EVALUATION OF BIOCHEMICAL AND ANTI-CANCEROUS ACTIVITIES OF MUSHROOMS

by

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled "**Evaluation of biochemical and anti-cancerous activities of mushrooms**" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Date: 30-07 -2018

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Certified that this thesis entitled **"Evaluation of biochemical and anti-cancerous activities of mushrooms"** is a record of research work done independently by Ms. Agnes Jose under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Agnes Jose

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LIST OF ABBREVIATIONS AND SYMBOLS USED

MTT	3-(4,5- dimethylthiazol-2,5- diphenyl tetrazolium bromide
et al.	And other co workers
B.E	Biological Efficiency
cm	Centi meter
CRD	Completely randomized design
CD	Critical difference
°C	Degree Celecius
DMEM	Dulbecos Modified Eagles Medium
DPPH	2,2- diphenyl-1-picrylhydrazyl
GAE	Gallic Acid Equivalent
g	Gram
h	Hours
kg	Kilo gram
µg	Micro gram
µl	Micro litre
µm	Micro meter
mg	Milli gram
mm	Milli meter

min	Minutes
viz.	Namely
pH	Negative logarithm of hydrogen ions
M	Molar
mM	Milli molar
N	Normal
nm	Nano meter
OD	Optical density
ppm	Parts per million
%	Per cent
PDA	Potato dextrose agar
QE	Quercetin Equivalent
rpm	Revolutions per minute
Sl. No.	Serial number
sp. or spp.	Species (Singular and plural)
S.Em±	Standard error of mean
temp	Temperature
i.e	That is
wt.	Weight

Introduction

1. INTRODUCTION

Mushrooms are fruiting bodies that produce spores, growing from the hyphae of fungi concealed in soil or wood. The emergence of newer commercial cultivars as well as the medicinal and nutritional benefits of mushroom boosted up the mushroom industry globally. Mushrooms have been considered as a source of healthy food for a long time and are rich in bioactive compounds such as polysaccharides, proteins, fats, ash, glycosides, alkaloids, volatile oils, phenolics, flavonoids, carotenoids, ascorbic acid, enzymes and organic acids with biological activities like immune-modulatory, anti-viral, anti-proliferative, anti-fungal and anti-bacterial effects (Masra, 2014). Recently, anti-cancer agents have also been identified from various medicinal and edible mushrooms. Polysaccharides and triterpenes are the most potent mushroom derived substances with anti-cancerous and immunomodulation properties. The anti-cancerous drugs currently available in market possess several side effect and complications in clinical management of the disease which emphasize the urgent need for novel, effective and less toxic therapeutic approaches (Patel *et. al.*, 2012).

Ganoderma lucidum (Curt. Fr.) P. Krast, commonly referred as Lingzhi, is well-known for its pharmacological benefits including immune-modulating, anti-inflammatory, anti-cancer, anti-diabetic, anti-oxidative, radical-scavenging and anti-aging effects. Both *in vitro* and *in vivo* studies have been conducted regarding the anti-cancer effects of *G. lucidum*. In addition, mushrooms also contain minerals such as potassium, phosphorus, calcium, magnesium and iron. Mushroom protein is reported to possess essential and non- essential amino acids. Numerous authors reported that triterpenes and polysaccharides are the major physiologically active components of *G. lucidum* which induce apoptosis in human cancer cell lines *via* mitochondria-dependent pathway. Cancer cells generate increased levels of free radicals compared to normal cells, further contributing to cancer progression. This cancer-inducing oxidative damage might be prevented or limited by mushroom derived anti-oxidants which directly react with or scavenge the free radicals (Kao *et al.* 2013).

The genus *Pleurotus* comprises a group of edible ligninolytic mushrooms with medicinal properties and environmental applications. The cultivation of *Pleurotus* sp. is economically important in food industry worldwide because it is considered to be rich in protein, fibre, carbohydrates, vitamins, minerals and is also cholesterol free. Besides, *Pleurotus sp.* is promising as medicinal mushrooms with numerous biological activities. The white oyster mushroom, *Pleurotus florida* (Mont.) is a commonly available edible mushroom having excellent flavour and taste. The bioactive compounds in *P. florida* such as polysaccharides and protein have health benefits including immunomodulatory, anticancer and hypocholesterolemic effects. Polysaccharides especially beta-glucan obtained from mushroom have been used for various purposes (Rout *et al.*, 2005).

The pink oyster mushroom, scientifically known as *Pleurotus djamor* (Fr.) Boedjn is an edible species of fungi that has a vibrant pink colour and ruffled appearance Nutritionally, it contains high levels of protein and essential elements such as vitamins, minerals, polysaccharides and a potential source of natural antioxidants (Chang and Miles , 2004). Oyster mushrooms are different from each other in nutritional composition although they are of same genus (Randive, 2012).

Hypsizygus ulmarius (Bull.Fr) Red Head is an edible mushroom, also known as elm oyster mushroom or blue oyster mushroom. *H. ulmarius* belongs to the family Tricholomataceae and grow in clusters on living elm trees or elm logs in the forests and thus named as elm oyster. The first successful cultivation of *Hypsizygus* was done during 1973. The cultural, physiological and spawn characters of this mushroom were studied by Wange and Patil (2007). *H. ulmarius* has high amount of protein, fibre and carbohydrate and thereby make mushroom as a low energy and healthy foodstuff. Hence mushroom is a promising food that may overcome protein energy malnutrition (Usha and Suguna, 2015). The mushroom *Calocybe gambosa* (Fr.) Singh is an edible and tropical milky mushroom cultivating in India. They are reported to possess many nutritive ingredients and are involved in free radical scavenging processes thereby enhancing antioxidant capacity (Mirunalini *et al.* 2012).

In this context, the present study was undertaken to assess the biochemical properties and preliminary studies on the anti-cancerous activities of the three commonly growing oyster mushrooms and one milky mushroom in comparison with the commonly used medicinal mushroom *G. lucidum*.

Review of literature

2. REVIEW OF LITERATURE

G. lucidum, the king of medicinal mushroom has been widely used as a traditional herbal medicine in Asian countries for centuries. It belongs to the phylum Basidiomycota, class Agaricomycetes, order Polyporales and family Ganodermataceae. This taxon is known in different names. The Chinese and Koreans call it as Lingzhi that means mushroom of herb and immortality, whereas the Japanese call this mushroom as Reishi. *G. lucidum* is popular around the world as an anti-cancer drug and symbol of happiness, good fortune, good health, longevity and even immortality. More than 250 taxonomic species have been reported worldwide from this genus, which include *G. adspersum, G. applanatum, G. australe, G. boninense, G. cupreum, G. incrassatum, G. lipsience, G. lobatum, G. lucidum, G. oerstedii, G. oregonense, G. pfeifferi, G. platense, G. resinaceum, G. sessile, G. sinense, G. tornatum, G. tsugae* and *G. webrianum.* Among these, species which are commonly used for medicinal purpose include *G. lucidum*, G. *tsugae and G. applanatum* (Moncalvo *et al.*,1995). In Latin, *lucidum* means shiny or brilliant which describe modelled, sculptured and varnished appearance of fruiting body. The genus *Ganoderma* is characterized by its unique laccate or non-laccate nature and particularly by its double-walled basidiospores (Moncalvo and Ryvarden 1997). *G. lucidum* is well-known for various pharmacological effects including immune-modulating, anti-inflammatory, anti-cancerous, anti-diabetic, anti-oxidative and radical scavenging. Polysaccharides and triterpenes are the major sources of pharmacological active constituents. All parts of *G. lucidum*, such as spore, mycelium and basidiocarp can be used for medicinal purpose (Sheena *et al.*, 2005).

Oyster mushroom is a common edible mushroom characterized with its oyster like shape. The genus has more than 50 well recognized species, out of which 25 have been reported from India of which 12 are cultivated in different parts of the country. The species which are commercially cultivated in different parts of the world are *P. ostreatus, P. flabellatus, P. florida, P. sajor-caju, P. sapidus, P. cystidiosus, P. eryngii, P. fossulatus, P. opuntiae, P. cornucopiae, P.*

yuccae, P. platypus, P. djamor, P. tuber-regium, P. australis, P. purpurea-olivaceous, P. populinus, P. levis, P. columbines, P. membanaceous etc. Its cultivation can be done on a number of agricultural wastes and organic waste materials. Different mushrooms require different climatic conditions for their mycelial growth and fruiting. *Pleurotus* spp. has been recognized as a highly potential converter of cheap cellulosics into valuable protein. *Pleurotus* sp. is promising as medicinal mushroom, exhibiting haematological, antiviral, antitumor, antibiotic, antibacterial, hypocholesterolemic and immunomodulation activities (Narayanasamy *et al.,* 2008). The cultivation of *Pleurotus* sp. is economically important worldwide which has expanded in the past few years. Nutritionally, it has unique flavour and aroma, and it is considered to be rich in protein, fiber, carbohydrates, vitamins and minerals with low cholesterol level (Menaga *et al.,* 2012). Oyster mushrooms are the second largest cultivated mushroom in the world (Sharma *et al.,* 2017).

P. djamor, commonly known as pink oyster mushroom belongs to the phylum Basidiomycota, class Agaricomycetes, order Agaricales and family Pleurotaceae. It was originally named as *Agaricus djamor* by the German botanist George Eberhard Rumphius, and sanctioned under that name by Elias Magnus Fries in 1821. The pink oyster mushroom is also known as salmon oyster mushroom, the strawberry oyster and the flamingo mushroom. This mushroom prefers tropical and subtropical regions and was known for its speed to fruiting, ability to flourish on a wide variety of substrates and high tolerance to temperature. *P. djamor* exhibits a wide range of colour and morphology for spores and fruit bodies. Stamets (2000) noted that colour of fruit body directly influences the colour of the spores. Pink mushrooms give pink spores. As the pink mushrooms fade with maturity, the spore colour also changes. *P. djamor* is an extremely fast growing mushroom that fruits easily on a wide range of lignocellulosic substrates such as hardwoods, wood by-products, cereal straws, corn cobs, sugarcane bagasse and coffee residues (Boulware *et al.*, 2014).

Blue oyster mushroom, *H. ulmarius* belongs to Phylum Basidiomycota, Class Agaricomycetes, order Agaricales and Family Lyophyllaceae. (Kirk *et al.*, 2001). This species was earlier named as *Pleurotus ulmarius* (Bull. ex Fr.) Kummer, later *Lyophyllum ulmarium* (Bull.:Fr.) Kuhner and most recently as *H. ulmarius* (Bull.:Fr.) Redhead. *H. ulmarius* often grow in clusters on elm trees and thus called as elm oyster mushroom. This mushroom is named "Blue oyster mushroom" because of blue coloured primordia. *H. ulmarius* is one of the important edible mushrooms in the world, popularly cultivated in Japan, China, North America and other Asian countries. Meera *et al.* (2011) reported that the mushroom is rich in antioxidants and known for its anti-diabetic, cardiovascular, anti-tumour and cholesterol controlling properties. The blue oyster mushroom has wide acceptability due to its unique flavour, nutritive value and medicinal properties. The fruiting bodies of elm oyster were larger, heavier and white to bluish grey in colour. It had a stipe that is off-centre to nearly central. Its gills were attached to stipe but not decurrent (Dixit and Shukla, 2012).

Milky mushroom, a tropical edible mushroom, belongs to the phylum Basidiomycota, class Agaricomycetes, order Agaricales and family Lyophyllaceae. It is becoming more popular due to its robust size, attractive colour, sustainable yield, delicious taste and unique texture (Amin *et al.*, 2010). It is appreciated for its large-sized milky white fruiting bodies. *C. indica* and *C. gambosa*, the two commonly cultivating milky mushroom species are rich in protein, lipids, mineral, fibre, carbohydrate and were abundant with essential amino acids (Sharma *et al.*, 2013).

C. gambosa commonly called St. George mushroom is a common mushroom species in Europe and Asia growing mainly in fields, grass verges, woodland edges, roadsides and grasslands and often in limestone rich areas. Its common name is reported to be derived from its first appearance in the UK on St. George's Day. This mushroom was previously classified in the genus *Tricholoma* and later named as *Tricholoma gambosum* before being reclassified as *C. gambosa*. The genus name '*Calocybe*' is derived from the ancient Greek

terms "*kalos*" means pretty, and "*cubos*" means head. The cap, stem and flesh can vary from white-creamy coloured to bright yellow. The gills are sinuate, white, densely packed, spore print is white to pinkish white and the cap has a slightly in-rolled margin and is often quite lumpy with a strong "floury" odour (Carluccio, 2003). Milky mushroom can be suitably grown in hot humid climate and cultivated almost throughout the year in India. Its cultivation is now spreading very fast in many states of the country like Tamil Nadu, Kerala, Odisha, Haryana and West Bengal due to its longer shelf life and adaptability to warm and humid conditions (Pani, 2011). Bheema variety of *C. gambosa* collected from natural habitat on coconut tree stump was released by Kerala Agricultural University in 2015. Bheema is a high yielding variety of milky mushroom with very big sized fruit bodies (Geetha and Jacob, 2013).

2.1. ISOLATION AND PURE CULTURING

2.1.1. *G. lucidum*

Venkatarayan (1935) reported that growth of *G. lucidum* was the best on malt extract agar medium. Similar result was given by Shukla and Uniyal, (1989) who reported the suitability of malt extract medium for the growth of *G. lucidum*. *G. lucidum* was grown on different culture media by Nasreen *et al.* (2005) and observed better mycelia growth on the potato dextrose medium (PDA) at pH 5 and temperature 25 ^{0}C, followed by malt extract agar (MEA). PDA has also been reported as good medium but it took more time for mycelial growth (Biley *et al.*, 2000). According to Sharma and Thakur (2010) the radial growth of *G. lucidum* was higher in MEA added with linseed extract medium. Varma (2013) reported fastest growth of *G.lucidum* on carrot agar media as compared with oat meal agar (OMA) and PDA. Rajesh *et al.* (2014) isolated two isolates of *Ganoderma* spp. using sterilized PDA by transferring small piece of surface sterilised mushroom fruiting body into the media. They observed the growth and colony characters of two fungal isolates in PDA. *Ganoderma* sp. DKR1 was white to pale orange coloured with 5 cm growth on 4th day and the colony appearance was even and

fatty. The *Ganoderma* DKR2 mycelia were felty to floccus, cottony and white in colour with colony growth of 6 cm on 4th day. Isolation and evaluation of *G. lucidum* was done by Rawat (2018) who reported that among the media tested MEA exhibited maximum average mycelial growth (7.64 cm) followed by PDA (5.64 cm).

2.1.2. Oyster mushrooms

Singh and Kushwaha (2007) reported maximum growth of *H. ulmarius* on malt extract agar as compared to PDA, OMA, corn meal agar, water agar, potato carrot agar, wheat extract agar, czapek's dox agar and yeast extract agar. *H. ulmarius* and *P. flabellatus* exhibited maximum mycelial growth on PDA media, while mycelial growth of *P. florida* was maximum on modified PDA (Patidar, 2008). Kushwaha *et al.* (2011) studied cultural variability of *H. ulmarius* and observed maximum growth of the mushroom on MEA and wheat extract agar followed by PDA. Sumi (2016) developed pure culture of *H. ulmarius* by tissue culture method. The surface sterilized mushroom tissue from the junction of stipe and pileus was used for inoculation in PDA. According to Kumar and Eswaran (2016), surface sterilisation of *H. ulmarius* using two per cent ethyl alcohol and sodium hypochlorite exhibited maximum tissue germination with minimum contamination. They also reported that PDA and potato malt extract agar (PMEA) were the best medium supporting mycelial growth of *H. ulmarius* out of nine different media tested.

Sharma (1999) evaluated six solid and liquid media, of which, maize extract and potato dextrose broth (PDB) gave maximum growth of *P. djamor*. Das *et al.* (2000) reported that *P. sajor-caju, P. florida, P. platypus* and *P. ostreatus* grew well in PDA and MEA. Rathod *et al.* (2002) observed superior mycelial growth of *P. florida* in yeast potato dextrose and PDA. Sardar *et al.* (2015) carried out an experiment to investigate the best medium for growth and development of *Pleurotus* sp. and proved the superiority of PDA for the mycelial growth of all *Pleurotus* spp. Ahmad *et al.* (2015) tested PDA, MEA, potato yeast dextrose agar

and yeast extract agar for culturing and suggested yeast extract medium as the best for growth of *P. djmour*. PDA medium was identified as suitable medium for isolation of *P. florida* (Karunakaran *et al.*, 2017).

2.1.3. Milky mushroom

Kumar *et al.* (2011) studied the mycelial growth of five strains of milky mushroom and found that maximum mycelial growth occurred in MEA followed by PDA and minimum in sugarcane bagasse extract agar medium after 8 days of incubation. According to Krishnamoorthy *et al.* (2015) seven days were needed for maximum mycelial growth of *C. indica* in PDA and 8 to 10 days in MEA. They also reported that the optimum pH and temperature for mycelial growth of milky mushroom as 5.5 - 8.5 and 30-35 °C respectively. Studies of Kerketta (2016) also revealed that maximum radial growth of *C. indica* was on PDA.

2.2. MORPHOLOGICAL AND CULTURAL STUDIES

2.2.1. Morphological studies

2.2.1.1. G. lucidum

Latiffah and Ho (2005) studied on the shape and size of *G. lucidum*. They found that the glossy basidiomata were sub-ungulate, flabellate or irregular with corrugations and concentric rings. It was either sessile or stipitate. The size of basidiomata ranged from 2.5 to 11.0 cm in diameter and the colour ranged from reddish brown to yellowish brown. According to Badalyan *et al.* (2012) *Ganoderma* strains exhibited species specific colony morphology on different culture media. They reported that *G. lucidum* had round-shaped clamps and numerous ovoid to ellipsoid chlamydospores in both agar and liquid media tested. Rakib *et al.* (2014) observed that spore length and diameter of *G. zonatum* ranged from 10.36 µm to 10.89 µm and 4.80 µm to 5.29 µm respectively. The shapes of *Ganoderma* spp. spores were either ellipsoid or narrowly ellipsoid. According to Rawat (2018) fruiting bodies of *G. lucidum* collected from Almora forest area were large, irregular in shape, copper red in colour, glossy appearance on lateral

surface, varnished, hard and thick. Basidiospores were ellipsoid, double walled with a size of 6.0 - 8.9 µm x 10 - 12 µm.

2.2.1.2. Oyster mushrooms

Junior *et al.* (2010) explained the morphological characters of *P. djamor*. The size of pileus was 7-13 cm x 5-10 cm with spatulate to flabelliform, pinkish when fresh, cream or yellowish when dry with smooth surface. Lamellae were decurrent, concolorous with the stipe and edges were smooth with lamellulae. Spore print was pink and became white when dry. The basidiospores were cylindrical, thin walled, hyaline and amyloid with dimensions of 10.0-11.2 µm X 3.7-5.0 µm. Cheilocystidia were present while pleurocystidia were absent in pink oyster mushroom. Dung *et al.* (2012) reported that the sporocarps of *P. fiorida* were offset, fleshy and unable to revive when remoistened. The pileus was smooth and laterally attached to the stipe. Radiating gills were present at the lower surface of cap which produced basidispores on a layer of tissue called hymenium.

Blue oyster mushroom was characterised with large fruiting body and blue coloured pinheads. It was reported to be high yielder, palatable with good flavour and attractive keeping quality (Rai, 2004). Biswas and Sanjeeb (2013) reported that the sporophore of *H. ulmarius* weighed an average of 7.98 g. According to Sumi (2016), *H. ulmarius* was dark blue coloured in pinhead stage later changed to creamy white. The pileus was convex in shape with irregular margin while stipe was eccentric and cylindrical in nature. Microscopic studies revealed that mycelium was septate, branched and hyaline with clamp connections.

2.2.1.3. Milky mushrooms

According to Purkayastha and Chandra (1974) the pileus of *C. indica* was found to be white with a diameter of 10 -14 cm. The pileus was convex in shape initially and later it became flat. The stipe was centric but sometimes eccentric in appearance. It was about 10 cm in length and width, showed variation from top to base *ie*, 1.8 cm thick at the top, 3.5 cm in the middle and 2.4 cm at the base.

Basidiospores were white, hyaline, broadly ellipsoidal, thin walled, with prominent apiculus, non-amyloid with dimension of 5.9-6.8 x 4.2-5.1 µm (Chandra and Purkayastha 1976). Heera (2006) observed that the length of its stipe ranges from 5.00-10.83 cm and that of pileus was 5.50-9.73 cm in *C. indica*. The maximum weight of milky mushroom was 70 g. Geetha and Jacob (2013) reported that Bheema is a high yielding variety of *C. gambosa* with attractive, robust and milky white coloured fruiting bodies. The distinguishing features of this variety are club shaped, stout and fleshy stipe and head like pileus. The weight of a single sporophore ranges from 250 g to 650 g.

2.2.2. Cultural Studies

2.2.2.1. G. lucidum

Veena and Pandey (2006) reported that during the cultivation of *G. lucidum*, the primordial initiation was fast at 30±2 °C and it was delayed at 24±2 °C temperature. According to Badalyan *et al.* (2012) favourable growing conditions for *Ganoderma* cultures were MEA with pH 6 at 25 to 30 °C temperature. Rawat (2018) found that among different temperatures evaluated, *Ganoderma* isolates showed maximum mycelial growth at 25 °C (8.72 cm) followed by 20 °C (7.34 cm) and minimum at 35 °C (3.58 cm) on 8th day after inoculation.

2.2.2.2. Oyster mushrooms

Singh and Kushwaha (2007) tested five levels of pH (4, 5, 6, 7 and 8) and reported that pH 7 is the best for the growth of *H. ulmarius*, whereas *P. sajor-caju, P. flabellatus* and *P. ostreatus* grow well at pH 5.5 (Suharban and Nair, 1994). Chandravanshi (2007) observed that the blue oyster mushroom exhibited maximum yield during the month of January when average temperature and relative humidity in the cropping room was 16.90 °C and 64.63 % respectively. In this study she also observed no spawn run during May month when the maximum temperature was 36 ^{0}C, minimum temperature 28 °C and relative humidity

68.52% existed in mushroom growing house. Kumar and Eswaran (2016) reported that maximum biomass production of *H. ulmarius* was at 25 °C- 30 °C and pH 7.

2.2.2.3. Milky mushrooms

According to Krishnamoorthy *et al.* (1998) milky mushroom grow well at 25-35 °C and relative humidity more than 80 %. Varshney (2007) studied on temperature requirement of *C. indica* and observed maximum mycelial growth at 28 °C followed by 32 °C and minimum at 20 °C. Shukla and Jaitly (2013) studied on the effect of temperature on mycelial growth of ten different strains of milky mushroom. They reported that the most suitable temperature for maximum mycelial impregnation and growth was 30 °C. Josaphine and Sahana (2014) reported that a temperature range of 20 °C to 35 °C was best suited for cultivation of milky mushrooms in India.

2.3. SPAWN PRODUCTION

2.3.1. *G. lucidum*

The concept of grain spawn was introduced by Sinden (1934). He used half cooked cereal grains along with calcium carbonate or calcium sulphate for spawn production. Mishra and Singh (2006) used different grains to check the response of grain spawn on yield of *G. lucidum* and concluded that wheat grain spawn resulted in the maximum yield which varied from 40-89 g per 500 g dry weight of substrate. Thakur and Yadav (2006) reported that sawdust and wheat bran combination was the best substrate for spawn development of *G. lucidum*. According to Perumal (2009) large scale production of spawn was achieved by cultivating *G. lucidum* in sawdust containing pots. Varma (2013) found that wheat grain was the best substrate for spawn production of *G. lucidum*, which required 13.50 days for fluffy mycelial spread. Joshi and Sagar (2016) observed that carbohydrate rich cereal grains and millets were best suited for spawn production and barley grain took only 8 days for complete mycelial run of *G. lucidum* as compared to other substrates.

2.3.2. Oyster mushrooms

Rathaiah and Shill (1999) used parboiled paddy grains for spawn production of oyster mushroom and paddy straw mushroom and found that parboiled paddy was equally good as wheat grains. Jiskani *et al.* (2000) reported sorghum grains as the best suited substrate for spawn run of *P. florida* as compared to maize, wheat and millet. Khatri and Agarwal (2002) evaluated different grains for spawn preparation of *P. florida* and found that jowar grains was ideal for early spawn preparation. According to Rathod *et al.* (2002), bajra and wheat grains were better substrates for commercial spawn production of *P. florida*. Sorghum and paddy grains supported colonization of *P. eous* in 10 and 16 days respectively whereas *P. florida* required an incubation period of 16 days to colonize on sorghum and paddy grains (Thulasi *et al.*, 2010). Dehariya and Vyas (2015) revealed that spawn of *P. sajor-caju* produced on wheat grain exhibited faster spawn running, pinhead formation, sporocarp development and increased yield as compared to maize and sorghum grains. Jayachandran *et al.* (2017) observed increased rate of mycelial running on paddy grain which needed 10 days for spawn development, while sorghum and wheat grains required 12 and 13 days respectively for spawn development of *P. florida*.

Chandrawanshi (2007) evaluated different grains for spawn preparation of *H. ulmarius* and found early spawn development on maize grains. Baghel (2017) reported minimum (10 days) period for spawn development on sorghum grains while paddy took maximum (17 days) period for spawn development of *H. ulmarius* followed by Bajra (11 days), maize (13 days) and wheat (14 days) grains.

2.3.3. Milky mushrooms

Prasuna (2002) studied on different grain substrates for spawn production of *C. indica* and reported early spawn development on wheat grain followed by jowar grains. Rana *et al.* (2004) evaluated cereal grains such as wheat, sorghum and pearl millet for spawn production of *C. indica*. Suman and Sharma (2007)

reported wheat grain as the appropriate substrate for production of spawn in *C. indica*. According to Subbiah and Balan (2015) sorghum or wheat grains were best substrates suited for spawn production of *C. indica*. Significantly minimum (14 days) time was required for spawn development on wheat grain by *C. indica* as compared to maize and sorghum grain (Kerketta, 2016).

2.4. CULTIVATION

2.4.1. *G. lucidum*

Studies have been conducted for artificial cultivation of *G. lucidum* in different hosts and better performance was observed in *Delonix regia* (Dadwal and Jamaluddin, 2004 and Tiwari *et al.*, 2004). According to Veena and Pandey (2006), biological efficiency was high while cultivating *G. lucidum* on sawdust amended with wheat bran. Peksen and Yakupoglu (2009) identified tea wastes as a new supplement for substrate mixture in *Ganoderma* cultivation. *G. lucidum* is generally cultivated on hardwood logs or sawdust based formulations but the current study demonstrated for the first time the cultivation of *G. lucidum* in paddy straw as substrate (Veena and Pandey, 2011). Among eight substrates and three combinations evaluated, Jain (2012) revealed sawdust (75 %) along with wheat bran (25 %) as the best substrate. Varma (2013) reported that sawdust (90 %) and rice or wheat bran (10 %) as the best medium for cultivation of *G. lucidum*. Complete mycelial colonization was reported within 18 days in the combination of substrates. Singh *et al.* (2014) observed a biological efficiency of 22 % while using billets of poplar as substrate for cultivation of *G. lucidum*. Joshi and Sagar (2016) reported a maximum yield of 65 g Kg^{-1} on oak sawdust substrate for the cultivation of *G. lucidum*. Wheat straw with moisture per cent 70 exhibited maximum mycelial growth (13.33 mm) followed by 80 per cent (11.63 mm) and 60±2 per cent (9.15 mm).The mycelial growth was minimum (7.13 mm) at 90 per cent moisture (Singh *et al.*, 2017).

2.4.2. Oyster mushrooms

According to Patidar (2008), the biological efficiency of *H. ulmarius* was 79.62 % and that of *P. florida* was 78.82 % when paddy straw was used as substrate for cultivation. According to Uddin *et al.* (2010) *P. ostreatus, P. florida, P. sajor-caju* and *P. high king* exhibited minimum days for primordial initiation, maximum number of fruiting bodies, biological yield and biological efficiency during December to February (14-27 ^{0}C, 70-80 % RH), whereas production was minimum during August to October. Chauhan (2013) recorded a biological efficiency of 72.26 % with *P. djamor* using wheat straw. Mago *et al.* (2014) reported that mycelial spreading and biological efficiency of *P. florida* and *P. sajor-caju* were the best on paddy straw compared to sawdust and leaf litter. Iqbal *et al.* (2016) reported the biological efficiency of *P. florida* on paddy straw as 123% and on wheat straw as 136 %. Sumi (2016) reported that blue oyster mushroom cultivated on paddy straw had 109.6 % biological efficiency as compared to *P. florida*, which had biological efficiency of 97.6 %. Patar *et al.* (2018) analysed comparative growth behaviour and yield potential of oyster mushroom *P. florida* and *P. sajor-caju* grown separately in wheat substrate and reported that *P. florida* showed faster spawn run period along with early pinhead initiation than in *P. sajor-caju*. The total crop periods in *P. florida* and *P. sajor-caju* were recorded as 43 days and 49 days respectively. The yield potential of *P. florida* (1363 g kg^{-1} of dry substrate) was superior to *P. sajor-caju* (940 g kg^{-1} of dry substrate).

2.4.3. Milky mushroom

Chakravarty *et al.* (1981) reported successful cultivation of *C. indica* on different substrates using sterilized and non-sterilized casing media. Krishnamoorthy *et al.* (1998) reported that paddy straw, sugarcane bagasse, millet stalks, palmarosa, vetiver grass, soyabean hay and groundnut haulms were suited for cultivation of milky mushroom. Amin *et al.* (2010) studied the effect of different substrates and casing materials on the growth and yield of *C. indica.*

They observed maximum biological efficiency on using cow dung and loamy soil (3 cm thick) as casing material and the paddy straw as substrate for commercial cultivation. Cultivation of milky mushroom using paddy straw was studied by Josaphine and Sahana (2014), who reported that *C. indica* recorded 14 days for spawn run, 7 days for pin head formation and 7 days for first harvest in paddy straw substrate. Bokariya *et al.* (2014) found that among the five substrates evaluated paddy straw, maize stalk and sorghum stalk recorded significantly higher yields followed by sugarcane bagasse and groundnut haulms. They also reported that casing with clay loam soil (pH 8.4) gave maximum yield per bed with more numbers of buttons as compared to peat soil and sandy soil. Vijaykumar *et al.* (2014) investigated the efficacy of six different substrates for cultivation of milky mushroom. They found that wheat straw was superior which recorded minimum days for spawn run, pinhead formation and for first harvest with highest number of fruiting bodies. Paddy straw was the next best superior substrate for cultivation of milky mushroom.

Purkayastha and Nayak (1981) used soil and sand at 1:1 ratio supplemented with 6, 12, and 20 % of calcium carbonate as casing material. They recorded the highest yield (315 g/15 kg substrate) with 12 % calcium carbonate, while no fruit body formation was observed with 20 % concentration. However, Krishnamoorthy *et al.* (1998) reported that sterilized garden soil was useful for casing of milky mushroom. Pani (2011) evaluated the effect of depth and time of casing on sporophore production of *C. indica* and found that 2 cm thick casing was ideal for early primordial initiation, higher sporophore number and maximum yield. Thickness beyond 2 cm gradually decreased the mushroom yield and delayed the fruiting.

2.5. DEVELOPMENTAL STAGES

2.5.1. *G. lucidum*

According to Singh *et al.* (2014) *G. lucidum* recorded 15 to 17 days for spawn run, 30 to 34 days for primordial formation, 40 to 46 days for antler

formation, 55 to 63 days for cap formation and 70 to 80 days for fruiting body development at 22 to 27 °C temperature with 70 to 80 % moisture using billets of poplar as substrate. *G. lucidum* required 23 days for complete spawn run and 32 days for primordial initiation on oak sawdust as substrate (Joshi and Sagar, 2016).

2.5.2. Oyster mushrooms

Elhami *et al.* (2008) reported that *P. florida* completed spawn run in 14.8 days, pinhead formation in 19.4 days and fruiting body formation in 22.5 days while using wheat straw as substrate. They observed the time period required for spawn run (19.5 days), pinhead formation (24.8 days) and fruit body formation (29.5 days) on sugarcane bagasse. According to Sahu (2012) 10.66 days were needed for complete spawn run and 14 days for primordial initiation of *P. eous* on wheat straw substrate while paddy straw required 10.33 days for spawn run and 14 days for primordial initiation. *P. djamor* recorded 11.33 days for spawn run and 15.7 days for primordial initiation when cultivated on wheat straw, saw dust (3:1) combination. The wheat straw, wheat bran (9:1) combination completed spawn run in 10.33 days and formation of primordia was observed in 14.67 days (Chauhan, 2013). Manimuthu and Rajendran (2015) reported that total cropping duration of *P. florida* using paddy straw as substrate was 43 days. Sporophore initiation took place in 19 to 23 days and harvesting was done at 23rd, 33rd and 43rd day after bed preparation. Iqbal (2016) observed 37 days for pinhead formation and 39 days for maturation of the oyster mushroom cultivated on paddy straw. According to Sumi (2016) *H. ulmarius* recorded 22.4 days for complete spawn run and 38.1 days for pinhead formation on paddy straw. The pinheads reached complete maturity after 5 days. Baghel (2017) reported earlier primordial formation of *H. ulmarius* during the month of October (2.75 days) followed by November (2.78 days), January (2.78 days) and December (3.00 days) while it was significantly delayed in April (3.88 days) and February (3.75 days).

2.5.3. Milky mushrooms

Bhat (2000) reported that *C. indica* initiated spawn run after 6 days of spawning on wheat straw and 80 per cent colonization was completed on 18th day while on maize straw spawn run was initiated on seventh day after spawning and 60 per cent spawn run was completed on 22nd day. The slowest initiation of spawn run was recorded on paddy straw after 10th day of spawning with 10 per cent colonization on 27th day. Heera (2006) studied about the effect of substrate sterilization on the mycelial growth and time taken for *C. indica* production. Who found that cultivation using paddy straw sterilized by boiling recorded 20.2 days for spawn run, 11.8 days after casing for primordial production and 34.6 days for harvest. Yadav (2006) analysed the yield performance of *C. indica* on substrates enriched with vermi compost and vermi meal and reported that days for spawn run ranged from 20 to 25 days depending on the substrates used. Case run took place 11 to 13 days from complete spawn run and harvesting was done after five to six days of case run. Geetha and Jacob (2013) reported the salient features of *C. gambosa* variety Bheema as a high yielding variety of milky mushroom with very big sized fruit bodies. The mushroom needed 32 days from spawning to first harvest. This variety is suited for cultivation in Kerala throughout the year on different agricultural wastes. The average yield recorded from one kg paddy straw is 1100 g of fresh mushroom and the biological efficiency being 110 per cent.

2.6. PEST AND DISEASE INCIDENCE

According to Sharma *et al.* (2007) oyster mushrooms were affected by various competitive moulds such as *Cladobotrym apiculatum, Gliocladium virens, Sibirina fungicolae* and bacterial contaminants like *Pseudomonas alcaligens, P. tolaasii, P. fluorescens* etc. The presence of fungal contaminants such as *Trichoderma harzianum, Aspergillus flavus* and *A. niger* resulted yield loss *in P. eous* (Anandh *et al.*, 1999). Sumi (2016) reported presence of *Aspergillus* sp., *Penicillium* sp., *Trichoderma* sp. and *Bacillus* sp. as contaminants during spawn production and cultivation of *H. ulmarius*.

Doshi *et al.* (1991) observed *T. viride, Sclerotium rolfsi, A. flavus* as competitors on beds of *Calocybe* sp. and managed them using 50 ppm carbendazin or Blitox 50 ppm. Pani (2000) reported *Coprinus* sp. as a major weed mould causing 80 per cent yield loss followed by *S. rolfsi* (74 per cent). *A. niger, A. flavus*, and *Trichoderma* sp. were the major contaminants of *C. indica* during spawn production (Raman *et al.*, 2005).

According to Krishnamoorthy *et al.* (1991) maggots of *Megaselia* sp. caused severe damage on the oyster mushroom beds by feeding on mycelia during spawn run. Deepthi *et al.* (2003) reported severe attack of sciarids and phorids during all the stages of mushroom development starting from spawn run till sporocarp formation. Naik (2015) reported presence of lepidopterous larvae during paddy straw mushroom production. Sumi (2016) observed the infestation of phorid flies, staphylinid beetles and springtails during the cultivation of *H. ulmarius*.

2.7. ANALYSIS OF NUTRITIONAL COMPONENTS

Edible mushrooms have long been known for their medicinal and nutritive value. Due to their high content of protein, vitamins, minerals and anti-oxidants mushrooms are considered as 'poor man's protein'. Proximate compositions of mushrooms were also varied within and among the species due to agro-climatic conditions and environmental factors (Pandey, 2004).

2.7.1. Protein

Takshak *et al.* (2014) reported that the crude protein content of *Ganoderma* spp. collected from different agro-climatic zones of Hariyana varied from 18 to 22 per cent on dry weight basis. The protein content in dried fruit bodies of domesticated *Ganoderma* sp. was reported as 18.2 per cent (Mhanda *et al.*, 2015). Sharif *et al.* (2016) recorded 15.04 % protein content in *G. lucidum*, 22.61 % in *Lentinus edodes* and 24.12 % in *Volvariella volaceae*.

Menaga *et al.* (2012) studied on the phytochemical characteristics of *P. florida* and reported 50.7 per cent protein content in the dried mushroom sample. Randive (2012) analysed the nutrient content in oyster mushroom and observed higher protein content in mushrooms cultivated on paddy straw (21 mg ml^{-1}) as compared to those on wheat straw (15 mg ml^{-1}). Khatun *et al.* (2015) found that protein content was highest in *P. florida* (23.8 %) followed by *P. citrinopileatus* (20.8 %) and *P. pulmonarius* (16.8 %). Usha and Suguna (2015) reported protein content of *H. ulmarius* TNAU strain and IIHR strain as 47.59 and 54.7 per cent respectively. According to Sumi (2016) protein content of *H. ulmarius* was 32 per cent on dry weight basis. Chauhan *et al.* (2017) estimated protein content of *H. tessellatus* as 28.67 %.

Sharma (1996) reported that mature fruit body of *C. indica* contained 17.26 per cent protein on dry weight basis while young pinheads contained 15 per cent protein. Krishnamoorthy (2003) reported the protein content of milky mushroom as 32.3 % .The crude protein content of *C. indica* was 28.87 % and 32.06 % when cultivated on wheat straw and sorghum stalks respectively (Yadav, 2006). Heera (2006) observed a maximum of 42 per cent protein content in *C. indica*. The protein content of *P. florida* was estimated as 23.18 per cent by Pushpa and Prushothama (2010). They also reported 21.60 per cent protein in *C. indica*.

2.7.2. Fat

Crude fat content of *Ganoderma* sp. was estimated within a range of three to five per cent by Takshak *et al.* (2014). According to Mahnda *et al.* (2015) fat and oil content of domesticated *Ganoderma* sp. was 1.9 per cent. *G. lucidum* was recorded with less fat content of 0.53 % as compared with *L. edodes, V. volvaceae* and *P. ostreatus* (Sharif *et al.*, 2016).

Fat content in *H. ulmarius, P. florida* and *P. flabellatus* were 3.10, 1.82 and 3.98 per cent respectively (Zape, 2003). Rathore and Thakore (2004) reported that fat content vary from 1.8 to 2.58 % on dry weight basis in *P. florida* when

grown in different substrates. Singh and Kushwaha (2007) observed 2.2 % fat content in blue oyster mushroom. According to Patidar (2008), fat content of *P. sajorcaju* was maximum (2.18 %) with wheat straw followed by gram straw and paddystraw. He also observed that fat content of *P. flabellatus* was maximum with soybean straw (2.07 %) and minimum was recorded in paddy straw (1.55 %). According to Pushpa and Purushothama (2010) *P. florida* had 1.54 per cent whereas *C. indica* had 4.96 % fat on dry weight basis. Menaga *et al.* (2012) recorded 0.6 % fat content in *P. florida*. Usha and Suguna (2015) reported fat content in *H. ulmarius* (CO2) as 3.55 % and in *H. ulmarius* IIHR (Hu1) as 4.8 %. Sumi (2016) estimated fat content in *H. ulmarius* as 2.86 %. The per cent fat content of *H. tessellatus* was 0.94 (Chauhan *et al.* 2017).

The fat content in milky mushroom was estimated as 4.1 % by Doshi *et al.* (1988). Bhavana and Thomas (2002) studied the nutritional composition of milky mushroom and recorded 3.2 per cent fat content. Yadav (2006) reported 2.62 % fat content in *C. indica* cultivated on wheat straw and 2.37 % on sorghum stalks. According to Kaur *et al.* (2015) the fat content in seven mutants of *C. indica* ranged from 1.24 to 2.46 per cent.

2.7.3. Fibre

According to Usman *et al.* (2012) proximate composition of crude fibre in 0.1 g crude powder of *G. lucidum* was 30.25 %. Takshak *et al.* (2014) reported variation in crude fibre content which ranged from 20 to 38 per cent in *Ganoderma* spp. Nile and Park (2014) analysed total soluble and insoluble dietary fibre contents of wild edible mushrooms and according to them the total dietary fibre content in *G. lucidum* was 35 per cent. The crude fibre content of *Ganoderma* sp. was estimated as 45 per cent (Mhanda *et al.*, 2015). Bhattachariya *et al.* (2015) estimated the fibre content in *P. ostreatus* as 20.53 per cent while cultivated on combination of sawdust. According to Usha and Suguna (2015) crude fibre content in blue oyster mushroom ranged from 17.45 % to 19.45 %. Sumi (2016) determined the crude fibre content of *H. ulmarius* as 17.69 per cent.

Dietary fibre content in *H. tessellatus* was estimated as 10.22 per cent by Chauhan *et al.*, 2017.

Heera (2006) observed that the fibre content in different isolates of *C. indica* ranged from 16.02 to 24.41 per cent. Crude fibre content of milky mushroom varied from 9.30 to10.43 per cent depending on the substrate used for cultivation (Yadav, 2006). Pushpa and Prushothama (2010) reported 23.18 per cent crude fibre in *P. florida* and 13.20 % in *C. indica*. Fibre content of fresh milky mushroom was found to be 3.24 g $100g^{-1}$ of dried sample (Anju, 2013).

2.7.4. Carbohydrate

Carbohydrate content in 0.1g of powdered *Ganoderma* sp. was estimated as 33.13 % (Usman *et al.*, 2012). Carbohydrate content in the range of 28 to 54 per cent was estimated in *G. lucidum* by Takshak *et al.* (2014). Mhanda *et al.* (2015) reported domesticated *Ganoderma* sp. as a good source of carbohydrate with 23 per cent carbohydrate in its dried fruiting body. According to Sharif *et al.* (2016) the mushroom contained 82.47 % carbohydrate on dry weight basis.

Carbohydrate content in *P. florida* was estimated as 32.08 per cent and in *C. indica* as 49.20 per cent by Pushpa and Purushothama (2010). *P. florida* was recorded with 26.6 per cent total carbohydrate content (Menaga *et al.*, 2012). Carbohydrate content in *P. ostreatus* cultivated on different sawdust varied from 39.67 to 42.36 % (Bhattachariya *et al.*, 2015). Carbohydrate content in *H. ulmarius* ranged from 28 to 34 per cent (Usha and Suguna, 2015). Sumi (2016) recorded 29 per cent carbohydrate in blue oyster mushroom. According to Chauhan *et al.* (2017) *Hypsizygus* sp. contained 43.37 per cent carbohydrate in oven dried sample.

Doshi *et al.* (1988) reported that *C. indica* had 64.26 per cent carbohydrate in matured sporocarp on dry weight basis. The carbohydrate content in milky mushroom was recorded as 57.18 % and 47.69 % when cultivated on wheat straw and sorghum stalk respectively (Yadav, 2006). Alam *et al.* (2008) reported that

carbohydrate content in 100 g dried sample of *C. indica* ranged from 46 to 51 g. Pushpa and Purushothama (2010) had also reported that milky mushroom contain 49.2 g of carbohydrate.

2.7.5. Moisture Content

Usman *et al.* (2012) reported that 0.1 g crude powder of *G. lucidum* contain 10.54 per cent moisture. Varma (2013) estimated the moisture content as 58.4 % and 53.4 % in two different isolates of *Ganoderma*. Takshak *et al.* (2014) studied on wild *G. lucidum* collected from different agro-climatic zones of Haryana state and found that moisture content of fresh mushroom ranged from 74.69 % to 79.93 %. Moisture content of 9.1 per cent was reported per 100 g of domesticated *Ganoderma* sp (Mhanda *et al.*, 2015).

According to Menaga *et al.* (2012) moisture content of *P. florida* was as 87.3 per cent. Randive (2012) reported that the moisture contents of grey oyster mushroom cultivated on wheat straw was 87.5 % and that on paddy straw was 77.5 %. For pink oyster mushroom the moisture content while cultivating on wheat straw was 92 % and on paddy straw was 86.11 %. Moisture content of 91.5 per cent was estimated in *H. ulmarius* by Sikha *et al.* (2012). Usha and Suguna (2015) studied on nutrient analysis of two strains of blue oyster mushroom and found that moisture content of the strains varied from 88.3 to 90.6 per cent. Sumi (2016) reported 90.37 per cent moisture content in blue oyster mushroom. Chauhan *et al.* (2017) observed moisture content in fresh sample of *Hypsizygus* sp. as 92.63 per cent.

According to Heera (2006) moisture content of milky mushroom ranged between 80.92 and 89.64 per cent. Yadav (2006) reported the moisture content of milky mushroom varied from 90.70 to 91.07 % when cultivated on wheat straw and sorghum stalks respectively. The moisture content of fresh *C. indica* varied in the range of 70 to 90 per cent depending upon the stage of harvest and environmental conditions while that of dried mushroom is about 10 to13 per cent

(Bhupinder and Ibitwar 2007). The moisture content of milky mushroom was reported to be 90.73 per cent by Anju (2013).

2.7.6. Amino Acid

Chirinang and Intarapichet (2009) reported that total amino acid content of *P. ostreatus* and *P. sajor-caju* was 21.11 mg gram^{-1} and 20.12 mg gram^{-1} respectively on fresh weight basis. Sharma *et al.* (2012) analysed the amino acid composition of five different species of genus *Lentinus* from northern India. According to them aspartic acid content ranged from 0.25 to 0.37 %, arginine content ranged from 0.21 to 0.29 %, alanine content from 0.09 to 0.15 %, Proline content from 0.01 to 0.06 % and tyrosine content from 0.16 to 0.24 %. Anju (2013) found that milky mushroom contained all the essential amino acids and the content of isoleucin, valine and threonine were exceptionally high when compared to reference protein. According to Ravikrishnan *et al.* (2015) *L. polychrous* collected from the Western Ghats forests of southern India contained all the essential amino acids, of which glutamic acid was predominant. Non-essential amino acids such as alanine, aspartic acid and serine were also present in it. Rana *et al.* (2015) identified 17 amino acids in the fresh samples of *Agaricus bisporus* and *P. pulmonarius*. The amino acid content in hot water extract of *P. sajor-caju* was estimated as 2.79 mg gram^{-1} by Devi and Krishnakumari (2015).

2.7.7. Ash content

Proximate chemical composition of 0.1g powdered *Ganoderma* mushroom recorded 5.93 % total ash content (Usman *et al.*, 2012). According to Takshak *et al.* (2014), the ash content of fresh *Ganoderma* sp. ranged from 3.66 to 9.70 per cent. Mhanda *et al.* (2015) reported 2.6 per cent ash content in *G. lucidum*. Sharif *et al.* (2016) studied on proximate composition and micronutrient mineral profile of wild *G. lucidum* and observed 2.01 % ash content in the dried sample. Slynko *et al.* (2017) revealed that ash content of *G. lucidum* as 6.01 per cent.

The ash content in *P. florida* was estimated as 8.72 per cent (Rathore and Thakore, 2004). Pushpa and Purushothama (2010) recorded ash content of *P. florida* as 9.41 % and that of *C. indica* as 12.70 %. Menaga *et al.* (2012) reported 2.1 % ash content in *P. florida*. According to Shikha *et al.* (2012) ash content of blue oyster mushroom was less (7.1 %) as compared to *P. florida, P. ostreatus* and *P. sajor-caju*. According to Usha and Suguna (2015) ash content of two strains of blue oyster mushrooms were 5.86 and 4.3 per cent. Bhattacharjya *et al.* (2015) observed that the ash content of *P. ostreatus* ranged from 8.5 to 13 per cent depending upon the substrates used for cultivation. Salami *et al.* (2017) found that ash content of *P. florida* varied with substrate used and they reported maximum ash content on corn straw (6.39 %) followed by corn cobs (5.94 %) while the least was on oil palm spadix (5.18 %).

Doshi *et al.* (1988) reported 7.43 per cent ash content in *C. indica*. The milky mushroom cultivated on fermented coir substrate recorded 16.6 per cent ash content (Bhavana and Thomas, 2002). Ash content of milky mushroom was reported as 9.03 per cent while grown on wheat straw and 7.67 per cent on sorghum stalks (Yadav, 2006).

2.7.8. Minerals

2.7.8.1. Sodium

Sodium is an important mineral that helps to control the balance of body fluid and help to send nerve impulses. It retains the pH and water balance of body. Anju (2013) studied sodium content of milky mushroom treated with different processing technique and she indicated mean values as 65.0 mg, 37.45 mg, 46.24 mg and 285.32 mg respectively on boiling, steaming, frying and drying the mushroom sample. The micronutrient mineral profile of wild *G. lucidum* and four commercial exotic mushrooms by Sharif *et al.* (2016) revealed that sodium content in *G. lucidum* was the lowest (20.50 mg $100g^{-1}$) among the mushrooms compared. Muthu and Shanmugasundaram (2016) reported 0.293 mg g^{-1} of

sodium in powdered *Agrocybe aegerita*. Chauhan *et al.* (2017) estimated sodium content in *Hypsizygus* sp. as 77.10 mg 100 g^{-1} of oven dried sample.

2.7.8.2 Phosphorous

The main function of phosphorus is the formation of bones and teeth. It also needed to make protein for the growth, maintenance and repair of cells and tissues. Kamugisha and Sharan (2005) evaluated nutritional composition of *C. indica* cultivated on paddy straw amended with ragi flour and recorded highest phosphorus content of 163.35 mg 100 g^{-1} on paddy straw supplemented with 20 % ragi flour in fresh weight basis. Phosphorus content of dried milky mushroom sample was recorded as 833.4 mg 100 g^{-1} (Anju, 2013). The phosphorous content in *P. florida* and *P. djamor* were analysed as 640.2 mg 100 g^{-1} and 743.2 mg 100 g^{-1} respectively by Mallikarjuna *et al.* (2013). Bhattachariya *et al.* (2015) reported that phosphorus content of *P. ostreatus* cultivated on sawdust ranged from 0.77 to 0.91 per cent. According to Sumi (2016) the phosphorus content in *H. ulmarius* was 0.68 per cent and that of *P. florida* was 0.67 per cent. Sharif *et al.* (2016) estimated the phosphorus content of *G. lucidum* as 502.5 mg 100 g^{-1}.

2.7.8.3. Potassium

Potassium content of milky mushroom was 415.6 mg 100 g^{-1} (Anju, 2013). Bhattachariya *et al.* (2015) evaluated *P. ostreatus* cultivated on six different combination of sawdust substrate and found that per cent composition of potassium ranged from 1.16 to 1.28. Sumi (2016) reported 1.98 per cent of potassium in *H. ulmarius* while 2.45 per cent in *P. florida.* Potassium play a significant role as an essential mineral which helps to maintain normal heart rhythm, fluid balance, muscle and nerve functions (Muthu and Shanmugasundaram, 2016). Potassium content of *G. lucidum* was recorded as 742.1 mg 100 g^{-1} on dry weight basis (Sharif *et al.*, 2016). Muthu and Shanmugasundaram (2016) estimated potassium content of *A. aegerita* as 0.085 mg g^{-1} of sample.

2.7.8.4. Calcium

The mineral calcium is well-known for its role in bone health. It maintains heart rhythm and muscle functions. Alam *et al.* (2008) reported that 100 g *C. indica* had 20.7 mg of calcium. Masamba and Kazombo-Mwale (2010) estimated the amount of calcium present in *P. florida* as 2.7 mg 100 g^{-1}. Calcium content in *P. ostreatus* was estimated between the range of 27.32 to 31.98 per cent by Bhattacharjya *et al.* (2015). The calcium content of *Ganoderma* sp. was found to be 23.2 g 100 g^{-1} by Mhanda *et al.* (2015). Sharif *et al.* (2016) reported 109.20 mg 100 g^{-1} of calcium in *G. lucidum*, which is superior to *P. osteatus, V. volvaceae* and *L. edodes*. Calcium content in *A. aegerita* was recorded as 0.273 mg g^{-1} by Muthu and Shanmugasundaram (2016). Kathiravan and Krishnakumari (2017) recorded 0.195 mg calcium content per 100 g of milky mushroom.

2.7.8.5. Magnesium

Magnesium is involved in biochemical reactions of the body such as strengthening of bones and improving heart health. According to Masamba and Kazombo-Mwale (2010) *P. florida* contained an amount of 9.0 mg magnesium per 100 g of sample. Bhattacharjya *et al.* (2015) reported 13.31 to 19.85 mg magnesium per 100 g of *P. ostreatus*. The magnesium content was recorded as 0.091 mg, 0.197 mg and 0.641 mg respectively in 100 g of *P. florida, H. ulmarius* and *P. eous*. Magnesium content in *G. lucidum* was estimated as 89.1 mg 100 g^{-1} of dried sample (Sharif *et al.*, 2016). Muthu and Shanmugasundaram (2016) analysed the mineral compositions of edible mushroom *A. aegerita*, and magnesium content was estimated as 0.096 mg g^{-1}. Kathiravan and Krishnakumari (2017) reported that *C. indica* contained 0.086 mg magnesium per gram.

2.8. ANALYSIS OF MEDICINAL COMPONENTS

The anti-cancer activities of *G. lucidum* have been demonstrated in both *in vitro* and *in vivo* studies and these have prompted its usage by cancer patients alongside

chemotherapy. Triterpenes and polysaccharides were the two main bioactive compounds reported for anti-cancerous activity.

2.8.1. Beta-glucan

Toledo *et al.* (2013) analysed beta-glucan concentration of edible and medicinal mushroom samples by enzymatic method and by high performance liquid chromatography (HPLC). *P. ostreatus* recorded 89.2 g kg^{-1} of beta-glucan while *P. sajor caju* recorded 48.7 g kg^{-1} using HPLC method. McCleary and Draga (2016) measured the beta-glucan using controlled acid hydrolysis with sulphuric acid and estimated 54 g 100 g^{-1} of *G. lucidum*. They concluded *G. lucidum* as the highest source of beta-glucan as compared to *Trametes versicolor, Grifola frondosa* and *Cordyceps militaris*. Avni *et al.* (2017) conducted quantitative analysis of glucan content in five different *Pleurotus* spp. The highest alpha and total glucan content was observed in *P. eryngii* with 4.5 % of alpha-glucans, 48.9 % of total glucan and 43.47 % for beta-glucan. In contrast *P. salmoneostramineus* exhibited the lowest glucan concentrations with 0.29 % of alpha-glucan, 17.09 % of beta-glucan and 17.34 % of total glucan. Sari *et al.* (2017) carried out screening of beta glucan content in commercially cultivated and wild growing mushrooms. Based on their analysis beta-glucan content of *P. djamor, P. citrinopileatus and P. pulmonarius* were recorded as 20.7, 15.54 and 17.46 g 100 g^{-1} respectively.

2.8.2. Glycoprotein

The presence of glycoprotein in *G. lucidum* was qualitatively confirmed by Tanaka *et al.* (1989). Ko *et al.* (1995) reported that glycoproteins isolated from *Flammulina velutipes* have inhibitory action against cancer cells. Hsu *et al.* (1997) and Liu *et al.* (2004) also confirmed the presence of glycoprotein in *V. volvacea* and *Xerocomus spadiceus* respectively. Tsai *et al.* (2013) reported that non-lectin glycoprotein present in *H. marmoreus* had inhibitory effect against leukemic cells

2.8.3. Terpenoid

Aqueous extract of *P. florida* recorded high level of terpenoid on qualitative estimation (Menaga *et al.*, 2012). Sasidhara and Thirunalasundari (2014) studied on phytochemicals and antioxidant potentials of *P. djamor* and confirmed the presence of terpenoid in powdered sample. Hamzah *et al.* (2014) conducted qualitative screening of phytochemicals in wild mushrooms and confirmed the presence of terpenoid in *P. pulmonarius, P. ostreatus, Auricularia aricula etc.* Terpenoid content in milky mushroom *C. indica* var APK-2 was estimated as 0.099 mg 100 g^{-1} by Sumathy *et al.* (2015). Parihar *et al.* (2015) observed presence of terpenoid in methanolic and aqueous extracts of *P. ostreatus*. Kinge *et al.* (2016) reported the presence of triterpenoid in *P. florida* and *P. ostreatus*. Taofiq *et al.* (2017) reported that terpenoid content in *Ganoderma* extract was 27.2 mg linalool equivalent per gram.

2.8.4. Polyphenol

Rawat *et al.* (2013) reported 9.24 mg catechol equivalent per g in the methanolic extract of wild *G. lucidum* collected from Himalayan hills of India. Total phenol content in *G. lucidum* was estimated as 9 mg g^{-1} (Rajoriya *et al.*, 2015). Total phenol content in *G. lucidum* was recorded as 22.28 µg mg^{-1} of sample (Acharya *et al.* 2015).

The total phenolic content in water and ethanol extracts of *P. sajor-caju* was estimated as 37.98 and 29.30 gallic acid equivalent (GAE) respectively per g dry weight of sample by Chirinang and Intarapichet (2009) . The total phenolic content of *P. florida* was estimated as 62.72 mg catechol equivalent. The total phenols content was higher in methanol extract followed by ethanol and aqueous extract which contained 59.5 mg and 58.1 mg of phenol respectively (Menaga *et al.*, 2012). Wandati *et al.* (2013) reported 675.56 mg GAE of polyphenol per 100 g of wild oyster mushroom sample. According to Hamzah *et al.* (2014) phenol content in *P. pulmonarius* and *P. ostreatus* were 223.11 mg g^{-1} and 248.8 mg g^{-1} respectively. Sasidhara and Thirunalasundari (2014) estimated phenolic content of

P. djamor as 32.55 mg g^{-1} of sample. Total phenolic content in *P. florida*, *P. citrinopileatus* and *P. pulmonarius* was estimated as 119, 83 and 64 µg catechol equivalent per g of powdered sample (Khatun *et al.*, 2015). Parihar *et al.* (2015) reported that methanolic extract of *P. ostreatus* contained maximum phenolic content of 24.01 mg GAE g^{-1} of dry extract. Boonsong *et al.* (2016) observed 12.34 mg GAE g^{-1} total phenolic content in the ethanolic extract of dried *P. sajor-caju* and 14.03 mg GAE in *P. eous*. Acharya *et al.* (2016) conducted the study of physical, chemical and biochemical properties of *C. indica* and observed 3.8 µg GAE of phenol per mg of dried mushroom sample. They also reported that mycochemical constituents were present in much more quantity in *C. indica* than that of *P. ostreatus*.

2.8.5. Beta-carotene

Acharya *et al.* (2015) reported 0.106 µg mg^{-1} of beta-carotene in methanolic extract of *G. lucidum*. Rajoriya *et al.* (2015) analysed beta carotene content in different *Ganoderma* spp. and reported that *G. lucidum* contained 3.63 mg g^{-1} and *G. applanatum* recorded 3.30 mg beta carotene per gram of dried sample.

Rajoriya *et al.* (2014) conducted comparative evaluation of biochemical properties in the mycelium of two *Pleurotus* sp. and reported that beta-carotene content of *P. sajor-caju* (0.038 mg g^{-1}) was higher as compared to *P. florida* (0.018 mg g^{-1}). Sumathy *et al.* (2015) studied on non-enzymatic anti-oxidants of *C. indica* var. APK2 and observed 112.73 µg 100 g^{-1} of beta carotene. Beta carotene content of *C. indica* was estimated as 0.234 µg mg^{-1} (Acharya *et al.*, 2016).

2.8.6. Flavonoid

The methanolic extract of *G. lucidum* collected from Himalayan hills of India was recorded with 2.14 mg quercetin equivalent (QE) of flavonoid (Rawat *et al.*, 2013). Acharya *et al.* (2015) analysed phytochemical concentration in

methanolic extract of *G. lucidum* and flavonoid content was estimated as 4.85 µg mg^{-1}. Rajoriya *et al.* (2015) reported 0.63 mg g^{-1} and 0.62 mg g^{-1} of flavonoid in *G. lucidum* and *G. applanatum* respectively.

According to Menaga *et al.* (2012), the total flavonoid content of *P. florida* was estimated as 17.71 mg of catechol equivalent in 100 ml of extract. Hexane and ethyl acetate extracts contained low flavonoid content as 7 mg and 9.1 mg respectively whereas ethanol and aqueous extract contain 15.89 mg and 15.32 mg. Wandati *et al.* (2013) reported 890.87 mg QE 100 g^{-1} of total flavonoid in wild oyster mushroom sample. Phytochemical screening of wild mushrooms revealed that flavonoid content of *P. pulmonarius* was 22.17 mg g^{-1} while that of *P. ostreatus* was 42.63 mg $^{-1}$g (Hamzah *et al.*, 2014). Flavanoid content of *P. djamor* was estimated as 1.53 mg g^{-1} of dried sample by Sasidhara and Thirunalasundari (2014). Rajoriya *et al.* (2014) reported 0.14 mg g^{-1} of flavonoid in *P. sajor-caju* and 0.11 mg g^{-1} in *P. florida*. Flavonoid content in aqueous extract of *P. sajor- caju* was recorded as 5.36 mg g^{-1} (Devi and Krishnakumari, 2015). Presence of flavonoid in methanolic and aqueous extracts of *P. ostreatus* was recorded by Parihar *et al.* (2015). Flavanoid content in *C. indica* APK -2 was estimated as 1.54 mg QE g^{-1} of mushroom sample (Sumathy *et al.*, 2015). Boonsong *et al.* (2016) studied on antioxidant activities of extracts from five edible mushrooms using different extractants and found that the total flavonoid contents varied from 1.06 to 9.05 mg of QE g^{-1} for ethanolic extracts of mushroom. The results indicated that the total flavonoid contents were greatest in *V. volvacea* (9.05 mg QE g^{-1}) and lowest in *P. sajor-caju* (1.06 mg QE g^{-1}).

2.9. DPPH RADICAL SCAVENGING PROPERTY

Rawat *et al.* (2013) conducted DPPH assay for the determination of free radical-scavenging activity of wild *G. lucidum* and 50 per cent inhibition concentration (IC_{50}) of methanolic extract was found to be 1.162 mg ml^{-1}. *In vitro* antioxidant activity of four *Ganoderma* spp. (*i.e. G. lucidium, G. tsugae, G. applanatum* and *Ganoderma* sp.) was studied by Rajoriya *et al.* (2015). Radical

scavenging activity was seen best in the *G. tsugae* (95.51 %) and *Ganoderma* sp. (94.43 %) at the IC_{50} value of 12 mg ml^{-1} and 10 mg ml^{-1} respectively.

Wandati *et al.* (2013) observed IC_{50} value of 61.86 mg 100 g^{-1} for oyster mushroom. Sasidhara and Thirunalasundari (2014) reported that methanolic extract of *P. djamor* exhibited DPPH free radical scavenging property. A concentration of 100 µg ml^{-1} mushroom extract resulted in 76.4 % inhibition with IC_{50} value of 64.72 µg ml^{-1}. According to Rajoriya *et al.* (2014) DPPH scavenging property of *P. sajor caju* was 45.53 per cent and that of *P. florida* was 9.95 per cent. Parihar *et al.* (2015) reported that at 12.5 to 150 µg ml^{-1} concentration of *P. ostreatus* ethanolic extract gave higher DPPH radical scavenging property. Antioxidant activities in five mushroom extracts were studied by Boonsong *et al.* (2016). They reported that ethanolic extract of *L. edodes* presented the strongest DPPH radical-scavenging activity of 64.34 % at 500 mg ml^{-1} as compared to *V. volvacea, P. eous, P. sajor-caju* and *A. auriculae.*

2.10. ANTI-CANCEROUS ACTIVITIES

The anti- cancerous properties of *G. lucidum* was reported by Patel and Goyal (2011). Tsai *et al.* (2013) reported that the crude extract from *H. marmoreus* in a concentration between 500 µg ml^{-1} and 100 % exhibited a growth inhibition rates between 28 % and 91 % on human leukemic cells after 48 h incubation. The ethanol extract of *G. lucidum* fruit body exhibited 50 per cent inhibition of low-grade bladder cancer cell line (MTC-11) at 129.3 µg ml^{-1} concentration after 24 h of incubation (Kao *et al.*, 2013). They worked on the potential anti-cancer mechanisms of *G. lucidum*. They extracted tritepenes in ethanol as it was the easiest approach to maintain the activity of tritepene extract and to scale up its production.

According to Tong *et al.* (2009) the methanol extract recorded two fold increase in cytotoxic activity of mouse myloma cells. The main side effect of radiation therapy was damage to the surrounding healthy tissues. Recent cytoprotective agents are having undesirable serious side effects that limit the

therapeutic applications. According to Smina *et al.* (2011) *G. lucidum* exhibit radio-protective effect in the normal cells and enhance recovery of cellular immune competence from gamma radiations. Studies conducted by Gao and Zhou (2016) revealed that cancer-preventive and tumoricidal properties of *Ganoderma* were ascribed to its anti-oxidative and radical-scavenging effects, enhancement of host immune function, induction of cell-cycle arrest and apoptosis.

Younis *et al.* (2014) conducted a study on anti- tumour activity of *A. bisporus*, *L. edodes* and *P. ostreatus* by different polar and non-polar solvents. They tested the effectiveness of mushroom extracts in inhibiting proliferation of three carcinoma cell lines including human liver carcinoma (Hep G2), the human colonic epithelial carcinoma (HCT 116) and the human cervical cancer cells (HeLa) using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Methanolic extract of lyophilized fruiting bodies of *A. bisporus* showed the highest cytotoxic effect with maximum inhibitory percentages of 87.3, 80.3 and 77.3 against HeLa, Hep G2 and HCT 116 cells and IC_{50} of 13.3, 17.4 and 14.4µg ml^{-1} respectively. The water extracts of lyophilized fruiting bodies in *L. edodes* showed highest cytotoxicity effect with maximal inhibition of 80.1, 70.2 and 81.3% against Hep G2, HCT 116 and HeLa cells and IC_{50} of 12.1, 15.8 and 15.3µg ml^{-1} respectively. The water extract of lyophilized fruiting bodies of *P. ostreatus* had the highest cytotoxic effect with maximal activity 86.2, 85.1 and 77.3 per cent against HeLa, Hep G2 and HCT 116 cells and with IC_{50} values of 12.1, 15.1 and 16.7 µg ml^{-1} respectively. On studying the effect of phytochemicals and antioxidant compounds from *C. indica var.* APK2 on proliferation of human MCF-7 breast carcinoma cells, Sumathy *et al.* (2015) observed decrease in the cell count from $40x10^4$ cells ml^{-1} (control) to $11x10^4$ cells ml^{-1} (1.0 mg ml^{-1}) after treatment with the mushroom extract for 24 h. This *in vitro* analysis revealed that aqueous extract of *C. indica*, has momentous anti-proliferative activity towards the human cancer cells. *G. frondosa, Cordyceps militaris, G. tsugae* var. *jannieae, Hericium erinaceus, Trametes versicolor, C. comatus,* and *Tremella fuciformis* were evaluated for their anticancer effect against human cancer (pancreatic

cancer, colon cancer, prostate cancer and breast cancer) cell lines. Among these mushrooms *C. militaris, T. versicolor* and *H. erinaceus* appeared as the best inhibitors of cell viability in a dose-dependent manner decreasing the cell viability by approximately 40 to 95 %. The treatment of all the cell lines with 250 to 500 $\mu g\ ml^{-1}$ of chloroform and ethyl acetate extracts from *G. tsugae* var. *jannieae* inhibited cell viability after 48 h and 72 h treatment. The most profound decrease in cell viability (85 to 95 %) was shown on colon cancer and prostate cancer cells (Asatiani *et al.*, 2018).

2.11. CYTOTOXICITY STUDIES

Cytotoxicity of hetero-polysaccharide from *A. blazei* was tested in osteosarcoma human osteoblast cells (HOS) as well as in normal human osteoblast cells. They showed significant inhibitory effect in HOS cell line by induction of apoptosis whilst showing no or little toxicity in a normal cell line (Wu *et al.*, 2012). Popovic *et al.* (2013) reported that the intracellular polysaccharides of *G. lucidum* have the ability to inhibit human hepato-carcinoma cell line (HepG2) in the first 48 h but it stimulated the cell growth after 72 h regardless of the concentration. These polysaccharides showed dose and time dependent inhibition of cancerous cells. They also observed the accelerated growth of normal human liver cells by the action of intracellular polysaccharides without causing any harm.

According to Asatiani *et al.* (2018) *C. militaris* exhibited direct cytotoxic activity against several kinds of cancer cells including lung carcinoma, melanoma, lymphocytic, prostate, breast, hepatocellular and colorectal while it did not show any cytotoxicity against normal cells.

Materials and methods

3. MATERIALS AND METHODS

3.1. ISOLATION AND PURE CULTURING

Five mushrooms namely *G. lucidum*, *P. florida*, *P. djamor*, *H. ulmarius* and *C. gambosa* were used for the study. The pest and disease free, medium aged, healthy mushrooms were collected and surface sterilized with ethyl alcohol. A small portion of the tissue from the junction of pileus and stipe was detached using a sterile inoculation needle after splitting it longitudinally. The detached tissue was placed in the petri plates containing sterile solidified PDA (Appendix-I) aseptically and incubated at room temperature. The pure mycelial growth observed was purified using hyphal tip method by transferring tips of fungal hyphae aseptically into PDA (Rangaswamy and Mahadevan, 2008).

The PDA was prepared and autoclaved at 1.02 kg cm^{-2} pressure for 20 min and allowed to cool. Twenty ml of sterilized medium was poured into sterilized petri plates and allowed to solidify. Inoculation was made by transferring a five mm disc of mycelial mat taken from the periphery of seven days old culture. The plates were incubated at 28±1 ^{0}C. Observations on colony characters and radial growth were recorded. Other cultural characters *viz.*, rate of growth, colour and diameter of mycelial growth were also recorded when it attained fifty per cent and hundred per cent growth. The experiment was conducted in Completely Randomised Design (CRD) with five treatments and four replications and the results were statistically analysed.

The composition and procedures for preparation of the media used in this experiment were followed as described by Ainsworth (1971) and Tuite (1969). The chemical composition of PDA is given in Appendix.

3.2. MORPHOLOGICAL STUDIES

The five mushrooms were observed for macroscopic characters such as colour and texture of pileus and stipe and nature of gills. Spore print was taken by

transferring the pileus from fully opened sporocarp on black paper sheets as per the method described by Deepa (2016). The detached pileus was placed upside down on a black paper with gills facing paper surface. A bell jar was placed over this to maintain humidity. After six hours, the bell jar and pileus were removed and the spore print thus obtained on paper was observed under microscope.

3.3. SPAWN PRODUCTION

The spawn production trials of five mushrooms were undertaken as per the standard technique given by Sinden (1934). The paddy grain was cooked in boiling water until the seed coat was just begin to split open and care was taken not to fully open the grains as it enhances the contamination problem. Then the excess water was drained off and the grains were spread on a clean cloth above the floor for drying. After sufficient drying paddy gain was mixed with calcium carbonate at the rate of 40 g kg^{-1} of grains. These were separately packed in polypropylene bags (12 x 6 inches) at the rate of 300 g per bag and sterilized by autoclaving at 1.02 kg cm^{-2} pressure and 121 ^{0}C for 2 h. After cooling, the bags were inoculated aseptically with mycelial bits of equal sizes from 10 days old culture of mushroom and incubated at 28 $\pm$ 2 ^{0}C for 15 days. The time taken for spawn run, nature of mycelial growth and presence of contaminants were observed and recorded. The experiment was conducted in CRD with five treatments and four replications and the results were statistically analysed.

3.4. CULTIVATION

3.4.1. Cultivation of *G. lucidum*

G. lucidum beds were prepared as per the method given by Geetha *et al.* (2012). Rubber saw dust was soaked in water (18 h) containing bavistin (75 ppm) and formalin (500 ppm) for chemical sterilization. The shade dried substrates then mixed thoroughly with 20 per cent rice bran and two per cent calcium carbonate. The substrate combinations were packed in polypropylene bag and autoclaved at 1.02 kg cm^{-2} pressure and 121 ^{o}C for one hour. Two kg of sterilized substrates were mixed with 300 g of spawn and packed in polythene bag of 60 X 30 cm size.

The bags were made compact, tied at the top and provided with 15 to 20 pinholes for air circulation. The bags were then transferred to an incubation chamber having adequate aeration, temperature (30 °C) and relative humidity (> 85 per cent). After complete spawn run one inch slits were put in polybags for the emergence of pinheads.

3.4.2. Cultivation of oyster mushrooms

Mushroom beds for *P. florida, P. djamor* and *H. ulmarius* were prepared as per the procedure described by Baskaran *et al.* (1978). Paddy straw was used as the substrate for oyster mushroom cultivation. The substrate after chemical sterilization spread over a silpaulin sheet under sun to reduce the moisture content to 60 %. The beds were prepared by polybag method by using polythene bags of 60 x 30 cm size. Paddy straw was placed in bag as twists and spawn laid in sides, over which again paddy straw twists were laid and spawning was done. Likewise four layers were prepared and the upper layer was fully covered with spawn. Then bags were made compact, tied at the top and provided with 15 pin holes for air circulation. The bags were then transferred to spawn running room.

3.4.3. Cultivation of milky mushrooms

Milky mushroom cultivation was done on paddy straw by polybag method (Baskaran *et al.*, 1978). Three layers of paddy straw twists were placed inside the bag one over the other and the spawn was laid over each layer. Then the bags were tied at the top and kept for incubation in spawn running room. After complete spawn run (20 days), the bags were opened and casing was done.

Milky mushroom beds were cased with sterilized casing mixture consisting of compost, soil and sand in the ratio 1:1:1. About 2.5 to 3.0 cm thick uniform layer of casing mixture was used. After casing, the bags were watered twice a day using knap-sac pump with nozzle to give misty spray.

The time taken for complete spawn run, pinhead formation, first flush, total yield, total crop period, average weight of sporocarp, number of sporocarps

and biological efficiency were recorded for each treatments. Pests and diseases incidence observed in the cultivation trials of five mushrooms throughout the production period and the per cent incidence was recorded. The experiment was conducted in CRD with five treatments and four replications and the results were analysed statistically.

3.5. DEVELOPMENTAL MORPHOLOGY

Developmental morphology of mushrooms was studied by observing the developmental changes of sporocarps from pinhead formation up to harvest. The observations recorded include colour, size and shape of the sporocarps and days taken for full bloom stage from pinhead formation.

3.6. ANALYSIS OF NUTRITIONAL COMPONENTS

Sample preparation

Healthy mushrooms at proper maturity were collected and cut into small longitudinal pieces to facilitate drying. The pieces were kept inside mechanical drier at 55 °C for 6 h. After complete drying, the samples were pulverized into coarse powder with the help of grinding machine.

3.6.1. Estimation of Protein

The protein estimation of the mushroom samples was done by Lowry's method (Lowry *et al.*, 1951). One g of grinded mushrooms were mixed with 10 ml of 0.1N NaOH and boiled for 30 min. The solution was cooled in room temperature and centrifuged at 1000 rpm for 10 min to separate the supernatant.

Aliquot of 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard were taken into a series of test tubes. Then 0.1 and 0.2 ml of the sample extract were taken in two test tubes and made up to one ml in all the test tubes. Five ml of alkaline copper solution (50 ml 2% Na_2CO_3 in 0.1 % NaOH) was added to each tube including the blank, mixed well and allowed to stand for 10 min. Then 0.5 ml of

Folin-ciocalteau reagent was added and incubated at room temperature in the dark for 30 min. Absorbance of the blue colour developed was read at 660 nm against reagent blank. Standard graphs were prepared using the bovine serum albumin. Using this graph, the concentration of protein content was estimated.

3.6.2. Estimation of Fat

Estimation of fat was carried out using Soxhlet extraction method (Lees, 1975). Five g of mushroom powder was taken in a thimble and placed inside the extractor. A piece of cotton wool was placed at the top of thimble for the proper distribution of solvent on the sample during extraction. Extraction of sample was then carried out with petroleum ether for 16 h. The extract was transferred into a pre-weighed beaker (w1), cooled in a desiccator and weighed (w2). The percentage of fat was determined using the following equation.

$$\text{Per cent of fat content} = \frac{w2-w1}{5} \times 100$$

3.6.3. Estimation of Fibre

Estimation of crude fibre content in mushroom was done as per the protocol of De, 1965.

Two g of powdered sample was extracted using petroleum ether to remove the fat content. Then the dried sample was boiled with 200 ml concentrated sulphuric acid (1.25 %) for 30 min with bumping chips. The digested sample was filtered through a muslin cloth and washed with boiling water until washings were no longer acidic. The sample was again boiled with 200 ml of sodium hydroxide solution for 30 min and filtered through muslin cloth and washed with 1.25 % sulphuric acid, three 50 ml portions of water and 25 ml alcohol. The residue was removed and transferred to pre-weighed ash dish (w1). Dried the residue for two h at 130 $\pm$ 2 ^{0}C. It was then cooled in a desiccator and recorded the weight (w2). The residue was further ignited for 30 min at 600 $\pm$ 15 ^{0}C, cooled in a desiccator and reweighed.

$$\text{Per cent crude fibre in ground sample} = \frac{\text{loss in weight}}{\text{weight of the sample}} \times 100$$

3.6.4. Estimation of Carbohydrate

Estimation of total carbohydrate content was done using anthrone method (Aminoff *et al.*, 1970). One hundred mg of mushroom powder was weighed in to boiling tube. It was hydrolysed by keeping in a boiling water bath for three h with 5 ml 2.5 N hydrochloric acid. It was cooled to room temperature and neutralised with solid sodium carbonate until the effervescence ceased. The volume was made to 100 ml and centrifuged at 5000 rpm for 10 min. The supernatant was collected and the aliquot was used for analysis. 0.5 ml aliquot was taken from the supernatant and made up to one ml by adding distilled water. Four ml anthrone reagent was added to the solution and heated for eight min in a boiling water bath. The solution was cooled rapidly and the absorbance was read at 630 nm in a spectrophotometer.

3.6.5. Estimation of Moisture Content

Hundred g (w_1) fresh samples of mushrooms were dried in an oven until a constant weight was obtained (w_2). Weight of dried sample was noted and the difference between fresh sample weight and dried sample weight gives the result which was converted into per cent (Geetha, 1993).

$$\text{Per cent of moisture content} = \frac{w1-w2}{w1} \times 100$$

3.6.6. Estimation of Amino Acid

Total free amino acids (Ninhydrin method) were determined according to the procedure given by Moore and Stein (1948). One ml of methanol extract of sample was mixed with one ml of ninhydrin in a test tube. Tubes were kept in boiling water bath for 20 min and then added 5 ml of diluent (equal volume of water and n-propanol) incubated at room temperature for 15 min and absorbance

was read at 570 nm against a reagent blank. The analysis was performed in triplicates and the results were expressed in percentage.

3.6.7. Estimation of Ash

Five g mushroom powder was transferred to a pre-weighed silica crucible (w1). It was then heated at low flame over a bunsen burner and when the substrate was charred, transferred the crucible to a muffle furnace. It was then heated to 500 ^{0}C for two h until a white ash was obtained. The weight (w2) was recorded after cooling in a desiccator. Per cent ash content was estimated using the following formula (Raghuramulu *et al.*, 1983).

$$\text{Per cent of ash content} = \frac{w2-w1}{5} \times 100$$

3.6.8. Estimation of Minerals

Digestion of sample

Digestion of the sample was carried out using Kjeldhal's digestion assembly. 0.5 g of powdered sample was kept inside Kjeldhal's distillation flask and added 10 ml of concentrated sulphuric acid containing salicylic acid (1 g salicylic acid in 30 ml concentrated sulphuric acid). The mixture was allowed to stand for overnight. Digestion was started initially on low flame for 10 to 15 min until the frothing stops. The digestion was continued at high flame for one to three h until the liquid became clear which indicated the complete digestion. The contents were allowed to cool, transferred to a 50 ml volumetric flask through a Whatmann No.1 filter paper and made up the volume to 50 ml.

3.6.8.1 Estimation of phosphorous

Working phosphorous standards of 2, 4, 6, 8 and 10 ppm were prepared by pipetting out 2, 4, 6, 8 and 10 ml of 50 ppm stock solution into 50 ml volumetric flasks and made up the volume. Ten ml of digested sample was transferred to a 50 ml volumetric flask and 10 ml of Barton's reagent was added. The volume was

made up to 50 ml with distilled water. It was allowed to stand for 30 minutes for colour development and the intensity of yellow colour was read in a spectrophotometer at 470 nm. The phosphorous concentration was found out with the help of standard curve. A blank was also prepared and read at 470 nm (Sadasivam and Manikam, 1992).

$$\text{Per cent of phosphorous} = x * \frac{50*50*100}{0.5*5}$$

X - Concentration of phosphorous from the graph

3.6.8.2 Estimation of Sodium &Potassium

Working standards of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ppm potassium was prepared from 100 ppm stock solution. Five ml of aliquot was pipetted out into a 50 ml volumetric flask from the digested sample and made up the volume with distilled water. The flame photometer was set up with aspirated working standards and aliquot. The readings were noted down and standard graph was prepared (Sadasivam and Manikam, 1992).

$$\text{Per cent of potassium} = x* \frac{50*50*100}{0.5*5}$$

X - Concentration of K from the graph

3.6.8.3 Estimation of Calcium and Magnesium

Wet ashing for determination of calcium and magnesium

Dried ground sample of 2.5 g from each mushroom variety was weighed and wetted afterwards with few drops of distilled water in a conical flask. 25 ml nitric acid was added and heated for 40 min. Thereafter 10 ml per chloric acid was added after cooling and heated again until white fumes appeared. 50 ml of distilled water was added after cooling to precipitate the remaining nitric acid. Then the contents were transferred into a 250 ml volumetric flask and filled to the mark with distilled water.

Determination of Calcium and Magnesium

Five ml of the aliquot were placed in a titration flask using a pipette and diluted to 100 ml with distilled water and subsequently 15 ml of buffer solution, ten drops of Eriochrome black T indicator and two ml of triethanolamine were added. The mixture was titrated with Ethylene-Diamine-Tetra-Acetate (EDTA) solution from red to clear blue. The minerals were calculated using the formula:

$$\text{Per cent of Ca} = \frac{a*N*0.02*25*100}{V*W}$$

$$\text{Per cent of Mg} = \frac{a*N*0.012*25*100}{V*W}$$

Weight of sample taken = W g

Volume of ammonium acetate made up = 25 ml

Volume taken for titration = V ml

Titrated volume of EDTA = a ml

Normality of EDTA =N

3.7. ANALYSIS OF MEDICINAL COMPONENTS

3.7.1. Estimation of beta-glucan

Extraction of beta-glucan

Beta-glucan content in mushroom was estimated using acidic extraction method (Ibrahim *et al*, 2017). Five g of powdered mushroom sample was weighed and mixed with 80 % ethanol in 4:1 ratio using magnetic stirrer for 2 h at room temperature (22 to 25 °C) and at 600 rpm. Then added one M sodium hydroxide in the ratio 1:7 (Mushroom: NaOH) and allowed to mix using magnetic stirrer (45 °C for 2 h at 250 rpm). Allowed to cool till it reaches 20 °C

and centrifuged (at 6000 rpm at 20 °C for 15 min). The supernatant was collected and centrifuged (at 6000 rpm for 15 min). Then added citric acid 15 per cent to the supernatant till pH reaches 3.5 at 20 °C. Allowed to cool till 4 °C and centrifuged (at 15000 rpm for 30 min at 4 °C). Then separated the supernatant and added 80 % ethanol in the ratio 1:2 (supernatant: ethanol). Incubated it at 4°C for 15 min and again centrifuged (at 6000 rpm for 15 min at 4 °C) to separate the sediment pellet. The pellets were transferred into a petri dish of known weight, and weighed the extract. Dried the pellets in a hot air oven at 42 °C for 16 h till they were completely dried and the colour is slightly dark and non-sticky. It was weighed out and ground well to get a powder.

The powdered pellets were dissolved in distilled water using magnetic stirrer for two h (at 25-30 °C and 700 rpm). Prepared the reagent (cold 86 % sulphuric acid, every 1 ml contains 0.7 mg L-cysteine). Then two ml of reagent was added to each 400 µl of dissolved extraction. Keep them directly in boiling water bath for 3 min and allowed to cool in room temperature and absorbance was read at 415 nm. The absorbance was compared with the standard curve to determine glucose concentration in the mushroom extract. Using the molecular weight of beta-glucan and glucose, the percentage of beta-glucan in the extract was determined.

3.7.2 Estimation of glycoprotein

Glycoprotein content in mushroom was estimated qualitatively using orcinol (1, 3-dihydroxy-5-methylbenzene) method (Koch *et al.*,1991). Prepared the orcinol reagent by mixing orcinol and sulphuric acid in 1:8 ratio. The purified mushroom extract was spotted on the thin layer chromatographic plate and air dried. Sprayed orcinol reagent on the thin layer plate and placed it at 100 °C for 20 min. Then allowed to cool and the presence of glycoprotein was confirmed qualitatively by the development of purple coloured spot.

3.7.3. Estimation of Terpenoid

Qualitative analysis

The qualitative phytochemical analysis for the presence of terpenoid was determined by the method described by Wadood *et al.* (2013). One g of mushroom sample was taken in a test tube and 10 ml of methanol was poured in it. The mixture was shaken well and filtered to take five ml extract of plant sample. Then two ml of chloroform were mixed in the extract of selected plant sample and three ml of sulphuric acid were added in selected sample extract. Formation of reddish brown colour indicated the presence of terpenoids in the mushroom.

Quantitative analysis

The previously prepared sample for qualitative analysis was transferred from assay tube to colorimetric cuvette [95 % (v/v) Methanol was used as blank] to read the absorbance at 538 nm. For the standard curve 200 µl of previously prepared linalool solution in methanol was added to 1.5 ml chloroform and serially diluted. In case of serial dilution total volume of 200 µl was made up by addition of 95 % (v/v) methanol, the total terpenoid content was determined by using standard curve.

3.7.4. Estimation of Polyphenol

The Folin-Ciocalteu method with modifications was followed to estimate total amount of polyphenol in the methanolic extracts of mushrooms (Ondo *et al.* (2013). Aliquots of 0.25 ml of extracts (1 mg ml^{-1}) were mixed with 1.25 ml Folin–Ciocalteu reagent (0.2 N diluted in methanol). A reagent blank using methanol instead of sample was prepared. After five min incubation at room temperature, one ml sodium carbonate solution (75 g L^{-1}) was added. Samples were incubated at room temperature for two h and the absorbance was measured at 765 nm. .

3.7.5. Estimation of beta-carotene

Five g of dried mushroom was powdered in a solution of 10 to 15 ml acetone and a few crystals of anhydrous sodium sulphate with the help of pestle and mortar. Supernatant was decanted into a beaker. This process was repeated twice and transferred the combined supernatant to a separating funnel. Ten to fifteen ml petroleum ether was added into it and mixed thoroughly. Two layers were separated out on standing. Discarded the lower layer and collected upper layer in a 100 ml volumetric flask, volume was made to 100 ml with petroleum ether and recorded optical density at 452 nm using petroleum ether as blank (Srivastava and Kumar, 2002).

$$\text{Beta- carotene } (\mu g\ g^{-1}) = \frac{O.D.* 13.7*104}{Wt.of\ sample * 560*1000}$$

3.7.6 Estimation of flavonoid

Flavonoid in methanol extract of mushroom was estimated by the method proposed by Jia *et al.*, 1954. One ml of the extract was mixed with 0.075 ml of 5% sodium nitrite solution and incubated at room temperature for 10 min. Then 10 % aluminium chloride and incubated at room temperature for 6 min. Then added 1 N sodium hydroxide and absorbance was read at 510 nm against a reagent blank. The analysis was performed in triplicates and the results were expressed as mg quercetin equivalent g^{-1} of sample.

3.8. DPPH RADICAL SCAVENGING PROPERTY

The total antioxidants of the extracts were evaluated by the phospho-molybdenum method according to the procedure described by Prieto *et al.* (1999). A 0.3 ml of extract was combined with three ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95 °C for 90 min. Then the absorbance of the solution was measured at 695 nm using a UV-VIS spectrophotometer against blank after cooling to room temperature. Methanol (0.3

ml) instead of the extract was used as the blank. The total antioxidant activity was expressed as the number of gram equivalent of ascorbic acid.

3.9. ANTI-CANCEROUS ACTIVITIES ON CERVICAL CANCER CELL

3.9.1. *In vitro* anti-proliferative effect determination by MTT assay

HeLa (cervical cancer) cell line was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained in Dulbecos modified Eagles medium (DMEM). The cell line was cultured in 25 cm^2 tissue culture flask with DMEM supplemented with L-glutamine, sodium bicarbonate and antibiotic solution (Penicillin or Streptomycin at 100 µg ml^{-1}). Cultured cell lines were kept at 37 °C in a humidified five per cent CO_2 incubator. The viability of cells was evaluated by direct observation of cells using inverted phase contrast microscope and MTT assay method.

3.9.1.1. Cells seeding in 96 well plate

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10 % growth medium, 100 µl cell suspension ($5x10^4$ cells/well) was seeded in 96 well tissue culture plate and incubated at 37 °C in a humidified 5 % CO_2 incubator.

3.9.1.2. Preparation of extracts and compound stock

One mg of ethanol mushroom extract was added to one ml of DMEM and dissolved completely by cyclomixer. Then the extract was filtered through 0.22 µm millipore syringe filter to ensure the sterility.

3.9.1.3. Anti-proliferative Evaluation

After 24 h of incubation the growth medium was removed and freshly prepared samples in 5 % DMEM were diluted to 100 µg, 50 µg, 25 µg and 12.5 µg in 100 µl of 5 % DMEM. Each concentration of 100 µl was added in triplicates to the respective wells and incubated at 37 °C in a humidified 5 % CO_2 incubator.

3.9.1.4. Anti-proliferative Assay by Direct Microscopic observation

Entire plate was observed at an interval of each 24 h up to 72 h in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

3.9.1.5. Antiproliferative Assay by MTT Method

Fifteen mg of MTT was reconstituted in three ml Phosphate buffer saline (PBS) until completely dissolved and sterilized by filter sterilization. After 24 h of incubation, the sample content in wells were removed and 30 µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well and incubated for 4 h. After the incubation period, the supernatant was removed and 100 µl of MTT solubilisation Solution (DMSO) was added and the wells were mixed gently by pipetting in order to solubilize the formazan crystals. The absorbance values were measured by using micro plate reader at a wavelength of 570 nm (Talarico *et al.*, 2004).

The percentage of growth inhibition was calculated using the formula:

$$\text{Per cent of viability} = \frac{\text{Mean OD Samples x 100}}{\text{Mean OD of control group}}$$

3.10. *IN VITRO* HEPATOTOXICITY DETERMINATION BY MTT ASSAY

Chang Liver Cell line was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained in DMEM. Preparation of mushroom extract and seeding into 96 wells plate was done as per the procedure in anti- proliferative assay. The cytotoxicity effect of mushroom extract on liver cell line was assessed by direct microscopic observation and MTT Method (Talarico *et al.*, 2004).

The percentage of growth inhibition was calculated using the formula:

$$\text{Per cent of viability} = \frac{\text{Mean OD Samples x } 100}{\text{Mean OD of control group}}$$

Results

4. RESULTS

Knowledge about the nutritional and medicinal properties of commercially cultivating mushrooms is of great importance. Hence, the current study was focussed on isolation, spawn production, cultivation, nutritional and medicinal aspects of four popular edible mushrooms of Kerala *viz. P. florida, P. djamor, H. ulmarius* and *C. gambosa* along with the leader medicinal mushroom, *G. lucidum*. This study was undertaken in College of Agriculture, Vellayani during 2016-18.

4.1. ISOLATION AND PURE CULTURING

The medium aged, disease and pest free, good quality mushrooms were collected and isolation was done by tissue culturing as per the standard method (Plate 1). Mycelial growth was started after 48 h of inoculation on PDA medium. *G. lucidum* recorded significantly minimum time for complete mycelial growth (6.25 days) followed by *H. ulmarius* (9 days) and *P. florida* (9.25 days) which were statistically on par. Maximum time for complete mycelial growth was observed on *C. gambosa* (10.75 days) and *G. lucidum* (11 days) which were statistically on par.

On ninth day of inoculation, maximum radial growth was observed in *G. lucidum* (9 cm) which was significantly not different from *H. ulmarius* (8.82 cm). *P. florida* and *C. gambosa* also were statistically on par with 8.4 cm and 7.52 cm radial growth respectively on ninth day. Slow mycelial growth was observed on *P. djamor* (6.25 cm) which is significantly different from other mushrooms.

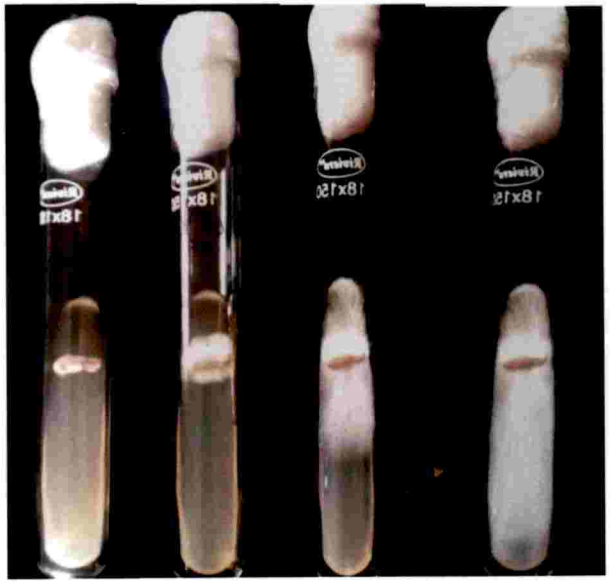
Flat, filamentous, translucent and creamy white mycelial growth was noticed in *G. lucidum*, while *P. florida* had characteristic flat, undulate, thick, white mycelia with irregular margin. White, very thick and fluffy mycelia were observed in *P. djamor*, *H. ulmarius* and *C. gambosa* (Table 1).

Table 1. Mycelial growth of mushrooms on PDA

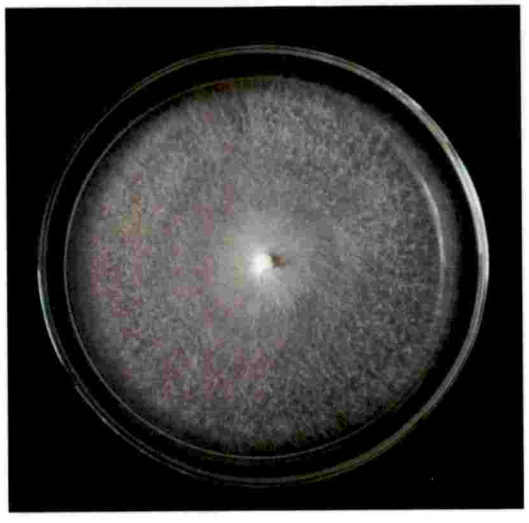
Sl. No.	Mushrooms	Days taken for complete mycelial growth *	Diameter of growth on 9th day (cm)*	Nature of mycelial growth	Colour of mycelia
1.	*G. lucidum*	6.25±0.43^{c}	9.00±0.00^{a}	Flat, filamentous and translucent	Creamy white
2.	*P. florida*	9.25±0.82^{b}	8.40±0.29^{b}	Flat, undulate with irregular margin	White
3.	*P. djamor*	11.00±0.70^{a}	6.25±0.18^{c}	Thick, fluffy with concentric zonation	Creamy white
4.	*H. ulmarius*	9.00±0.70^{b}	8.82±0.20^{a}	Thick, fluffy	Milky white
5.	*C. gambosa*	10.75±0.82^{a}	7.52±0.31^{b}	Smooth, fluffy	White
	CD(0.05)	1.246	0.398		
	SEm ±	0.847	0.506		

* Mean ± SD of four replications

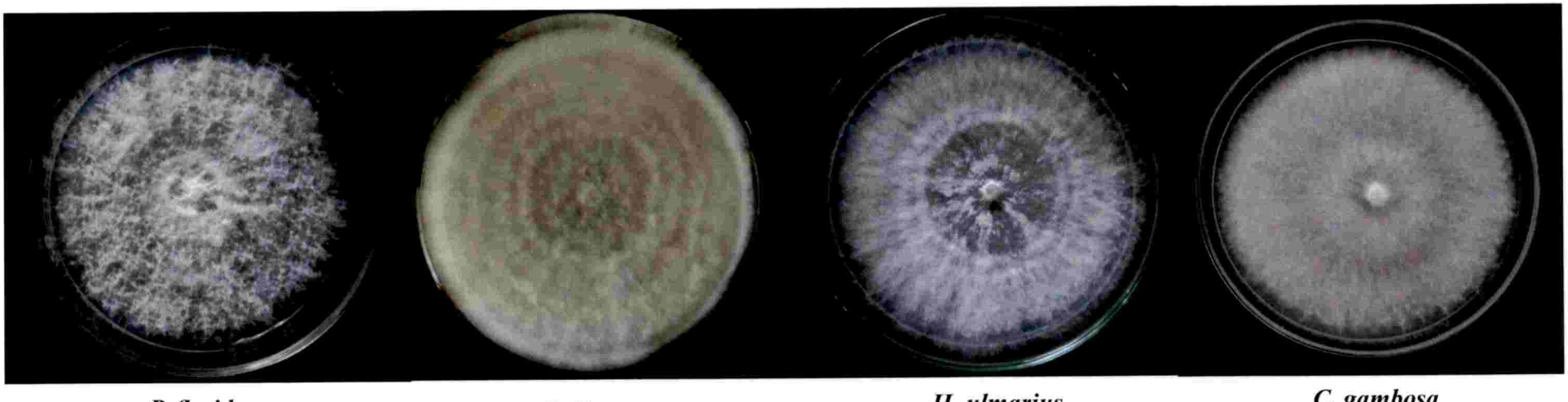
Values followed by similar superscripts are not significantly different at 5% level



Day-1 Day-3 Day-5 Day-7



G. lucidum
Full plate growth on PDA (Day-6)



P. florida
Day-9

P. djamor
Day-11

H. ulmarius
Day-9

C. gambosa
Day-11

Plate 1. Isolation by tissue culturing and purification of mushrooms

4.2. MORPHOLOGICAL STUDY

Macroscopic characters like colour, shape and size of stipe and pileus were studied (Table 2). The sporophore of *G. lucidum* was thick, hard and glossy. Length of stipe ranged from 1.5 cm to 3.5 cm and dark brown coloured. The pileus was 5 cm - 11 cm x 2.5 cm - 5 cm in size, laccate and shallow waved with reddish to yellowish brown colour and concentric zonation (Plate 2). Spore print was brown coloured (Plate 3).

P. florida was creamy white in colour, oyster shaped, soft and fleshy. The stipe was 3 cm - 4.5 cm in length with lateral attachment. Size of pileus ranged from 4.5 cm - 7.5 cm x 2.5 cm – 3 cm which is spatulate in shape with white coloured spore print.

P. djamor was characterized with pink coloured primordia which later faded to light pink. Stipe was non-prominent with 0.9 cm to 1 cm long. Pileus was 6 cm - 9 cm x 3.5 cm - 4.5 cm in size with wavy margin. White or pinkish white spore print was observed in pink oyster mushroom.

H. ulmarius, the blue oyster mushrooms were bluish initially and turned to creamy white on maturity. Sporocarps were soft, fleshy and tongue shaped with laterally attached stipe which was 2.5 cm - 3.5 cm long. The size of pileus ranged from 7.5 cm- 12 cm x 5 cm - 6.5 cm. Spore print was white coloured.

The sporophores of *C. gambosa* were milky white with stout stipe and umbrella shaped pileus. Stipe length was 12 cm to 16 cm, robust with centric attachment. Pileus was convex shaped with a dimension of 9 cm - 11.5 cm x 11.5 cm - 9 cm. The spores were white in colour.

4.3. SPAWN PRODUCTION

Five mushrooms were evaluated for production of spawn on paddy grain based on time taken for spawn run, nature of mycelial growth and presence of contaminants (Table 3). Among the five mushrooms, *P. djamor* took significantly lesser time (12.25 days) for spawn run followed by *H. ulmarius* and *P. florida*.

Table 2. Morphological studies of mushrooms

Sl. No.	Mushrooms	Length (cm) & nature of stipe	Size (l cm x b cm) & nature of pileus	Margin pattern of pileus	Surface pattern of pileus
1.	*G. lucidum*	1.5 - 3.5 Dark brown	5.0 - 11 .0 x 2.5 - 5.0 Reddish to yellowish brown	Smooth	Laccate, shallow waved with well-developed concentric zones.
2.	*P. florida*	3.0 - 4.5 Lateral to central	4.5 - 7.5 x 2.5 - 3.0 White	Entire	Spathulate
3.	*P. djamor*	0.9 - 1.0 Not prominent	6.0 - 9.0 x 3.5 - 4.5 Pink coloured primordia fade colour on maturity	Wavy	Spathulate to flabelliform
4.	*H. ulmarius*	2.5 - 3.5 Long, thick, Eccentric or lateral	7.5 - 12 x 5 - 6.5 Blue coloured primordia turned creamy white on maturity	Irregular shaped, depressed towards the base	Tongue shaped
5.	*C. gambosa*	12 - 16 Robust , centric	9.0 - 11.5 x 11.5 - 9.0 Milky white	Entire	Convex in shape

G. lucidum

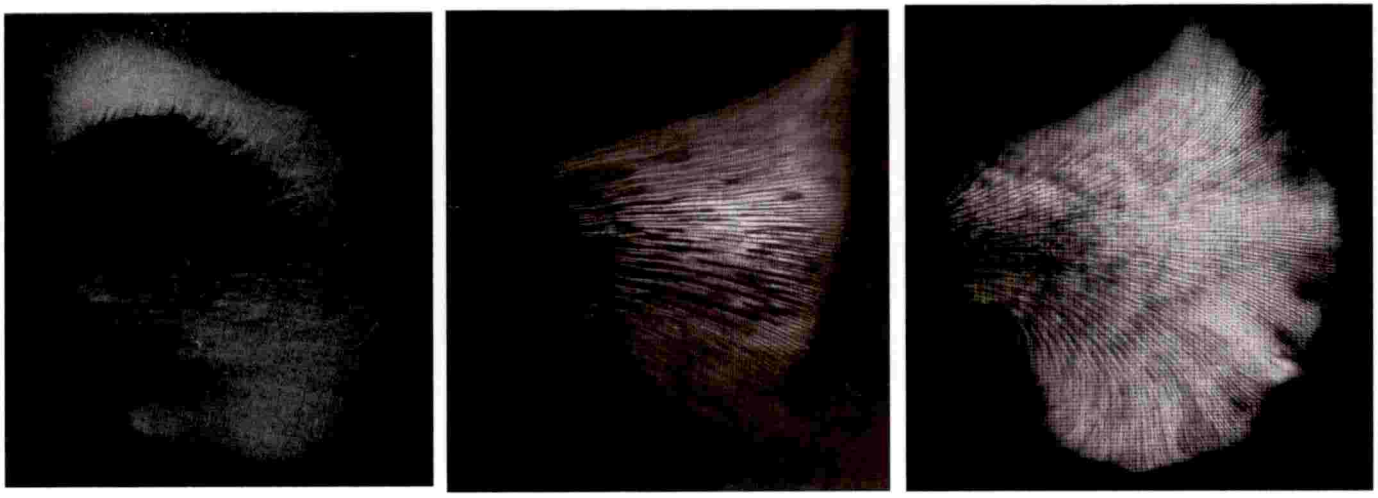
P. florida

P. djamor

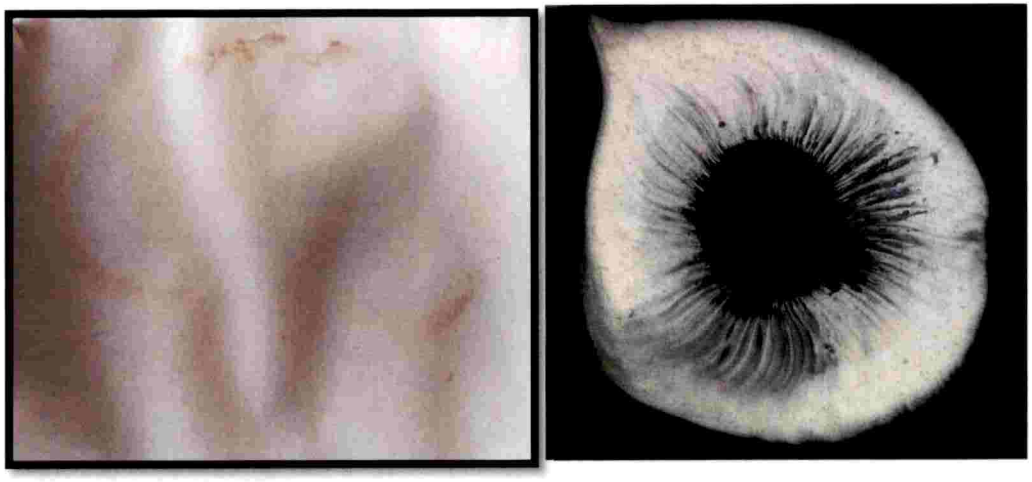
H. ulmarius

C. gambosa

Plate 2. Mature sporocarp



H. ulmarius *P. florida* *P. djamor*



G. lucidum *C. gambosa*

Plate 3. Spore print of mushrooms

Table 3. Evaluation of spawn production on paddy grain

Sl.no.	Mushrooms	Days taken for spawn run*	Nature of mycelial growth	Shelf life of spawn(days)*	Contamination (%)	Contaminants
1	*G. lucidum*	19.00± 1.00[a]	Thick and fluffy	178.75±5.41[a]	16.25	*Trichoderma sp. Penicillium sp. Aspergillus sp. Bacillus sp.*
2	*P. florida*	15.50± 0.50[b]		88.75±5.44[c]	13.75	
3	*P. djamor*	12.25± 0.43[c]		83.00±2.12[d]	10.00	
4	*H. ulmarius*	15.50±0.50[b]		89.25±5.62[c]	11.25	
5	*C. gambosa*	20.00± 1.41[a]		151.25±7.39[b]	15.00	
	CD (0.05)	1.492		9.53	NS	
	SEm±	1.388		19.625		

* Mean ± SD of four replication

Values followed by similar superscripts are not significantly different at 5% level

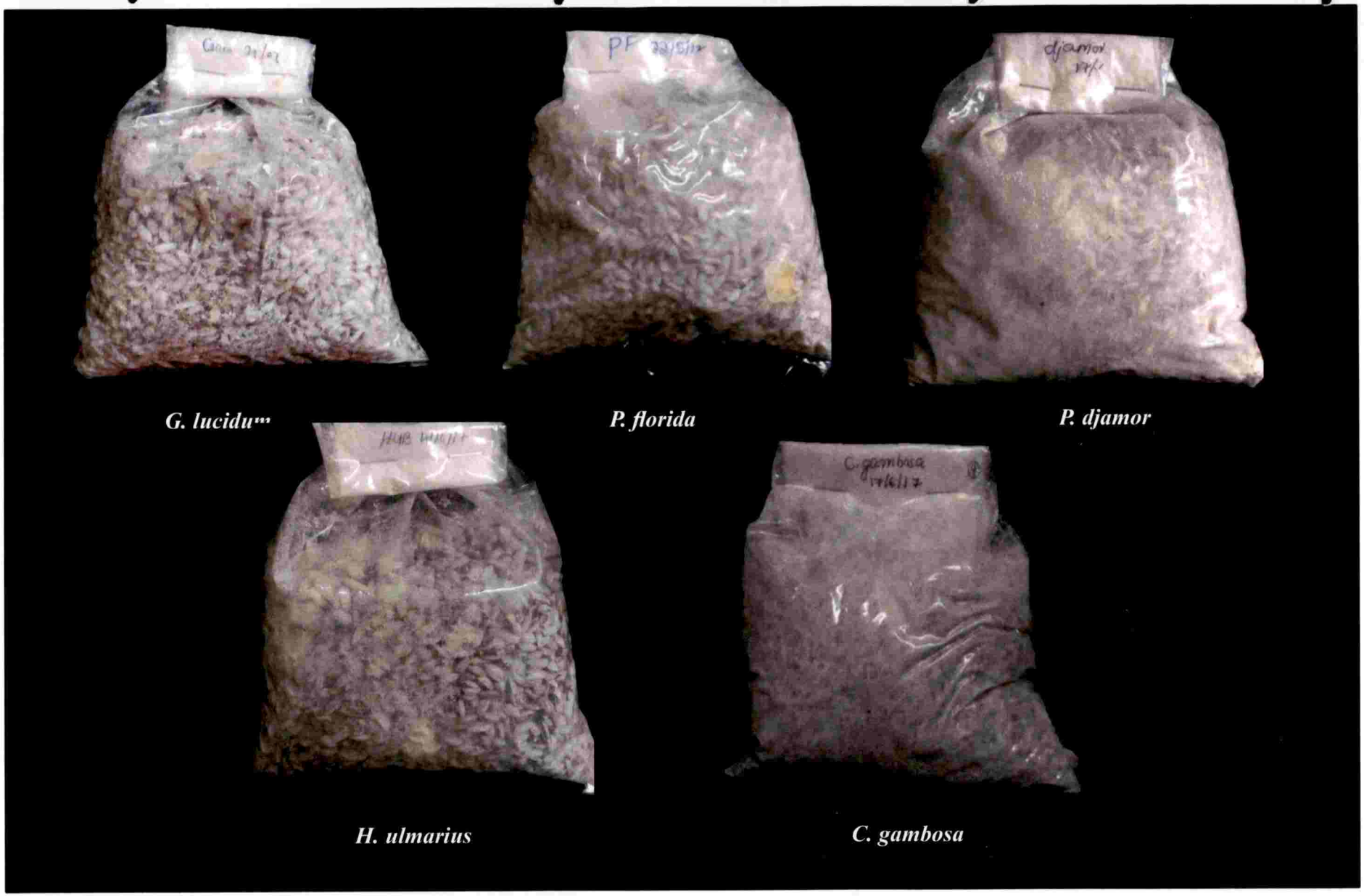
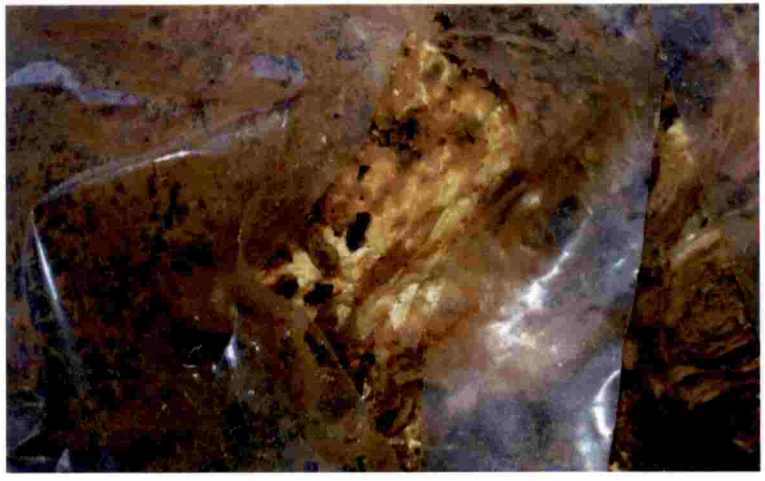
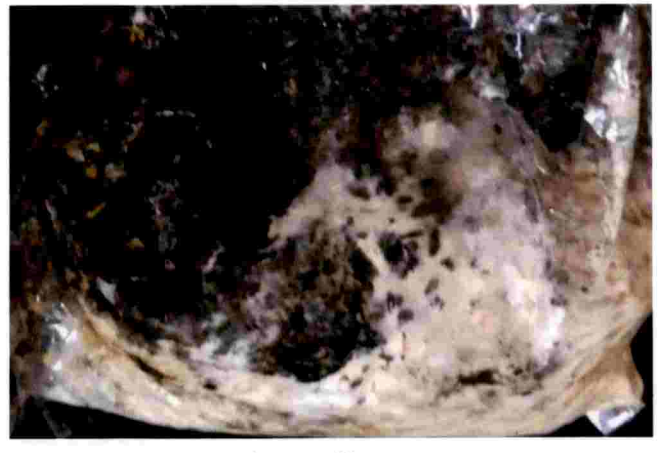


Plate 4. Spawn production of mushrooms on paddy grain



Bacterial contamination

Trichoderma sp.



Aspergillus sp.

Plate 5. Contaminants in spawn production

Both of these mushrooms completed spawn run after 15.50 days of inoculation. *G. lucidum* differed significantly from all other mushrooms which recorded maximum time (19 days) for completing the spawn run. Thick and fluffy growth of mycelia was observed in all the mushroom spawns produced (Plate 4). Contaminants such as *Trichoderma* sp. *Penicillium* sp. *Aspergillus* sp. and *Bacillus* sp. were observed during spawn production (Plate 5). The contamination percentage was recorded maximum in *G. lucidum* (20 %) followed *H. ulmarius* (15 %) and *P. florida* (15 %) and least contamination percentage was observed on *P. djamor* (10 %) and *C. gambosa* (10 %). Spawns of *G. lucidum* recorded maximum shelf life (178.75 days) followed by *C. gambosa* (151.25 %). The Shelf life of *H. ulmarius* and *P. florida* were recorded 89.25 days and 88.75 days respectively, which were statistically on par. Least shelf life was observed on *P. djamor* (83 days).

4.4. CULTIVATION

Cultivation of oyster mushrooms and milky mushrooms was done on paddy straw while rubber wood saw dust was used as substrate for cultivation of *G. lucidum* (Plate 6 and Plate 7). Among the five mushrooms, *P. djamor* recorded significantly lesser time for complete spawn run (10.5 days), pinhead formation (14.5 days), days for first harvest (16.5 days) and minimum crop period. *C. gambosa* took 20 days for spawn run, 29.5 days for pinhead formation, 35.25 days for first harvest and 60.75 days of total crop period. *P. florida* (24.75 days) and *H. ulmarius* (22 days) recorded maximum days for spawn run which were statistically on par. *G. lucidum* took 15.75 days for complete spawn run (Table 4).

H. ulmarius recorded significantly more number of days for pinhead formation (36.5 days) followed by *P. florida* (30.5 days) and *C. gambosa* (29.5 days). *G. lucidum* recorded 24.75 days for pinhead formation. *H. ulmarius* recorded maximum days for first harvest (40.25 days) followed by *C. gambosa* (35.25 days) which was statistically on par with *P. florida* (33.75 days) and *G. lucidum* (33.5 days). Maximum crop period was recorded in *G. lucidum* (88.75

Table 4. Growth stages and biological efficiency (BE) of mushrooms

Sl. No.	Mushroom	Days for complete spawn run	Days for pinhead formation	Days for first harvest	Total crop period (days)	Total yield (g/Kg)	BE (%)
1.	*G. lucidum*	15.75±1.29^{c}	24.75±1.70^{c}	33.50±1.65^{b}	88.75±5.44^{a}	115.00 ±7.08^{d}	11.50 ±0.353^{d}
2.	*P. florida*	24.75±0.43^{a}	30.50±0.86^{b}	33.75±0.43^{b}	55.00±3.53^{c}	845.00 ±31.81^{c}	84.50 ±3.18^{c}
3.	*P. djamor*	10.50±0.86^{d}	14.50±1.29^{d}	16.50±0.50^{c}	38.75±4.14^{d}	812.00 ±8.48^{c}	81.20 ±0.84^{c}
4.	*H. ulmarius*	22.00±1.41^{a}	36.50±0.86^{a}	40.25±1.08^{a}	63.75±4.14^{b}	960.00 ±14.14^{b}	96.00 ±1.41^{b}
5.	*C. gambosa*	20.00±1.41^{b}	29.50±0.86^{b}	35.25±0.82^{b}	60.75±2.558^{bc}	1037.25±36.29^{a}	103.72 ±3.62^{a}
	CD (0.05)	2.008	2.154	1.757	7.097	39.789	3.978
	SEm±	2.500	3.670	4.020	8.086	164.716	16.35

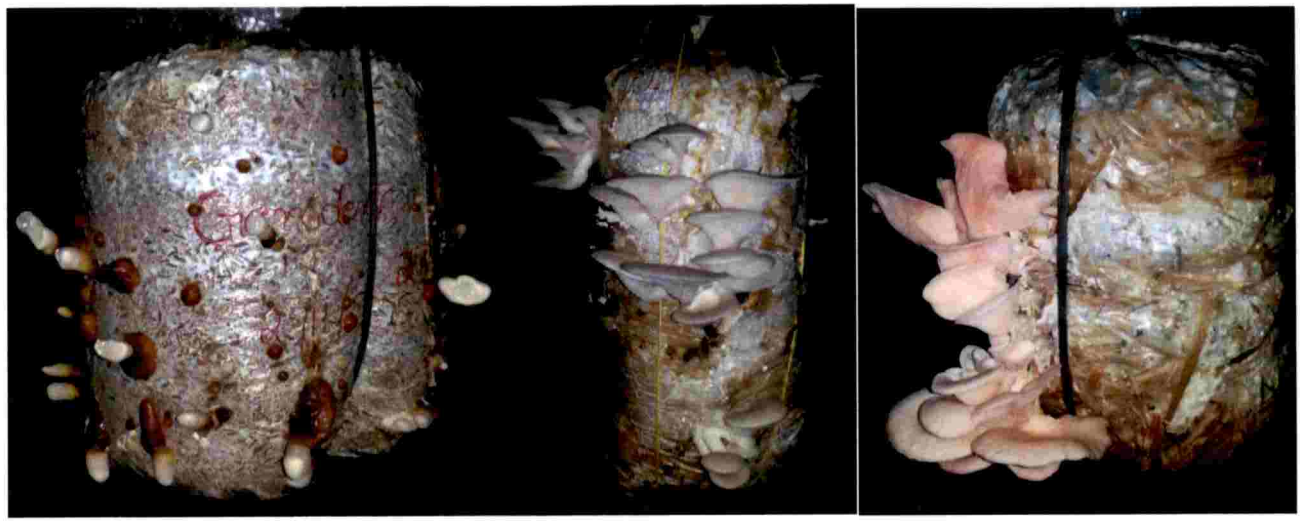
* Mean ± SD of four replications

Values followed by similar superscripts are not significantly different at 5% level

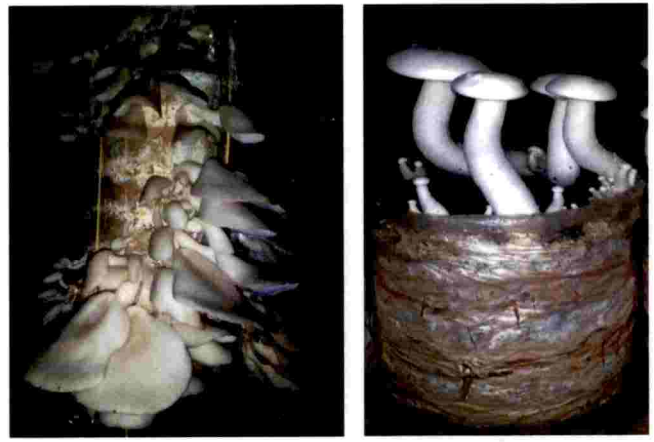
Milky mushroom beds

Oyster mushroom beds

Plate 6. General view of mushroom house



G. lucidum *P. florida* *P. djamor*



H. ulmarius *C. gambosa*

Plate 7. Production of sporocarp

days). *H. ulmarius*, *C. gambosa* and *P. florida* recorded 63.75, 60.75 and 55 days respectively for completion of crop period and these were not significantly different. Among the five mushrooms, the highest yield (1037.25 g) and biological efficiency (103.72 %) was recorded with *C. gambosa*, while *G. lucidum* recorded the lowest yield (115 g) and biological efficiency (11.5 %). *H. ulmarius* recorded 96 % biological efficiency with 960 g total yield per kg of substrate followed by *P. florida* and *P. djamor* with biological efficiency of 84.50 and 81.20 per cent respectively which were statistically on par.

4.5. DEVELOPMENTAL STAGES

G. lucidum recorded an average of nine days from pinhead formation to fully mature sporocarp (Plate 8). Creamy white coloured round primordia developed initially on the bed surface which later developed brown colouration and became darker with maturity. Finally, the stipe and pileus became distinct with reddish brown coloured, shiny pileus and dark brown stipe. Pileus had characteristic concentric zonation with white margin. The sporocarps were produced singly on the bed and were of woody consistency.

The sporocarps of *P. florida* recorded three days from pinhead formation to attain harvesting maturity (Plate 9). The pinheads were white coloured with distinct circular pileus and bulged stipe. On maturity pileus became spatulate with short and slender stipe. The sporocarps were produced in bunches. *P. djamor* recorded three days from pinhead formation to complete maturity (Plate 10). The pinheads were pink coloured with short stipe. The colour of primordia faded and became pale pink coloured on maturity. On the third day, stipe became non-prominent and had spatulate pileus with wavy margin. Mushrooms appeared either singly or as a bunch. *H. ulmarius* attained complete maturity on fifth day after pinhead formation (Plate 11). Blue coloured pileus and creamy white coloured short stipe were observed on the primordial stage. The stipe length and thickness increased gradually up to third day while on maturity stipe length reduced and pileus became irregular shaped with creamy white colour.

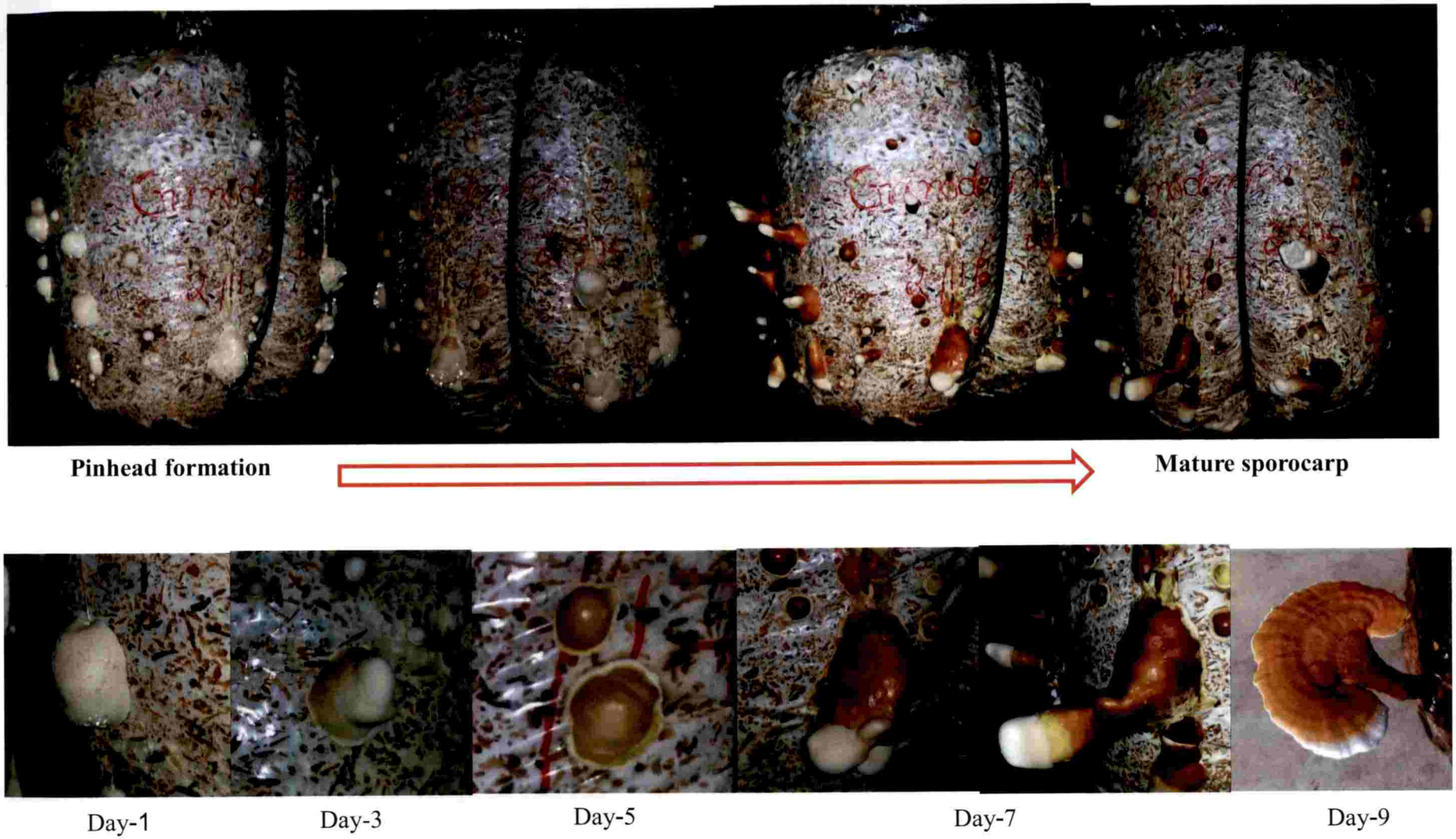
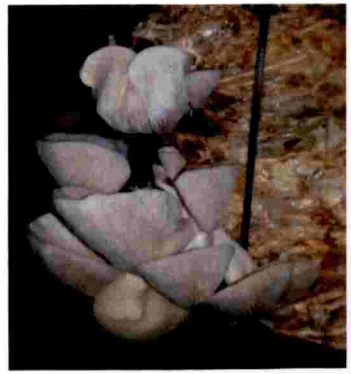


Plate 8. Developmental stages of *G. lucidum*

Pinhead formation → Mature sporocarp

Day-1

Day-2



Day-3

Plate 9. Developmental stages of *P. florida*

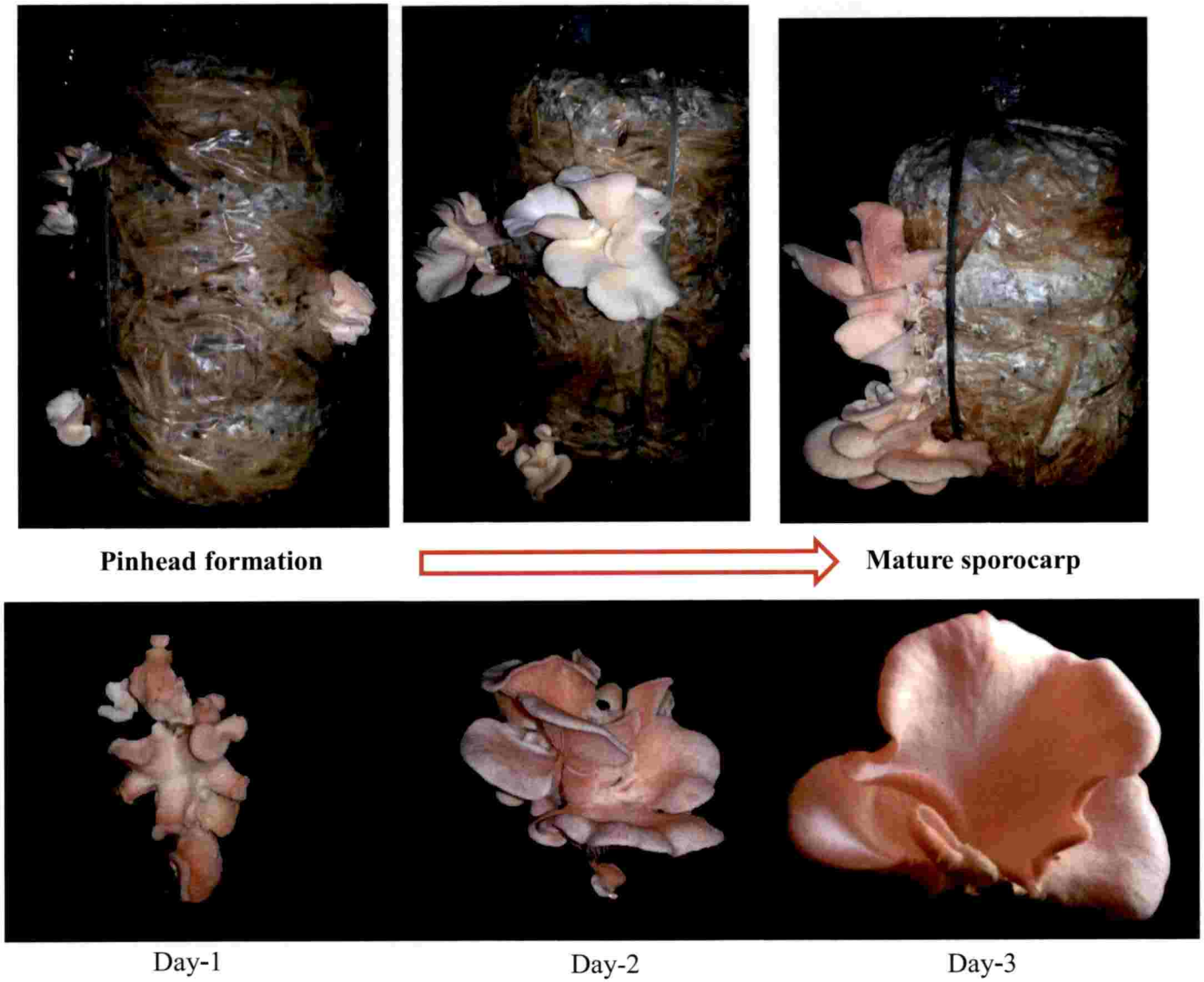


Plate 10. Developmental stages of *P. djamor*

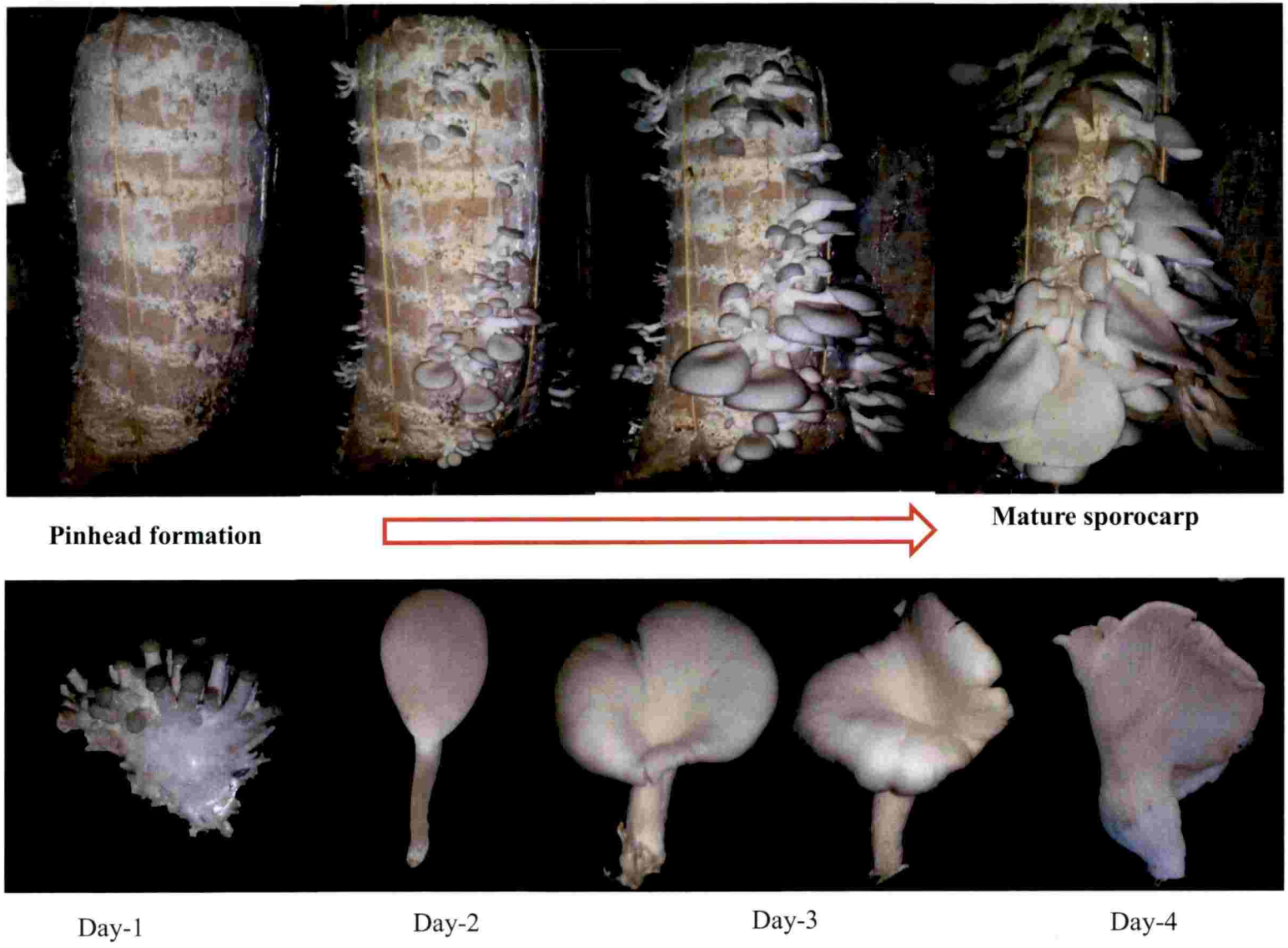


Plate 11. Developmental stages of *H.ulmarius*

Plate 12. Developmental stages of *C. gambosa*

Sporocarps of *C. gambosa* recorded five days from pinhead formation till complete maturity (Plate 12). Numerous pinheads (approximately 20-32 numbers) appeared initially whereas 5 to 6 sporocarps get matured. The sporophores of *C. gambosa* were characterised with distinct stipe and pileus. Stipe was robust and bulged at centre. Pileus was milky white with convex shape.

4.6. PEST AND DISEASE INCIDENCE

Incidence of pests such as phorid flies, thrips, sciarid flies and snail were observed during cultivation trials (Plate 13). *H. ulmarius* and *P. florida* recorded maximum (30 %) pest incidence followed by *C. gambosa* (20%). *P. djamor* and *G. lucidum* recorded least (10) incidence of pest among the five mushrooms.

Fungal contaminants such as *Aspergillus sp., Coprinus sp., Trichoderma sp.* and *Penicillium sp.* were also observed on mushroom beds (Plate 14). Maximum contamination percentage (30 %) was recorded on *P. florida* beds followed by *H. ulmarius* and *C. gambosa* (20 %) which were statistically on par. *G. lucidum* and *P. florida* recorded minimum fungal contamination (10 %).

4.7. ANALYSIS OF NUTRITIONAL COMPONENTS

The nutritional, mineral and medicinal components of five mushrooms were determined on dried dry weight basis (Plate 15).

4.7.1. Protein

The protein content of five mushrooms was estimated on dry weight basis (Table 7) and *G. lucidum* recorded the maximum (30.91 %) followed by *C. gambosa* (26 %). *P. florida* and *P. djamor* recorded 22.16 % and 21.98 % protein respectively. The five mushrooms recorded significantly different amount of protein and *H ulmarius* recorded the least protein content (20.76 %).

4.7.2. Fat

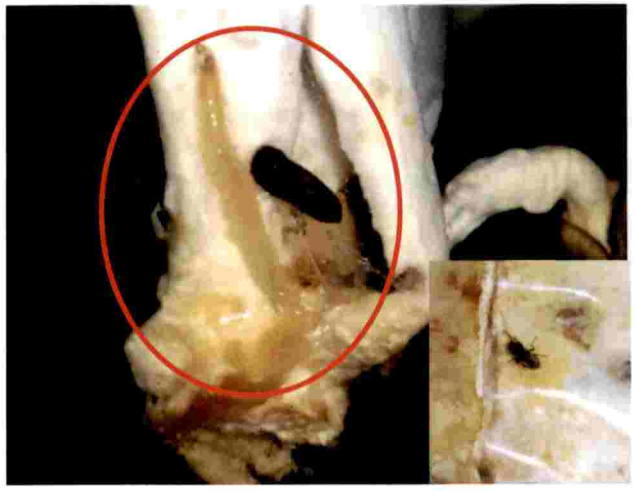
Estimation of fat content in the powdered mushroom samples was done using soxhlet extraction method (Table 7). Maximum fat content was found in *C.*

Table 5. Infestation by pests in mushroom production.

Sl. No.	Mushrooms	Beds infested by pests (%)	Major pests observed
1.	*G. lucidum*	10.00	Phorid flies Thrips Sciarid fly Snail
2.	*P. florida*	30.00	
3.	*P. djamor*	10.00	
4.	*H. ulmarius*	30.00	
5.	*C. gambosa*	20.00	

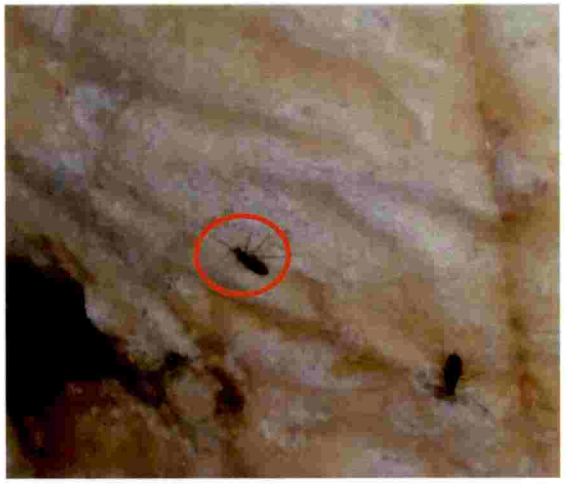
Table 6. Incidence of infection by competitor moulds in mushroom production

Sl. No.	Mushrooms	Contamination (%)	Contaminants
1.	*G. lucidum*	10.00	*Aspergillus* sp. *Coprinus* sp., *Trichoderma* sp. *Penicillium sp.*
2.	*P. florida*	30.00	
3.	*P. djamor*	10. 00	
4.	*H. ulmarius*	20.00	
5.	*C .gambosa*	20.00	

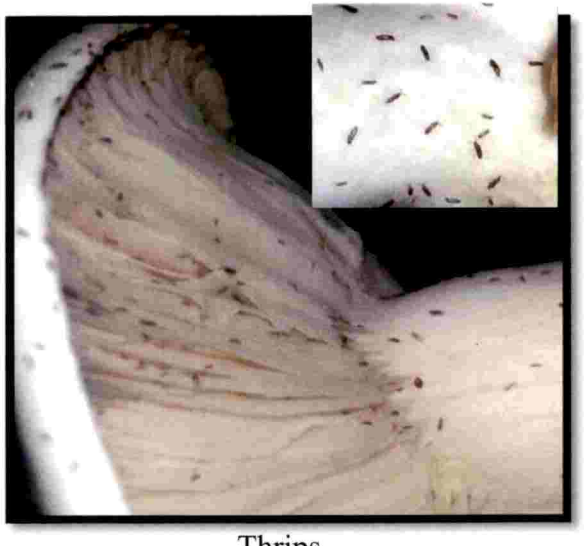


Phorid fly- *Megaselia* sp.

Snail



Sciarid fly- *Lycoriella* sp.

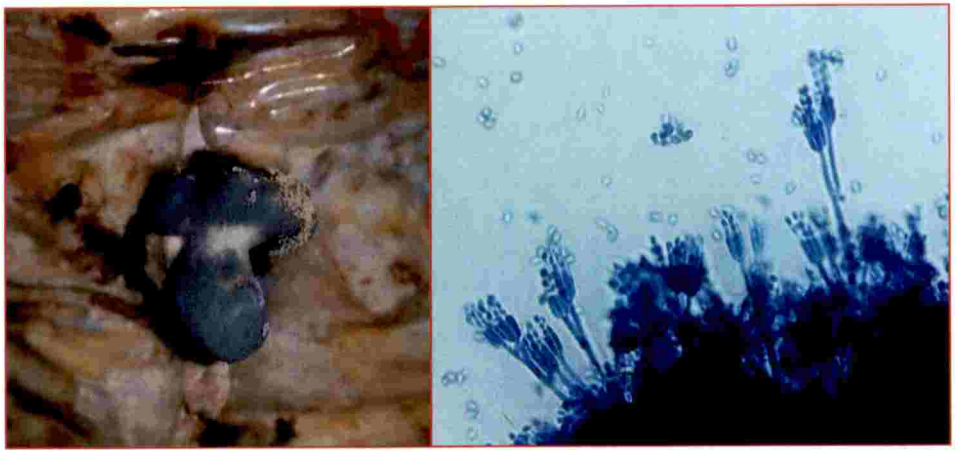


Thrips

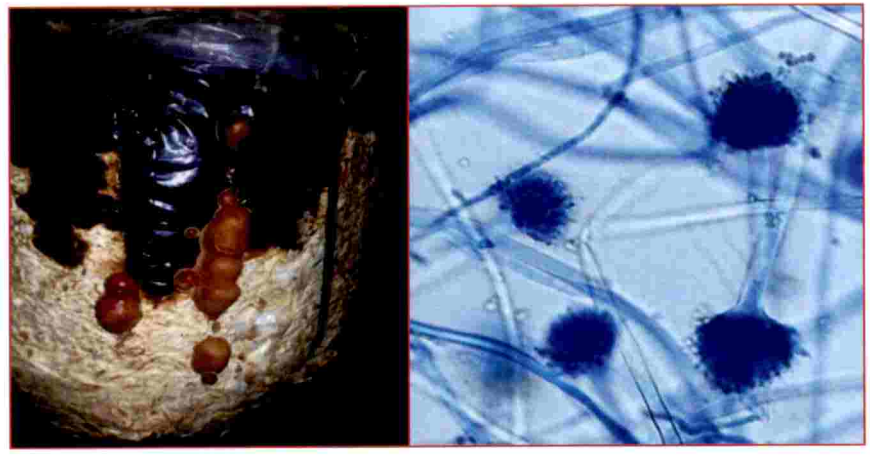
Plate 13. Pests observed on mushroom cultivation

Coprinus spp.

Trichoderma sp.



Penicillium sp.



Aspergillus sp.

Plate 14. Fungal contaminants

gambosa (2.79 %) followed by *P. florida* (2.28 %). *P. djamor* contained 2.10 % fat while *H. ulmarius* recorded 1.72 %. The minimum fat content was recorded in *G. lucidum* (1.33 %) as compared to other mushrooms. All the five mushrooms differed significantly in their fat content.

4.7.3. Fibre

The crude fibre content was recorded significantly high in *G. lucidum* (49.33 %) followed by *P. djamor* (30.59%), *P. florida* (24.38 %) and *H. ulmarius* (17.06%) while *C. gambosa* recorded the least fibre content (15.56 %) which was significantly different from other mushrooms (Table 7).

4.7.4. Carbohydrate

The carbohydrate content in dried mushroom samples was determined by anthrone method (Table 7). Among the five mushrooms *C. gambosa* was significantly high in carbohydrate (46.17 %) followed by *G. lucidum* (39.19 %). *H. ulmarius* recorded 30.87 % carbohydrate while *P. florida* and *P. djamor* were statistically on par with 26.68 % and 26.59 % of carbohydrate respectively.

4.7.5. Moisture Content

The moisture content of mushrooms was determined on fresh weight basis (Table 7). *C. gambosa*, *H. ulmarius* and *P. djamor* contained 91.10 %, 89.28 % and 88.38 % of moisture which were not significantly different. *P. florida* recorded 84.92 % moisture whereas the minimum moisture content was observed in *G. lucidum* (66.95 %) which was significantly different from other mushrooms.

4.7.6. Amino Acid

Estimation of total amino acid content in dried mushroom samples was done by ninhydrin method (Table 7). The maximum amino acid content was observed in *C. gambosa* (23.90 %) followed by *G. lucidum* (19.67 %). The amino acid content in *P. djamor* and *P. florida* were 17.10 % and 14.75 % respectively. *H. ulmarius* recorded the minimum amino acid content of 10.62 % as compared to

Table 7. Per cent composition of nutritional components in five mushrooms

Sl. No	Mushroom	Moisture (%)	Protein (%)*#	Fibre (%)*#	Carbohy-drate (%)*#	Total amino acid (%)*#	Ash (%)*#	Fat (%)*#
1.	*G. lucidum*	66.95^{c}	30.91^{a}	49.33 ^{a}	39.19^{b}	19.67 ^{b}	10.00^{b}	1.33^{e}
2.	*P. florida*	84.92^{b}	22.16^{c}	24.38 ^{c}	26.68 ^{d}	14.75 ^{d}	9.10^{b}	2.28^{b}
3.	*P. djamor*	88.38^{a}	21.98^{d}	30.59 ^{b}	26.59 ^{d}	17.10 ^{c}	9.00^{bc}	2.10 ^{c}
4.	*H. ulmarius*	89.28^{a}	20.76^{e}	17.06 ^{d}	30.87^{c}	10.62 ^{e}	7.25^{c}	1.72^{d}
5.	*C. gambosa*	91.10^{a}	26.00^{b}	15.56 ^{e}	46.17 ^{a}	23.90 ^{a}	13.5^{a}	2.79 ^{a}
	CD(0.05)	3.129	0.502	0.487	0.603	1.243	1.99	0.051
	SEm±	4.409	1.858	3.805	3.829	2.238	2.01	0.248

Dry weight basis

* Mean of four replications

Values followed by similar superscripts are not significantly different at 5% level

Plate 15. Dried mushroom samples

other mushrooms. All the mushrooms recorded significantly different quantities of amino acid content.

4.7.7. Ash content

The ash content was significantly high in *C. gambosa* (13.5 %) while *G. lucidum*, *P. florida*, *P. djamor* and *H. ulmarius* were statistically on par with 10 %, 9.1 %, 9 % and 7.25 % ash content respectively.

4.7.8. Minerals

Mineral components such as sodium, phosphorus, potassium, calcium and magnesium were estimated in powdered samples of mushrooms (Table 8). The digestion of the sample was carried out using Kjeldhal's digestion assembly.

4.7.8.1. Sodium

Sodium content of mushrooms was relatively less in quantity as compared with other minerals. Among the five mushrooms, sodium content was found significantly more in *P. djamor* (0.46 %), whereas the content in other mushrooms such as *C. gambosa* (0.029 %), *P. florida* (0.028 %), *H. ulmarius* (0.024 %) and *G. lucidum* (0.02 %) were statistically on par.

4.7.8.2 Phosphorous

The phosphorus content of mushrooms was estimated on dry weight basis. *P. florida* (0.225 %) recorded maximum phosphorus content, which is not significantly different from *P. djamor* (0.217 %) and *G. lucidum* (0.203 %). *H. ulmarius* recorded 0.194 % phosphorous content which was statistically on par with *C. gambosa* (0.178 %).

4.7.8.3. Potassium

Among the five mushrooms, *C. gambosa* recorded significantly higher potassium content (1.44 %). *G. lucidum* (0.76 %) was not significantly different

Table 8. Per cent composition of minerals in five mushrooms.

Sl. No.	Mushroom	Na (%)*#	P (%)*#	K (%)*#	Ca (%)*#	Mg (%)*#
1.	*G. lucidum*	0.020^{b}	0.203^{bc}	0.760^{b}	0.130^{a}	0.090^{a}
2.	*P. florida*	0.028^{b}	0.225^{a}	0.390^{d}	0.100^{b}	0.040^{c}
3.	*P. djamor*	0.096^{a}	0.217^{ab}	0.720^{bc}	0.080^{c}	0.050^{b}
4.	*H. ulmarius*	0.024^{b}	0.194^{cd}	0.700^{c}	0.080^{c}	0.040^{c}
5.	*C. gambosa*	0.029^{b}	0.178^{d}	1.440^{a}	0.080^{c}	0.050^{b}
	CD (0.05)	0.013	0.018	0.046	0.006	0.002
	SEm±	0.051	0.008	0.172	0.009	0.009

Dry weight basis

* Mean of four replications

Values followed by similar superscripts are not significantly different at 5% level

from *P. djamor* (0.72 %) which is statistically on par with *H. ulmarius* (0.70 %). Potassium content was significantly lower in *P. florida* with 0.39 %.

4.7.8.4. Calcium

The calcium content was recorded significantly higher in *G. lucidum* (0.13 %) followed by *P. florida* (0.10 %). *H. ulmarius*, *P. djamor* and *C. gambosa* were statistically on par with 0.08 per cent calcium content.

4.7.8.5. Magnesium

G. lucidum recorded significantly higher magnesium content (0.09 %) followed by *P. djamor* and *C. gambosa* with 0.05 % magnesium. The magnesium content of *H. ulmarius* was not significantly different from *P. florida* with 0.04 % on dry weight basis.

4.8. ANALYSIS OF MEDICINAL COMPONENTS

The medicinal properties of mushrooms were due to the presence of certain components present in mushrooms such as beta-glucan, terpenoid, polyphenol, beta-carotene and flavonoid which were estimated qualitatively and quantitatively on dry weight basis (Table 9).

4.8.1. Beta-glucan

Beta-glucan content was estimated by using acid extraction method. *G. lucidum* (38.58 g 100 g^{-1}) recorded significantly high beta-glucan followed by *C. gambosa* (35.88 g 100 g^{-1}). *P. djamor*, *H. ulmarius* and *P. florida* recorded 32.15 g, 26.62 g and 20.80 g of beta-glucan respectively per 100 g of dried mushroom sample which differed significantly from each other (Table 9).

4.8.2. Glycoprotein

Qualitative estimation glycoprotein was done by using orcinol reagent. Presence of glycoprotein was confirmed in all the five mushrooms based on purple spot formation.

4.8.3. Terpenoid

C. gambosa was found with significantly high terpenoid content (12.41 mg g^{-1}) followed by *G. lucidum* (11.14 mg g^{-1}). Terpenoid content of *H. ulmarius* was 10.13 mg and that of *P. djamor* was 9.78 mg respectively per gram of dried sample (Table 9). *P. florida* (9.22 mg g^{-1}) was recorded with minimum terpenoid content. All the mushrooms were significantly different in their terpenoid content.

4.8.4. Polyphenol

Total polyphenol content in mushrooms was determined using Folin-ciocalteu method and the result revealed that *G. lucidum* (23.80 mg GAE g^{-1}) was found with significantly high polyphenol content followed by *C. gambosa* (22.46 mg GAE g^{-1}). *H. ulmarius*, *P. florida* and *P. djamor* recorded 20.14 mg, 19.14 mg and 14.56 mg of total polyphenols respectively which were not statistically on par (Table 9).

4.8.5. Beta-carotene

Beta-carotene content in one gram dried mushroom samples was estimated and *P. djamor* (355µg g^{-1}) recorded significantly higher quantity among the five mushrooms (Table 9). Beta-carotene in *G. lucidum* was recorded as 246.46 µg which was statistically on par with *C. gambosa* (187.68 µg) which in turn on par with *H. ulmarius* (195.58 µg). *P. florida* recorded the least content (125.24 µg) of beta-carotene.

4.8.6. Flavonoid

Flavanoid content in *C. gambosa* (3.33 mg QE g^{-1}) was significantly higher followed by *G. lucidum* (3.02 mg QE g^{-1}). *H. ulmarius*, *P. djamor* and *P. florida* were recorded with 2.83 mg, 2.64 mg and 1.14 mg quercetin equivalent respectively per gram of dried mushroom sample (Table 9).

Table 9. Composition of medicinal ingredients in five mushrooms.

Sl.No.	Mushrooms	β- glucan (g/100g)*#	Glycoprotein (Present/ absent)*#	Flavonoid (mgQE/g)*#	Terpenoid (mg/g)*#	Polyphenols (mg GAE/g)*#	β-carotene (µg/ g)*#
1	*G. lucidum*	38.58^{a}	+	3.02^{b}	11.14^{b}	23.80^{a}	246.46^{b}
2.	*P. florida*	20.80^{e}	+	1.14^{e}	9.22^{e}	19.14^{d}	125.24^{d}
3.	*P. djamor*	32.15^{c}	+	2.64^{d}	9.78^{d}	14.56^{e}	355.04^{a}
4.	*H. ulmarius*	26.62^{d}	+	2.83^{c}	10.13^{c}	20.143^{c}	195.58^{cd}
5.	*C. gambosa*	35.84^{b}	+	3.33^{a}	12.41^{a}	22.46^{b}	187.68^{bc}
	CD (0.05)	1.029		0.127	1.657	0.332	60.84
	SEm±	3.203		0.371	1.600	4.323	38.42

Dry weight basis + present

* Mean ± SD of four replication

Values followed by similar superscripts are not significantly different at 5% level

4.9. DPPH RADICAL SCAVENGING PROPERTY

The anti-oxidant properties of mushrooms were analysed using DPPH assay. Among the five mushrooms *G. lucidum* was the mushroom with significant free radical scavenging property (Table 10). *G. lucidum* extract at 740 μg ml^{-1} exhibited fifty per cent inhibition (IC_{50}) of free radical. While *P. florida, P. djamor, H. ulmarius* and *C. gambosa* did not showed significant anti-oxidant properties even at higher concentration.

4.9. ANTI-CANCEROUS ACTIVITIES

The cytotoxicity effect of five mushrooms against human cervical cancer cell lines (HeLa) was studied. Control sample showed 100 per cent cell viability while percentage viability of cancer cells got reduced with increase in concentration of mushroom extract. The concentrations ranging from 12.50, 25, 50, 100 and 200 μg ml^{-1} decreased the cell viability to 87.80, 82.84, 72.80, 62.60 and 54.85 per cent respectively by *G. lucidum* extract (Table 11). Microscopic changes such as shrinking of cells, granulation and vacuolization in the cytoplasm of cells were also observed as an indicator of cytotoxicity (Plate 16).

C. gambosa extract decreased cell viability to 86.66, 78.44, 72.02, 69.38 and 60.92 per cent with increase in concentrations (Table 15). Morphological changes were also observed in the cancerous cells treated with higher concentrations. The activity of *P. djamor* mushroom extract on cancer cell line was dosage dependent and as the concentration increased, the per cent viability of cells reduced (Table 13). The per cent viability of cells got reduced to 69.47 per cent at 200 μg ml^{-1} concentration. The per cent viability of cervical cancer cell line decreased to 65.62 at 200 μg ml^{-1} concentration in *P. florida* (Table 12), whereas in *H. ulmarius* per cent cell viability reduced to 65.89 at 200 μg ml^{-1} (Table 14). Per cent viability of the cells were not reduced further even with increase in concentration above 150 μg per ml in both *P. florida* and *H. ulmarius*. Among the five mushrooms *G. lucidum*, *C. gambosa* and *P. djamor* showed potent anti-cancerous properties.

Table 10. DPPH *– Antioxidant analysis of *G. lucidum*

Sample	Concentrat-ion ($\mu g\ ml^{-1}$)	OD at 515 nm	Inhibition of free radical (%)
Control at 0 mins	-	0.531	
Control at 15 min	-	0.528	
Ganoderma – ethanol extract	200	0.464	12.68
	400	0.389	26.89
	600	0.348	34.65
	800	0.225	57.95

*DPPH- (1,1- diphenil-2-picrylhydrazyl)

Table 11. MTT* assay on anti- cancer property of *G. lucidum*

Concentration ($\mu g\ ml^{-1}$)	Average OD at 540 nm	Viability of cancer cells (%)
Control	1.1309	100
12.5	0.9932	87.823
25	0.9369	82.845
50	0.8234	72.809
100	0.708	62.605
200	0.6203	54.850

MTT*: 3-(4,5-dimethyl-2- thiazolyl)-2,5- diphenyl-tetrazolium bromide

Table 12. MTT assay on anti- cancer property of *P. florida*

Sample Concentration (µg ml^{-1})	Average OD at 540nm	Viability of cancer cells (%)
Control	1.1894	100
12.5	0.9211	78.60
25	0.9191	76.11
50	0.8901	74.83
100	0.8241	69.28
200	0.7806	65.62

Table 13. MTT assay on anti- cancer property of *P. djamor*

Sample Concentration (µg ml^{-1})	Average OD at 540nm	Viability of cancer cells (%)
Control	1.1372	100
12.5	0.98855	86.928
25	0.91923	80.832
50	0.8702	76.521
100	0.8394	73.812
200	0.7901	69.477

Table 14. MTT assay on anti- cancer property of *H. ulmarius*

Sample Concentration (µg ml^{-1})	Average OD at 540 nm	Viability of cancer cells (%)
Control	1.1991	100
12.5	0.9454	79.842
25	0.9291	76.151
50	0.8804	73.421
100	0.8147	67.942
200	0.7901	65.891

Table 15. MTT assay on anti- cancer property of *C. gambosa*

Sample Concentration (µg ml^{-1})	Average OD at 540 nm	Viability of cancer cells (%)
Control	1.1372	100
12.5	0.9855	86.660
25	0.8921	78.447
50	0.8191	72.027
100	0.7891	69.389
200	0.7611	60.927

4.10. CYTOTOXICITY STUDIES ON HEALTHY LIVER CELLS

The cytotoxicity effect of five mushroom extracts were assessed on healthy human liver cells obtained from National Centre for Cell Sciences, Pune. *G. lucidum* extract exhibited 33.69 per cent decrease in viability of normal hepatic cells at 200 µg ml^{-1} concentration which is less as compared to per cent reduction in viability of cancerous cells (45.15 %).

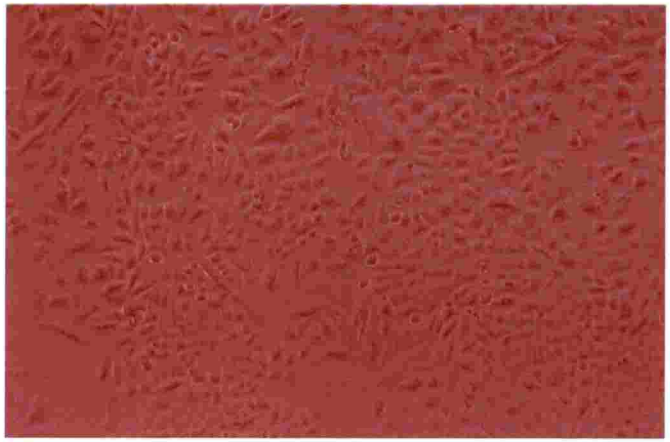
P. florida, P. djamor, H. ulmarius and *C. gambosa* extracts recorded 27.28, 25.48, 20.98 and 26.75 per cent reduction in liver cell viability respectively at 200 µg ml^{-1} concentration as against 30.53, 34.38, 34.21 and 39.08 per cent reduction in viability of cancer cells (Table 17).

Table 16. Comparison of anti- cancerous activity of mushrooms

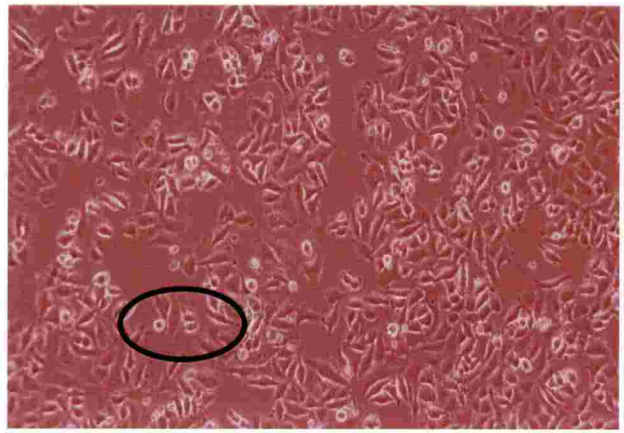
Sl. No.	Mushroom	Per cent viability of cancer cells at 200 µg ml^{-1}
1.	*G. lucidum*	54.850
2.	*P. florida*	65.621
3.	*P. djamor*	69.477
4.	*H. ulmarius*	65.891
5.	*C. gambosa*	60.927

Table 17. Cytotoxicity study of mushroom extracts on human liver cells

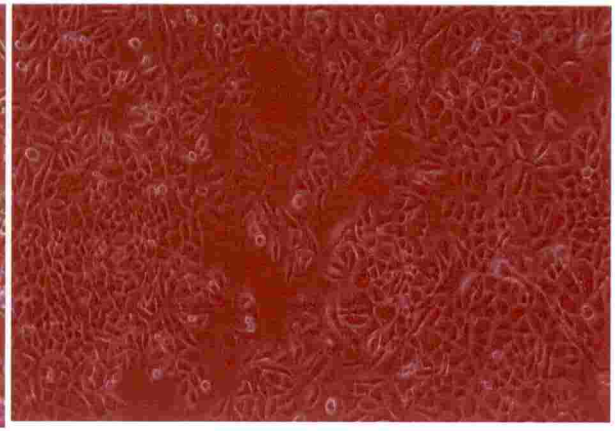
Sl. No.	Mushroom	Per cent inhibition of liver cells at 200 µg ml^{-1}
1.	*G. lucidum*	33.69
2.	*P. florida*	27.28
3.	*P. djamor*	25.48
4.	*H. ulmarius*	20.98
5.	*C. gambosa*	26.75



Control (HeLa Cell lines)



G. lucidum (200 µg/ml)



H. ulmarius (200 µg/ml)

P. florida (200 µg/ml)

P. djamor (200 µg/ml)

C. gambosa (200 µg/ml)

Plate 16. Microscopic observation of cervical cancer cells (Inverted phase contrast microscope 40X)

Discussion

5. DISCUSSION

Mushrooms have a long history of use in oriental medicines and of later they have emerged as wonderful source of nutraceuticals, anti-oxidants, anti-cancer, prebiotic, anti-microbial and anti-diabetic compounds. The present research project is aimed to analyse the pharmacologically active compounds present in four commercially cultivating mushroom species including three species of oyster mushrooms *viz*, *P. florida* the white oyster mushroom, *P. djamor* the pink oyster mushroom, and *H. ulmarius* the blue oyster mushroom along with one species of milky mushroom, *C. gambosa* variety Bheema in comparison with the leader of medicinal mushroom *G. lucidum*.

5.1. ISOLATION AND PURE CULTURING

Pure culturing is of paramount importance in mushroom cultivation since for a good spawn, a medium impregnated with mycelium serving as the seed for mushroom cultivation is necessary. Among the different methods of culturing, tissue culturing is attempted in the present study. The tissue from the junction of stipe and pileus is taken for oyster mushroom species and *Ganoderma sp.* whereas the upper part of central stalk is taken for *C. gambosa* since this is the zone of rapid cell elongation and no genetic segregation or variation will be present in tissue cultures. The yield from such cultures is more reliable and stable in the present study, mushrooms collected from healthy, pest and disease free high yielding beds were taken for tissue culturing. In all the mushrooms tried, the mycelial growth started after 48 h of inoculation on PDA slant. Purification of mushroom cultures was done by hyphal tip method in which the growing mycelial tip is transferred to petri plates containing PDA medium and the pure cultures of five mushrooms were maintained.

The growth of five species of mushrooms was assessed by growing on PDA medium as PDA was reported as the best medium for healthy growth of mushrooms (Singh *et al.*, 2000; Mishra *et al.*, 2015). In the present study, *G. lucidum* recorded faster rate of growth which completed mycelial growth in 6.25

days in PDA medium (Table 1). The mycelial growth of *G. lucidum* was flat, filamentous, with creamy white in colour. This is in accordance with the report of Rajesh *et al.* (2014) in which the growth of *Ganoderma* sp. DKR1 in PDA was observed as white to pale orange coloured, even and felty colonies with 6 cm size on fourth day. Nasreen *et al.* (2005) also reported PDA as the best medium for growth of *G. lucidum*, while Venkatarayan (1935) and Sharma and Thakur (2010) recorded maximum growth of *G. lucidum* in MEA.

The oyster mushrooms such as *P. florida*, *P. djamor* and *H. ulmarius* showed white, thick and fluffy mycelial growth on PDA media which took 9.25 days, 11 days and 9 days respectively for complete mycelial growth (Figure 1). Sumi (2016) and Kumar and Eswaran (2016) also observed faster growth of *H. ulmarius* in PDA media with 9 cm growth on 10^{th} day. Das *et al.* (2000) reported that *P. sajor caju*, *P. florida* and *P. ostreatus* grew faster in PDA and MEA. Sardar *et al.* (2015) proved the superiority of PDA for the growth and development of oyster mushrooms.

In the present study, *C. gambosa* took 10 days for completing mycelial growth in petri plates while culturing on PDA. This is in accordance with the work of Krishnamoorthy *et al.* (2015) who reported the maximum mycelial growth of *C. indica* in PDA within 7 to 10 days. Kerketta (2016) also recoded maximum radial growth of *C. indica* in PDA.

5.2. SPAWN PRODUCTION

The mycelium of mushroom growing in its substratum and prepared for the purpose of mushroom production is called spawn. The success or failure of mushroom cultivation depends on the availability of good quality spawn. The yield and quality of a spawn is governed by the genetic makeup of the strain and technology used for spawn productions which include the nature of substrate also (Kumar, 1995). In the present study paddy grain was trying as substrate for spawn production of five mushrooms since earliness in spawn run and best mycelial

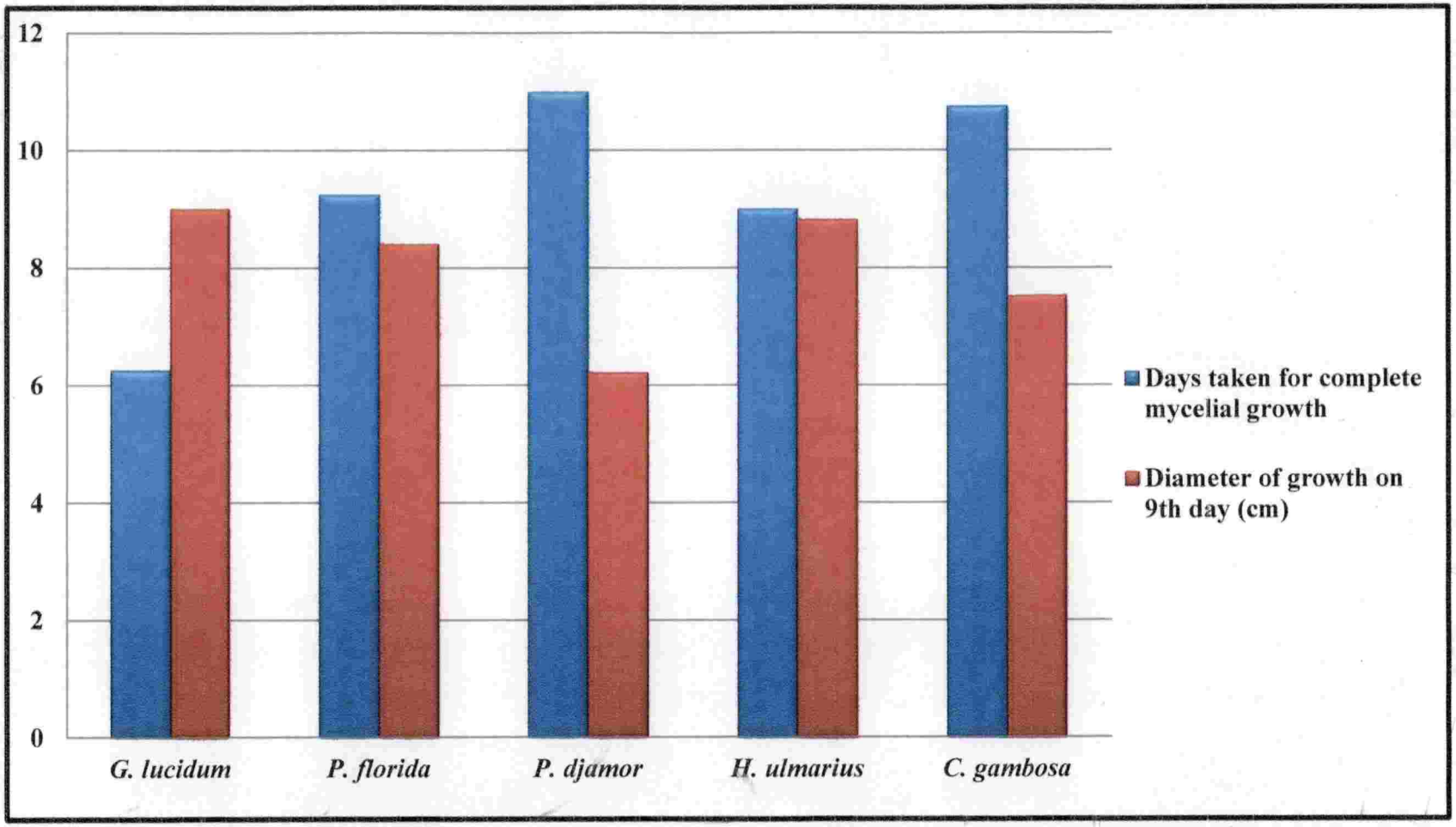


Fig 1: Mycelial growth of mushrooms on PDA

growth was observed in grain spawn as reported earlier by Chandravanshi, 2007; Kapoor, 2011; Sumi, 2016).

G. lucidum recorded 19 days for complete spawn run in paddy grain substrate in the present study which is in accordance with the findings of Varma (2013) who reported 19.42 days for spawn run in paddy grain, but a lesser time was taken in wheat grain (13.50 days). Significantly lower time was recorded in barley grain which took 8 days for complete spawn run of *G.lucidum* (Joshi and Sagar, 2016). The suitability of grain spawn may be due to the richness of carbohydrate and protein in cereals and millets.

Among the *pleurotus* spp. P. *djamor* took least time for spawn run (12.25 days) while both *P. florida* and *H. ulmarius* were recorded with 15.50 days for spawn run in paddy grain substrate in the present study (Table 2). Spawn development period for *P. djamor* varied from 8 to 16 days depending upon the cereal grain substrate used (Chauhan, 2013). Jayachandran *et al.* (2017) reported that *P. florida* required 12 days for spawn run whereas Thulasi *et al.* (2010) observed 16 days for spawn production of *P. eous* and *P. florida* on paddy grain. Sumi (2016) reported that *H. ulmarius* complete spawn run after 15.50 days of inoculation while Baghel (2017) revealed that *H. ulmarius* recorded 17.60 days for spawn production in paddy grain.

C. gambosa recorded a maximum of 20 days for spawn production in the present study but the growth was not as thick as that of oyster mushroom spawn and it took 25 to 30 days to reach full maturity and ready for spawning. This is in conformity with the work of Panda (2015) who reported that *C. indica* completely colonized the wheat grain substrate in 18.33 days. Similar observation was made by Heera (2006) who reported that ten different *C. indica* isolates required 18.33 to 26.67 days for complete spawn run and the mycelial growth was thick and fluffy. Geetha and Jacob (2013) reported complete spawn run of *C. gambosa* variety Bheema on paddy straw in 18.33 days.

5.3. CULTIVATION AND DEVELOPMENTAL STAGES

Successful production of mushroom is correlated with the nature and quality of substrate, cellulose content, quality of spawn and the environmental conditions such as temperature, humidity, light and aeration prevailing in the mushroom house. The cultivation of *G. lucidum* was tried on a combination of rubber sawdust (80 %) amended with rice bran (20 %) which was reported to be the best for its cultivation by Veena and Pandey (2006), Varma (2013) and Geetha and Jacob (2013). *G. lucidum* completed spawn run in 15.65 days and the mycelial growth was creamy white in the beginning which later changed to yellowish brown colour. Creamy white pinheads started developing on the beds nine days after complete spawn run. This turned to distinct dark brown coloured thick stipe and reddish brown, shiny pileus in ten days (Figure 2). The pileus was characterised with concentric rings and white margin. Pores were observed on the under surface of the pileus. These results were similar with morphology of *G. lucidum* reported by Varma (2013). Rawat (2018) reported irregular shaped, copper red coloured, varnished, hard and thick fruit bodies of *G. lucidum*. The first harvesting was done in 33.50 days and a total of three harvests produced 115.00 grams of mushroom with 11.50 per cent biological efficiency. Similar trials were conducted by Veena and Pandey (2006) who reported that *G. lucidum* cultivated on rice bran enriched sawdust completed spawn run within 14 to 21 days. Varma (2013) also reported the suitability of sawdust along with rice bran for cultivation of *G. lucidum* with 6.59 per cent biological efficiency. Among the five mushrooms cultivated in the present study *G. lucidum* exhibited least biological efficiency. According to Jain (2012) sawdust-wheat bran combination is the best substrate for *G. lucidum*, although various other substrates such as paddy chaff, rice bran and paddy straw were reported to be suitable for its cultivation by Geetha *et al.* (2012). Singh *et al.* (2014) observed a biological efficiency of 22 per cent while cultivating on billets of poplar. Triratana and Chaiprasert (1991) and Rai (2004) reported sawdust as the best substrate for the

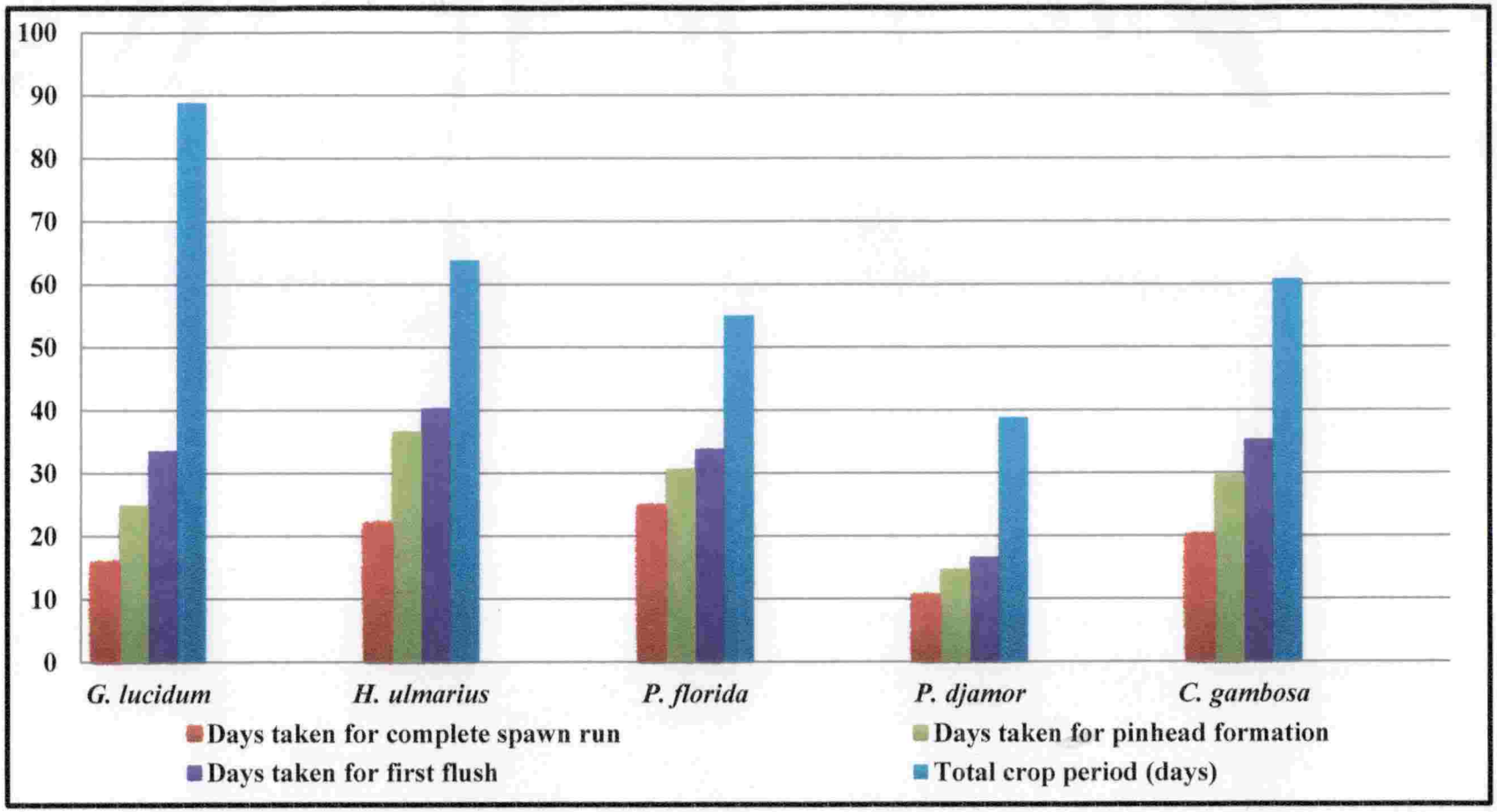


Fig. 2. Duration of growth stages in mushrooms

cultivation of *G. lucidum* on which a biological efficiency ranging between 10 to 15 per cent.

Cultivation of *Pleurotus* spp. on natural habitat like tree stumps and logs was first described at the beginning of twentieth century. The foundation for commercial production of *Pleurotus* on different substrates was laid by Kurtzman, 1979. In India, the polybag technology for the production of oyster mushroom on paddy straw was standardised by Baskaran *et al.* (1978). Oyster mushrooms such as *P. florida*, *P. djamor* and *H. ulmarius* were cultivated on paddy straw. Polybag method was reported as the best method for cultivation of oyster mushroom by Balakrishnan and Nair (1995). Mago *et al.* (2014) and Patidar (2008) reported that maximum biological efficiency of *P. florida* was observed while cultivating on paddy straw substrate. The spawn run was completed in 24.75 days and pinheads were formed on 30.50 days after incubation. The total yield from three harvests recorded a biological efficiency of 84.50 per cent. *P. florida* started developing pinheads six days after complete sawn run (Table 4). The pinheads were white in colour and numerous. On the third day after pinhead formation mature fruit bodies of *P. florida* were observed. The sporocarps appeared white in colour, fleshy and decurrent. The pileus has radiating gills at the lower surface and was laterally attached to the stipe. Dung *et al.* (2012) and Krishnapriya *et al.* (2017) had observed similar morphological characters in *P. florida*. Mondal *et al.* (2010) observed 24.25 days for complete spawn run and Ahmed *et al.* (2009) recorded 83.43 per cent biological efficiency on rice straw substrate. Cultivation trials of *P. florida* conducted by Iqbal *et al.* (2016) on paddy straw recorded similar results indicating 27 days for spawn run and 37 days for pinhead formation while biological efficiency was 123 per cent which is higher than current findings.

In the present study the pink oyster *P. djamor* completed spawn run within 10.50 days and pinheads were formed within 14.50 days. *P. djamor* recorded a biological efficiency of 81.20 per cent. Kathiravan *et al.* (2016) reported the cultivation of *P. eous* and *P. sajor-caju* on paddy straw substrate. According to them, *P. eous* took 10.67 days for spawn run and 12.33 days for pinhead

formation. Chaubey *et al.* (2010) and Chauhan (2013) reported 72 per cent and 74.76 per cent biological efficiency respectively on *P. djamor* cultivated on wheat straw.

The blue oyster mushroom *H. ulmarius* recorded 22 days for complete spawn run and 40.20 days for first harvest in the present study. Compared to *P. florida* the mycelial growth was thin and feeble in *H. ulmarius*. Similar finding was reported by Sumi (2016) with a spawn run period of 22.60 days and first harvesting was done in 48 days. *H. ulmarius* recorded an average of five days from pinhead formation to complete maturity. Pileus of the pinheads formed on beds was dark blue coloured and stipe was short and creamy white in colour. The colour gets faded on maturity. The stipe length and thickness increased gradually up to third day of pinhead formation. On the third day, stipe became bulged at the centre and the length of stipe gets reduced. Sporocarps were produced either singly or as a bunch. The production of sporocarp was greatly influenced by the presence of high humidity and low temperature. This was in accordance with findings of Sumi (2016) who reported that the pileus of *H. ulmarius* was convex shaped with irregular margin and depressed towards the base. Baghel (2017) reported 84.75 per cent biological efficiency of blue oyster mushroom on paddy straw substrate while 96 per cent was recorded in present study. According to Chandravanshi (2007) *H. ulmarius* recorded a biological efficiency of 96.8 per cent and 95.2 per cent during the months of February and October respectively. The above results also support the current findings.

C. indica was cultivated for the first time by Purkayastha and Chandra (1974). Krishnamoorthy *et al.* (1998) and Bokariya *et al.* (2014) reported successful cultivation of milky mushroom on sterilized paddy straw. Unlike other mushrooms *C. gambosa* require casing for higher biological efficiency. In the present trial *C. gambosa* was cultivated on paddy straw substrate and the casing mixture used was 1:1:1 combination of sand, soil and vermi compost with 2 per cent calcium carbonate. *C. gambosa* recorded 20 days for spawn run and primordia started appearing on the mushroom bed after 10 days of spawn run.

Approximately 20 to 30 numbers of pinheads were appeared initially, whereas 5 to 6 sporocarps get matured after five days of primordial initiation. The first harvest was on 35.25 days with 103.72 per cent biological efficiency. *C. gambosa* was characterised with convex shaped pileus of 9 cm to 11cm diameter attached with robust stipe at centre. Purkayastha and Chandra (1974) studied the morphological characters of *C. indica* and reported almost similar characters. Heera (2006) had also reported morphology of milky mushrooms in accordance with current result. Kudada *et al.* (2017) observed a shortest period of 20.50 days for complete mycelial growth of *C. indica* with 105.64 per cent biological efficiency on using jowar grain spawn. Similar results were given by Vijayakumar *et al.* (2014) in which they recorded 17.67 days for spawn run, 32.33 days for pinhead formation and 37.33 days for first harvest with 132.4 per cent biological efficiency.

5.4. PEST AND DISEASE INCIDENCE

The yield and productivity of mushrooms can be adversely affected by pest and diseases. The mushrooms grown under hot and humid climate is more prone to competitor mould diseases. Beside this mushroom are infested with large number of insect pests, mites, millipedes, grubs, nematodes, earthworms and they cause maximum damage of the mycelium and young growing fruiting bodies (Naik, 2015).

Phorid flies, thrips and sciarid flies were the major pests observed during spawn running and sporocarp formation during cultivation of oyster mushrooms such as *P. florida*, *P. djamor* and *H. ulmarius* (Table 5). Deepthi *et al.* (2003) reported severe attack of sciarids and phorids which resulted in decaying of oyster mushroom bed. Pest infestation was noticed during all the stages of mushroom production from spawn run to sporocarp formation. This is similar with the findings of Krishnamoorthy *et al.* (1991) who reported the damage caused by maggots of *Megaselia* sp. on the oyster mushroom beds. Maggots were feeding on mycelia during spawn run and caused further decay by bacteria. According to

Sumi (2016) phorid flies, staphylinid beetles and springtails were the major pest during the cultivation of *H. ulmarius*. Staphylinid grubs were causing damage on developing sporocarps by inhabiting between the gills. This is in accordance with results of Balakrishnan (1994) and Sumi (2016). Lepidopterous larvae have been observed to cause damage by feeding on *G. lucidum* fruitbodies. These are polyphagous in nature and when they arrive by chance on mushroom bed feed on the mushrooms in absence of their preferred hosts. Snails were numerous in *C. gambosa* beds cultivated on paddy straw. Naik (2015) also reported the presence of lepidopterous larvae in paddy straw mushrooms.

Competitor moulds were also adversely affected the spawn run and caused damage of fruiting bodies at various stages of crop growth in mushrooms producing distinct disease symptoms. It may cause complete crop failure depending upon stage of infection, quality of substrate used for cultivation and environmental conditions. High relative humidity inside mushroom production room can help the fungi for its multiplication and spread (Sharma *et al.*, 2007). Fungal contaminants such as *Aspergillus* sp., *Coprinus* sp., *Trichoderma* sp. and *Penicillium* sp. were observed during spawn running and sporocarp formation (Table 6) which suppress the spread of mushroom mycelium and affect the yield. *Trichoderma sp.* was the serious competitor during cultivation causing severe yield loss. This result is compatiable with findings of Sumi (2016) who reported that *Trichoderma* spp., *Aspergillus* sp. and *Coprinus* spp. were the major competitors during cultivation of *H. ulmarius*. Similar findings were given by Doshi *et al.* (1991) and Raman *et al.* (2005). They reported that *Trichoderrma* spp. and *Aspergillus* spp. were the most severe contaminants observed in milky mushroom cultivation. Pest infestation and contamination percentage were comparatively lesser in *G. lucidum* bed which might be attributed to its woody appearance.

Bacterial contamination was observed on the mushroom beds during spawn running stage, which appeared as water soaked areas which later resulted in brown discolouration. A foul smell is also found emitting from infected beds

which ultimately resulted in complete damage. The incidence of bacterial rot on *P. sajor-caju* and *H. ulmarius* as water soaked areas was reported by Biswas *et al.*, 1983; Sumi, 2016.

5.5. ANALYSIS OF NUTRITIONAL COMPONENTS

Some mushrooms are food of good nutritional value, some have medicinal value as dietary supplement and some have both of these properties. In this way mushrooms can be considered as functional food (medicinal food or nutritional food). Thus mushrooms have a role in disease suppression or bridging about suppression or remission of diseased state. Mushroom production has increased remarkably in recent decades with the realization that they are good source of delicious food with nutrient attributes and medicinal values. The sources of energy food are carbohydrates and fats and the sources of body building materials are proteins. In addition, the accessory food factors, vitamins and inorganic compounds together with water are indispensable to good health. The determination of nutritional value involves the analysis of proximate composition and a study of the spectrum of amino acid and fatty acids, vitamins, minerals and nucleic acid present. In the present study five mushrooms were analysed for protein, fat, fibre, carbohydrate, amino acid and medicinal components (Figure 3).

5.5.1. Protein

The protein content of commercially cultivated mushrooms ranged from 1.75 to 3.63 per cent on their fresh weight and the value can be as high as 5.9 per cent. Recently certain mushrooms species are known for a group of fungal immune-modulatory protein (FIPs). These include LZ-8 (Ling Zhi-8) from *G. lucidum*, 'FIP-vvo' from *V. volvaceae* and 'FIP-gts' from *G. tusgae*. The FIPs can suppress autoimmune diabetes and has a significant effect on cellular immunity (Chang and Miles, 2004). In the present study the protein content of *G. lucidum* was estimated as 30.91 per cent which was the highest among the five mushrooms. Tashak *et al.* (2014) reported that protein content of *G. lucidum* varied from18 to 22 per cent. According to Swati *et al.* (2016) and Varma (2013),

the protein content of *G. lucidum* was estimated as 18.22 per cent and 20 per cent respectively.

P. florida contained 22.16 per cent protein on dry weight basis (Table 7) which is in accordance with findings of Khatun *et al.* (2015) who recorded 23.8 per cent protein in *P. florida* and 20.8 per cent protein content in *P. citrinopileatus*. Kinge *et al.* (2016) reported higher protein content of 29.45per cent while cultivating *P. florida* on sawdust substrate. The protein content of *P. djamor* was found to be 21.98 per cent in the current study which is in accordance with results of Khan *et al.* (2013). They recorded 21.03 to 21.89 per cent protein in *P. djamor* cultivated on sawdust of different woods. *H. ulmarius* was recorded with 20.76 per cent protein. Sumi (2016) reported 32 per cent while Chauhan *et al.* (2017) observed 28.67 per cent protein in the dried fruit bodies of blue oyster mushroom.

The milky mushrooms contained higher amount of protein which is estimated as 26 per cent. The crude protein content of *C. indica* varied from 28.87 to 32.06 per cent based on the substrate used (Yadav, 2006). According to Pushpa and Purushothama (2010), protein content of *C. indica* was 21.60 per cent. Heera (2006) found 42 per cent protein in milky mushroom which is a deviation from current study. The protein content of mushrooms varied depending on composition of substrate, size of pileus, harvest time and species of mushrooms (Bano and Rajarathnam, 1988). Hence deviation in the values might be due to one of these reasons. However mushrooms in general have higher protein content as compared with most of the vegetables. Mushrooms contain 19 to 35 per cent proteins on dry weight basis while rice, wheat and soyabean contain 7.3, 12.7 and 38.1 per cent respectively (Bano and Rajarathnam, 1988).

5.5.2. Fat

Mushrooms are known for their low fat contents. The fat content of different species of mushrooms ranges from 1.1 to 8.3 per cent on dry weight basis with an average content of 4 per cent. The fat present in mushroom fruiting

bodies are dominated by unsaturated fatty acids (Thatoi and Singdevsachan, 2014). At least 72 per cent of total fatty acid was found to be unsaturated in each of the mushrooms. Unsaturated fatty acids are essential in our diet and its presence in higher proportion along with high per cent of linoleic acid is a significant factor in regarding mushrooms as a health food. Crude fat content in *G. lucidum* was recorded as 1.33 per cent on dry weight basis (Table 7). This is in accordance with the report given by Mhanda *et al.* (2015). According to them domesticated *G. lucidum* contained 1.9 per cent fat. Fat content in *G. lucidum* was recorded as three to five per cent by Swati *et al.* (2016) and Takshak *et al.* (2014).

In the present study fat content of *P. florida*, *P. djamor* and *H. ulmarius* recorded 2.28, 2.10 and 1.72 per cent fat content respectively. Similar results were given by Zape (2003) who reported the fat content in *H. ulmarius*, *P. florida* and *P. flabellatus* as 3.10, 1.82 and 3.98 per cent respectively. Sumi (2016) recorded fat content in *H. ulmarius* and *P. florida* as 2.96 and 1.55 per cent respectively on paddy straw substrate. Huang *et al.* (1985) reported 1.6 per cent fat content in *P. sajor caju* on dry weight basis.

The fat content of *C. gambosa* on dry weight basis was 2.79 per cent in the current study. Yadav (2006) reported 2.62 per cent fat in *C. indica* cultivated on wheat straw while 2.37 per cent on sorghum stalks. According to Kaur *et al.* (2015) fat content of milky mushroom ranged from 1.24 to 2.46 per cent.

5.5.3. Fibre

The fibre content ranges from 7.4 to 27.6 per cent in *Pleurotus* sp. as compared to 10.4 per cent in *A. bisporus* and 4 to 20 per cent in *V. volvaceae*. Fibre is considered to be an important ingredient in a balanced and healthy diet. Anderson and ward, 1979 reported that feeding diabetic patient with high fibre diets reduces their daily insulin requirement and stabilises blood glucose profile possibly by decreasing the rate of glucose absorption and delaying gastric emptying. In the present study crude fibre content of *G. lucidum* was estimated as 49.33 per cent which was highest among five mushrooms (Table 7 and Figure 3).

Similar result was given by Mhanda *et al.* (2015) in which they reported 45 per cent fibre content in domesticated Namibian *Ganoderma* sp. Usman *et al.* (2012) recorded 30.25 per cent whereas Nile and Park (2014) recorded 35 per cent crude fibre in the dried samples of *G. lucidum*. Deviation from present study was reported by Varma (2013) with 10.76 per cent crude fibre.

The fibre content of *P. florida*, *P. djamor* and *H. ulmarius* were recorded as 24.38, 30.59 and 17.06 per cent respectively. This is in accordance with the results of Bhattachariya *et al.* (2015) who reported 20.53 per cent fibre content in *P. ostreatus*. According to Pushpa and Purushothama (2010) crude fibre content in *P. florida* was 23.18 per cent. The fibre content of *P. florida* varied from 16.92 to 19.78 per cent depending on the substrate used (Paul *et al.*, 2016). *H. ulmarius* was recorded with 17.69 per cent and *P. florida* with 10.49 per cent fibre content (Sumi, 2016). Usha and Suguna (2015) estimated that fibre content of blue oyster mushroom ranged from 17.45 to 19.45 per cent. Among the oyster mushrooms, *P. djamor* recorded the maximum fibre content. This is in agreement with Nile and Park (2014) who recorded 37 per cent fibre in *P. djamor*.

The tropical milky mushroom *C. gambosa* was recorded with 15.56 per cent fibre content on dry weight basis which is the least among five mushrooms studied. The result was in accordance with findings of Heera (2006) in which fibre content ranged from 16.02 to 24.41 per cent. Pushpa and Purushothama (2010) reported 13.20 per cent fibre in *C. indica*. The result of Anju (2013) was found to be 32.4 per cent fibre in *C. indica* which is two times more than the present result the variation may be due to the increased fibre content of the matured sporophores used for analysis.

5.5.4. Carbohydrate

Crisan and Sandes (1978) reported that pentoses, methyl pentoses, hexoses, disaccharides, amino sugars, sugar alcohols and sugar acids are the constituents of mushroom carbohydrates. *Pleurotus* spp. contains carbohydrate ranging from 46.6 to 81.8 per cent whereas *A. bisporus* contain 60 per cent on dry

weight basis. Water-soluble polysaccharide components obtained from mushroom fruiting bodies have the ability to inhibit tumour growth. The carbohydrate content of *G. lucidum* was estimated as 39.19 per cent on dry weight basis. Similar findings were given by Usman *et al.* (2012) who recorded 33.13 per cent carbohydrate. Takshak *et al.* (2014) reported that the carbohydrate content of *G. lucidum* ranged from 28 to 54 per cent. Carbohydrate content in *G. lucidum* was estimated as 82.47 per cent by Sharif *et al.* (2016) which is contradictory to present findings. Manikandan (2011) reported that total carbohydrate content varies from 26 to 82per cent on dry weight basis in different mushrooms.

According to Menaga *et al.* (2012) *P. florida* contain 26.60 per cent carbohydrate on dry weight basis. This is in accordance with current study in which 26.68 per cent carbohydrate was recorded in *P. florida* and 26.59 per cent in *P. djamor*. In contrary to this Pushpa and Purushothama (2010) recorded 32.08 per cent and Ahmed *et al.* (2015) reported 55.50 per cent carbohydrate content in *P. florida*. In the present study, carbohydrate content of *H. ulmarius* was recorded as 30.87 per cent. Similar results were given by Sumi (2016) with 29 per cent carbohydrate in blue oyster mushroom. The carbohydrate content in *H. ulmarius* ranged from 28 to 34 per cent (Usha and Suguna, 2015).

C. gambosa recorded 46.17 per cent carbohydrate which was recorded as the highest among the five mushrooms analysed. According to Yadav (2006), the carbohydrate content in milky mushroom ranged from 47.69 to 57.18 per cent depending on the substrate used for cultivation. Pushpa and Purushothama (2010) had also reported that milky mushroom contain 49.20 per cent carbohydrate on dry weight basis. These results supported current findings.

5.5.5. Moisture Content

Moisture content is a quality factor of food products and of great significance in the stability and quality of foods. Generally moisture contents of the mushroom are high, indicating that mushrooms are highly perishable and susceptible to microbial growth. Moisture content in the fresh samples of *G.*

lucidum was estimated as 66.95 per cent in the current study (Table 7 and Figure3). The results were almost similar with finding of Varma (2013) who recorded 58.4 per cent and 53.4 per cent moisture content in two different isolates of *G. lucidum*. The moisture content of wild *Ganoderma* samples ranged from 74.69 to 79.93 per cent (Takshak *et al.*, 2014).

In the present study, moisture content of *P. florida* and *P. djamor* were 84.92 per cent and 88.38 per cent respectively. This is in accordance with the results of Menaga *et al.* (2012) who recorded moisture content of *P. florida* as 87.30 per cent. Similar result was given by Kinge *et al.* (2016) with 86 per cent moisture content in *P. florida* grown on sawdust substrate. Randive (2012) recorded that the moisture content of pink oyster mushroom ranged from 86.11 to 92 %. The moisture content in fresh samples of *H. ulmarius* was estimated as 89.28 per cent. Similar results were recorded by Sikha *et al.* (2012) with 91.5 % and Chauhan *et al.* (2017) with 92.63 % moisture content. The findings of Sumi, 2016; Usha and Suguna, 2015 were also supporting the current study.

The moisture content of *C. indica* was estimated as 91.10 per cent in the current study, which is in accordance with previous studies of Heera (2006) who reported moisture content of milky mushrooms ranged from 80.9 to 89.64 per cent. According to Yadav (2006) moisture content of *C. indica* ranged from 90.70 to 91.07 per cent.

5.5.6. Amino Acid

Mushrooms are very useful for vegetarians because they contain some essential amino acids which are found in animal proteins. Mushrooms contain all the essential amino acids required for an adult. Gupta and Sing (1991) reported 41.40 per cent essential amino acids in *P. pistillaris*. Estimation of crude protein is an indirect assay for total amino acids because proteins are made up from over 20 amino acids in varying amount. Food products of animal origin provide a better balanced protein than that of plant origin, because it lacks some of the important amino acids. The proteins of commercially cultivating mushrooms contain all the

nine essential amino acids needed for human beings. Among the amino acids common in mushrooms, lysine is the most abundant one while tryptophan and methionine are in least quantity (Chang and Miles, 2004).

According to Rana *et al.* (2015) total free amino acid content in dried samples of *A. bisporus* and *P. pulmonarius* were 301.20 mg g^{-1} (30.12 %) and 255.20 mg g^{-1} (25.50 %) respectively. They also reported that all the nutraceuticals and essential amino acids present in the mushrooms were retained in the samples even after drying. Chirinang and Intarapichet (2009) recorded 211.1mg g^{-1} (21.10 %) and 201.2 mg g^{-1} (20.12 %) total amino acid content in dried samples of *P. ostreatus* and *P. sajor-caju* respectively. Contradictorily, in the present study the amino acid contents of *P. djamor* (17.10 %), *P. florida* (14.75 %) and *H. ulmarius* (10.62%) were less. However *C. gambosa* and *G. lucidum* recorded higher (23.9 % and 19.6 %) amino acid content on dry weight basis (Table 7 and Figure 3). Anju (2013) reported that milky mushroom contain all the essential amino acid and the content of isoleucine and threonine were exceptionally high compared to reference protein. The deviation in values may be due to climatic variation during cultivation as amino acid content is an environment dependent factor (Sharma *et al.* 2012).

5.5.7. Ash content

The ash content is the residue remaining when all the moisture has been removed and organic matter has been burnt away. Total ash content is a useful parameter of the nutritional value of foods and feeds. In the present study, ash content of *G. lucidum* was estimated as 10 per cent on dry weight basis. Takshak *et al.* (2014) reported that ash content of *Ganoderma* sp. ranged from 3.66 to 9.70 per cent while Mhanda *et al.* (2015) and Sharif *et al.* (2016) recorded 2.60 % and 2.01 % ash content respectively in *G. lucidum.*

The ash content of *P. florida* was recorded as 9.10 per cent and that of *P. djamor* was 9 per cent. Similar results were given by Pushpa and Purushothama (2010) who recorded the ash content of *P. florida* as 9.41 per cent. Bhattachariya

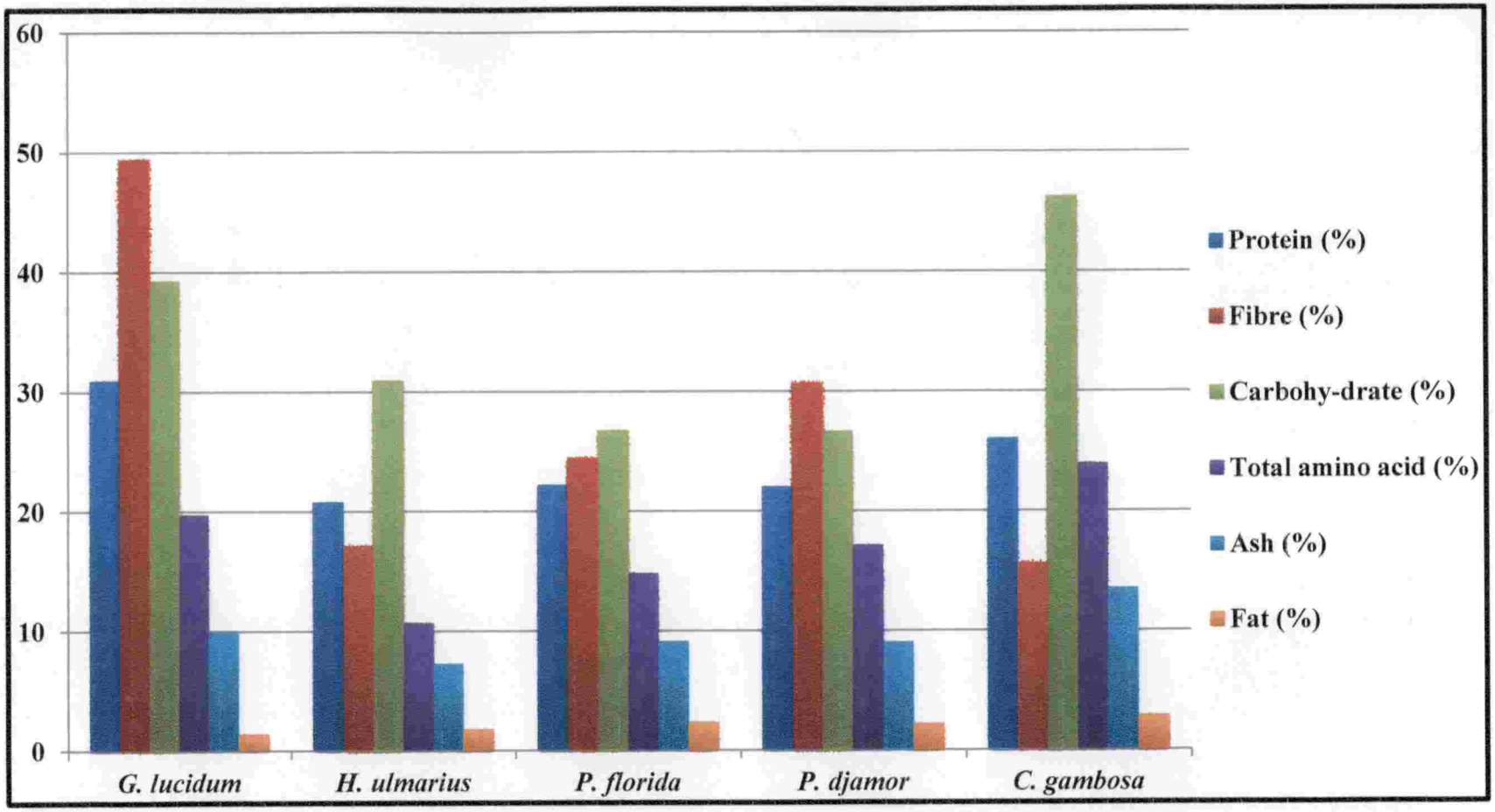


Fig. 3. Per cent composition of nutritional components in five mushrooms

et al. (2015) reported that the ash content of *P. ostreatus* ranged from 8.5 to 13 per cent depending upon the substrate used for cultivation. *H. ulmarius* was recorded with 7.25 per cent ash content which is less as compared with other oyster mushrooms (Table 7 and Figure 3). This is in accordance with results of Shikha *et al.* (2012) who reported the ash content of blue oyster mushroom as 7.1 per cent. Sumi (2016) reported 8 per cent ash content in *H. ulmarius*. Deviation from the result was observed by Usha and Suguna (2015) who recorded the ash content of two strains of oyster mushrooms as 5.66 % and 4.30 %.

C. gambosa contained 13.55 per cent ash on dried sample which is almost similar with the findings of Bhavana and Thomas (2002) with 16.60 per cent ash content in *C. indica*. Doshi *et al.* (1988) reported 7.43 per cent and Yadhav (2006) reported 9.03 per cent ash content in milky mushroom sample which is contradictory to current result.

5.5.8. Minerals

Mushroom is an important source of minerals such as sodium, potassium, phosphorus, calcium and magnesium. These minerals were removed by mushroom mycelium from the substrate used for cultivation, being supplied during growth of mycelium and translocated to the fruiting body during its formation process (Chang and Miles, 1989). The mineral composition varied in different mushroom species. Environmental conditions and nutrient availability were some other factors which influence the mineral composition of mushroom (Kathiravan and Krishnakumari, 2017). As in higher plants, mushrooms contain potassium in higher amount followed by phosphorous.

5.5.8.1. Sodium

Mushrooms are generally low in sodium concentration. The low sodium and high potassium concentration is of great significance because low Na to K ratio (<0.6) suggests that mushrooms are suitable for healthy diet. Sodium and potassium are important in the maintenance of cell osmotic and the interstitial

fluid balance in animal systems (Nieman *et al.*1992). In the current study *G lucidum* recorded 0.020 per cent sodium content on dry weight basis which was the least as compared to oyster and milky mushrooms (Table 8 and Figure 4). The result was compatible with results of Sharif *et al.* (2016) who reported sodium content of *G. lucidum* as 0.020 per cent on dry weight basis.

Sodium content of *H. ulmarius* and *P. florida* were recorded as 0.024 and 0.028 per cent respectively which is in accordance with results of Muthu and Shanmugasundaram (2016) who recorded 0.029 per cent sodium in the powdered sample in *Agrocybe aegerita*. *P. djamor* recorded higher sodium content of 0.096 per cent in the current study which is almost similar with Sodium content of in *H. ulmarius* (0.077%) reported by Chauhan *et al.* (2017). *C. gambosa* recorded 0.029 per cent sodium in the present study as against the report of Anju (2013) who recorded the sodium content of milky mushroom treated with different processing techniques as 65, 37.45, 46.24 and 285.32 mg respectively on boiling, steaming, frying and drying the mushroom sample.

5.5.8.2 Phosphorous

Phosphorus containing compounds have important roles in cell structure such as maintenance of cell membrane integrity and nucleic acids synthesis. Phosphorus is required for generation of ATP, regulation of subcellular processes and maintenance of acid-base homeostasis. Phosphorus plays a major role in the mineralization of bone and teeth (Alizadeh and Reilly, 2010). The phosphorus content of *G. lucidum* was estimated as 0.203 per cent (Table 8 and Figure 4) while the research finding of Sharif *et al.* (2016) showed slight variation from who reported 0.502 per phosphorous in *G. lucidum*.

The phosphorus and potassium are the main constituents of ash of *Pleurotus* spp. and the phosphorus content ranges 0.76 per cent in *P. sajor caju* to 1.85 per cent in *P. florida*. In the present study phosphorous content of *H. ulmarius*, *P. florida* and *P. djamor* were recorded as, 0.194, 0.225 and 0.210 per cent respectively. Among the five mushrooms *P. florida* recorded the maximum

phosphorus content on dry weight basis which was similar to those reported by Chang and Miles (1989) and Bano and Rajarathnam (1982). Mallikarjuna *et al.* (2013) estimated the phosphorus content of *P. florida* and *P. djamor* as 0.640 per cent and 0.743 per cent respectively. According to Sumi (2016) *H. ulmarius* and *P. florida* recorded 0.69 per cent and 0.68 per cent respectively. These results showed deviation from the present study. The reason for deviation in values might be attributed with variation in mineral uptake by the mushrooms from the substrate at different developmental stages.

The phosphorus content of *C. gambosa* was recorded as 0.178 per cent which is in accordance with that of Kamugisha and Sharan (2005) who reported that *C. indica* cultivated on paddy straw contain 0.163 per cent phosphorus. Similar results were given by Gaur *et al.* (2016) who studied the phosphorus content of five different mushrooms such as *A. bisporus* (0.345 %), *C. indica* (0.056 %), *L. edodes* (0.465 %), *P. sajor-caju* (0.412 %) and *Macrocybe gigantea* (0.601 %).

5.5.8.3. Potassium

Potassium is the most abundant mineral element in various species of edible mushrooms. Potassium play a significant role as an essential mineral which helps to maintain normal heart rhythm, fluid balance, muscle and nerve functions (Muthu and Shanmugasundaram, 2016). The potassium content ranges from 1.246 per cent in *L. edodes* to 4.762 per cent in *A. compestris*.

In the present trial potassium content of *G. lucidum* was estimated as 0.760 per cent which is in accordance with the findings of Sharif *et al.* (2016) who reported 0.742 per cent potassium in dried *G. lucidum*. The potassium content is high in oyster mushroom species. Bano and Rajarathnam (1982) reported 3.26 % potassium in *P. sajor caju*, 4.57 % in *P. eous* and 4.66 % in *P. florida*. In the present study potassium content of *H. ulmarius* was estimated as 0.70 per cent. The potassium content of *P. florida*, *P. djamor* and *C. gambosa* were estimated as 0.390, 0.720 and 1.44 per cent respectively (Table 7 and Figure 4). Sumi (2016)

recorded 1.98 per cent phosphorus in *H. ulmarius* and 2.45 per cent in *P. florida*. Almost similar result was given by Anju (2013) who reported potassium content of milky mushroom as 0.415 per cent.

5.5.8.4. Calcium

Calcium plays a major role in the circulatory system critical for mediating vascular contraction and vasodilatation. The muscle function, nerve transmission, intracellular signalling and hormonal secretion in the tissue were regulated by calcium. Bone tissue serves as a reservoir for and source of calcium for these critical metabolic needs through the process of bone remodelling (IOM, 2011).

In the present study *G. lucidum* was recorded with 0.130 per cent calcium content which is compatible with result given by Sherif *et al.* (2016) who recorded calcium content of *G. lucidum* as 0.109 per cent which was higher in quantity as compared to *P. ostreatus*, *V. volvaceae* and *L. edodes*. Calcium content of *A. aegerita* was recorded as 0.027 per cent by Muthu and Shanmugasundaram (2016). The calcium content of *H. ulmarius*, *P. djamor* and *C. gambosa* were estimated as 0.080 per cent while that of *P. florida* was 0.100 per cent (Table 8 and Figure 4). Similar result was given by Gaur *et al.* (2016) who recorded 0.068 per cent calcium content in *L. edodes*.

5.5.8.5. Magnesium

Magnesium acts as an important co-factor for certain enzymes in numerous biochemical pathways and help to maintain functions of nerves and muscles. Besides these, magnesium is an important mineral that supports healthy immune system and keep bones strong. Mushrooms are wonderful source of magnesium.

Magnesium content in *G. lucidum* was recorded as 0.090 per cent in the current study which is in agreement with results of Sharif *et al.* (2016) who recoded 0.089 per cent magnesium. Kathiravan and Krishnakuumari (2017) reported magnesium content of mushroom species such as *H. ulmarius* and *P.*

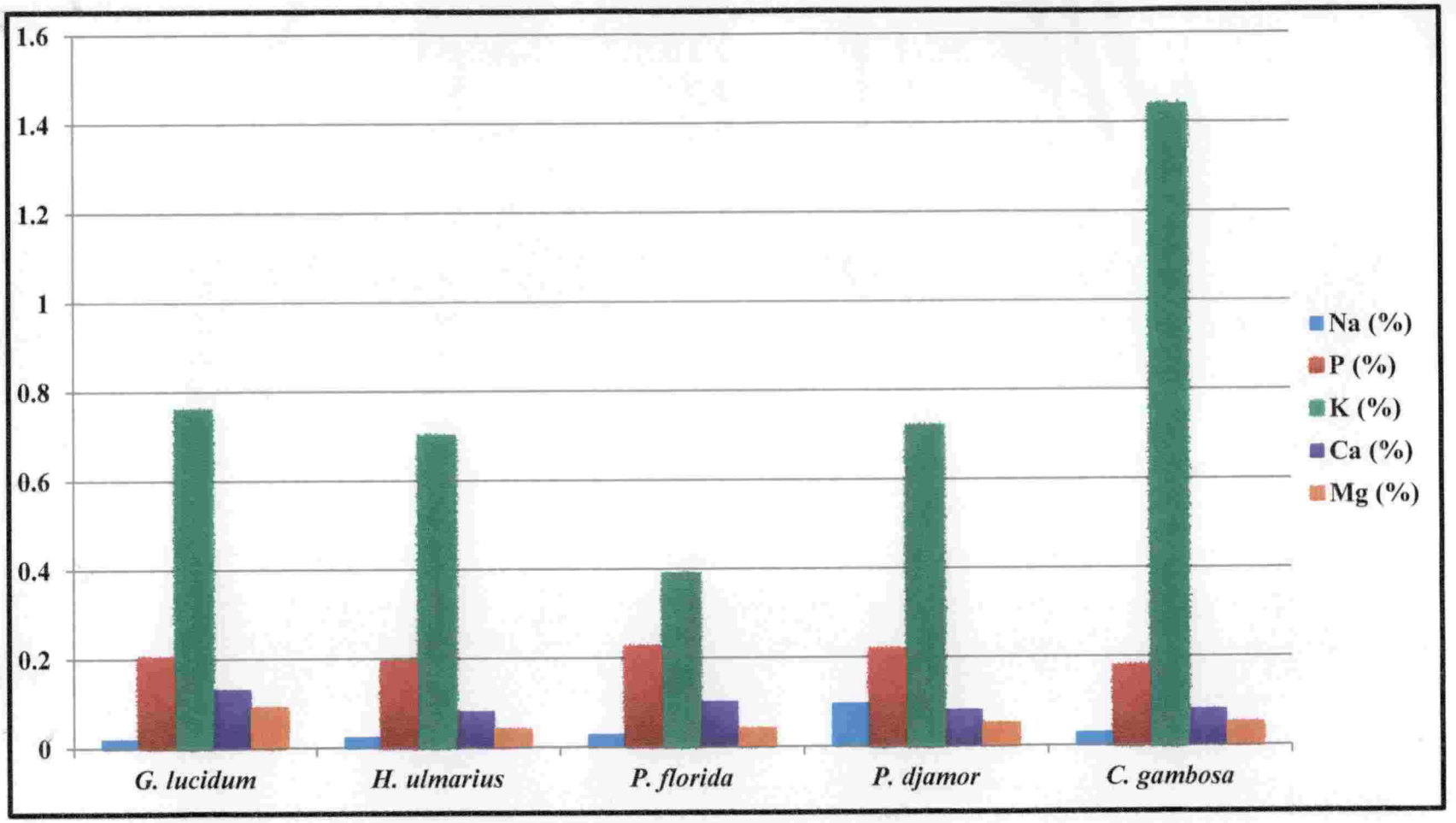


Fig. 4. Per cent composition of mineral components in five mushrooms

eous as 0.019 and 0.064 per cent respectively. This is almost in accordance with present study in which magnesium content of *H. ulmarius* and *P. florida* were estimated as 0.040 per cent while that *P. djamor* and *C. gambosa* were 0.050 per cent (Table 8 and Figure 4). According to Bhattachariya *et al.* (2015) the magnesium content of *P. ostreaus* ranged from 0.133 to 0.198 per cent depending on the changes in substrate combination whereas Muthu and Shanmugasundaram (2016) recorded 0.009 per cent magnesium content in *A. aegerita*. The variation in value may be due to difference in cultivar or environmental factors.

5.6. ANALYSIS OF MEDICINAL COMPONENTS

Mushrooms have potential medicinal value and many bioactive immunomodulation components have been isolated recently from mushrooms. These include polysaccharides, glycoproteins, terpenoid and immune-modulatory proteins, which are effective in retarding the progress of cancer and other diseases through immune stimulation. Another important advantage of mushroom extract over other drugs is the low toxicity on regular consumption (Chang and Miles, 2004).

5.6.1. Beta-Glucan

Beta-glucans are polysaccharides which represent fundamental building blocks in fungi since their cell walls are composed of polymers namely chitin and beta-glucan. It is known for immunomodulation activity mainly dependent on single helix glucan structure capable to interact with immunoglobulin present in blood serum. The mechanism behind antitumor activities of beta-glucan include stimulation of hematopoietic stem cells, activation of the alternative complement pathway and activation of immune cells such as lymphocytes, macrophages, T cells and B cells (Popovic *et al.* 2013)

G. lucidum recoded maximum beta-glucan content of 38.58 per cent on dry weight basis (Table 9 and Figure 5). Similar result was given by McCleary and Draga (2016) who recorded the beta-glucan content of *G.lucidum, L. edodes,*

P. ostratus and *A. bisporus* as 54, 23.50, 32.30 and 6 per cent respectively. They also reported that *G. lucidum* as the highest source of beta-glucan. The beta-glucan content of *H. ulmarius*, *P. florida*, *P. djamor* and *C. gambosa* were estimated as 26.62, 20.80, 32.15 and 35.64 per cent respectively. This was in agreement with findings of Sari *et al.* (2017) who estimated the beta-glucan content of *P. djamor* (20.70 %), *P. citrinopileatus* (15.54 %) and *P. pulminarius* (17.46 %). Avni *et al.* (2017) reported that beta-glucan content of various *Pleurotus* sp. ranged between 17.09 per cent and 48.90 per cent.

5.6.2. Glycoprotein

A few mushroom glycoproteins have been reported to be inhibitory against cancer cells including the glycoprotein fraction from *Flammulina velutipes* (Ko *et al*, 1995). Only limited studies are available regarding the analysis and medicinal qualities of glycoprotein.

In the present study presence of glycoprotein content in all the five mushrooms including *G. lucidum, P. florida, P. djamor, H. ulmarius* and *C. gambosa* were observed qualitatively (Table 9). This is in agreement with previous studies conducted by Tanaka *et al.* (1989) who reported presence of glycoprotein in *G. lucidum*. Tsai (2013) reported that non-lectin glycoprotein present in *H. marmoreus* have inhibitory effect against leukemic cells. Similar results were given by Hsu *et al.* (1997) and Liu *et al.* (2004) who confirmed the presence of glycoprotein in *V. volvacea* and *Xerocomus spadiceus* respectively.

5.6.3. Terpenoid

Triterpene compounds from *G. lucidum* such as ganodermic acids A and B were first isolated by Kubota *et al.* (1985). These compounds can be isolated from fruit bodies, spores as well as mycelia. Triterpenes act as an important intermediate in the biosynthetic pathway of steroids. These have cytotoxic, hepatoprotective and hypolipidemic properties (Chang and Miles, 2004)

In the present study terpenoid content of *G. lucidum* was estimated as 11.14 mg g^{-1} which is contradictory to previous reference. Taofiq *et al.* (2017) recorded the terpenoid content of *G. lucidum* as 27.2 mg g^{-1} on dry weight basis whereas terpenoid content of 0.009 per cent was determined in APK-2 variety of *C. indica* by Sumathy *et al.* (2015). Triterpenes extracted from *G. lucidum* have anti-oxidative properties *in vitro* and can reduce oxidative damage by scavenging free radicals generated in the cell (Smina *et al.*, 2011). Terpenoid content of *P. florida, P. djamor, H. ulmarius* and *C. gambosa* were estimated as 9.22, 9.78, 10.13 and 12.41 mg g^{-1} respectively (Table 9 and Figure 5). Menaga *et al.* (2012) and Kinge *et al.* (2016) were also qualitatively confirmed the presence of terpenoid in *P. florida*. The qualitative estimation of terpenoid content in *P. ostreatus* was done by Hamzah *et al.* (2014) and Parihar *et al.* (2015).

5.6.4. Polyphenol

Polyphenol compounds are large group of secondary metabolites with a wide range of medicinal properties. Among the anti-oxidant compounds, polyphenols are of more importance due to the free radical scavenging, metal chelation and enzyme modulation activities. Anti-bacterial and anti-inflammatory activities of mushrooms were attributed with polyphenol content in it (Li *et al.*, 2012).

In the present study, phenolic content in *G. lucidum* was estimated as 23.80 mg gallic acid equivalent (GAE) g^{-1} which is the highest among five mushrooms followed by *C. gambosa* which recorded 22.46 mg GAE g^{-1}(Table 9 and Figure 5). Comparatively low polyphenol content was recorded by *H. ulmarius, P. florida* and *P. djamor* (20.143, 19.14 and 14.56 mg GAE g^{-1}) content respectively. The findings of Rawat *et al.* (2013) and Rajoriya *et al.* (2015) were contradictory to the current study in which polyphenol content in powdered *G. lucidum* was very low (9.24 and 9.00 mg g^{-1}) respectively. The total polyphenol content in the ethanolic extract of *P. sajor caju* was recorded as 29.30 mg GAE g^{-1} of dried mushroom sample (Chirinang and Intrapichet, 2009). According to

Sasidhara and Thirunalasundari (2014) phenolic content of *P. djamor* was 32.55 mg g^{-1}. Phenolic content of *P. eous* was recorded 14.03 mg g^{-1} by Boonsong *et al.* (2016).

5.6.5. Beta-carotene

Beta-carotene, the precursor of vitamin A is one of the major compound contributing anti-oxidant properties of mushroom. These act as principle compound in biosynthesis of several molecules (Ullah *et al.*, 2011).

In the present study beta-carotene content of *G. lucidum* was recorded as 246.46 µg g^{-1} (Table 9). This is almost similar to the findings of Achariya *et al.* (2015) they reported 106 µg g^{-1} of beta-carotene in *G. lucidum*. Maximum beta-carotene content was recorded in *P. djamor* (355.04 µg g^{-1}). This may be due to the pink coloured sporocarps which contain more carotenoid pigments. *H. ulmarius*, *P. florida* and *C. gambosa* recorded with 195.58, 125.24 and 187.68 µg g^{-1} of beta-carotene in dried mushroom sample. According to Acharya *et al.* (2016) beta-carotene content of *C. indica* was 234 µg g^{-1}which is compatible with current findings. Rajoriya *et al.* (2015) analysed the beta-carotene content and estimated 3630 µg g^{-1} of *G. lucidum*. The beta-carotene content of *P. florida* was recorded as 18 µg g^{-1} (Rajoriya *et al.* 2014). These results were contradictory to the current results.

5.6.6. Flavonoid

Flavonoids are wide group of phenolic compounds commonly found in plants and mushrooms. The total flavonoid content of mushrooms ranges from 0.4 to 17 mg g^{-1} of dried matter. The quantity can be varying depending on the mushroom species, genetic and environmental factors. These are medicinally important with amelioration of age-related diseases and of anti-oxidant properties (Villares, 2012).

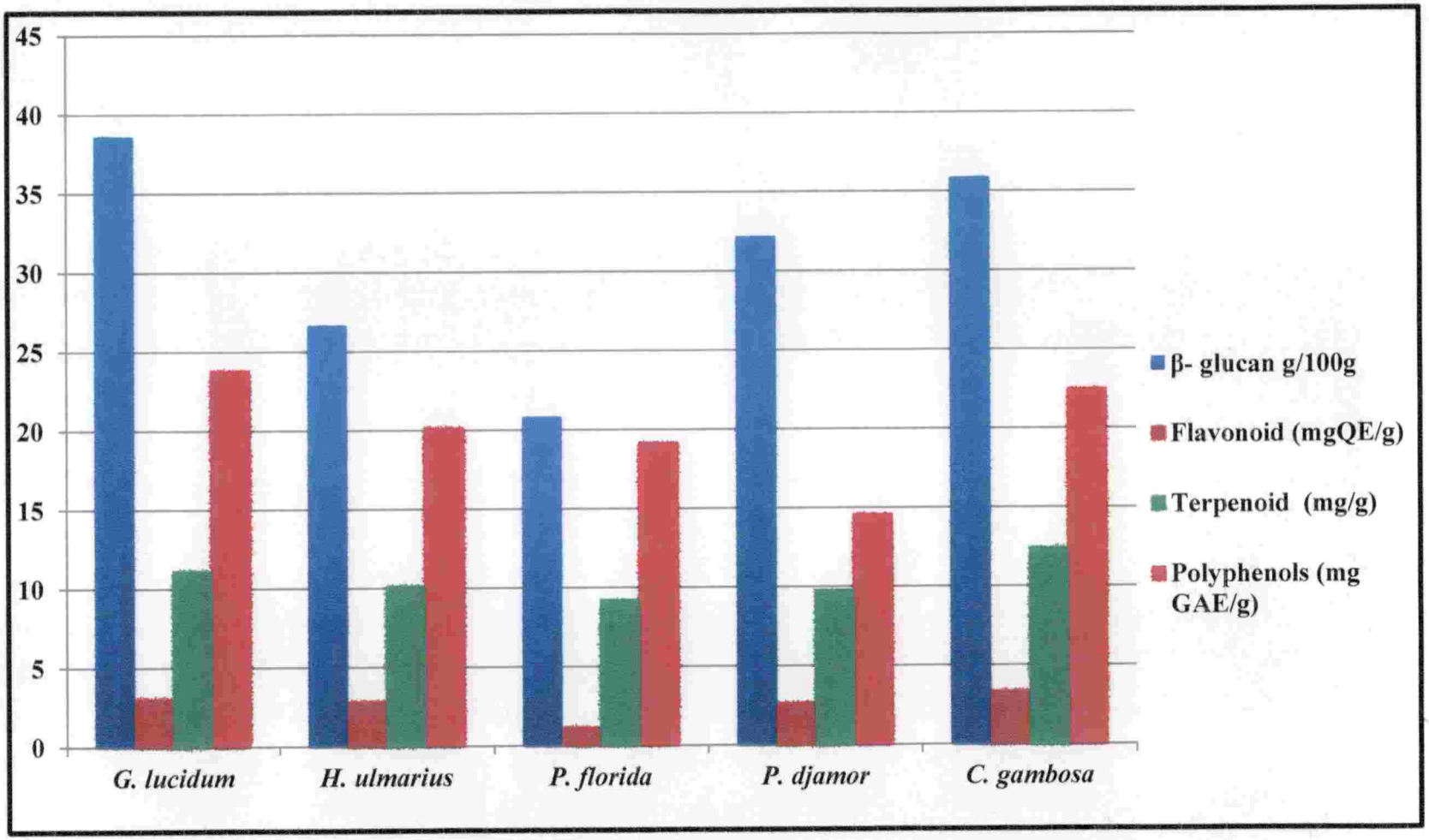


Fig. 5. Composition of medicinal ingredients in five mushrooms

In the present study *G. lucidum* was recorded with 3.02 mg QE g^{-1} of flavonoid which is in accordance with Rawat *et al.* (2013) who reported the flavonoid content in the methanolic extract of *G. lucidum* as 2.14 mg QE g^{-1}. Achariya *et al.* (2015) recorded 4.85 mg g^{-1} of flavonoid in *G. lucidum*. The flavonoid content of oyster mushrooms such as *P. florida*, *P. djamor* and *H. ulmarius* were estimated as 1.14, 2.64 and 2.83 mg g^{-1} of dried sample (Table 9 and Figure 5). Similar results were given by Boonsong *et al.* (2016) they estimated the flavonoid content of five mushroom extracts varied from 1.06 to 9.05 mg QE g^{-1}. *C. gambosa* recorded 3.3 mg g^{-1} of flavonoid, while Sumathy *et al.* (2015) reported 1.06 mg QE g^{-1} in APK-2 variety of *C. indica* which is contradictory to current result.

5.7. DPPH RADICAL SCAVENGING PROPERTY

The ability of mushroom derived preparations to prevent oxidative damage of cellular DNA is considered as anti-oxidant properties. The anti-oxidant components present in mushrooms are polyphenol, flavonoid, terpenoid, beta-carotene etc. which can prevent oxidative damage related to aging and diseases such as atherosclerosis, diabetes, cancer and cirrhosis (Chang and Miles, 2004).

In the present study *G. lucidum* extract exhibited 50 per cent inhibition of DPPH free radicals at 740 µg ml^{-1} concentration (Table 10 and Figure 6). Rawat *et al.* (2013) reported 50 per cent inhibition of free radicals at 1162 µg ml^{-1} concentration of *G. lucidum* extract. Rajasekaran and Kalaimagal (2011) recorded maximum per cent inhibition (72.24 %) at 250 mg ml^{-1} concentration of *G. lucidum* extract. The extracts of oyster mushrooms (*P. florida*, *P. djamor* and *H. ulmarius*) and milky mushroom (*C. gambosa*) even at higher concentration did not reached fifty per cent inhibition of free radicals in the current study. The result does not match with findings of Sasidhara and Thirunalasundari (2014) they reported IC_{50} value of 64.72 µg ml^{-1}. Wandati *et al.* (2013) also observed radical scavenging activity of oyster mushroom at 61.86 mg ml^{-1} concentration. Rajoriya

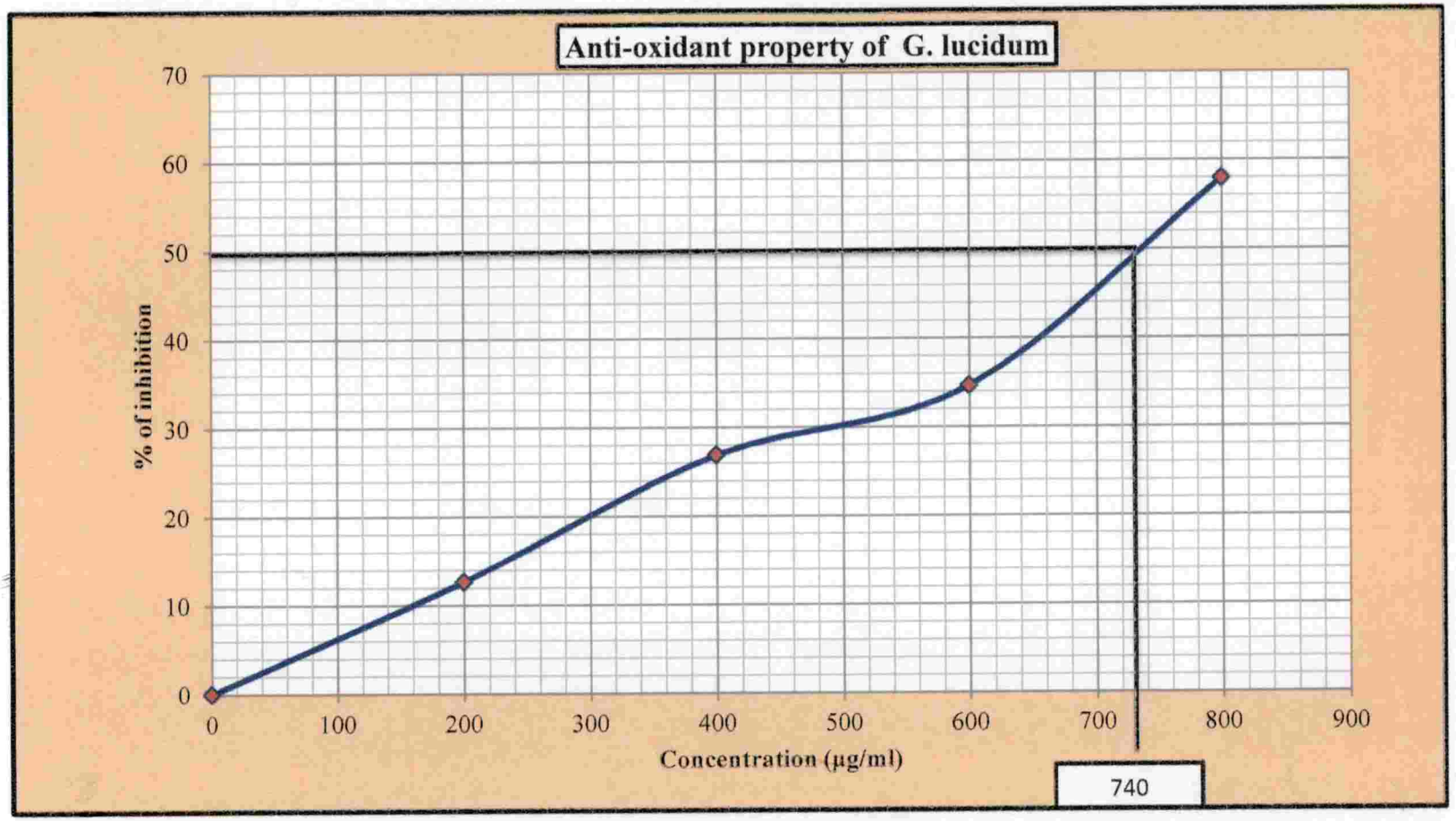


Fig. 6. Antioxidant analysis of *G. lucidum*

et al. (2014) and Boonsong *et al.* (2016) were also reported the anti-oxidant properties of *P. sajor caju*.

5.8. ANTI-CANCEROUS ACTIVITIES

Antitumor activity of polysaccharides derived from mushrooms has been extensively studied during last decades. A variety of polysaccharides from mushroom varieties have been reported to enhance the immune system. These polysaccharides are mainly concentrated in the fruit bodies, mycelia and culture broth of mushrooms. The main sources of antitumor polysaccharides are cell wall components such as beta-glucan, chitin and cellulose (Chang and Miles, 2004). All of these have shown significant antitumor activity as a result of their ability to activate the host immune system rather than direct cytotoxicity. The mushroom polysaccharides appear to be well tolerated and compatible with chemotherapy and radiation therapy (Daba and Ezeronye, 2003).

In the present study, the anti-cancerous activities of ethanolic extract of *G. lucidum, P. florida, P. djamor, H. ulmarius* and *C. gambosa* were assessed against cervical cancer cell lines (HeLa) *in vitro* by MTT assay (Table 16 and Figure 12). The *G. lucidum* extract at 200 µg ml^{-1} concentration reduced the viability of cancer cells to 54.85 per cent in the current study which was the maximum inhibition of cancerous cells as compared with other mushrooms under study (Table 11 and Figure 7). *C. gambosa* extract at 200 µg ml^{-1} decreased the viability of cancerous cells to 60.92 per cent (Table 12 and Figure 11). Anti-cancerous properties of *G. lucidum* were reported by Kao *et al.* (2013) who observed 50 per cent inhibition of low-grade bladder cancer cell line at 129.3 µg ml^{-1} concentration. Patel and Goyal (2011) and Gao and Zhou (2016) also reported the cancer preventive properties of *G. lucidum* extract. Studies demonstrated that *G. lucidum* extracts interfere with cell cycle progression, induce apoptosis and suppress angiogenesis in human cancer cells thereby act as anticancer agents (Hu *et al.*, 2002). Sumathy *et al.* (2015) studied the anti-proliferative property of *C. indica* extract on breast cancer cell lines.

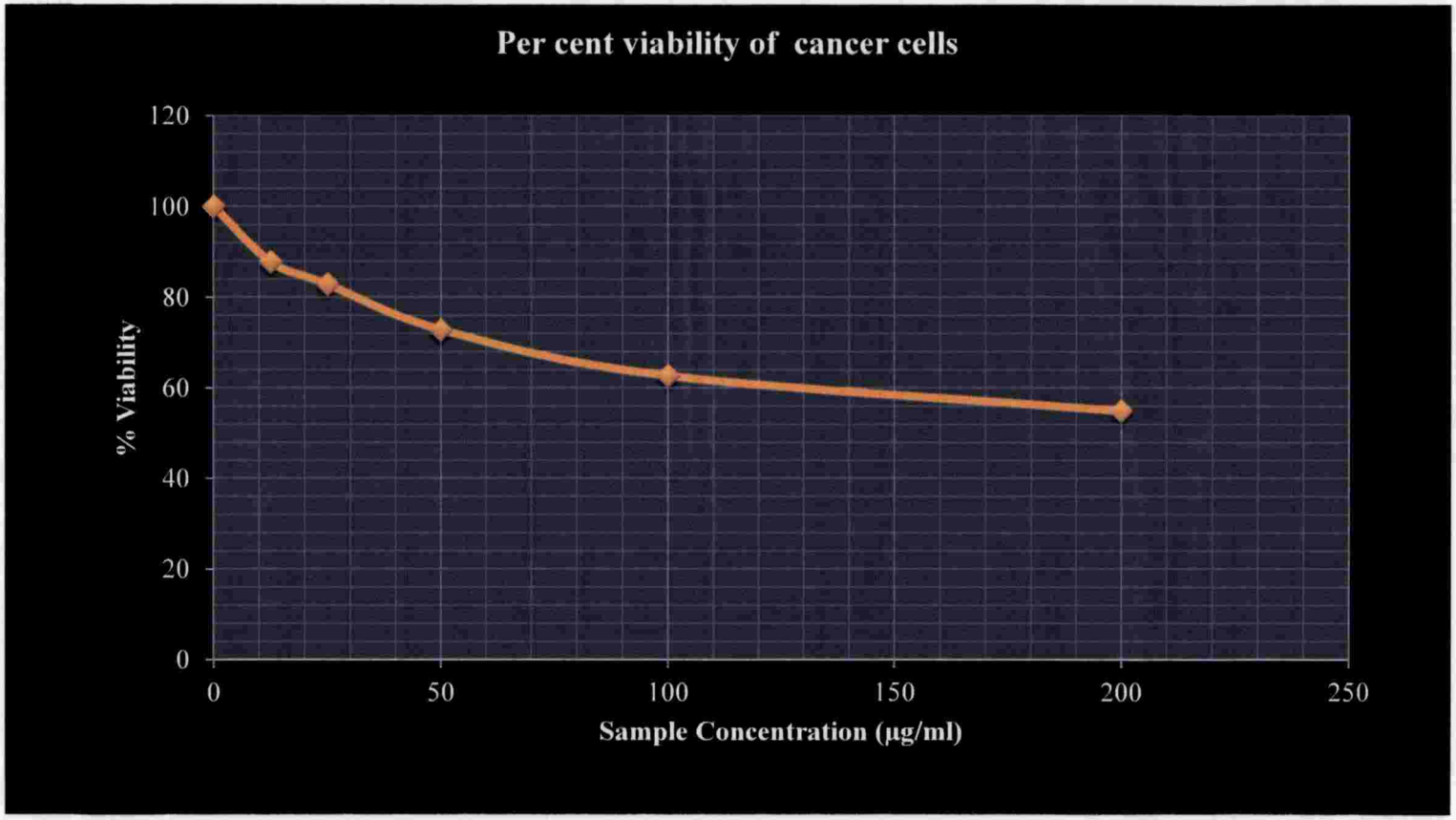


Fig. 7. Cytotoxic effect of *G. lucidum* extract on HeLa cell line

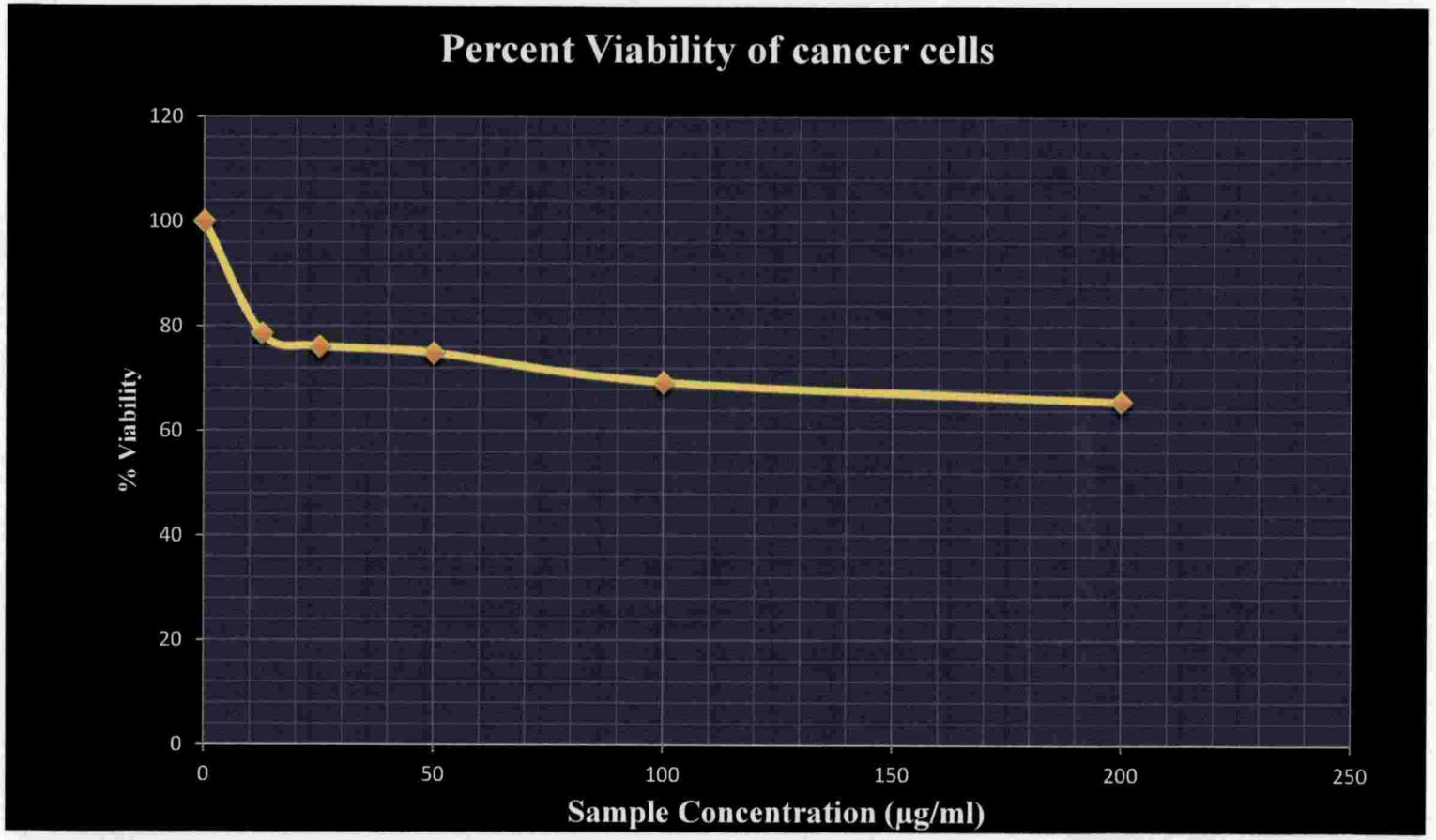


Fig. 8. Cytotoxic effect of *P. florida* extract on HeLa cell line

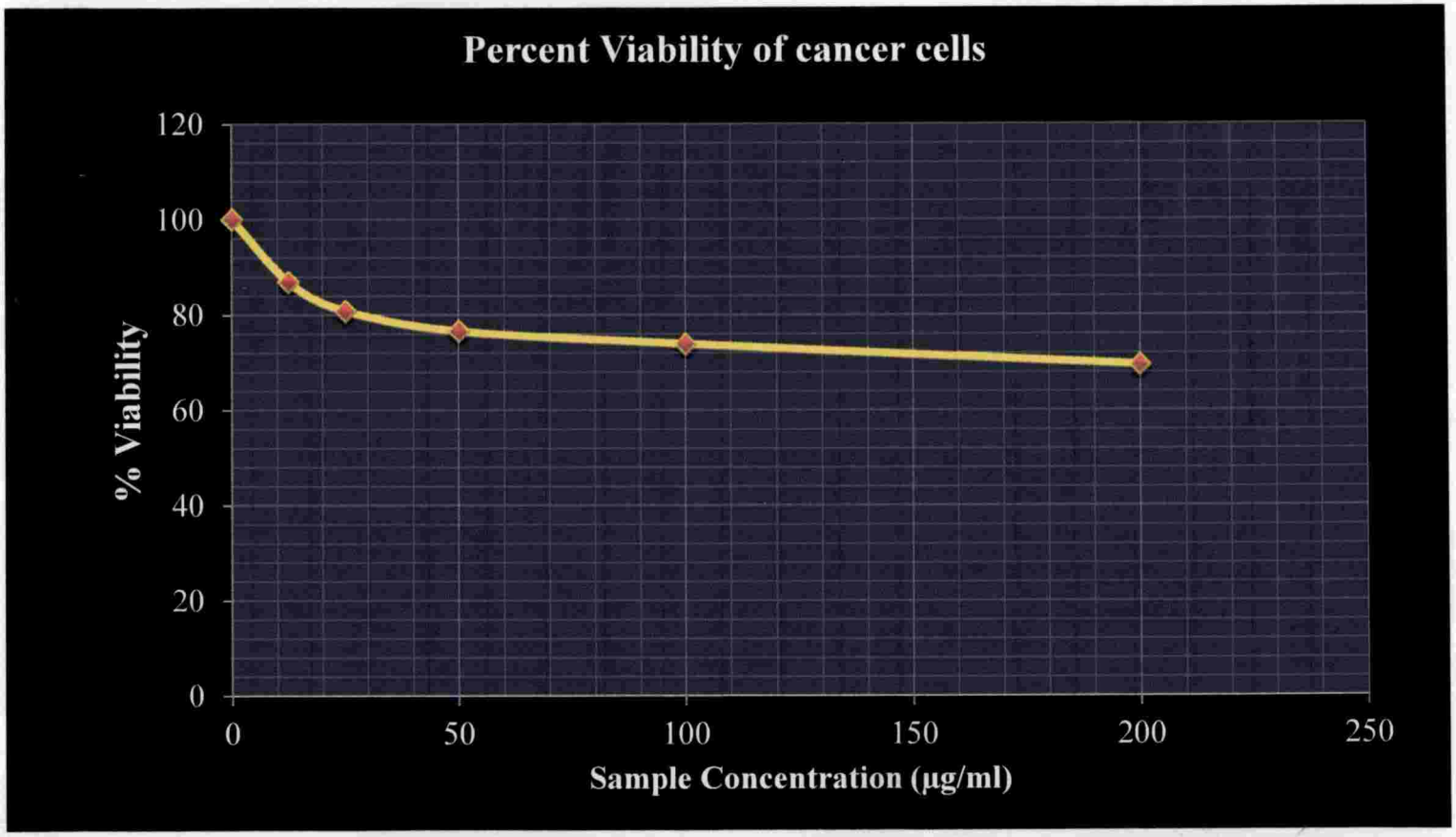


Fig. 9. Cytotoxic effect of *P. djamor* extract on HeLa cell line

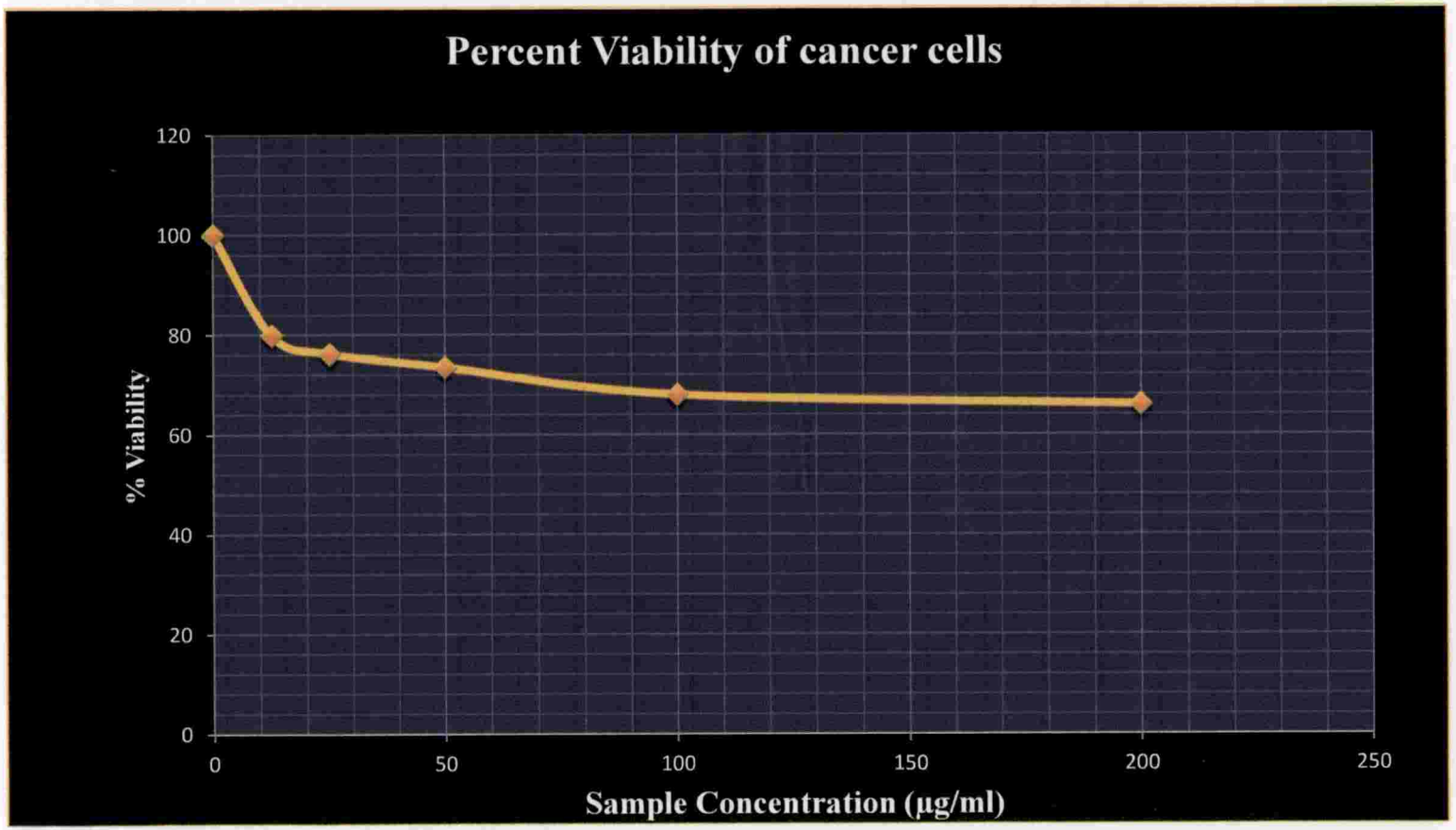


Fig. 10. Cytotoxic effect of *H. ulmarius* extract on HeLa cell line

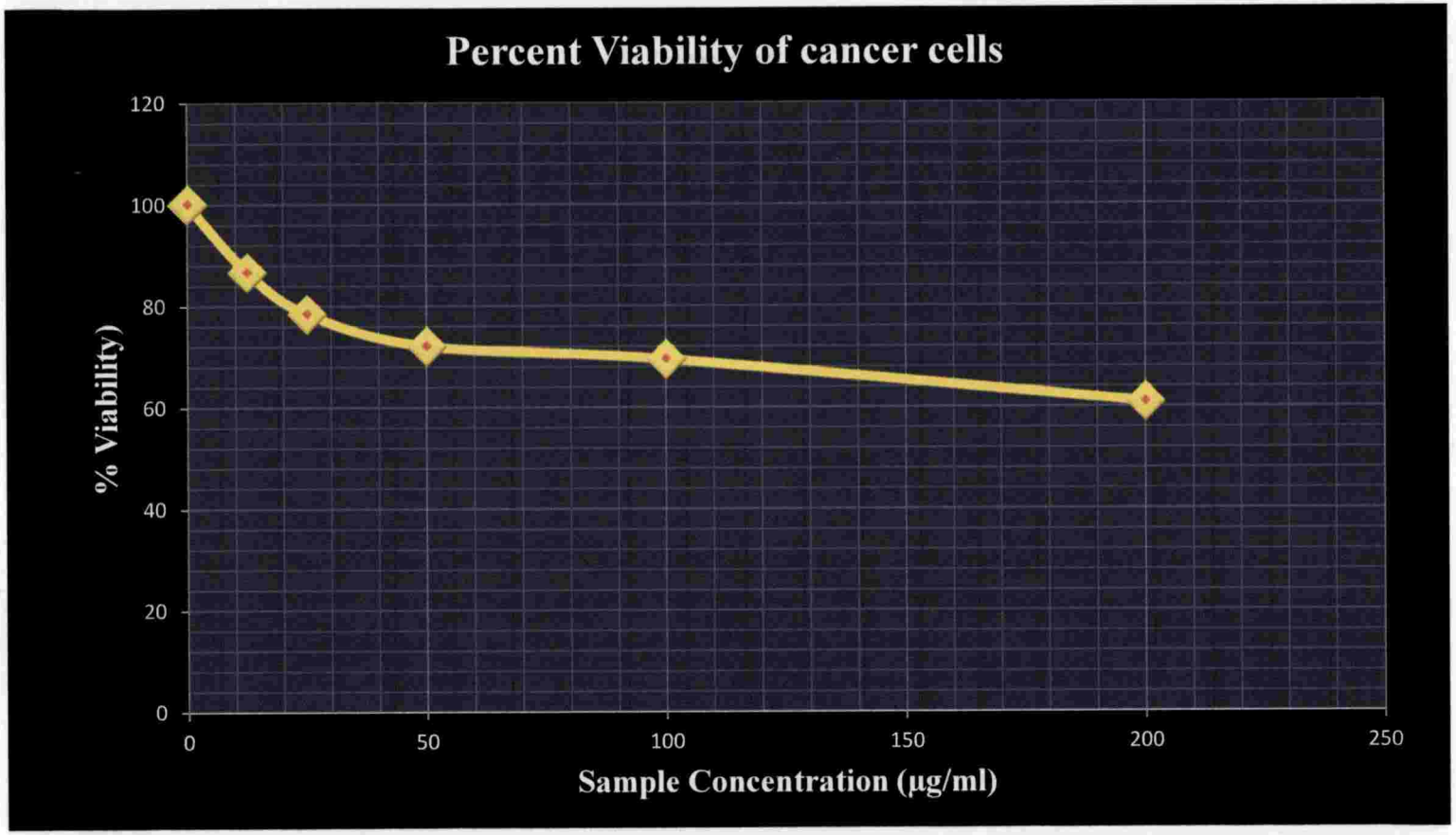


Fig. 11. Cytotoxic effect of *C. gambosa* extract on HeLa cell line

In the present study *P. florida, P. djamor* and *H. ulmarius* extracts exhibited decrease in viability of cervical cancer cell lines to 65.52, 69.47 and 65.69 per cent respectively at 200 µg ml^{-1} concentration (Table 13, 14, 15 and Figure 8, 9, 10) . Fifty per cent inhibition of human cancer cell lines by 500 µg ml^{-1} concentration of *L. edodes* extract was reported by Fang *et al.* (2006). Lavi *et al.* (2006) reported that aqueous polysaccharide extract from *P. ostreatus* induced anti-proliferative and pro-apoptotic effects on colon cancer cells. *P. pulmonarius* significantly reduced the *in vitro* and *in vivo* cancer cell proliferation and enhanced the drug-sensitivity to chemotherapeutic drug (Xu *et al.,* 2012). Finimundy *et al.* (2013) reported that aqueous extract of *P. sajor-caju* showed inhibitory activity against the proliferation of human laryngeal carcinoma cells and cervical adenocarcinoma cells with IC_{50} values ranged from 0.23 to 1.21per cent.

5.9. CYTOTOXICITY STUDIES ON HUMAN LIVER CELLS

The cytotoxic effects in the polysaccharide extracts of mushrooms are due to the active generation of reactive oxygen species (ROS). ROS mediate signal transduction and regulation of diverse processes such as phagocyte activation, cell proliferation, migration and apoptosis. Molecular damage caused by ROS in normal cells induces repair mechanisms, while in tumour cells ROS activate cell death processes through apoptosis (Durgo *et al.* 2013). The cytotoxicity effect of five mushroom extracts were assessed on liver cells which are more susceptible to the damage caused by toxins.

In the present study, *G. lucidum* extract exhibited 33.69 per cent reduction in the viability of normal hepatic cells at 200 µg ml^{-1} concentration which is low as compared with per cent reduction in viability of cancerous cells (Table 17 and Figure 13). Popovic *et al.* (2013) reported that extracellular polysaccharides from *G. lucidum* performed high inhibition on human hepato carcinoma cell line, but also exerted certain toxicity in normal liver cell line which is in accordance with present study. *P. florida, P. djamor, H. ulmarius* and *C. gambosa* extracts

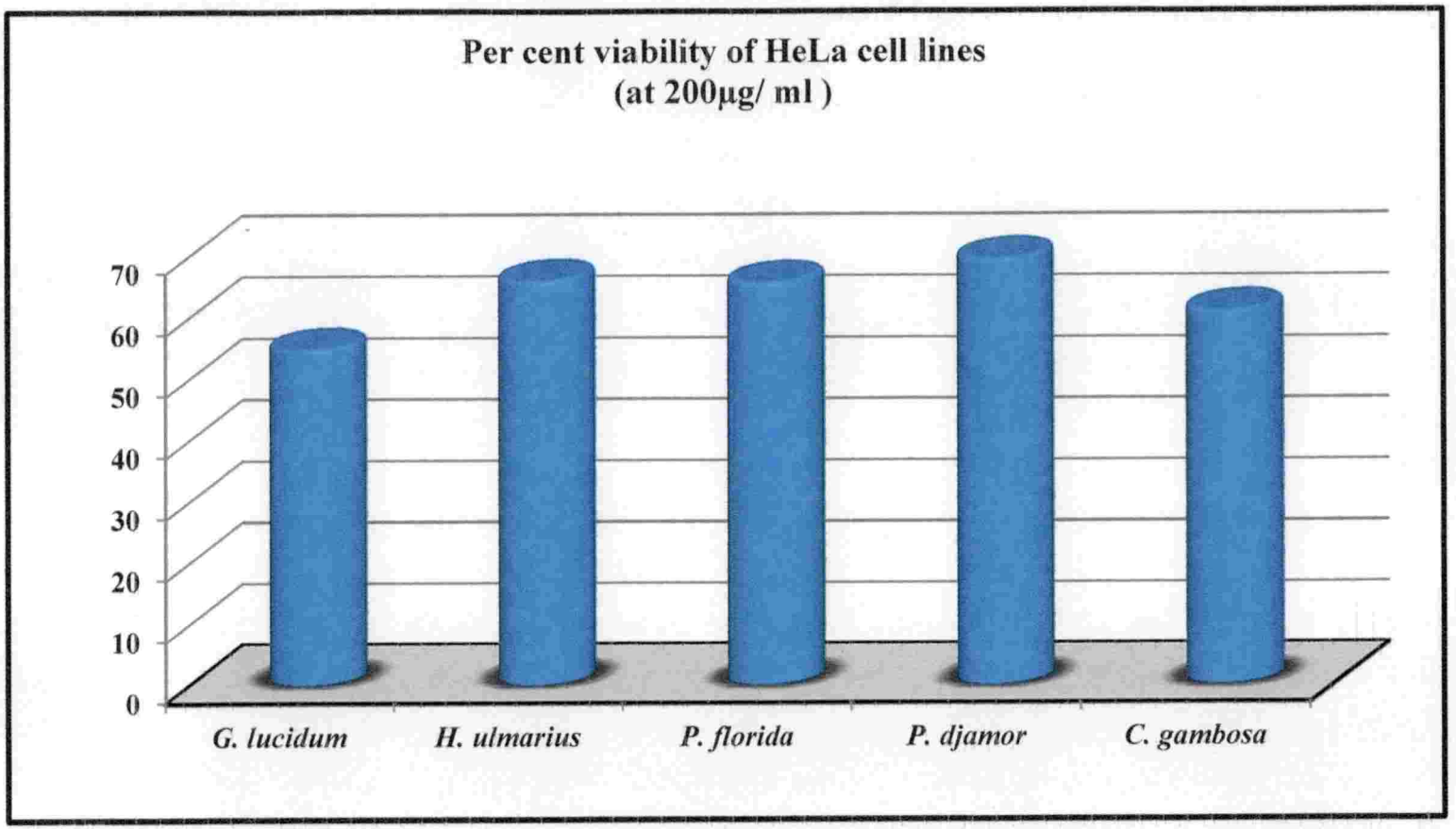


Fig. 12. Cytotoxic effect of mushroom extracts on HeLa cell line at 200µg/ ml

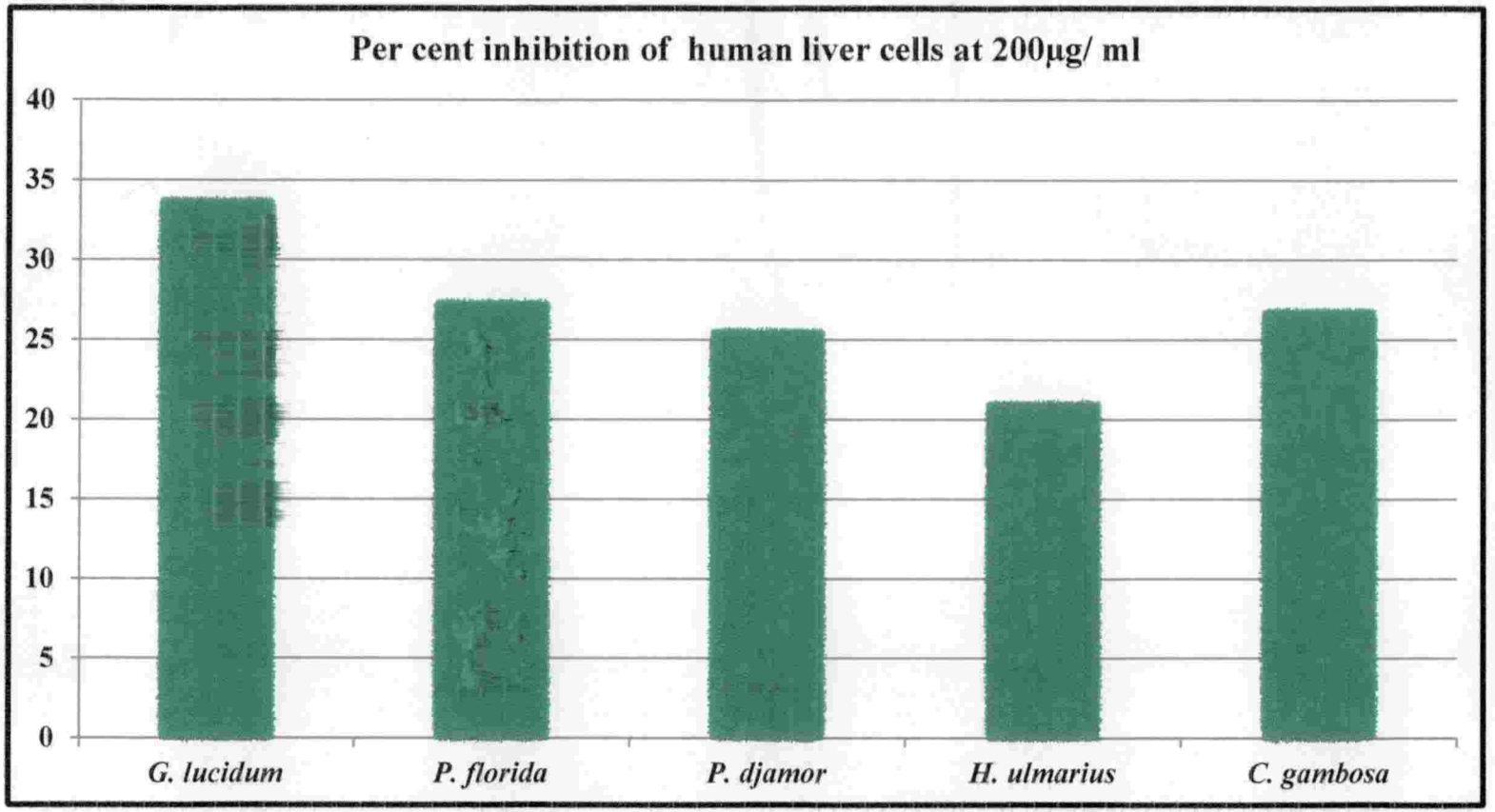


Fig. 13. **Cytotoxic effect of mushroom extracts on human liver cells at 200µg/ ml**

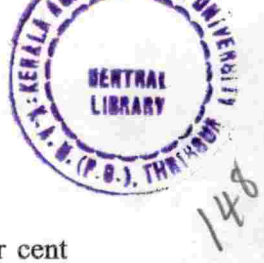
recorded 27.28, 25.48, 20.98 and 26.75 per cent reduction in liver cell viability respectively at 200 µg ml^{-1} concentration in the present study. Contradictory results were given by Wu *et al.* (2012) who reported significant inhibitory effect of *A. blazei* on human osteosarcoma cell line by induction of apoptosis while showing no or little toxicity in a normal cell line. According to Senthilraja and Kathiresan (2015) the cell free extracts of *Saccharomyces cerevisiae* at 37.25 µg ml^{-1} concentration exhibited 50 per cent inhibition of human breast carcinoma cells while African green monkey kidney normal cell line showed 50 per cent inhibition of growth at higher concentration of 250 µg ml^{-1}. Asatiani *et al.* (2018) also reported that *C. militaris* did not show any cytotoxicity against normal cells. However, this is only a preliminary study and further *in vitro* and *in vivo* clinical trials are necessary before its recommendation as drug.

Summary

6 SUMMARY

The present research project was aimed to analyse the nutritionally and pharmacologically active compounds present in four commercially cultivating mushroom species such as *P. florida, P. djamor, H. ulmarius*, *C. gambosa* and *G. lucidum*, the leader of medicinal mushrooms. Fruit bodies of the mushrooms were obtained from the mushroom unit of Instructional Farm, College of Agriculture, Vellayani. Isolation and purification of the fungi were done on PDA and growth of the mushrooms was assessed. Growth of *G. lucidum* was the fastest which completed mycelial growth in 6.25 days, while the oyster mushrooms such as *P. florida, P. djamor* and *H. ulmarius* took 9.25 days, 11 days and 9 days respectively for the white, thick and fluffy mycelia to grow.

The spawn production study of five mushrooms was conducted on paddy grain substrate since it promoted earliness in spawn run and mycelial growth. Among the *Pleurotus* spp., *P. djamor* recorded least time for spawn run (12.25 days) while *C. gambosa* recorded the maximum of 20 days for spawn production in paddy grain substrate. The cultivation of *G. lucidum* was tried on a combination of rubber sawdust (80 %) amended with rice bran (20 %). The first harvesting was done in 33.50 days and a total of three harvests produced 115 g of mushroom with 11.50 per cent biological efficiency. Oyster mushrooms such as *P. florida, P. djamor* and *H. ulmarius* and milky mushrooms were cultivated on paddy straw by polybag method. Among the mushrooms, *P. djamor* recorded minimum time for spawn run (10.50 days) and pinheads formation (14.50 day) with a biological efficiency of 81.20 per cent from total of three harvests. *P. florida* took maximum time for spawn run (24.75 days) and pinheads formation (30.50 days). The total yield from three harvests recorded a biological efficiency of 84.50 per cent. *C. gambosa* required casing for higher biological efficiency. During the cultivation study, casing was done using 1:1:1 combination of sand, soil and vermi compost with 2 per cent calcium carbonate. *C. gambosa* recorded 20 days for spawn run and primordia started appearing on the mushroom bed after 10 days of spawn run.



C. gambosa was recorded with highest biological efficiency of 103.70 per cent from three harvests.

Phorid flies, thrips and sciarid flies were the major pests observed during the cultivation of oyster mushrooms such as *P. florida*, *P. djamor* and *H. ulmarius*. The pest infestation was comparatively less in *C. gambosa* and *G. lucidum* beds. Competitor moulds also adversely affected spawn run and caused damage of fruit bodies at various stages of crop growth. Fungal contaminants such as *Aspergillus* sp., *Coprinus* sp., *Trichoderma* sp. and *Penicillium* sp. were observed during spawn running and sporocarp formation since they suppress the growth of mushroom mycelium and affect the yield.

The nutritional analysis revealed that mushrooms are potential source of proteins, carbohydrate, amino acids, vitamins and minerals with less fat content. The protein content of *G. lucidum* was estimated as 30.91 per cent which was highest among the five mushrooms followed by *C. gambosa* (26 %). *P. florida* recorded 22.16 per cent protein on dry weight basis and *P. djamor* contained 21.98 per cent. *H. ulmarius* was recorded with the least protein content (20.76 %). Mushrooms were found with low fat contents. Crude fat content was the least in *G. lucidum* with 1.33 per cent on dry weight basis. *P. florida*, *P. djamor* and *H. ulmarius* recorded 2.28, 2.10 and 1.72 per cent fat content respectively. The fat content of *C. gambosa* was 2.79 per cent which was the maximum among five mushrooms. Fibre is considered to be an important ingredient in a balanced and healthy diet. The crude fibre content of *G. lucidum* was estimated as 49.33 per cent which was highest among five mushrooms while the tropical milky mushroom *C. gambosa* recorded 15.56 per cent fibre content on dry weight basis which is the least among the five mushrooms studied. *G. lucidum* was estimated with 39.19 per cent carbohydrate on dry weight basis. Low content of 26.68 per cent carbohydrate was recorded in *P. florida* and 26.59 per cent in *P. djamor*. Carbohydrate content of *H. ulmarius* was recorded as 30.87 per cent. *C. gambosa* recorded 46.17 per cent carbohydrate which was recorded as the highest among the five mushrooms analysed.

The moisture content in the fresh samples of *G. lucidum*, *P. florida*, *P. djamor and H. ulmarius* were estimated as 66.95, 84.92, 88.38 and 89.28 per cent respectively. *C. gambosa* was estimated with more moisture content (91.10 %) as compared to *Ganoderma* and oyster mushrooms. Amino acid contents of *P. djamor* (17.10 %), *P. florida* (14.75 %) and *H. ulmarius* (10.62 %) were less. *C. gambosa* and *G. lucidum* recorded higher (23.9 and 19.6 per cent) amino acid content on dry weight basis. The ash content of *G. lucidum* was estimated as 10 per cent on dry weight basis. The ash content of *P. florida* was recorded as 9.10 per cent and that of *P. djamor* was 9 per cent. *H. ulmarius* was recorded with 7.25 per cent ash content which is less as compared to other mushrooms. *C. gambosa* contained 13.55 per cent ash on dried sample.

Mushrooms were found as an important source of minerals such as sodium, potassium, phosphorus, calcium and magnesium. Studies conducted on mineral composition of mushrooms revealed that *G. lucidum* was recorded with 0.020 per cent sodium content which was the least as compared to oyster and milky mushrooms. Sodium content of *H. ulmarius*, *P. florida* and *C. gambosa* were found to be 0.024, 0.028 and 0.029 per cent respectively. *P. djamor* recorded higher sodium content of 0.096 per cent. The phosphorus content of *G. lucidum*, *H. ulmarius* and *P. djamor* were recorded as 0.203 per cent, 0.194 per cent and 0.21 per cent respectively. Among the five mushrooms *P. florida* recorded the maximum phosphorus content (0.255 %) and *C. gambosa* recorded the minimum (0.178 %). Potassium was the most abundant mineral element in the mushrooms analysed. In the present trial potassium content of *C. gambosa* was the highest (1.44 %). The potassium content of *G. lucidum*, *P. florida*, *P. djamor* and *H. ulmarius* were recorded as 0.760, 0.390, 0.720 and 0.70 per cent respectively. The calcium (0.130 %) and magnesium (0.090 %) contents were the maximum in *G. lucidum.*

The studies regarding medicinal properties confirmed that mushrooms were rich in bioactive immunomodulation components such as polysaccharides, terpenoids and flavonoids. *G. lucidum* recoded maximum beta-glucan content of

38.58 per cent on dry weight basis. The beta-glucan content of *H. ulmarius*, *P. florida*, *P. djamor* and *C. gambosa* were estimated as 26.62, 20.80, 32.15 and 35.64 per cent respectively. The presence of glycoprotein in all the five mushrooms were analysed qualitatively. Estimation of terpenoid content of *G. lucidum* recorded 11.14 mg g^{-1} which had anti-oxidative properties *in vitro* and reduced oxidative damage by scavenging free radicals generated in the cell. Terpenoid content of *P. florida, P. djamor*, *H. ulmarius* and *C. gambosa* were estimated as 9.22, 9.78, 10.13 and 12.41 mg g^{-1} respectively. Polyphenol compounds were secondary metabolites with a wide range of medicinal properties. The phenolic content of *G. lucidum* was estimated as 23.80 mg GAE g^{-1} which is the highest among five mushrooms followed by *C. gambosa* which recorded 22.46 mg GAE g^{-1}. Comparatively low polyphenol content was recorded by *H. ulmarius, P. florida* and *P. djamor* (20.143, 19.14 and 14.56 mg GAE g^{-1}) content respectively. Maximum beta-carotene content was recorded in *P. djamor* (355.04µg g^{-1}). Flavonoids were considered as phenolic compounds found in mushrooms. The king of medicinal mushroom, *G. lucidum* was recorded with 3.02 mg QE g^{-1} of flavonoid. The flavonoid content of oyster mushrooms such as *P. florida*, *P. djamor* and *H. ulmarius* were estimated as 1.14, 2.64 and 2.83 mg g^{-1} of dried sample. *C. gambosa* recorded 3.3 mg g^{-1} of flavonoid.

Analysis of anti-oxidant property of mushroom by DPPH assay revealed that *G. lucidum* extract exhibited 50 per cent inhibition of free radicals at 740 µg ml^{-1} concentration, whereas the extracts of oyster mushrooms (*P. florida*, *P. djamor* and *H. ulmarius*) and milky mushroom (*C. gambosa*) even at higher concentration did not reach fifty per cent inhibition of free radicals in the current study. The anti-cancerous activities of ethanolic extract of *G. lucidum, P. florida, P. djamor, H. ulmarius* and *C. gambosa* were assessed against cervical cancer cell lines (HeLa) *in vitro* by MTT assay. The *G. lucidum* extract at 200 µg ml^{-1} concentration reduced the viability of cancer cells to 54.85 per cent which was the maximum inhibition of cancerous cells as compared to other mushrooms under study followed by *C. gambosa* (60.92 %). The extracts of *P. florida*, *P. djamor*

and *H. ulmarius* exhibited decrease in viability of cervical cancer cell lines to 65.52, 69.47 and 65.69 per cent respectively at 200 µg ml^{-1} concentration. The cytotoxicity of mushroom extracts on normal human liver cells was less as compared to the effect on cancerous cells. This is a baseline study on the medicinal properties and anti-cancerous activities of mushrooms. Further *in vivo* and clinical studies are needed in this field.

The present study recommends the use and inclusion of oyster and milky mushrooms in our daily diet which is in agreement with the recommendation of Food and Agriculture Organisation as well as nutritional experts who recommend use of 50 to100 g of mushroom per day in diet for a healthy and immune body (Mayett *et al.* 2006).

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Abstract

EVALUATION OF BIOCHEMICAL AND ANTI-CANCEROUS ACTIVITIES OF MUSHROOMS

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ABSTRACT

The present study entitled "Evaluation of biochemical and anti-cancerous activities of mushrooms" was conducted at College of Agriculture, Vellayani during 2016-2018, with the objective to undertake the cultural, spawn production and cultivation trials of five mushrooms namely, *Ganoderma lucidum* (Curtis) P. Karst., *Pleurotus florida* (Mont.), *Pleurotus djamor* (Fr.) Boedjn, *Hypsizygus ulmarius* (Bull.:Fr.) Redhead and *Calocybe gambosa* (Fr.) Singh and to evaluate their biochemical and anti-cancerous activities. The cultures of these mushrooms were isolated from the mushroom beds maintained in the mushroom unit of Instructional Farm, Vellayani through tissue culture method and purified by hyphal tip method.

Studies on mycelial growth of five mushrooms on potato dextrose agar revealed that *G. lucidum* had the maximum radial growth (9.00 cm) in shorter period of time (6.25 days). The nature of mycelial growth of *G. lucidum* and *P. florida* was flat and filamentous, while that of *P. djamor, H. ulmarius* and *C. gambosa* was thick and fluffy. The colour of mycelium of all the mushrooms was white to creamy white. Spawn production trials of five mushrooms on paddy grain indicated the minimum time for spawn run of 12.25 days for *P. djamor* followed by *H. ulmarius* and *P. florida.*

Cultivation trials of *P.djamor, P. florida, H. ulmarius* and *C. gambosa* were undertaken on paddy straw substrate while that of *G. lucidum* was done on rubber wood sawdust. *P. djamor* recorded minimum time for spawn run (10.50 days), pinhead formation (14.50 days) and first harvest (16.50 days). The milky mushroom, *C. gambosa* recorded the maximum yield of 1037.25 g kg^{-1} from three harvests with 103.72 per cent biological efficiency (BE) followed by *H. ulmarius* with yield of 960 g kg^{-1} and BE of 96 %.

Infestation of pests *viz.* phorid flies and staphylinid beetles as well as fungal contaminants such as *Trichoderma sp., Aspergillus sp.,* and *Coprinus spp.* were found in all the mushrooms during sporocarp formation.

Dried and powdered samples of mushrooms were used for nutritional analyses. *G. lucidum* recorded maximum protein (30.91 %) and fibre content (49.33 %), whereas *C. gambosa* recorded maximum carbohydrate, total amino acids, ash and fat content. The present study indicated the high nutritive value of milky mushroom *C. gambosa*

Mineral components like sodium, potassium, phosphorus, calcium and magnesium were determined in the study. Among the five mushrooms *C. gambosa* recorded the maximum potassium while calcium and magnesium were the maximum in *G. lucidum*. The phosphorus content was not found to be significantly different among the five mushrooms.

Analysis of medicinal components of mushrooms indicated that mushrooms were rich in beta-glucan, polyphenols, flavonoids and terpenoid. *G. lucidum*, the king of medicinal mushrooms recorded maximum beta-glucan (38.58 %) and polyphenols (23.80 mg GAE g^{-1}) followed by *C. gambosa*. Flavonoid and terpenoid contents were recorded maximum in *C. gambosa* followed by *G. lucidum*. The pink oyster mushroom, *P. djamor* recorded the maximum beta-carotene content (355µg g^{-1}).

Preliminary trials on the anti-cancer activities of mushroom extracts were conducted by direct microscopic studies and MTT assay. The results revealed that percentage viability of cervical cancer cell lines decreased with increase in concentration of mushroom extracts. However *G. lucidum* extract exhibited maximum cytotoxic effect on cancer cell lines even at lower concentration (200 µg ml^{-1}) followed by *C. gambosa*. The present study indicated that all the five mushrooms are rich source of protein, carbohydrate, fibre and minerals. *P. florida, P. djamor, H. ulmarius* and *C. gambosa* were nutritionally and medicinally

superior. The commercial cultivation as well as clinical studies of these medicinally important mushrooms must be undertaken in large scale.

Appendices

APPENDIX-I

Composition of Potato Dextrose Agar

Potato	: 200 g
Dextrose	: 20 g
Agar- agar	: 20 g
Distilled water	: 1000 ml