2

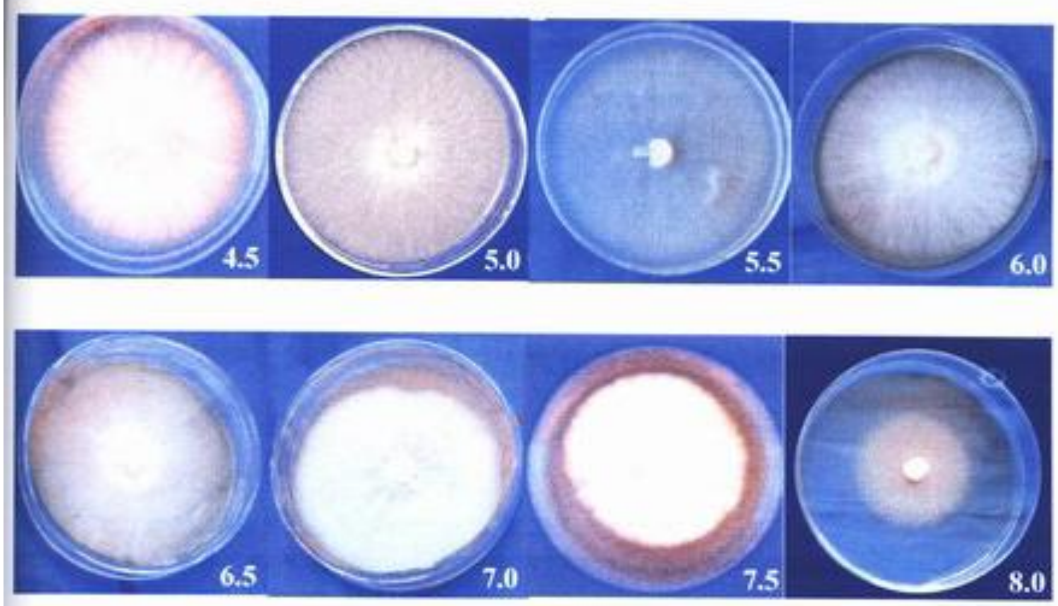


Effect of different temperature on the radial growth of *L. edodes*

PLATE 3



Strain L_1



Strain L_2

Effect of different pH on the radial growth of *L. edodes*

was 9.9 and 8.6 mm/day respectively for both the strains. The cultivation of mycelium was successful at all the pH tested, but as we go on the alkaline side there was a decrease in growth.

In this case, the maximum radial growth was observed at pH 5.0. Khan *et al.* (1991) and Balazs *et al.* (1996) also reported the mycelial growth of *L. edodes* at acidic pH and observed that the most suitable pH for growth of the fungus was 5.0. Campbell (1930 and 1932) worked on the chemical aspect of wood rots had led to the conclusion that acid production and enzyme action are closely related. He suggested that acids might be formed by the action of an oxidase on lignin and pentosans and then these react together with the oxidase to bring about the later stages of decay in which cellulose is decomposed.

4.4 Morphology of Fruit Bodies

The structures of fruit body like diameter and thickness of the pileus, length and diameter of stipe were measured as described in 'Materials and Methods'. The data recorded are summarized in Table 4.4 and Fig. 4.4.

The data in Table 4.4 shows that the highest pileus diameter (87.9 mm) was recorded in strain L_1. There was significant difference among the treatments. The lowest pileus diameter was (77.4 mm) recorded in L_2.

Table 4.4 : Morphological measurements of L_1 and L_2

Strains	L_1	L_2	CD at 5%
Pileus			
Diameter (mm)	87.9	77.4	6.23
Thickness (mm)	20.6	16.6	4.1
Stipe			
Length (mm)	39.0	33.0	1.6
Diameter (mm)	9.9	8.2	1.2
Fruit body length (mm)	92	76	1.4
Fruit body weight (g)	376	300	58.4

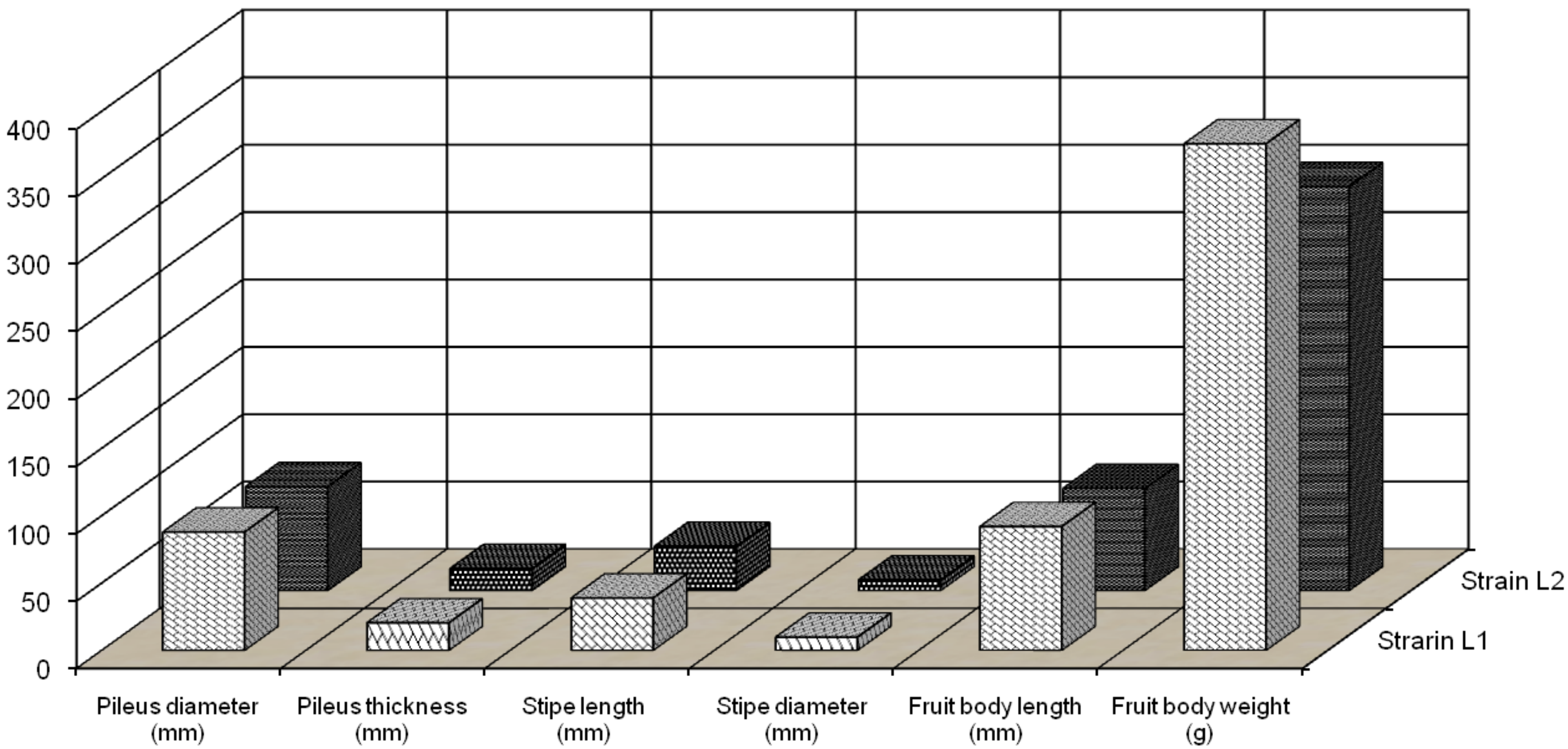


Fig. 4.4 : Morphological measurements of L_1 and L_2

The thickness of pileus did not vary significantly. There was significant difference in the thickness of pileus among the treatments. Maximum thickness (20.6 mm) was recorded in L_1. There was significant difference in length of stipe among the treatments. Maximum stipe length (39.0 mm) in L_1 and (33.0 mm) in L_2 was recorded. Significant difference was observed in the diameter of stipe among the treatments. Maximum and minimum diameter for stipe was found in strain L_1 (9.9 mm) and L_2 (8.2 mm), respectively. There was significant difference in fruit body length among the treatments, the average length of fruit body was maximum in strain L_1 (92.0 mm) and minimum in strain L_2 (76.0 mm).

The wheat straw substrate gave largest heaviest sporocarps with pileus diameter and fruit body weight 75-90 mm and 60-85 g, respectively. However, the substrate of poplar sawdust and teak sawdust individually, produced smallest fruiting bodies (pileus diameter- 20-35 mm and fruit body weight- 18-24 g).

Significant difference was recorded in fruit body weight among the treatments, higher fruit body weight was recorded in L_1 strain (37.6 g) in comparison to L_2 strain.

Purkayastha and Chandra (1985) recorded the pileus diameter and stipe length of Shittake mushroom utpo 11.0 cm and 3.0-4.0 × 0.8-1.3 cm long, respectively. Pegler (2003) recorded the diameter of pileus 5-15 cm across and Gaitan *et al.* (2004) recorded the pileus diameter ranging from 5 to 20 cm. Our results are in accordance with the previous workers.

4.5 Effect of Supplementation on the Yield of *L. edodes*

The experiment was conducted to validate the yield of *L. edodes* on wheat straw which was the best substrate in terms of yield, with the supplementation of wheat bran at 5 and 10 per cent, alongwith check. Wheat bran supplemented @ 10 per cent gave significantly higher yield (80.4 g/500 g dry substrate) over all the substrates and minimum yield was observed in case of check (Table 4.5, Fig. 4.5 and Plate 4). The strain L_1 gave higher yield and found to be best. L_2 gave yield of 61.1 g/500 g dry substrate. In the present investigation, the biological efficiencies recorded for both strains were 45.9 and 34.9 per cent, respectively. On the basis of above results, it is obvious that among the supplements evaluated for yield of *L. edodes* strains, 10 per cent supplementation of wheat bran was found to be the best in terms of yield and biological efficiency. Royse (1985) cultivated Shiitake on the mixture of maple and birch (60:40) sawdust substrate with 10 per cent spring wheat bran and 10 per cent millet and it was found the best formula for nutritional components. Royse *et al.* (1990) advocated that regardless of the main ingredient used, starch based supplements such as wheat bran (10-40% dry weight) serve as nutrients to provide an optimum growing medium. Silva *et al.* (2005) also found that eucalyptus residues supplemented with cereal brans supported fast growth of

Table 4.5 : Effect of supplementation of wheat bran on wheat straw substrate on the yield of *L. edodes* strains

S. No.	Substrates	Strains/yield (g)			
		L_1		L_2	
		Weight (g)	**Biological efficiency (%)**	**Weight (g)**	**Biological efficiency (%)**
1.	Wheat straw	41.2	23.5	32.6	18.6
2.	Wheat straw + wheat bran (5%)	48.0	34.7	34.9	24.8
3.	Wheat straw + wheat bran (10%)	80.4	45.9	61.1	34.9
	CD at 5%				
	Strains (A)	10.3	–	10.3	–
	Substrate (B)	12.6	–	12.6	–
	A × B	17.8	–	17.8	–

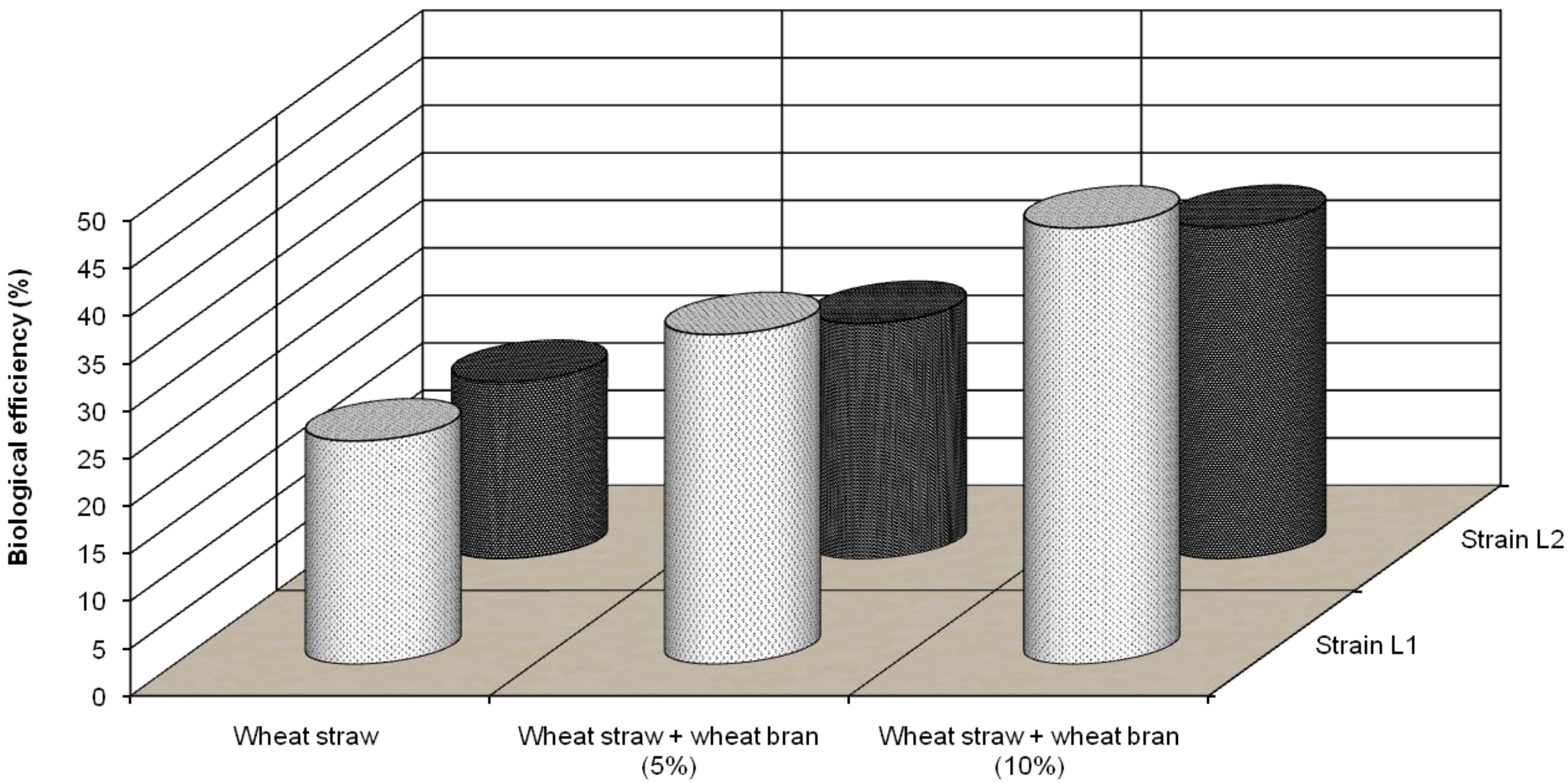


Fig. 4.5 : Effect of supplementation of wheat bran on wheat straw substrate on the yield of *L. edodes* strains

PLATE 4

Wheat straw (Check)

Wheat straw + 5% wheat bran

Wheat straw + 10% wheat bran

Cultivation of *L. edodes* over wheat straw using different supplementation of wheat bran

L. edodes indicating that mycelium extension is related to the bioavailability of nitrogen. The type and concentration of nutrient supplement has a considerable effect both on substrate colonization and on the type of hydrolytic and oxidative enzymes produced. These characteristics may be useful for mushroom growing.

4.6 Evaluation of Different Substrates for Production of *L. edodes* Strains

The experiments were conducted to screen the different substrates (Plate 5) viz., wheat straw, coirpith, poplar sawdust, teak sawdust, sal sawdust, wheat straw and coirpith (1:1), wheat straw and poplar sawdust (1:1), wheat straw and teak sawdust (1:1), wheat straw and sal sawdust (1:1), poplar sawdust and teak sawdust (1:1), poplar sawdust, and sal sawdust (1:1), poplar sawdust and coirpith (1:1), teak sawdust and coir pith (1:1), teak sawdust and sal sawdust (1:1) and sal sawdust coipith (1:1) for production of *Lentinula edodes*. The results were presented in Table 4.6, Fig. 4.6 and Plate 6. The results revealed that all the substrates showed significant difference to one another in terms of yield. Wheat straw gave significantly higher yield among all the substrates evaluated. Minimum yield was observed in popular sawdust. Intermediate yield was recorded in wheat straw and poplar sawdust, wheat straw and teak sawdust, poplar sawdust and teak sawdust and teak sawdust.

Table 4.6 : Screening of substrates for production of *L. edodes* strains/yield (g)

S. No.	Substrates	L_1		L_2	
		Weight (g)	**Biological efficiency (%)**	**Weight (g)**	**Biological efficiency (%)**
1.	Wheat straw + 10% wheat bran	80.4	45.9	61.1	40.7
2.	Wheat straw + poplar sawdust + 10% wheat bran	77.4	33.2	73.2	31.2
3.	Wheat straw + teak sawdust + 10% wheat bran	23.2	9.9	21.4	9.2
4.	Wheat straw + coirpith + 10% wheat bran	0.0	0.0	0.0	0.0
5.	Wheat straw + sal sawdust + 10% wheat bran	0.0	0.0	0.0	0.0
6.	Poplar sawdust + 10% wheat bran	19.0	8.14	11.4	4.9
7.	Poplar sawdust + teak sawdust + 10% wheat bran	56.6	24.2	22.8	9.8
8.	Poplar sawdust + coirpith + 10% wheat bran	0.0	0.0	0.0	0.0
9.	Poplar sawdust + sal sawdust + 10% wheat bran	0.0	0.0	0.0	0.0
10.	Teak sawdust + 10% wheat bran	20.3	5.8	13.17	3.8
11.	Teak sawdust + coirpith + 10% wheat bran	0.0	0.0	0.0	0.0
12.	Teak sawdust + sal sawdust + 10% wheat bran	0.0	0.0	0.0	0.0
13.	Coirpith + 10% wheat bran	0.0	0.0	0.0	0.0
14.	Coirpith + sal sawdust + 10% wheat bran	0.0	0.0	0.0	0.0
15.	Sal sawdust + 10% wheat bran	0.0	0.0	0.0	0.0
	CD at 5%				
	Strain (A)	2.7	–	2.7	–
	Substrate (B)	7.3	–	7.3	–
	Strain × Substrate	10.3	–	10.3	–

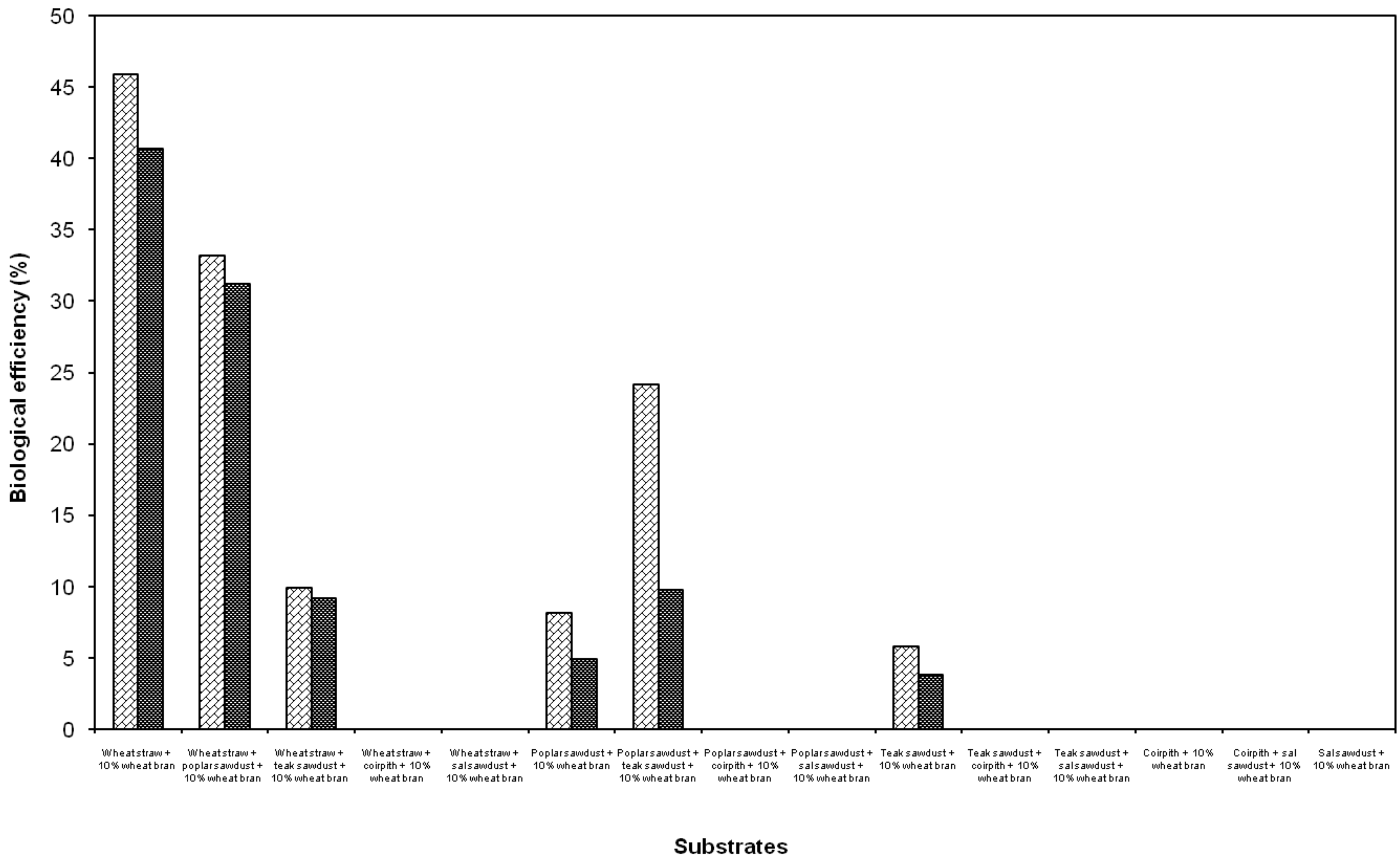


Fig. 4.6 : Screening of substrates for production of *L. edodes* strains/yield (g)

PLATE 5

Strain L_1

Strain L_2

Experimental trails of *L. edodes* cultivation

PLATE 6

Poplar sawdust + teak sawdust

Wheat straw + Poplar sawdust

Wheat straw + Teak sawdust

Wheat straw

Poplar sawdust

Teak sawdust

Screening of the substrate for yield performance of *L. edodes*

In the sawdust of poplar both strains, L_1 and L_2 gave yield (11.4 g/500 g dry substrate and 19.0 g/500 g dry substrate, respectively). In the mixture of wheat straw and coirpith, poplar sawdust and coirpith, teak sawdust and coirpith, sal sawdust and coirpith, alone, the mycelium completely colonized the above substrates and a few primordia also appeared but they failed to develop into fruiting bodies. In the mixture of wheat straw and sal sawdust; poplar sawdust and sal sawdust, the mycelium colonized the substrate after a much longer time but produced no primordia even under favorable conditions. The sawdust of sal and mixture of sal and teak sawdust was not colonized completely with the mycelial growth and stopping after a few days and producing no primordial.

The colonization of different sawdust substrates was achieved at durations varying from 55-85 days. The minimum time for colonization (55 days) was recorded in the mixture of wheat straw and poplar sawdust and maximum (85 days) in the mixture of sal and coirpith. In fact, the sawdust of sal was never colonized by the fungus completely, the growth stopping after sometime. The wheat straw and sawdust of poplar, when used individually were colonized in a much longer period than when they were used in mixture.

In general, strain L_1 was superior than the L_2 in terms of yield. The yield of L_1 ranged from 19.0 to 80.4 g/500 g dry substrate whereas the yield of L_2 ranged from 11.4 to 61.1 g/500 g dry substrate. The biological efficiency of L_1 ranged from 5.8 to 45.9 per cent in comparison to L_2 (3.8 to 40.7%).

On the basis of the above experimental results, it was obvious that among the different substrates screened for yield performance of *L. edodes* strains, wheat straw was found to be superior in terms of yield and biological efficiency. In present investigation, the substrate sawdust gave only 3.8-24.2 per cent biological efficiency which was lower than that of wheat straw (9.2 to 45.9%).

Chou-Chou (1984) reported maximum yield (569 g/kg) with 56.9 per cent biological efficiency on tawa sawdust supplemented with barley, whereas the yield obtained from the pure *P. radiata,* poplar and beach had been reported to be negligible. Kaur and Lakhanpal (1995) used *Populus* sawdust alone obtained a very low yield with 6 per cent biological efficiency in comparison to the sawdust mixture. Kovacsne and Kovacs (2000) and Zervakis *et al.* (2001) also reported wheat straw as a most suitable substrate for production *L. edodes*. Variable ranges in biological efficiencies of Shiitake hare been reported by different workers with different sawdusts. Philippoussis *et al.* (2003) reported the production of heavier basidiomata on wheat straw and oakwood sawdust substrates with biological efficiency 54.17 per cent whereas, Gaitan *et al.* (2004) reported biological efficiency wheat straw substrate ranged from 24.8 to 55.6 per cent.

Our findings are in accordance with the previous workers in case of wheat straw and poplar sawdust used as a substrate. We have recorded the yield of *L. edodes* using teak sawdust, wheat straw and poplar sawdust, wheat straw and teak sawdust and poplar and teak sawdust as substrate. There is no any evidence in literature in using above mentioned substrates for production of *L. edodes*. The present study has not been tried by anyone else so far so, the results are not absolutely comparable. But the biological efficiency recorded on these substrates is comparable with those recorded by others.

4.7 Total Sugars, Reducing Sugars and Non-Reducing Sugars in *Lentinula edodes* Strains

The biochemical components like total sugars, reducing sugars and non-reducing sugars were estimated from two strains of *L. edodes* and results are given in Table 4.7.

The data revealed that total soluble sugars varied between 22.33-22.38 per cent on dry weight basis. There were non significant differences between the strains. The maximum total soluble sugars content was found in L_1 (22.38%).

Reducing sugars content varied between 0.030-0.031 per cent on dry weight basis. The difference in reducing sugars content of two strains does not vary significantly.

Non-reducing sugars varied between 22.30-22.35 per cent on dry weight basis. The higher content of non-reducing sugars were found in

Table 4.7 : Total sugars, reducing sugars and non-reducing sugars contents (% dry weight) in *L. edodes* strains

Components / Strains	Total soluble sugars (%)	Reducing sugars (%)	Non-reducing sugars (%)	Protein content (%)
L_1	22.38	0.031	22.35	10.03
L_2	22.33	0.030	22.30	11.11
Mean	22.36	0.031	22.33	10.57
CD at 5%	0.18	0.006	0.18	0.92

L_1 (22.35%). Both strains were non-significantly different in terms of non-reducing sugar contents.

The data revealed that protein content was maximum in L_2 (11.11%) which was significantly different from other strain, L_1 (10.57%).

In the present investigation, the fruiting body of *L. edodes* contained 22.36 per cent total soluble sugars, 0.03 per cent reducing sugars and 22.33 per cent non-reducing sugars with 10.57 per cent protein content. These results are in accordance with Mishra (2005) in Ganoderma.

SUMMARY

The present investigation was carried out on "Studies on cultural, biochemical characterization and production technology of *Lentinula edodes* (Berk.) Pegler" at Mushroom Research and Training Centre of the G.B. Pant University of Agriculture and Technology, Pantnagar. The trails were conducted on prevailing crop room conditions during 2005-2006.

The strains of *L. edodes* varied extensively in their cultural, morphological and biochemical characters along with yielding abilities. The findings observed under present investigations are summarized as follows :

1. Among the different media tested, potato dextrose agar (PDA) medium was found to be superior in terms of radial growth (8.7 mm/day). No growth was obtained on water agar medium.
2. Among the temperatures tested, the fungus showed maximum radial growth at 25°C (9.4 mm/day). A reduction in radial growth was obtained on increasing or decreasing the temperature.
3. The cultivation of mycelium was successful at all the pH tested. The fungus preferred acidic medium (pH 5.0, 9.9 mm/day) for their growth. There was a decrease in growth when the pH of the medium increased.

4. On the basis of morphological (fruit body weight, fruit body length, pileus diameter, pileus thickness, stipe length and stipe diameter) as well as cultural and yield characters, strain L_1 was superior than L_2.

5. The minimum time for colonization of substrate (55 days) was recorded in the mixture of wheat straw and poplar sawdust whereas the mixture of sal and coirpith took maximum time for colonization (85 days).

6. Increasing the rate of supplements resulted in higher yield. Wheat bran @ 10 per cent gave significantly higher yield (80.4 g/500 g dry substrate) with 45.9 per cent biological efficiency.

7. Among the different substrates used for yield of *L. edodes* strains, wheat straw was found to be superior (80.4 g/500 g dry substrate for L_1 and 61.1 g/500 g dry substrate for L_2). This substrate also showed the highest biological efficiency (45.9% for L_1 and 34.9% for L_2) in comparison to other substrates. The coirpith and sal sawdust alone and in combination also gave no satisfactory yield.

8. The fruiting body of *L. edodes* contained 22.38 per cent total soluble sugars, 0.031 per cent reducing sugars and 22.35 per cent non-reducing sugars on dry weight basis. The estimated sugar contents of both the strains do not vary significantly. The amount of these sugars contents was found to be higher in strain L_1.

9. The protein content in *L. edodes* ranged from 10.57-11.11 per cent. It varied significantly in both the strains. The amount of protein was higher in strain L_2.

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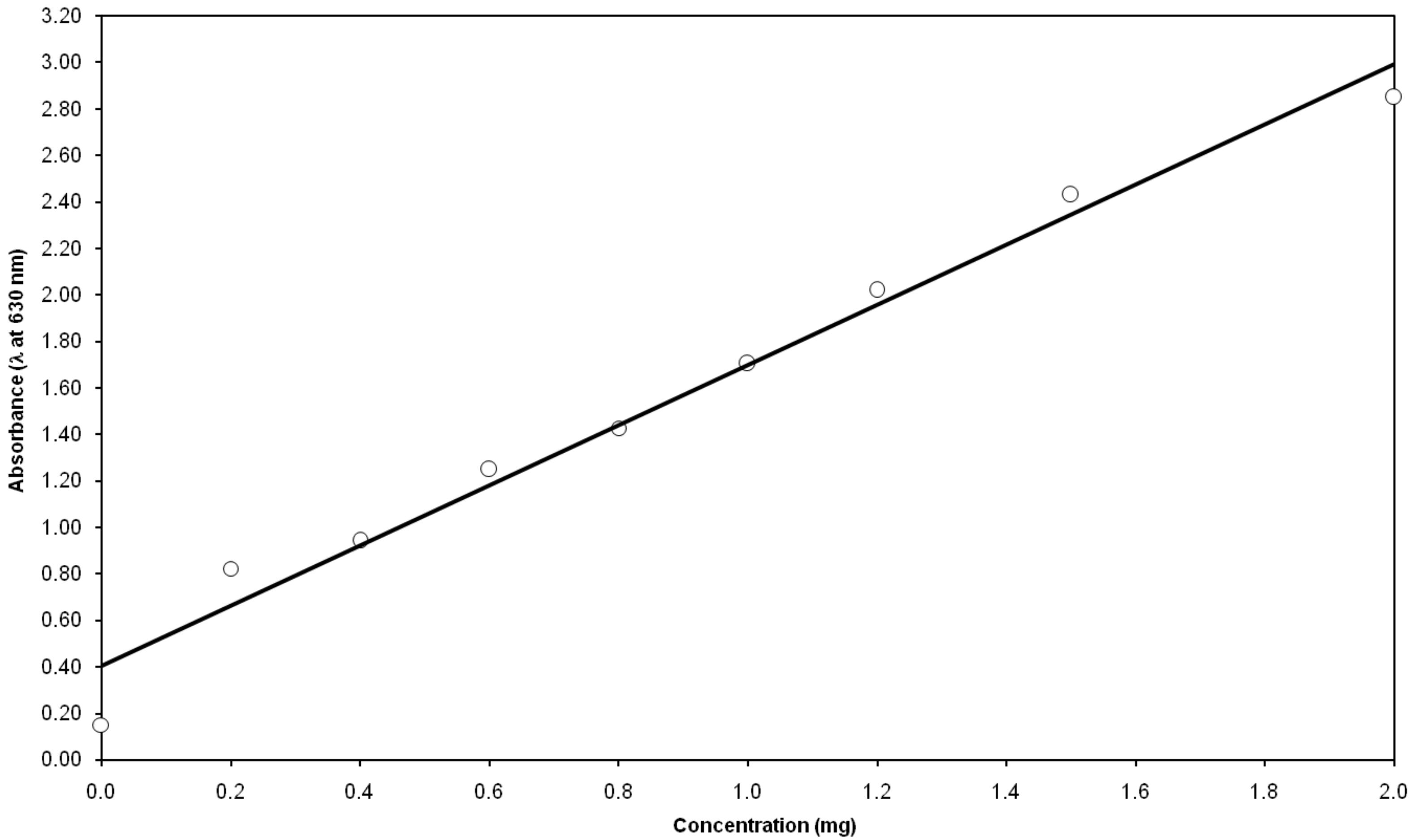
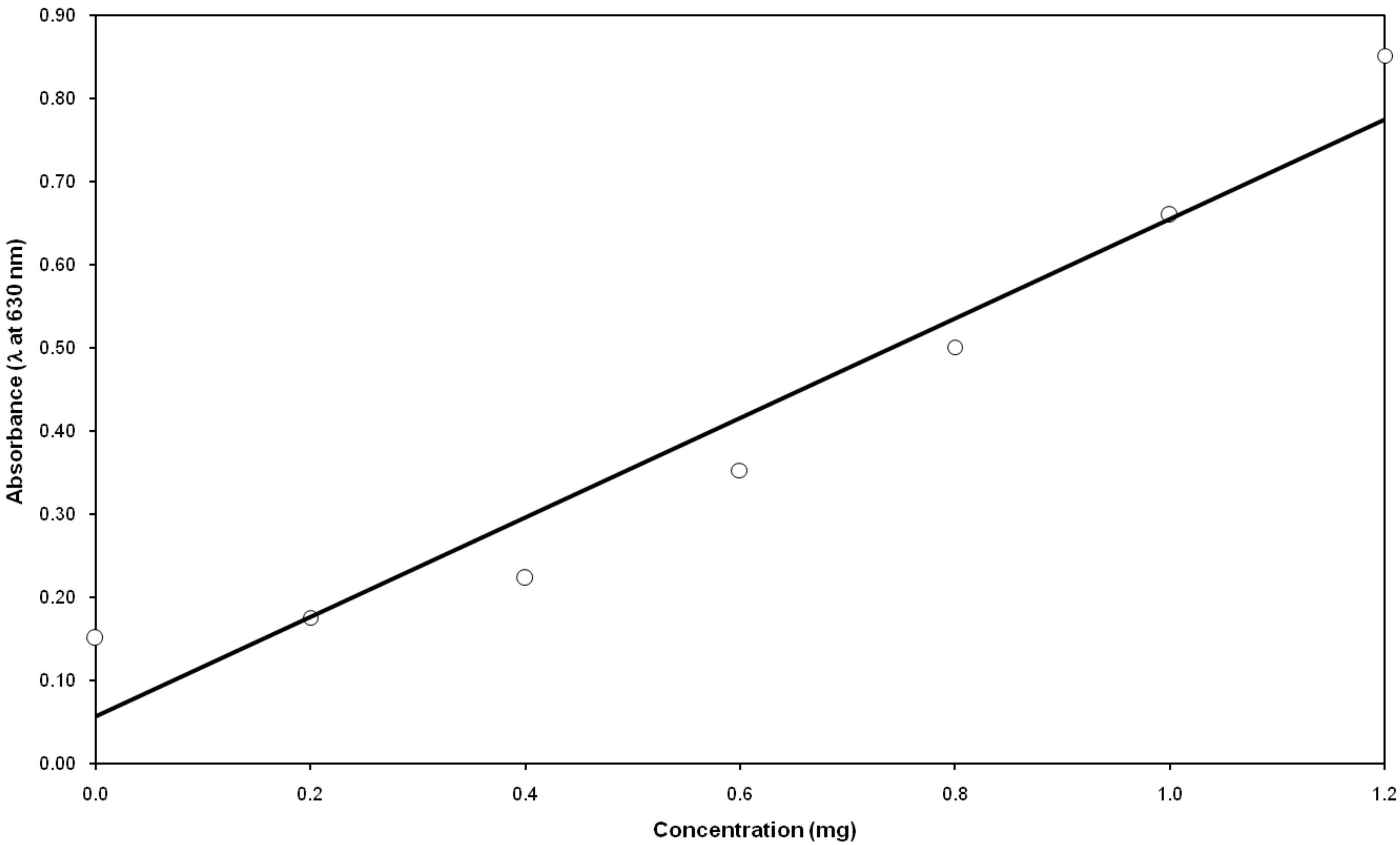
Appendix I



Appendix I : Calibration curve for total soluble sugars

Appendix II



Appendix II : Calibration curve for non reducing sugars

Appendix III

I. Composition of reagents used in estimation of reducing sugars

(A) Alkaline copper tartarate

(a) Dissolve 2.5 g anhydrous sodium carbonate, 2 g sodium bicarbonate, 2.5 g potassium sodium tartarate and 20 g anhydrous sodium sulphate in 80 ml water and make up to 100 ml.

(b) Dissolve 15 g copper sulphate in a small volume of distilled water. Add one drop of sulphuric acid and make upto 100 ml.

Mix 4ml of (b) and 96 ml of solution (a) before use.

(B) Arsenomolybdate reagent

Dissolve 2.5 g ammonium molybdate in 45 ml water. Add 2.5 ml sulphuric acid and mix well. Add 0.3 g disodium hydrogen arsenate dissolved in 25 ml water. Mix well and incubate at 37°C for 24 to 48 h.

II. Composition of alkaline copper solution used in estimation of protein

1. Solution A : 2% sodium carbonate in 0.1 N NaOH
2. Solution B : 0.5% copper sulphate in 1% sodium potassium tartarate (prepare fresh).
3. Solution C (alkaline copper solution) : Mix 50 ml of solution A with 1 ml of solution B just prior to use.

Vita

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ABSTRACT

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Lentinula edodes (Berk.) Pegler also known as Shiitake, is a white rot wood decay fungus, that produces flavourful brown sporocarps with medicinal properties. According to a Chinese folkfare, this mushroom is a "elixir of life", capable of generating stamina, curing colds, improving circulation, lowering blood pressure and prevent premature aging. Many biologically active substances, in particular polysaccharides (Lentinan and lps) have been isolated from *L. edodes* and a number of products prepared from it, are sold throughout the world as dietary supplements. Many countries have developed production technology of this mushroom but detailed accounts are not available in literature. Therefore, present investigations were undertaken to cultivate, to know its cultural characters along with the biochemical makeup of different strains.

The cultural characters of two strains were studied for their radial growth on different media, pH and at different temperatures. Different substrates were used to study the yield performance and biological efficiency of strains. The biochemical makeup of both the strains were studied by estimating total soluble sugars, reducing sugars and non-reducing sugars.

The findings indicate that the strains vary extensively in their cultural, morphological and biochemical characteristics along with yielding abilities. Potato dextrose agar medium, temperature 25°C and acidic pH 5.0 were best suited to *L. edodes* strains. Among the substrates evaluated, wheat straw supplemented with 10 per cent wheat bran gave the maximum yield (80.4 g) and biological efficiency (45.9%) in case of strain L_1. The total soluble sugars, reducing sugars and non-reducing sugars contents do not vary significantly in the strains. Total soluble sugars, reducing sugars and non-reducing sugars content are found to be 22.38, 0.03 and 22.35 per cent, respectively, in the fruiting bodies.

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