STRATEGIES FOR IMPROVEMENT IN CULTIVATION PRACTICES OF OYSTER MUSHROOM IN NORTH BENGAL

Thesis submitted to the
University of North Bengal for the award of
Doctor of Philosophy in Botany

Submitted By

Somnath Roy

Under the Supervision of

Professor Bishwanath Chakraborty

Immuno-Phytopathology Laboratory
Department of Botany
University of North Bengal
Raja Rammohunpur, Siliguri

July, 2016

DECLARATION

I declare that the thesis entitled "**STRATEGIES FOR IMPROVEMENT IN CULTIVATION PRACTICES OF OYSTER MUSHROOM IN NORTH BENGAL"** has been prepared by me under the guidance of Professor Bishwanath Chakraborty, Immuno Phytopathology Laboratory, Department of Botany, University of North Bengal. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

Somnath Roy.

(Somnath Roy)
Department of Botany
University of North Bengal
Raja Rammohanpur, P.O. – NBU
Dist.- Darjeeling, Pin - 734013

Date:

UNIVERSITY OF NORTH BENGAL
Department of Botany

Professor B. N. Chakraborty
Ph.D, FPSI, FNRS, FISMPP, FISPC, FMSI, FIMS
FAScT, FRSC (Lond.), FNAAS

ENLIGHTENMENT TO PERFECTION

IMMUNO-PHYTOPATHOLOGY LABORATORY
PO. N.B.U 734013, Siliguri, West Bengal, India
Phone : 0353-2776337 (O), 09800266298 (M)
Fax : 0353-2699001
E-mail : bncnbu@gmail.com
Website: www.iplnbu.ac.in

CERTIFICATE

This is to certify that Mr. Somnath Roy has carried out his research work under my supervision in the Department of Botany, University of North Bengal. His thesis entitled **"STRATEGIES FOR IMPROVEMENT IN CULTIVATION PRACTICES OF OYSTER MUSHROOM IN NORTH BENGAL"** is based on his original work and is being submitted for the award of Ph. D degree in Botany, in accordance with the rules and regulations of the University of North Bengal

(B.N. Chakraborty)
(Professor B. N. Chakraborty)
Department of Botany
University of North Bengal

ABSTRACT

Oyster mushroom is one of the most popular edible mushroom cultivating throughout the world. Different species of *Pleurotus* are now in cultivation practices and North Bengal is one of the main source of oyster mushroom production. Four species of Pleurotus were selected for the study. *Pleurotus ostreatus, P sajor-caju, P. djamor* and *P florida*. Formerly in North Bengal, only *P ostreatus, P sajor-caju* and *P florida* was commonly cultivated. A new species of oyster mushroom *P djamor* the pink oyster mushroom recently introduced and this species is now being cultivated throughout North Bengal.

Morphological and histopathological characterization was done to assess the differentiation between four species of Pleurotus along with their gill structure as well as the spore characteristics. Mycelial growth pattern of Pleurotus species were also studied using various medium and it was found that the growth rate varies in different medium. Molecular detection of the four fungal isolates IPL/MC/PO-1, IPL/MC/PS-01, IPL/MC/PD-01 and IPL/MC/PF-01 were carried out using 18S rDNA sequencing using ITS1 and ITS 4 primers. The sequences of the identified species were submitted in NCBI GenBank under the accession number KT768095, KT818506, KT 768094 and KT826605 for *P. ostreatus, P sajor-caju, P. djamor* and *P florida* respectively.

Seasonal productivity of all the selected *Pleurotus* species was chased and it was observed that the variable seasonal productivity throughout the year. Depending upon the temperature, relative humidity and rainfall, the productivity was found to be different. In case of *P. ostreatus,* February to September was found to be suitable while in case of *P sajor-caju,* temperature and relative humidity was quite higher and thus it found to be suitable to cultivate during May to September. While in case of *P florida* and *P djamor* it was found to be quite similar depending upon the temperature, relative humidity and rainfall required for the growth and fruiting body initiation. It was observed that at higher temperature, the fruiting initiation required very long time and also the productivity decreased during May to September. Various containers like waste bottles, glassware and boxes were also used to evaluate their effect on yield.

Biochemical constituents such as moisture, total sugar, reducing sugar, protein and lipid content were also estimated of cultivated *Pleurotus* species. All four species cultivated

using paddy straw, wheat straw and saw dust were found to be very high amount of protein, total sugar and reducing sugar content. Results also revealed that mushroom possess very low amount of lipid content. Antioxidant activity of selected *Pleurotus* species were also estimated and it was found that all four species showed very high amount of antioxidant activity such as DPPH scavenging activity, ferric reducing antioxidant power activity as well as flavonoid activity. All the Pleurotus species showed very high DPPH scavenging activity, Ferric reducing antioxidant power activity as well as flavonoid content. In vivo antidiabetic activity were also evaluated and oral treatment of *Pleurotus* species helps in reducing plasma glucose level as well as helps in regaining the body weight.

Spent mushroom substrate is an important by product of *Pleurotus* cultivation and this spent mushroom substrate were applied as fertilizer for crop improvement. *Capsicum chinense, C annuum, Solanum lycopersicum* and *Amaranthus* sp. were tasted successfully and the effect of SMS on different crop plants helps in growth and better yield.

Short term processing of *Pleurotus* species was done which include Packaging of mushroom for sell as well as different recipes were tasted in laboratory like mushroom pakora, soup, curry, scrambled mushroom. Long term processing of *Pleurotus* was also practiced in the form of drying, powder and also preparing mushroom biscuits. Promotion of mushroom cultivation and marketing were also done. Several unemployed youth, post graduate students as well as mushroom growers on North Bengal were trained about the seasonal productivity, spawn preparation as well as post-harvest processing of *Pleurotus* species.

Contaminants were found to be a very serious problem during the cultivation of *Pleurotus* species. Several fungal species like *Coprinus*, *Fusarium* as well as *Trichoderma*; bacterial species like *Pseudomonas* and pastes like Sciarid, phorid and Beetle fly drastically affected the growth and yield of *Pleurotus*. Management strategies like application of bavistin, phenyl, carbandazium and formaldehyde were adopted which reduces the effect of the fungal as well as bacterial genera. Racks of the mushroom production unit were covered with nylon nets which helped in reducing the attack of flies on the substrates.

Promotion of mushroom cultivation and marketing were also done. Several unemployed youth, post graduate students as well as mushroom growers on North Bengal were trained about the seasonal productivity, spawn preparation as well as post-harvest processing of *Pleurotus* species. Promotion of *Pleurotus* cultivation was also done at the Krishi Mela organised by the Department of Agriculture, Govt. of West Bengal. Several unemployed youth, retired persons as well as students of different colleges and women of self-help groups were trained successfully and established their own cultivation unit. They are also very much efficient in spawn production and thus the growers produce their own spawn for cultivation. Economic efficiency of mushroom production and marketing were also studied and it was found that the growers selling mushroom at very high price and the cost benefit ration become very high which results in economic upliftment of different economically weaker people.

PREFACE

At the very outset, I express my profound sense of gratitude and indebtedness to my supervisor *Professor Bishwanath Chakraborty*, Immuno-Phytopathology Laboratory, Department of Botany, University of North Bengal for his constant guidance throughout my research work. Without his encouragement and tireless mentorship, I would not have completed my thesis.

I would also like to present my sincere gratitude to *Professor Usha Chakraborty*, Department of Botany for her constant guidance and supportive attitude whenever I was in need of her help. Thank you, Madam, for your support.

I would also like to thank *Dr. Aniruddha Saha*, Head, and all the past Heads of the Department for all administrative support during the whole time of the course. I would also like to acknowledge support of *Professor Abhaya Prasad Das, Professor Prabir Kumar Sarkar, Dr. Arnab Sen, Dr. Subhash Chandra Roy, Dr. Palash Mandal* and *Dr. Manoranjan Chowdhury* Department of Botany, NBU. I trust all my teachers would accept my respectful offer of gratitude. I also wish to extend my thanks to all the office staffs and Laboratory assistants of the Department.

I am also indebted to the Professor Somnath Ghosh, Honourable Vice Chancellor, University of North Bengal for letting me to carry out my work in this University. My sincere thanks are also due to The Registrar, NBU for his positive response during my registration and submission of Ph.D. Thesis.

Without the financial support from the University Grant Commission, Govt. of India, my work would start and end in nothingness and hence I sincerely thank the UGC, for providing me resource to study.

During the years of my research work, I had to take help from various other esteemed institutions of India namely Directorate of Mushroom Research, ICAR Solan, Himachal Padesh for providing me the fungal cultures and training on mushroom cultivation; Credora Life Sciences, Kerala for fungal identifications; Arbro Pharmaceuticals, New Delhi for food value analysis. I am indebted to all concerned in these laboratories for their help. I wold also like to pay my sincere thanks to M/s Drugs and Chemicals, M/ SRN Scentific, M/s MJ Enterprises for providing me the required chemicals and BioNet for supplying me the albino rats for conducting the experiments.

I would also like owe special thanks to the mushroom growers for allowing me to their respective mushroom farms.

I want to express my warm personal gratitude to all my friends of the Immuno-Phytopathology Laboratory especially Dr. Pannalal Dey, Dr. Kiran Sunar, Dr. Arka Pratim Chakraborty, Dr. Utanka Kumar De, Dr. Sanjita Allay, Ms. Amrita Acharya, Ms. Sweata Khati, Mr. Shibu Barman, Ms. Priyanka Bhattacharjee and the other research scholars of Plant Biochemistry laboratory such as Ms. Nishika Jaishee, Ms. Jayanwita Sarkar and others for all their ideas that we argued about and constant support and assistance in completion of this thesis. Thanks indeed. I wish to thank the field assistants especially Mr. Sambhu Banik, Mr. Nimai Roy and Ms. Doly Mahato for their effortless help in field work.

Finally, I would like to thank my parents and other family members for their constant support and effortless care without which it will not be possible for me to complete my work

(Somnath Roy)

Immuno-Phytopathology Lab
Department of Botany
University of North Bengal
Date:

CONTENT

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

CHAPTER 1
INTRODUCTION

Mushrooms are fruit bodies of macroscopic, filamentous fungi and they are made up of hyphae which form interwoven web of tissues known as mycelia in the substrate upon which the fungi feed. Collection of food from nature dates back to early history. Even today parallel to food production, people are engaged in collection and discovery of new sources of food. Best of such new sources of food is supposed to be the mushroom which grows naturally on organic and has excellent of taste and nutritional value. Initially mushrooms were collected from nature; however, due to efforts of scientists, it is now possible to cultivate mushroom artificially.

Demand for proteinaceous food is rapidly increasing along with expanding human populations throughout the world. The field of mushrooms has opened up new possibilities of exploring resources of nutritious and delicious food. Edible mushroom is undoubtedly a good source of vegetable protein. Besides, a large number of wild edible mushrooms are also being consumed by the local people and sometimes being exported to other countries.

Mushrooms are rich in protein, minerals and vitamins. However, the awareness of mushroom as a healthy food and as an important source of biological active substances with medicinal value has only recently emerged (Cheung *et al.,* 2003). Mushrooms are considered as healthy food because they are low in calories and fat but rich in proteins and dietary fibers (Manzi *et al*., 1999). The mushroom protein contains all the nine essential amino acids –phenylalanine, histidine, leucine, lysine, arginine, tryptophan, threonine, valine, isoleucine required by humans. In addition to their good protein content, mushrooms are a relatively good source of the nutrients like phosphorus, iron and vitamins, including thiamine, riboflavin, ascorbic acid, ergosterol, and niacin (Barros *et al*., 2008). The moisture content of fresh mushrooms varies within the range of 70-95% depending upon the harvest time and environmental conditions, whereas it is about 10-13% in dried mushrooms. The protein content of the cultivated species ranges from 1.75 to 5.9 % of their fresh weight. Hence mushrooms have been recommended by the FAO as food contributing to the protein nutrition of the developing countries

depending largely on cereals. The digestibility of mushroom protein ranges between 60-70%. Comparatively food value of dry mushroom shows its superiority with other common protein foods.

Mushroom is also an important food item concerning human health, nutrition and disease prevention. Besides the above nutritional aspects, the following medicinal importance of mushroom is noteworthy: (a) Carbohydrates of mushrooms do not contain starch and sucrose and is present in very small quantity, so it is good food for diabetic patients, (b) Many vegetables and cereals are deficient in some of the essential amino acids compared to egg proteins, so it will help in overcoming amino acid deficiency, (c) The mushroom protein is easily digestible and its quality is intermediate between vegetables and meat protein, (d) Mushroom can recover vitamin deficiency (particularly vitamin B) as it is a good source of vitamins.

The Indian subcontinent is known worldwide for its varied agro-climatic zones with a variety of habitats that favour rich mushroom biodiversity (Thakur *et al*, 2011). Mushroom cultivation is the most economical and relatively short biological process for the biotransformation of lognocellulosic materials into protein rich food (Thakur and Singh, 2013; Thakur, 2014). In India, major share (80%) goes to button mushroom production, while rest of the share (20%) goes to tropical mushrooms such as oyster, milky and paddy straw mushrooms. China is the largest producer, consumer and exporter of mushroom in the world followed by USA and Netherland. In North Bengal, the environmental conditions, climate and temperature are very suitable for cultivation of different types of mushrooms all-round the year.

The genus *Pleurotus* (oyster mushroom) comprises some most popular edible mushroom species (*Pleurotus sajor-caju*, *P. ostreatus*, *P. sapidus*, *P. flabellatus*, *P. cystidiosus*, *P. florida*, *P. eous*), due to their favourable organoleptic and medicinal properties as well as vigorous growth. *Pleurotus* species are widely cultivated throughout the world especially in Asia and Europe because of low cost and simple production technology. They have high production and biological efficiency (Khan *et al*, 2014). *Pleurotus* species are rich in carbohydrate, minerals (high in P, K, Ca, Fe, folic acid and low in Na) and fiber as compared to other foods. They are also a rich source of essential and non-essential amino acids such as lysine which is low in cereals. *Pleurotus* species have ability to grow efficiently in a variable range of temperature

conditions (15-30^{0}C), so they can ideally be cultivated throughout the year in different agro ecological zones (Chakraborty *et al*, 2014). Owing to efficient lignin-degraders, it can be cultivated on log and a wide variety of agro-cellulosic wastes, for the production of food, feed, enzymes and medicinal compounds, or for waste degradation and detoxification. Besides, mushroom cultivation a prosperous venture for improvement of livelihood of poor tribal in Tripura have been demonstrated by Biswas *et al*. (2015) and cultivation was optimized in Ethiopia by Tesfaw *et al*. (2015). Abiotic stresses affect the growth and development of organisms both qualitatively and quantitatively. One of the common abiotic stresses is high temperature in growing season. At temperatures over the optimal level for growth, organism usually displays two major damages, oxidative stress and irreversible protein aggregation (Mittler, 2002).

Pleurotus florida has been reported to have antioxidant and antitumor activities (Manpreet *et. al*., 2004), while *P. ostreatus* possess antitumor activity (Yoshioka *et.al.*, 1985). Antihyperglycemic action of oyster mushroom (*P. ostreatus*) has also been demonstrated by Ghaly *et.al* (2011). These medicinal properties might be due to the presence of some important substance in dietary mushrooms. Antimicrobial and antioxidant properties of *Pleurotus ostreatus* cultivated on different tropical woody substrates have been demonstrated (Chaturvedi *et al*, 2011; Saha *et al*, 2012; Oyetayo and Ario, 2013). Chemical analysis of a wild edible mushroom found in the northern tropical moist deciduous forests of Tripura have also been documented (Roy Das *et al*, 2014). Though different types of oral hypoglycemic agents are available along with insulin for the treatment of Diabetes, there is increasing demand by patients to use natural product with antidiabetic activity. Insulin cannot be used orally and continuous use of the synthetic antidiabetic drugs causes side effects and toxicity. The treatment of diabetes often involves medication to control blood glucose levels; some of these medications have undesirable side-effects. Several vegetables have been found to have therapeutic or ameliorating effects in lowering blood glucose levels by reducing insulin resistance and improving glucose tolerance (Yadav *et.al,* 2002). An extensive search for traditional plant treatments for diabetes has concluded that recognized edible mushrooms are an ideal food for the dietetic prevention of hyperglycemia because of their high dietary fiber and protein and low fat content (Alarcon Aguilara *et al.,* 1998). The most common animal models used for the study of the hypoglycemic effects of mushrooms are rats and mice with insulin-dependent diabetes mellitus (IDDM) induced

by Streptozotocin (STZ) and genetically diabetic mice with non-insulin dependent diabetes mellitus (NIDDM) (Beattie *et al.*,1980; Swanston-Flatt *et al.,* 1989).

Keeping in mind the above information about edible mushroom, attempts have been made to develop suitable strategies for improvement in cultivation practices of oyster mushroom in this region, so that the cultivation can easily be continued throughout the year under present changing environmental condition. Its nutrition as well as medicinal values must be retained for the benefit of mankind. In order to full fill the above criteria the present investigation has been undertaken with the following objectives

Objectives

I. Selection of different species of oyster mushroom suitable for cultivation in North Bengal.

II. Molecular characterization of selected *Pleurotus* species

III. Development of cultivation technology of selected *Pleurotus* species with special reference to spawn production, selection and standardization of substrates for higher yield in North Bengal region.

IV. Strategies for management of contaminants during cultivation process.

V. Biochemical characterization and comparison of nutritional value of *Pleurotus* species cultivated in North Bengal.

VI. Evaluation of *in vivo* antidiabetic properties of *Pleurotus* species.

VII. Development of post-harvest technology for better preservation.

VIII. Promotion of mushroom marketing

CHAPTER 2
LITERATURE REVIEW

Edible Mushrooms

Mushrooms are the fleshy and edible fruit bodies of several species of macro fungi (fungi which bear fruiting structures that are large enough to be seen with the naked eye). They can appear either below ground (hypogeous) or above ground (epigeous) where they may be picked by hand. Mushrooms have been used as food and medicine in many parts of the world. Although mushrooms are often grouped with vegetables and fruits, they are actually belongs to the fungal kingdom. They are macro-fungi which belong either to Basidiomycetes or Ascomycetes and they are very distinct from plants, animals and bacteria as described by Mushigeni and Chang (2001). Chang and Miles (1991) stated that mushroom with their great variety of species, constitutive a cost effective means of both supplementing the nutrition to human. 4-5 different mushrooms are now industrially cultivated throughout the world and in India three mushrooms are now being cultivated such as *Agaricus bisporus, Pleurotus* sp. and *Volvoriella volvocea*. *Pleurotus ostreatus*, the oyster mushroom, is a common edible mushroom. More than 2000 species of edible mushrooms are known, out of which only few species have been cultivated commercially by preparing beds. Among the various edible mushroom types, *Pleurotus* species have become more popular and widely cultivated throughout the world particularly in Asia and Europe as they have simple and low cost production technology shows higher bio-efficiency. Mushrooms are the source of extra ordinary power and virility and are used in the preparation of many continental dishes and have medicinal properties like anticancerous, anticholesteral, antitumorous. Cultivation of edible mushroom obtains nutritive food materials by decomposing various agricultural waste materials due to its saprophytic nature. Cultivation of edible mushroom is a biotechnological process, which reduces and equally protects the environment from excess solid waste pollution (Mshandete and Cuff, 2008; Sánchez, 2010). Miles and Chang (1997) reported that out of about 70,000 described species of fungi, it has been suggested that around 14,000-15,000 species produces fruiting bodies of sufficient size and suitable structures to be considered as macro fungi. Of these, about 5,000 species are considered to possess varying degrees of edibility and more than 2,000 species from 31 genera are regarded as prime edible mushrooms. But only

100 of them are experimentally grown, 50 economically cultivated and 30 commercially cultivated and only about 6 to have reached an industrial scale of production in many countries (Table 1). Furthermore, about 1,800 are known to possess medicinal properties. They also reported that the number of poisonous mushrooms are relatively very small (approximately 10%), of these about 30 species are considered to be lethal.

Table 1: Present status of world production of mushroom

Countries	Total Productions (Metric Ton)		
	1997	2007	2012
China	5,62,194	15,68,523	51,58,773
United States of America	3,66,810	3,59,630	3,88,450
Netherlands	2,40,000	2,40,000	3,07,000
Poland	1,00,000	1,60,000	2,20,000
Spain	81,304	1,40,000	1,46,000
France	1,73,000	1,25,000	1,16,574
Italy	57,646	85,900	7,85,000
Ireland	57,800	75,000	67,063
Canada	68,020	73,257	82,000
United Kingdom	1,07,359	72,000	73,100
Japan	74,782	67,000	61,500
Germany	60,000	55,000	52,907
Indonesia	19,000	48,247	40,659
India	**9,000**	**48,000**	**52,350**
Belgium	NA	43,000	42,000
Australia	35,485	42,739	46,493
Korea	13,181	28,764	26,000
Iran	10,000	28,000	87,675
Hungary	13,599	21,200	19,330
Viet Nam	10,000	18,000	23,000
Denmark	8,766	11,000	10,700
Thailand	9,000	10,000	6,820
Israel	1,260	9,500	10,000
South Africa	7,406	9,395	14,284
New Zealand	7,500	8,500	9,884
Switzerland	7,239	7,440	7,977
Other Countries	85,911	59,279	61,346
Total Production	**21,86,222**	**34,14,392**	**79,16,885**

*Source: Table 50, World Mushroom and Truffles: Production. 1961-2007; United Nations, FAO, FAOSTAT (8/28/2009) and FAOSTAT | © FAO Statistics Division 2015 | 14 July 2015

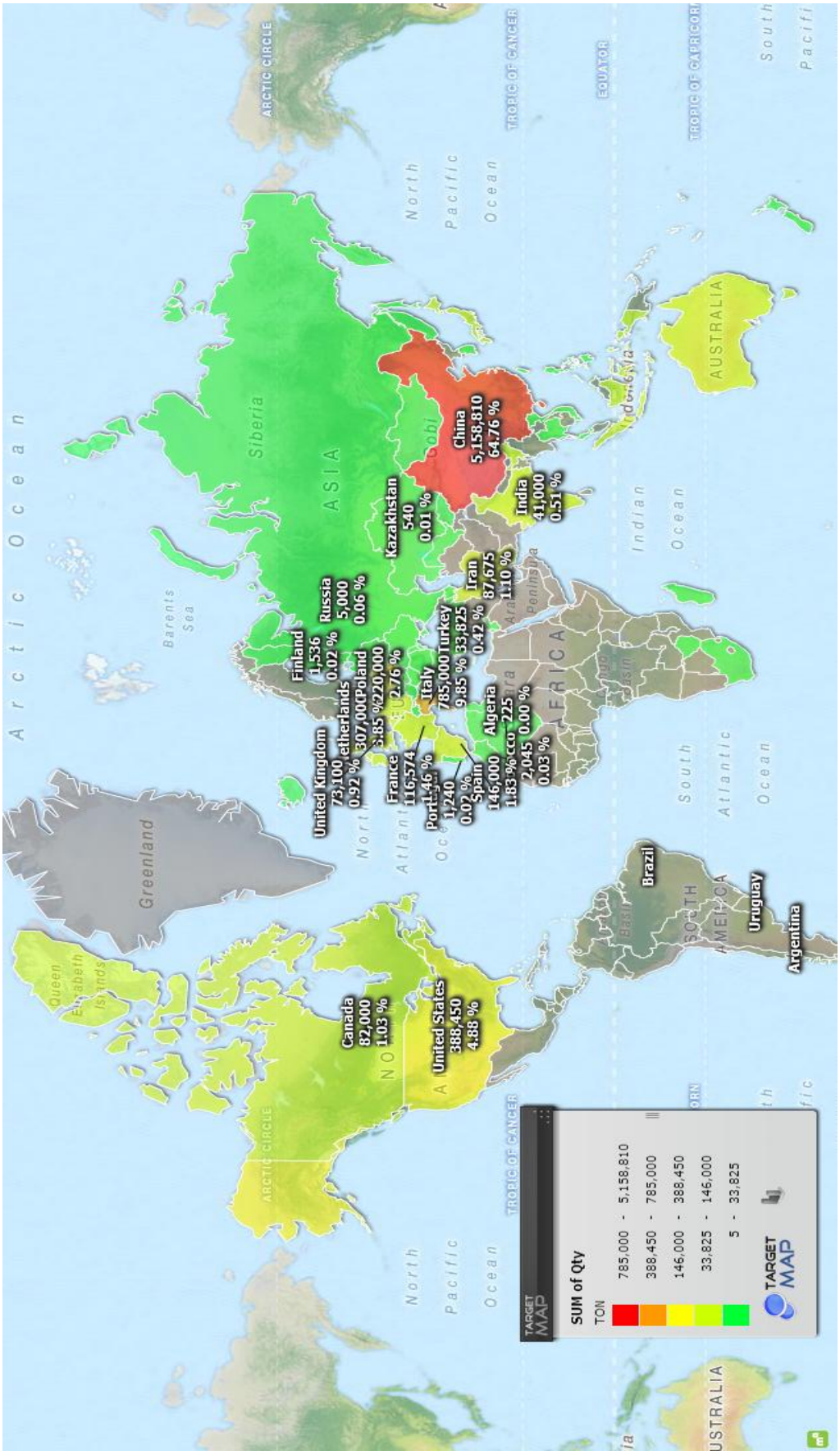


Figure 1: Global scenario of mushroom production

Oyster Mushroom

Oyster mushroom (i.e. *Pleurotus* spp.) is one of the major cultivated mushroom of India is commonly called as Dhengri because of its oyster like shape. The genus *Pleurotus* belongs to family Tricholomataceae. About 40 different species are now cultivated popularly throughout the world out of which 12 species are cultivated in different parts of India. Chang *et. al*. (1991) and Erkal (1992) stated that in terms of its yield and production *Pleurotus* is the second large cultivated mushroom right next to *Agaricus bisporus* throughout the world (Figure 1 and Table 1). Cultivation of oyster mushroom increased greatly throughout the worlds during the last few decades and it's accounted about 14.2% of the total world edible mushroom as described by Chang (1999). Cultivation of oyster mushroom is gaining popularity because of its simplicity in technique and less investment. In India, mushroom production rate was also quite higher and almost all the states are producing different edible mushroom out of which, *Agaricus bisporus* and *Pleurotus* species widely distributed (Table 2).

Cultivation Technology of oyster mushroom

Mushroom cultivation represents the only current economically viable biotechnology process for the conversion of waste plant residues from forests and agriculture (Wood and Smith, 1987). Cultivation viable processes for the bioconversion of lignocellulosic wastes (Bano *et al*., 1993; Cohen *et al*., 2002). Singh *et al*., (1990) reported that the cultivation of oyster mushroom is gaining importance in tropical and subtropical regions due to its simple way of cultivation and high biological efficiency. Das and Mukherjee (2007) stated that different species of *Pleurotus* are usually found to be most efficient in the degradation of lignocellulose substrates among all types of white rot fungi. Weinheim, (2006) suggested that cultivation of mushroom also decrease the air pollution with turning agricultural wastes also help in the management in pastes and other fungal inoculum through the edible mushroom cultivation. He also stated that the cultivation of edible mushroom also help in solving many problems like improving the economic condition of rural people as well as in improvement in the health of people as mushroom considered as a valuable health food which contains considerable amount of protein, calories and minerals. Sawdust and sugarcane bagasse were the best substrates for growing of oyster mushroom than other agro-based substrates (Ahmed, 1998). Wide spread malnutrition with ever increasing protein gap in our country has necessitated the

search for alternative source of protein because the production of pulses has not kept pace with our requirement due to high population growth. Sanchez *et al.,* (2002) stated that the cultivation of *Pleurotus* sp. is one of the major techniques for the bioconversion of agricultural wastes into a protein rich food which will sustain the food security for the people of developing countries. Several technologies has been developed for the cultivation of oyster mushroom to increase the productivity of mushroom as described by Holker *et al.* (2004).There are different methods of oyster mushroom cultivation like bag, shelf, log, bottle, tray, jar and grid-frame as described by Stamets (2000) but the most adopted cultivation technique is bag and shelf cultivation (Choi, 2003). Thomas *et al*., (1998) stated that various agricultural wastes rich in cellulose can be used as substrates for cultivation of dhingri mushrooms and about 200kinds of wastes in which this mushroom can be grown as reported by Poppe (2000). *Pleurotus* species requires about 20 to 30°C temperature both for its vegetative growth and reproductive phase in natural habitat (Chang and Miles, 2004).Oyster mushrooms have ability to grow at wide range of temperatures and utilizing various lignocellulose substrates (Khan and Garcha, 1984). Hassan *et al*., (2011) suggested that oyster mushroom can be grown on various substrates including paddy straw, maize stalks, vegetable plant residues, sugarcane bagasse etc. and this substrates influences its growth, yield and composition (Iqbal *et al*., 2005; Kimenju *et al*., 2009; Khare *et al*., 2010). However, an ideal substrate should contain nitrogen as supplement and carbohydrates for rapid mushroom growth. Oyster mushroom can be cultivated in different waste materials and a very wide range of waste materials have been used. Several report on the suitability of various substrates for mushroom production suggested rice straw (*Oryza sativa*), wheat straw (*Triticum aestivum*), ragi straw (*Elucine coracana*), bajra straw (*Pennisetum typhoides*), sorghum straw (*Sorghum vulgare*), maize stalk (*Zea mays*) reported by Bano *et al*.,1987; Goswami *et al*.,1987 and Gupta and Langar,1988. Woods of poplar (*Populus robusta*), oak (*Quercus leucotrichopora*), horse chest nut (*Aesculus indica*), *Acasia* sp., chopped banana pseudo stem, cotton stalk, pea shells and poplar saw dust has also been used for cultivation (Philippoussis *et al*., 2001; Zervakis *et al*., 2001). Pavlik (2005) also stated that the production of *Pleurotus* mushroom varies with the species of trees used for cultivation and investigation revealed that the production ranges from 21% biological efficiency for beech wood to 3% BE for alder woods. The growth of oyster mushroom also requires high humidity (80-90%) and temperature (25-30°C) for the vegetative growth called spawn running and a lower temperature (18-25°C) for fruit body

formation as described by Onyango *et al*., (2011) and like other mushrooms, oyster mushroom can be grown on various agricultural waste with the use of different technologies. According to Zhang *et al* (2002), different types of straw can be used for *Pleurotus* cultivation and straw can be used as composted or pasteurized form. Higher yield was observed of *Pleurotus sajor-caju* when it was grown in ground straw than the chopped straw. It was also observed that 10% more yield obtained in case on paddy straw and using more quantity of spawn also improves the biological efficiency. Obodai *et al.* (2003) also reported that paddy straw is the best substrate for the cultivation of *Pleurotus ostreatus* supplemented with banana leaves, corn husks, rice husks and elephant grass. On the contrary, wheat straw supplemented with *Lolium perenne* grass chaff stimulate the fruit body formation and yield of *P. pulmonarius* (Domondon *et al;* 2004) Studies of Ahemad *et al* (2009) revealed that cultivation of *Pleurotus florida* on different agro wastes showed significant better result and it was found that using soybean straw give higher yield, crude protein content and phosphorus content in compare to paddy straw and wheat straw. While maximum water content and crude fibre was found in case of paddy straw cultivated oyster mushroom. Sawdust is one of the main substrate and studies revealed that biological yield was highest found in case of mango plant saw dust followed by mahogany and sirish as described by Islam *et al* (2009). Cost benefit analysis revealed that the Mango sawdust and Shiris sawdust were promising substrates for the growing of oyster mushroom (*Pleurotus flabellatus*).

Table 2: Mushroom production in India

Sl. No.	State	Amount of Mushroom Production (tons)				
		Button Mushroom	Oyster Mushroom	Milky Mushroom	Other Mushroom	Total Production
1.	Andhra Pradesh	2,992	15	15	0	3,022
2.	Arunachal Pradesh	20	5	0	1	26
3.	Assam	20	100	5	0	125
4.	Bihar	400	80	0	0	480
5.	Chhattisgarh	0	50	0	0	50
6.	Goa	500	20	0	0	520
7.	Gujrat	0	5	0	0	5
8.	Haryana	7,175	0	3	0	7,178
9.	Himachal Pradesh	5,864	110	17	2	5,993
10.	Jammu & Kashmir	565	15	0	0	580
11.	Jharkhand	200	20	0	0	220
12.	Karnataka	0	15	10	0	25
13.	Kerala	0	500	300	0	800
14.	Maharashtra	2,725	200	50	0	2,975
15.	Madhya Pradesh	10	5	0	0	15
16.	Manipur	0	10	0	50	60
17.	Meghalaya	25	2	0	0	27
18.	Mizoram	0	50	0	0	50
19.	Nagaland	0	75	0	250	325
20.	Orissa	36	810	0	5,000	5,846
21.	Punjab	58,000	2,000	0	0	60,000
22.	Rajasthan	100	10	0	10	120
23.	Sikkim	1	2	0	0	3
24.	Tamil Nadu	4,000	2,000	500	0	6,500
25.	Tripura	0	100	0	0	100
26.	Uttarakhand	8,000	0	0	0	8,000
27.	Uttar Pradesh	7,000	0	0	0	7,000
28.	**West Bengal**	**50**	**50**	**0**	**0**	**100**
Union Territories						
1.	A & N Island	0	100	0	0	100
2.	Chandigarh	0	0	0	0	0
3.	Dadar & Nagar Haveli	0	0	0	0	0
4.	Daman & Diu	0	0	0	0	0
5.	Delhi	3,000	50	20	0	3,070
6.	Lakshadweep	0	0	0	0	0
7	Puducherry	0	0	0	0	0
	Total Production	**1,00,683**	**6,399**	**920**	**5,313**	**1,13,315**

Source: RMCU, Directorate of Mushroom Research, ICAR, Solan.

Nutritional value of oyster mushroom

Mushrooms are rich in proteins, minerals and vitamins and it is popularly known as the vegetarian's meat. Kurtzman (1976) stated that mushroom protein is considered to be intermediate between that of animal and vegetables. It also contains essential amino acids required for human health (Hayes and Haddad, 1976). Shah *et al.,* (2004) stated that mushrooms has high nutritive and medicinal value and contributes to a healthy diet because of its rich source of vitamins, minerals and proteins. As reported by Sharma and Madan (1993), the oyster mushroom (*Pleurotus ostreatus*) is an edible mushroom having excellent flavour and taste and it is characterized by its high protein content (30–40% on dry weight basis). Mushrooms contains about 85-95% water, 3% protein, 4% carbohydrates, 0.1% fats, 1% minerals and vitamins as reported by Tewari, (1986). According to Eswaran and Ramabadran, (2000) mushrooms have been identified as a good source of food for alleviate malnutrition in developing countries for its flavour, texture, food value and its production capacity per unit area. A high nutritional value of oyster mushrooms has been reported by Stanley (2011) which states that oyster mushroom contains about 25-50% protein, 2-5% fat, 17-47% sugars, 7-38% myco-cellulose and minerals (potassium, phosphorus, calcium, sodium) of about 8-12%. Edible mushrooms are also rich in vitamins such as niacin, riboflavin, vitamin D, C, B1, B5 and B6 as recorded by Syed *et.al*. (2009).

According to Manzi *et.al.* (2001), cultivated mushrooms have higher protein contents and minerals, low in fat and rich in vitamins B, vitamin D, vitamin K and sometimes vitamins A and C. In 1991, Peter reported that mushroom a food of high quality, flavour and nutrition value have high content of protein, low content of fat (4%), vitamins (B1, B2, C, niacin, biotin etc), minerals (P, Na, K, Ca) and high content of fiber and carbohydrates. Pandey and Ghosh, (1996) also recorded the chemical composition of the fresh fruiting bodies of oyster mushroom which clearly states that a large quantity of moisture (90.8%), whereas fresh as well as dry oyster mushrooms are rich in proteins (30.4%), fat (2.2%), carbohydrates (57.6%), fiber (8.7%) and ash (9.8%) with 345 K (cal) energy value on 100 g dry weight basis; while vitamins such as thiamine (4.8 mg), riboflavin (4.7 mg) and niacin (108.7 mg), minerals like; calcium (98 mg), phosphorus (476 mg), ferrous (8.5 mg) and sodium (61 mg) on 100 g dry weight basis was also found to be present. Biochemical studies revealed that many bioactive compounds such

as hemicellulose, polysacchirides, glycoproteins, lipopolysacchirides, peptides triterpinoides has een isolated from variour species of oyster mushroom (Asfors and Ley., 1993; Tzianabos, 2000; Daba and Ezeronye, 2003 and Lindequist *et al*, 2005) According to Bauh *et al.*(2010), oyster mushroom also contains appreciable amounts of potassium, phosphorus, copper and iron but have low levels of calcium. Mushroom protein is intermediate between that of animals and vegetables. Oyster mushroom has no starch, low sugar content and high amount of fibre, hence it serves as the least fattening food (Osei, 1996).

Medicinal and Therapeutic value of mushrooms

Mushrooms are not only the sources of nutrients but also a good source of therapeutic food useful in preventing diseases such as hypertension, diabetes, hypercholesterolemia and cancer as reported by Bobek *et. al.* (1995, 1999). Among the large resources of fungi, higher basidiomycetes especially the mushrooms are unlimited source of therapeutically useful biologically active agents (Table 3). There are approximately 700 species of higher basidiomycetes that have been found to possess significant pharmacological activities as reported by Mizuno (1995) and Wassar (2002). Zaidman (2005) reported that the modern study on medicinal mushroom have expanded exponentially during last two decades and it not only restricted in China, Japan and Korea only but also spread over the USA and it was scientifically explained that compounds derived from the mushrooms have important function in human system. Medicinal mushrooms have an established history of use in traditional oriental medicine and many of them are *Auricularia, Flammulina, Ganoderma, Grifola, Lentinus, Pleurotus, Agaricus* which possess a wide range of medicinal properties. According to Manzi *et. al*. (2001) these functional characteristics are mainly due to the presence of dietary fibre and in particular chitin and glucans. The significant pharmacological effect and physiological properties of mushrooms are bio-regulations, maintenance of homeostasis and regulation of biorhythm, cure of various diseases and prevention and improvement from life threatening diseases such as cancer, cerebral stroke and heart disease. Manzi *et al* (2001) also explained that mushroom have been reported to be of therapeutic value, useful in preventing disease such as hypertension, hypercholesterolemia, cancer and also having antibacterial and antiviral properties and these functional characteristics are mainly of their chemical compositions.

Table 3: Major medicinal compounds extracted from different medicinal mushrooms

Name of the mushrooms	Name of the Compounds	References
Ganoderma lucidum	Polysaccharide (GI-PS)	Zhang and Lin, (2004)
Ganoderma applanatum	Exopolymer (GAE)	Yang *et al* (2007)
Agaricus subrufescens	Beta glucans and oligosaccharides (AO)	Kim *et al*. (2005)
Cordyceps sinensis	Polysaccharide CSP-1	Li *et. al*. (2006)
Grofola frondosa	Alpha-glucan (MT-alpha glucan)	Kubo *et al*., (1994) Konno *et al*., (2001)
Pleurotus ostreatus	Ostreolysin	Berne *et al* (2002)
Pleurotus eryngii	Eyringin and Eryngiolisin	Wang and Ng (2004); Ngai and Ng (2006)

Oyster mushroom as a source of medicinal value

Oyster mushrooms are considered to be as functional food because of their positive effect on human being in various ways (Sadler and Saltmarsh; 1998), which comprises many microbial, plant and animal origin containing several biologically active compounds beneficial for human health and reducing the risk of chronic diseases. It also contains dietary supplements, medicinal foods, phyto-chemicals as described by Hasler (1996).There is a common saying that "medicines and foods have a common origin" (Kaul, 2001). Chang (2007) reported that mushrooms possess a wide range of metabolites consists of pharmaceutical values e.g. antitumour, immunomodulatory, antioxidant, anti-inflammatory, hypocholesterolaemic, antihypertensive, anti-hyperglycaemic, antimicrobial and antiviral activities. Major medicinal properties attributed to mushrooms include anticancer, antibiotic, antiviral activities, immunity and blood lipid lowering effects. *Pleurotus* spp. are also rich in medicinal values. Various studies have been performed to evaluate the medicinal properties and it was found that *Pleurotus florida* has antioxidant and antitumor activities (Nayana and Janardhanan, 2000; Manpreet *et al*., 2004), *Pleurotus sajor-caju* has hypertensive effects through its active ingredients which affect the renin- angiotensin system (Chang, 1996), *P. ostreatus* possesses antitumor activity (Yoshioka *et al*., 1985) and hypoglycaemic effects in experimentally diabetic induced rats (Chorvathova *et al*.,

1993). Oyster mushrooms are very effective in reducing the total plasma cholesterol and triglyceride level (Alam *et al*., 2007) and thus reduce the chance of atherosclerosis and other cardiovascular and artery related disorders. Fruiting body as well as actively growing mycelia of different species of *Pleurotus* also contains a number of therapeutic values such as anti-inflammatory, immunomodulatory, immunostimulatory (Asfors and Ley; 1993), anticancer activity (Wasser; 2002) and ribonuclease activity (Wang and Ng; 2000). *Pleurotus* mushroom produces a wide range of bioactive compounds which actively helps in improving human health and the medicinal properties are as follows:

Antimicrobial properties

Oyster mushroom has been explored to active against several microbes and thus helps in defense mechanisms. Gerasimenya *et al* (2002) reported that the water extract of *Pleurotus ostreatus* can inhibit the growth of *Aspergillus niger* while some other extracts of *P. ostreatus* can inhibit the growth of *Bacillus sp, E. coli, Vibrio cholera* and *Salmonella typhi* (Periasamy, 2005). It also helps to reduce the pathogenic effect of *Eschrichia coli, Staphylococcus epidermidis, S. aureus* (Akyuz *et al.,* 2010) and species of *Candida* (Wolff *et al.,* 2008), *Streptococcus, Enterococcus* (Kotra and Mobashery, 1998; Sandven, 2000; Thomson and Moland, 2000). Antimicrobial and antifungal activity of *Pleurotus* sp depends upon the nature of the solvent, ether extract were more active against *Bacillus subtilis, E. coli* and *Saccharomyces cerevisiae* as compared to acetone extract as described by Iwalokun (2007). Another study by Nithya and Raghunathan (2009) revealed that *P. sajor-caju* showed higher antimicrobial activity against *Pseudomonas aeruginosa* and *E. coli* when compared with *S. aureus* (Table 4). On the contrary, it was reported that bioactive compound 'Eryngin' and 'Eryngiolysin' isolated from *P. eryngii* actively inhibit the growth of *Fusarium oxysporum* and *Mycosphaerella archidicola* (Wang and Ng, 2004) *Bacillus* spp. (Ngai and Ng, 2006) respectively.

Table 4: Antimicrobial activity of *Pleurotus* sp.

Species	Compound extracted	Effective against	References
P ostreatus	Crude extract from fermentation broth	Gram positive, Gram Negative and *Aspergillus niger*	Gerasimenya *et al* (2002)
	Various extracts; two main unidentified extracts	*Bacillus sp., E. Coli, Vibrio cholerare, Salmonella typhi*	Periasamy (2005)
	Hexane dichloromethane extract containing p-anesaldehyde	*Bacillus subtilis, Pseudomonas aeruginosa, Aspergillus niger* and *Fusarium oxysporum*	Okamoto *et al.* (2002)
P. sajor-caju	12kDa ribonuclease	*Fusarium oxysporum, Pseudomonas aeruginosa* and *staphylococcus aureus*	Ngai and Ng (2004)
P. eryngii	Eyringin- an antifungal peptide	*Fusarium oxysporum* and *Mycospaerella arachidicola*	Wang and Ng (2004)
	Eyringiolysin- a haemolysin	*Bacillus sp*	Ngai and Ng (2006)

Antitumor activity

The most significant medicinal effect of mushrooms and their metabolites that have attracted the attention is their antitumor properties. Lucas has first demonstrated the antitumor activity of higher basidiomycetes in 1957 (Lucas *et. al.* 1957). Wasser, (2002) stated that there are approximately 650 species of higher Basidiomycetes that have been found to possess antitumor activity. Various crude extracts of *Pleurotus* sp have been shown to possess strong antitumor activity. Jose and Janardhanan (2000, 2002) reported that the methanolic extract of the fruit body of *P. florida* and *P. pulmonaris* significantly reduces the tumors in mice. Li *et al* (2008) revealed that Lectin isolated from *P. citrinopileatus* showing a potent antitumor activity in mice bearing sarcoma S-180. *In vitro* studies revealed that extracts of *P. ostreatus* active against cancer cell. This type of cytotoxic effect was due to the presence of higher content of flavonoids in the fruiting bodies. Several components have been isolated from the extracts of different *Pleurotus* sp and among the isolated polysaccharides some are known to be a potent antitumor and immunomodulating substances (Wassar, 2002; Zhang *et al.,* 2007). Maiti *et al* (2011) reported that Cibacron blue affinity of purified protein, protein fraction extracted from *P. ostreatus* having a higher antitumor activity against different tumour inducing agents. Zhang *et al* (2004) reported that the polysaccharides extracted from the fruiting body and mycelia of *P. tuber-regium* effectively inhibit the solid tumour proliferation in mice. Wang *et al.* (2000) has reported that a lectin isolated from the *P. ostreatus* successfully inhibit the growth of sarcoma and hepatoma in mice. Antiproliferative effect was also observed in case of

another lectin isolated from *P. eous* without causing any cytotoxicity as described by Mahajan *et al* (2002). Thekkuttuparambil *et al.,* (2007) was found that *Pleurotus florida, Pleurotus pulmonaris* and *Ganoderma lucidum* possessed profound antioxidant and antitumor activities.

Antioxidant activity

In the year 2001, Kaul quoted a very important statement that "medicines and food have a common origin". Chang (1996) also stated that mushroom is an important food item and a very good source of nutrition as well as therapeutic properties concerning human health. Mushrooms can be a producer of such bioactive compounds with free radicle scavenging activity as mushroom fruiting body can be produced very early as well as the mycelia can be grown in very short time and also the medium can be designed to produce optimal level of bioactive compounds (Chang R., 1996; Chang S., 1999; Lindequist *et. al*., 2005; Wassar, 1999). There are several species of *Ganoderma, Grifola* and *Pleurotus* having antioxidant activities and it was scientifically proved that *P. ostreatus* having great antioxidant value (Kaul, 2001; Zhang *et. al.* 2002; Khan *et al.* 2010). Bhatnagar *et. al.* (2008) reported that hypercholesterolemia has an important role in inducing the oxidative stress and it is related to diabetes. More over the oxidative stress causes a wide range of disease such as Alzheimer's disease, neurodegeneration, ageing etc. and most importantly cancer as suggested by Nunomura *et al.* (2006), Wood-Kaczmar *et al.* (2006) and Khan *et al* (2010). Klein and Perry (1982) explained that the wild mushrooms exerts vitamin C which is very effective against heart disease, neurological disorders, cataractogenesis, cancer and the compounds were found through HPLC reaction against 2,6-dichlorophenolindophenol. Thekkuttuparambil *et. al.* (2007) and Wassar SP (2002) reported that the medicinal mushrooms namely *Ganoderma lucidam, Pleurotus pulmonaris, Pleurotus florida* possess to profound the medicinal activities. Methanolic extract of the mushrooms effectively reduces the ferric ions in FRAP assay as well as scavenged DPPH radicles. Kasuga *et al.* (1995) suggested that mushrooms are considered to be a good source of proteins and phenolic compounds, such as variegatic acid and diboviquinone for determination of antioxidant activity antioxidant compounds (phenolics and flavonoids) content, scavenging capacity on $DPPH^{\cdot}$ and reducing power have been investigated. Oxygen allows aerobic organisms to use energy stored in foodstuffs, such as carbohydrates, fats, and protein. Halliwell B,

Gutteridge, (1989) revealed that it is experimentally proven that this catabolic process can generate oxygen free radicals and other reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet-}$), hydroxyl radical, and hydrogen peroxide (H_2O_2). Under normal physiological conditions, the majority of free radicals are produced in the mitochondrial electron transport chain since 90% of the oxygen consumption by the body is reduced to water in the mitochondria as reported by Ames *et al* (1995). ROS have a strong tendency to extract electrons to reach a chemically more stable structure; therefore, they are capable of eliciting oxidative damage to various cellular components (Yu, 1994). Antioxidants from natural sources play a vital role in helping endogenous antioxidants to neutralize oxidative stress. Fruit bodies of mushrooms are appreciated, not only for texture and flavour, but also for their chemical and nutritional properties. Wild edible mushrooms are traditionally used in many Asian countries in both food and medicine was also proposed by Isildak *et al*, (2004); Sanmee *et al*, (2003); Manzi *et al* (1999)

Anti-Inflammatory activity

Oyster mushroom possesses a wide range of medicinal properties and different extracts of *Pleurotus pulmonarius* and *Pleurotus florida* helps on lowering the acute as well as chronic inflammation as reported by Josh *et al* (2002, 2004). Jose *et al.*, (2002) explained that the Methanolic extract of fruiting bodies of *Pleurotus pulmonaris* reduces carrageenan-induced and formalin-induced pawedema in mice when compared to the diclofenac (10 mg/kg). Bobek *et al* (2001) reported one compound 'Pleuran' isolated from oyster mushroom showing anti-inflammatory activity. Sano *et al.* (2002) demonstrated that oral or percutaneous administration of *Pleurotus eryngii* successfully suppresses the inflammation in delayed type allergy response in mice.

Antidiabetic property

Diabetes is one of the major disease worldwide and Wild *et. al.* (2004) reported that in the year 2000 about 171 million people or 2.8% of the world population suffers from severe diabetic. It causes severe morbidity and increasing cardiovascular mortality and also enhances the development of nephropathy, neuropathy as well as retinopathy (Zimmet *et. al*. 1997). There are two types of diabetes out of which type I diabetes occurs due to less amount of insulin and as a result of destructive lesions of pancreatic β-cells where as in case of type II diabetes the pancreatic β-cells and their function are preserved to some extent and injection is seldom to sustain life as reported by Kuzua *et.*

al.(2002) and Kobayashi, 1994. Windholz (1983) demonstrated that the compound Guanide related to bi-guanide class of oral antidiabetic drug was isolated from *Pleurotus* showing antihypoglycemic effect. Kim *et al* (1997) also reported that endo polymer from submerged culture of *P. ostreatus* possess hypoglycemic effect (Table 5). Aqueous extract of *P. pulmonarius* upon oral administration decrease serum glucose level in aloxan induced diabetic rats (Sachin *et al*., 2006). Lee *et al.,* (2007) and Hu *et al*., (2006) demonstrated that mushrooms represent a major source of powerful pharmaceutical products and they are exemplary sources of natural medicines. *Pleurotus citrinopileatus,* an edible mushroom belonging to the Pleurotaceae family, has some physiological effects, including antitumor, immune enhancement, anti-hyperglycaemia.

Table 5: Different species of mushroom showing hyperglycaemic activity against different animal models

Sl No	Organism	Extract/ Fraction and Dose	Experimental model	Observations	References
1	*Agaricus bisporus*	Dehydrated fruiting body extracts 400mg/kg body wt.	Streptozotocin induced diabetic rats	Serum glucose levels decreased by 29.68 % and insulin levels increased to 78.5 %	Yamac *et al* (2010)
2	*Agaricus bisporus*	Powdered fruiting bodies 200 mg/kg for 3 weeks, p.o.	STZ-induced diabetic male Sprague-Dawley rats	Significantly reduced plasma glucose, total cholesterol, low-density lipoprotein (LDL), levels	Jeong *et al* (2010)
3	*Agaricus subrufescens*	β-glucans and enzymatic-ally produced oligo-sacchari-des	Diabetic rats	Anti-hyperglycemic; anti-hypertrigly-ceridemic, anti-hypercholes-terolemic, and anti-arteriosclerotic activity	Kim *et al,* (2005)
4	*Auricularia auricula-judae*	Water-soluble poly-saccharide from fruiting bodies 30 g/kg; in diet	Genetically diabetic KK-Ay mice	Significant effect in lowering plasma glucose, insulin, urinary glucose, and food	Yuan *et al* (1998)

				intake; increased tolerance to intraperitoneal glucose loading and the hepatic glycogen content	
5	*Cordyceps militaris*	Exo-polymers produced from submerged mycelia cultures 50mg/kg for 7 days, p.o.	STZ-induced diabetic rats	Significantly decreased levels of plasma glucose, total cholesterol, triglyceride and plasma glutamate-pyruvate transaminase (GPT)	Kim *et al*, (2001)
6	*Cordyceps sinensis*	Polysaccharide fraction CSP-1, isolated from cultured mycelia 200 and 400mg/kg/ day for 7 days, p.o.	Normal; alloxan and STZ-induced diabetic rats	Significant drop in blood glucose levels and increased serum insulin levels, stimulation of pancreatic release of insulin and/or reduced insulin	Li *et al* (2003;2006)
7	*Ganoderma lucidum*	Aqueous extract of fruiting bodies 500 and 1000 mg/kg, p.o.	Alloxan induced and normal Wistar rats	Significant hypoglycemic and antihyper-glycemic effects	Mohammed *et al* (2007)
8	*Grifola frondosa*	Powdered fruiting body 1g/day, p.o.	Genetically diabetic mouse (KK-Ay)	Reduced levels of blood glucose, insulin and triglycerides	Kubo *et al* (1994)
9	*Grifola frondosa*	Fermented G. frondosa rich in vanadium i.g. route	Alloxan- and adrenalin-induced hyperglycemic mice	Significant decrease in blood glucose levels	Cui *et al* (2009)
10	*Lentinula edodes*	Exopolymers produced from mycelia cultures 50 mg/kg for 7 days, p.o.	STZ-induced diabetic rats	Significant reduction in plasma glucose, total cholesterol	Kim *et al* (2001)
11	*Phellinus baumii*	Crude exopolysaccharides from submerged mycelial cultures 200 mg/kg, p.o.	STZ-induced diabetic rats	Hypoglycemic effect with substantially reduced plasma glucose levels	Hwang *et al*, (2005)

12	*Phellinus baumii*	Exopolysacch-arides (EPS) produced by submerged mycelial culture 200 mg/kg for 52 days, p.o.	ob/ob mice	Reduced plasma glucose levels, increased glucose disposal, reduced blood triglyceride levels	Cho *et al* (2007)
13	*Pleurotus citrinopileatus*	Water-soluble polysaccha-rides (WSPS), extracted from submerged fermented medium 0.4 g/kg, in diet	STZ-induced diabetic rats	Reduced fasting blood glucose levels	Hu *et al*, (2006)
14	*Tremella fuciformis*	Glucuronoxyloman nan (AC) from the fruiting bodies Oral administrat-ions of the AC solution	Normal and STZ-induced diabetic mice	Significant dose-dependent hypo-glycemic activity	Kiho *et al* (1994)
15	*Pleurotus pulmonarius*	Aqueous extract of fruiting bodies 250, 500, and 1000 mg/kg, p.o.	Normal and Alloxan-induced diabetic mice	Antihyper-glycemic effect (increased glucose tolerance in both normal and diabetic mice)	Badole *et al*, (2008)
16	*Pleurotus eryngii*	Freeze-dried, powdered fruiting body Diet containing 5% freeze dried mushroom	Male db/db mice	Reduced total cholesterol, triglyceride levels, and increased high density lipoprotein cholesterol levels with improved insulin sensitivity	Kim *et al* (2010)
17	*Pleurotus ostreatus*	Powdered fruiting bodies Diet containing 4 % mushroom	Type 2 diabetic rats	Significantly lower basal and postprandial glycaemia.	Chorváthová *et al* (1993)
18	*Pleurotus ostreatus*	Ethanol extract of fruiting bodies 100 and 200 mg/kg for 30 days, p.o.	STZ - induced diabetic rats	Significant decrease of blood glucose levels, genetic alterations	Ghaly *et al* (2011)
19	*Wolfiporia extensa*	Crude extract containing dehydro- tumulosic acid, dehydro-trametenolic acid and pachymic acid	STZ-induced diabetic mice	Insulin sensitizer activity	Sato *et al*, (2002) Li *et al*, (2011)

Anti-hyperlipidemic activity

Hyperlipidemia is now a days a very high risk factor for atherosclerosis. Total lipid and cholesterol excretion increases by feeding mushroom powder. Gunde-Cimerman *et al* (2001) and Hossain *et al* (2003) explained that Mevonolin- a polysaccharide present in the fruiting body of *Pleurotus ostreatus* and *P. citrinopileatus* shows higher antihyper cholesterolemic activities. Badole *et al* (2008) also reported that *P. pulmonaris* also exhibits synergistic antihyper cholesterolemic activity when used in combination with Glyburide. According to Chang and Buswell (1996), Lovastatin isolated from the oyster mushroom an active chemical component is used in San Francisco to reduce LDL cholesterol in hypercholesteraemic patients.

Immunomodulatory activities of oyster mushroom

Oyster mushroom has been reported to have a wide range of medicinal activities and recent studies revealed that many compounds extracted from different species of oyster mushroom showing immunomodulatory activities on humoral and cell mediated immunity of human beings. Wang *et al* (2005) reported that water soluble polysaccharide extracted from the fermented *Pleurotus citrinopileatus* administered in albino mice that helps in increasing the number of macrophages, T-cells and $CD4^+$ and $CD8^+$ cells. *In Vitro* studies of mouse macrophages actively responses against the phagocytic response by the glucans isolate from *P. florida* as described by Rout *et al.* (2005) and significantly induced the proliferative response as well as phagocytic activity of fish leukocytes *in vitro* (Kamilya *et al* 2006). Sarangi *et al* (2006) stated that the proteoglycans isolated from the *Pleurotus ostreatus* showing immunomodulatory activity by elevating mouse natural killer cell cytotoxicity and by macrophage stimulation. Ngai and Ng (2004) revealed that a ribonuclease extracted from *Pleurotus sajor-caju* fruiting bodies exerts antiproliferative effect against murine splenocytes.

Antiviral Activity of oyster mushroom

In the year 2000, Brandt and Piraino stated that mushroom contains substances that show indirect antiviral effects as an immunostimulatory activity. Wang *et al* (2007) demonstrated that hot water extract of *Pleurotus sajor-caju* and *Pleurotus pulmonarius* active against human immunodeficiency virus (HIV1) reverse transcriptase. Anti-HIV activity was also demonstrated for ubiquitin like protein isolate from the fruiting body of *Pleurotus ostreatus* (Wang and Ng; 2000).

Anti-Ageing Effect

Extracts of *Pleurotus* is a very potent antiaging effect Shashoua and Adams (2004) revealed that the extract of *Pleurotus abalonus* elevate the levels of Vitamin C and E which increase the activity of catalase, superoxide dismutase and glutathione peroxidase in aged rats. Similar results were also found in case of *P. ostreatus* as reported by Jaykumar *et al* (2007).Different extracts (Water, ethanol and Methanol) of *Pleurotus* can improve the antioxidant status during ageing leads to reduce the occurrence of age associated disorders like stroke, atherosclerosis, diabetes and cirrhosis.

Hepatoprotective activity

Liver damage by hepatotoxic agents is of vital consequence because chronic liver injury leads to fibrosis, end stage cirrhosis and hepato-carcinoma. Many species of *Pleurotus* contains some active compounds like glucan, phenol and vitamin C that increase the activity of antioxidant enzymes like catalase, glutathione reductase are responsible for reduction of hepatic cell necrosis (Bobek *et al*., 1997; Fu *et al.*, 2009). Koyama *et al* 2006 reported that hepatoprotective activity of this mushroom is exerts through the increased levels of serum aminotransferase enzymes in animals. Recently Refaie *et al* (2009) suggested that the hepatoprentive and therapeutic activity of hot water extract of *P. ostreatus* by mechanism of inhibition through preventive regimen caused less leakage of alkaline phosphatase, less pronounced increase inhepatic malondialdehyde concentration, less notable reduction in hepatic total protein activities. It was also observed that water soluble polysaccharides extracted from *P. eryngii* removes the free radicals and also increase the activities of antioxidant enzymes in liver injury mouse model (Chen *et al.*, 2012).

Antimutagenic Effect

Mutagenesis also can be influenced by the edible mushrooms and Filipic *et al* (2002) demonstrated that *Pleurotus cornucopiae* showed most effective against the antigenotoxic and bio-antimutagenic activity against *E. coli* and *S. typhimurium.* Bohi *et al* (2005) reported that dried *P. ostreatus* in diet reduces pathological changes in dimethyl hydrazine induced rats and it also mitigated genotoxicity through suppression of DNA damage induced different mutagens in Drosophila DNA repair test (Taira *et al.,* 2005).

Hypotensive activity

Miyazawa *et al.* (2008) explain that the antihypertensive activity varies in different mushroom and oyster mushroom possesses blood sugar lowering activity. In the year 2003 Miyazawa *et al* reported that the hot water extract of *P. nebrodensis* shows their activity in prevention of hypertension.

Oyster Mushroom as processed food

Human being consumes very wide range of food materials like plant, fish meat etc as well as fungi or edible mushrooms. Edible mushrooms are consumed for their nutritional as well as medicinal properties (Chocksaisawasdee *et al.,* 2010; Wan-Rosli *et al.*, 2011). Mushrooms are versatile and may be eaten freshly or cooked entirely. It is very popular for its texture, unique flavour and for its great nutritional importance. Mushrooms are very useful in reducing cholesterol lowering blood pressure and improve immune system against many diseases as suggested by Regula and Siwulski (2007). Sanchez (2010) stated that mushrooms are of great demand for their taste, texture and for the economical as well as ecological importance. He also reported that *Pleurotus ostreatus* is the second most cultivated mushroom in the world followed by *Agaricus bisporus* which constitute about 25% of total world's production. Chye *et al* (2008) explained that freshly oyster mushroom contains low fat content about 0.38-2.28% indicated low calorific value and consisting about more than 80% moisture content. Strmiskova *et al* (1992) stated that oyster mushroom is a good source of biologically valuable substances for human nutrition as it contains about 90% water, protein, all types of vitamin wide range of mineral contents and very low amount of fat. On the other hand, Kotawaliwale *et al* (2007) reported that dry mushrooms consists about more that 25% proteins less than 3% lipid and almost 50% total carbohydrates. Singh *et al* (1995) stated that mushrooms have a good potential of due to its high amount of protein, dietary fibres vitamins (dry weight basis) and minerals and thus it can be dried, powdered and can be used for fortification in baked products like bread biscuits.

Application of spent mushroom Substrate for crop improvement

Mushroom cultivation is an eco-friendly process to convert the waste biological materials to a nutritive food. However after harvesting of the mushroom, the waste

mushroom substrates can be harmful for environment for pollution stated by Bayers (1996). Uncontrolled disposal of SMC may pose a problem to the environment. This problem may include foul odour and other problems associated with air pollution. It is therefore, necessary to recycle this so called useless material to an utilizable .The demand for organic residues and compost has also increased several folds considering the ill effects of synthetic inorganic fertilizers. Jonathan *et al*, (2011) defined spent mushroom substrate (SMS) as the leftover of wastes after different flushes of mushrooms have been harvested. Fasidi *et al.,* (2008) also defined spent substrate as the bi-product of mushroom industry, after several flushes harvested. The weathered SMC are those that undergo further decomposition for several weeks before their utilization by farmers as soil conditioner (Jonathan *et al*., 2006). Ahlawat *et al.,* (2005) stated that the recycling of spent mushroom substrate for its utilization as manure is the alternative way to get rid of the environmental contamination due to spent mushroom substrate. Recycling of spent substrate as manure is now very popular for cultivation of different cereals and horticultural crops. Gupta *et al* (2004) and Pill *et al* (1993) explained that spent substrates are rich in organic matter and moderate nutrient load near neutral pH and the presence of beneficial microbial population make the spent substrate suitable for crop improvement. Ahlawat *et al.,* (2007) incorporation of composted SMS not only improves the soil quality, neutralize the soil acidity but it also helps in cultivation in the bare lands. Yohalem *et al* (1996) stated that SMS is a potential biocontrol agent against certain foliar and soil borne diseases and also a potential to bioremediate several agricultural grade fungicides and pesticides (Ahlawat *et al.,* 2010). Ahalawat *et al* (2009) also stated that spent mushroom substrate also helps in increasing the yield and improvement in plant health while used as singly or in combination of other organic fertilizers. The spent mushroom substrate was evaluated against some farm yard manure and chemical fertilizer on *Pisum sativum* and it was observed that SMS influences the growth of the plant in compare to other treatments and also the quality as well as the quantity of pods increase (Ahlawat *et al.*, 2011).

CHAPTER 3
MATERIALS AND METHODS

3.1. Fungal Culture

The pure culture of *Pleurotus ostreatus*, *Pleurotus sajor-caju*, *Pleurotus djamor* and *Pleurotus florida* was obtained from the Directorate of Mushroom Research, ICAR Solan, Himachal Pradesh, India. The cultures were maintained on Potato Dextrose Agar (PDA) and 2% Malt Extract Agar (MEA) slants and stored at 4^0 C (Fritsch 1978 and Jong 1978). Sub-culturing was done after every 15 days intervals.

A. Potato Dextrose Agar

Sr. No	Material	Quantity
1.	Potato	400gm
2.	Dextrose anhydrous	20gm
3.	Agar agar Type I	20gm
4.	Distilled Water	1000ml

B. Malt Extract Agar

Sr. No	Materials	Quantity
1.	Malt extract	30gm
3	Peptone	5gm
4	Agar agar Type I	20gm
5	Distilled water	1000ml

C. Water Agar

Sr. No	Materials	Quantity
4	Agar agar Type I	20gm
5	Distilled water	1ltr.

3.2. Histopathological characterization of *Pleurotus* sp.

Transverse sections of the mushroom gills were washed with sterile distilled water and stained with lacto phenol - cotton blue (1:1) and incubated for 5-10 min for staining. After staining mounted with cover slip and observed in the Leica DM3200 microscope using 20X and 40 X magnifications. Photographs were taken using Leica Application Suit (LAS Version 4.4.0) software.

3.3. Preparation of Spawn

Wheat grains were used to prepare the spawn for cultivation. Wheat grains were boiled for 20 min and then water drained off. Then the grains were allowed to dry for 4-5 hours in a clean and dry place. After drying, 0.5% (W/W) $CaCO_3$ and 2% (W/W) $CaSO_4$ was added and mixed well. The grains were then filled in the polypropylene bags (250 gm/bag) and autoclaved at 121^0 C for 1 hour. The grains were then inoculated with actively growing mycelia of *Pleurotus ostreatus*, *P. sajor-caju*, *P. djamor* and *P. florida* and incubated at $25\text{-}28^0$ C for 12-14 days until the mycelia fully cover the grains (Sanchez, 2010) (Figure 2).

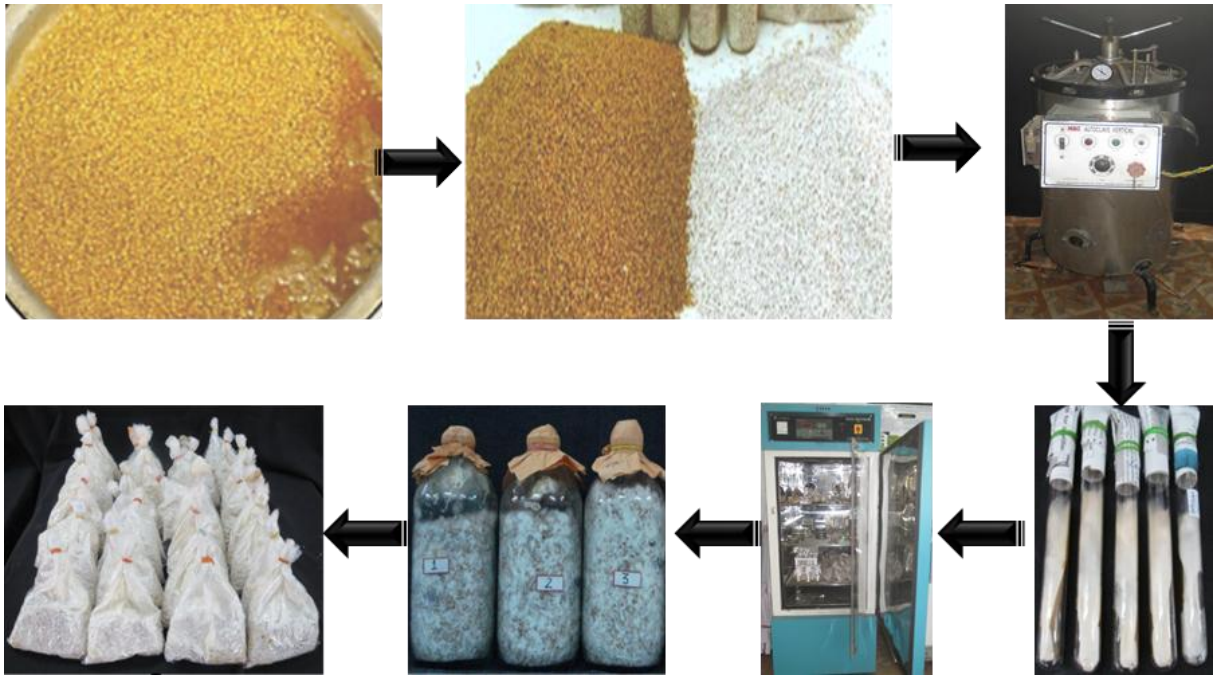


Figure 2: Preparation of grain spawn for the cultivation of *Pleurotus* sp.

3.4. Cultivation of Oyster mushroom

3.4.1. Preparation of substrates

Different agro wastes were used for the cultivation of different species of oyster mushroom. Paddy straw, wheat straw, saw dusts were used either singly or in combination with other substrates. Substrates were collected from the local markets. Straw were dried and chopped in 2-5cm long pieces and washed 3-4 times with tap water. Then the substrates were allowed to soak in water for overnight. Again it was again cleaned 2-3 times until the brown water drained off fully. After cleaning, the substrates were pasteurized at 55-65^0 C for 20-30 min and allowed to cool at room temperature.

3.4.2. Spawning

Spawning was done using different types of containers such as polypropylene bags (12 inch X 18 inch), plastic waste bottles (12 inch X 3 inch dia.), broken laboratory glass goods and paper boxes (30 inch X 12 inch X 12 inch) for the cultivation. Cooled pasteurized substrates were used singly or mixed and layer spawning was done using the fully grown spawn at 100gm/kg substrates following the methodology Sarkar *et al* (2007). The containers were closed tightly and small pores were made for aeration. The containers were then incubated at room temperature in the Mushroom Production Unit, Immuno-Phytopathology Laboratory, NBU (Figure 3) for 10-12 days. Mycelia cover the substrate and the polypropylene bags or the containers were removed and water sprayed over the fully grown mycelia to maintain the 80-90% humidity. After 3-4 days of removal of the container, the pinhead initiation was observed.

3.4.3. Initiation of fruiting body

The pinhead appeared after 4-5 days of opening the bags, lids of bottles or the box cover. Fruiting body was developed at room temperature and 80-90% relative humidity. The fruiting body was harvested from the base carefully so that there will be no damage on the fully grown mycelia. Humidity was again maintained by spraying water after the harvest of the mushrooms for more flushes (Figure 3).

Figure 3: Mushroom production unit for large scale production of *Pleurotus* sp established at Immuno phytopathology laboratory, Department of Botany, NBU

3.5. Biochemical analysis

3.5.1. Determination of moisture content

100 gram of fresh mushroom was weighed and dried in hot air oven at 100-105^{0}C and then cooled in room temperature. Moisture content of mushroom were estimated using the methodology described by Raghuramulu *et al.,* (2003). The moisture content was calculated as following equation

Moisture content (%) = (initial weight − final weight) × 100/weight of sample.

3.5.2. Extraction and estimation of Carbohydrates

Extraction of carbohydrate form the mushroom was done following the methodology of Harbone (1973). 1gm of sample was homogenated using 95% ethanol and then it was kept on boiling water bath for evaporation of the alcoholic fraction. The sample was then resuspended in distilled water. Then it was centrifuged at 10000 rpm for 15 min and the supernatant collected for further analysis.

3.5.2.1. Estimation of total sugar

Total sugar was estimated following the methodology described by Plummer (1973). 1ml of the supernatant was taken and to it 4ml of anthrone reagent (0.2gm anthrone in 100 ml H_2SO_4) was added and then it was kept in boiling water bath for 10 min. the sample was then cooled in tap water and observed at 620 nm weave length.

3.5.2.2. Estimation of reducing sugar

Reducing sugar of mushroom was estimated using the Nelson-Somogy's methodology. 1 ml of supernatant was taken and to it, 1 ml of alkaline copper tartarate added and warmed in water bath for 20 mins. Then it was cooled in tap water and 2 ml of arseno molybdate was added and then observed at 510nm in colorimeter.

3.5.3. Extraction and estimation of soluble protein

3.5.3.1. Mycelia

Mushroom protein was prepared following the protocol as outlined by Chakraborty and Saha (1994). For extraction of protein, different stages of fruiting body were harvested and incubated at -20^0C for 2 hours. The fruiting body was crushed with sea sand using a chilled mortar and pestle and homogenized with cold 0.05 M Sodium phosphate buffer (PH-7.2) supplemented with 0.85% NaCl, 10 mM sodium metabisulphite, PVPP (polyvinyl pyrollidine phosphate) and 0.5mM magnesium chloride in ice bath. The homogenated mixture was kept for 2h or overnight at 4ºC and then centrifuged at10,000 rpm for 30 min at 4ºC to eliminate cell debris. The supernatant was equilibrated to100% saturated ammonium sulphate under constant stirring in ice bath and kept overnight at 4ºC. After this period, the mixture was centrifuged (10,000 rpm) for 30 min at 4º C; the precipitate was dissolved in the same buffer (pH 7.2).

3.5.3.2. Leaf

Soluble protein was extracted from leaves following the method of Chakraborty *et al.*, (1994). Leaf tissues were frozen in liquid nitrogen and ground in 0.05mM sodium phosphate buffer (pH 7.2) containing 10 mM $Na_2S_2O_5$, 0.5 mM $MgCl_2$ and 2mM PVP was added during crushing and centrifuged at 4ºC for 20 min at 12000rpm.The supernatant was used as crude protein extract.

3.5.3.3. Estimation of soluble protein content

Soluble proteins were estimated following the method as described by Lowry *et al*, (1951). To 1ml of protein sample 5ml of alkaline reagent (1ml of 1% $CuSO_4$ and 1ml of 2% sodium potassium tartarate, added to 100 ml of 2% Na_2CO_3 in 0.1 NaOH) was added. This was incubated for 15 min at room temperature and then 0.5ml of 1N Folin-ciocalteau reagent was added and again incubated for further 15 min following which optical density was measured at 720 nm. Quantity of protein was estimated from the standard curve made with bovine serum albumin.

3.5.3.4. SDS-PAGE analysis of soluble proteins

Sodium dodecyl sulphate polyacrylamide gel electrophoresis was performed for detailed analysis of protein profile following the method of (Laemmli, 1970). For the preparation of gel the following stock solution were prepared.

3.5.3.4.1. Preparation of stock solution

Following stock solutions were prepared

A. Acrylamide and N'N' – methylene bis acrylamide

Stock solution containing 29% acrylamide and 1% bis-acrylamide was prepared in warm water, as both of them are slowly dominated to acrylic and bis acrylic acid by alkali and light. The pH of the solution was kept below 7.0 and the stock solution was then filtered through Whatman No. 1 filter paper and kept in brown bottle, stored at 4ºC and used within one month.

B. Sodium Dodecyl Sulphate (SDS)

A 10% stock solution of SDS was prepared in warm water and stored at room temperature.

C. Tris Buffer

i) 1.5M Tris buffer was prepared for resolving gel. The pH of the buffer was adjusted to 8.8 with concentrated HCl and stored at 4ºC for further use.

ii) 1.0 M Tris buffer was prepared for use in the stacking and loading buffer. The pH of this buffer was adjusted to 6.8 with conc. HCl and stored at 4ºC for use.

D. Ammonium persulphate (APS)

Fresh 10% APS solution was prepared with distilled water each time before use.

E. Tris- glycine electrophoresis buffer

Tris running buffer consists of 25mM Tris base, 250mM Glycine (pH 8.3) and 0.1% SDS. A 1X solution was made by dissolving 3.02 g Tris base, 18.8 g Glycine and10 ml of 10% SDS in 1000 ml distilled water.

F. SDS gel loading buffer

This buffer contains 50 mM Tris –HCl (pH 6.8), 10 mM ß- marcaptoethanol, 2%SDS, 0.1% bromophenol blue, 10% glycerol. A 1x solution was prepared by dissolving 0.5ml of 1M Tris buffer (pH 6.8), 0.5ml of 14.4 M ß- mercaptoethanol, 2 ml of 10%SDS, 10 mg bromophenol blue, 1ml glycerol in 6.8 ml of distilled water.

3.5.3.4.2. Preparation of gel

Mini slab gel (plate size 8cm x10cm) was prepared for analysis of proteins patterns through SDS-PAGE. For gel preparation, two glass plates (8 cmx10 cm) were washed with dehydrated alcohol and dried to remove any traces of grease. Then 1.5 mm thick spacers were placed between the glass plates at the two edges and the three sides of the glass plates were sealed with gel sealing tape or wax, clipped tightly to prevent any leakage and kept in the gel casting unit (table 6). Resolving and stacking gels were prepared by mixing compounds in the following order and poured by pipette leaving sufficient space for comb in the stacking gel (comb +1cm). After pouring the resolving gel solution, it was immediately over layered with isobutanol and kept for polymerization for 1h. After polymerization of the resolving gel was complete, overlay was poured off and washed with water to remove any unpolymerized acrylamide. Stacking gel solution was poured over the resolving gel and the comb was inserted immediately and over layered with water. Finally the gel was kept for polymerization for 30-45 minutes. After polymerization of the stacking gel the comb was removed and the wells were washed thoroughly. The gel was then finally mounted in the electrophoresis apparatus. Tris-glycine buffer was added sufficiently in both upper and lower reservoir. Any bubble trapped at the bottom of the gel, was removed carefully with a bent syringe.

Table 6: Composition of resolving and stacking gel for SDS-PAGE

Sl No	Chemical	Resolving Gel (ml)	Stacking gel (ml)
1	Distilled water	2.85	2.10
2	30% acrylamide	2.55	0.5
3	Tris*	1.95	0.38
4	10%SDS	0.075	0.030
5	10%APS	0.075	0.030
6	TEMED**	0.003	0.003

*For 1.5 M tris pH 8.8 in resolving gel and for 1M Tris pH 6.8 in stacking gel

** N,N,N‘,N‘ –Tetramethyl ethylene diamine.

3.5.3.4.3. Sample preparation

Sample (50µl) was prepared by mixing the protein extract (35 µl) with 1xSDS gel loading buffer (16 µl) in cyclomixer. All the samples were floated in a boiling water bath for 3 min to denature the proteins samples. After boiling, the sample was loaded in predetermined order into the bottom of the well with T-100 micropipette syringe. Along with the protein samples, a marker protein consisting of a mixture of six proteins ranging from high to low molecular mass (Phosphorylase b-97,4000: Biovine Serum Albumin-68,000; Albumin-43,000; Carbolic Anhydrase-29.000; Soybean Trypsin inhibitor-20,000; Lysozyme -14,300) was treated as the other samples and loaded in separate well.

3.5.3.4.4. Electrophoresis

Electrophoresis was performed at 18mA current for a period of two to three hours or until the dye reached the bottom of the gel.

3.5.3.4.5. Fixing and staining

After completion of electrophoresis, the gel was removed carefully from the glass plates and the stacking gel was cut off from the resolving gel and finally fixed in glacial acetic acid: methanol: water (10:20:70) for overnight. The staining solution was prepared by dissolving 250mg of Coomassie brilliant blue (Sigma R 250) in 45 ml of methanol. When the stain was completely dissolved,45 ml of water and 10ml of glacial acetic acid were added. The prepared stain was filtered through Whatman No.1 filter paper. The gel was removed from the fixer and stained in this stain solution for 4 h at 37ºC with constant shaking at low speed. After staining the gel was finally destained with

destaining solution containing methanol, water and acetic acid (4.5: 4.5:1) at 40ºC with constant shaking until the background become clear.

3.5.4. Determination of total lipid content

Total lipid was determined following the methodology described by Folch *et al.* (1957). Five gram of grinded mushroom was suspended in 50 ml of chloroform**:** methanol (2:1 v/v) mixture then mixed thoroughly and kept for 72 hours. The solution was filtrated and centrifuged at 10000 rpm for 15 min. The upper layer of methanol was removed by pasteur pipette and chloroform was evaporated by heating. The remaining was the crude lipid.

3.5.5. Determination of dietary fibre

Dietary fibre content was estimated from the Arbro Pharmaceuticals, New Delhi following the methodology AOAC (1995). 10 gm mushroom dried powder was taken in a beaker and 200 ml of boiling 0.255 N H_2SO_4 was added. The mixture was boiled for 30 minutes keeping the volume constant by the addition of water at frequent intervals. The mixture was then filtered and washed with hot water until the total acid removed from the residue. Then 200 ml of boiling 0.313 N NaOH was added and boiled for 30 minutes and again the mixture was filtered and washed with hot water till free from alkali. It was then dried overnight at 80-100^{0}C and weighed (We) in an electric balance. Then the sample was heated in a muffle furnace at 600^{0}C for 5-6 hours, cooled and weighed again (Wa). The difference in the weights (We- Wa) represents the weight of crude fibre.

Crude fiber (g/100 g sample) = [100 − (moisture + fat)] × (We-Wa)/Wt of sample

3.6. Application of spent mushroom substrate in crop plants

3.6.1. Experimental Design

Spent mushroom substrate was applied in pot as a soil conditioner for improvement of plant health status. To experiments were performed using the spent mushroom in two species of chili plant. The experimental design is as follows

A. The oyster mushroom (*Pleurotus ostreatus*) was cultivated using four different substrates separately and the spent substrates were dried completely under sunlight and mixed with the soil for experiment. In this experiment, soil was

prepared using different spent mushroom substrates as per the cultivation design of oyster mushroom. The design was like T1=paddy straw, T2= wheat straw, T3= paddy straw+ wheat straw, T4= paddy straw + sawdust and a control (untreated) pot without any treatments. No other organic or inorganic fertilizers were used in the growing media. Seedlings of *Capsicum chinense* were collected from the local market and seedlings were then transferred to the pre-treated pots.

B. In this experiment, seven different treatments were done using the spent mushroom substrate of oyster mushroom and button mushroom. The experiment was performed to compare the effect of spent substrate of different mushroom. Spent mushroom compost of button mushroom was collected from the immuno-phytopathology laboratory, Department of Botany, NBU. The treatments were such as Control (only soil), T1=Soil + Oyster mushroom leachate (100ml/kg soil), T2=Soil + oyster mushroom substrate fresh (250gm/kg soil), T3= Soil + Button mushroom leachate (100ml/kg soil), T4= Soil + Button mushroom spent compost fresh (250gm/kg soil), T5= Soil + Weathered Spent oyster mushroom substrate (250gm/kg soil), T6= Soil + Weathered Button mushroom compost (250gm/kg soil), T7= Soil + Fresh oyster mushroom substrate + Fresh button mushroom spent compost (250gm/kg soil). The experiment was designed in a complete randomized design which was set up in 3 replicates. Pots were placed in open field condition and no other organic or inorganic fertilizer used in the growing media.

C. Application of spent mushroom substrate of *Pleurotus* sp was also performed in *Solanum lycopersicum* and *Amaranthus* sp. Treatment of spent mushroom substrates were done in field condition in two plots (4ftX 4ft) using the freshly harvested spent mushroom substrate. Spent mushroom substrate (1kg) was amended in each plot and kept for 7days. 10day old seedling of *S. lycopersicum* was sowed and seed of *Amaranthus* sp were spread in field. Another two plots were also prepared and no treatment was done which was considered as untreated control plots.

3.6.2. Evaluation of growth promotion of plant

Effect of spent mushroom substrate was evaluate primarily on the morphological growth like plant height, number of leaves, and number of branches were recorded in regular intervals after the transfer of plants. Data was also collected during the flowering and fruiting period.

3.6.3. Extraction and quantification of phosphate from soil, root and leaf

Soil samples (1g, air dried) or plant materials (1g, oven dried) were suspended in 25 ml of the extracting solution (0.025N H_2SO_4, 0.05N HCl) and activated charcoal (0.01 g) was also added. The extracted samples were shaken for 30 min in a rotary shaker and filtered through Whatman No. 2 filter paper. Quantitative estimation was carried out following ammonium molybdate-ascorbic acid method as described by Knudsen and Beegle (1988).

3.6.4. Extraction and estimation of chlorophyll content

Total chlorophyll of plant leaf was estimated following the methodology as described by Harborne (1973). 1 gm of leaf sample was grinded using 10 ml of 80% acetone and then it was filtrated using the Whatman No 1 filter paper. The absorbance was taken spectrophotometrically at 663 nm and 645 nm and calculated using the following formula

$Chlorophyll_a = [12.7X\ A_{663}] – [2.67\ X\ A_{645}]$ µg/gm tissue

$Chlorophyll_b = [22.9\ X\ A_{645}] – [4.68\ X\ A_{663}]$ µg/gm tissue

Total Chlorophyll = $[20.2\ X\ A_{645}] + [8.02\ X\ A_{663}]$ µg/gm tissue

3.6.5. Extraction and estimation of carotenoid

Carotenoid content was also estimated from the fruit and leaves of the plant treated with spent mushroom substrate. 1gm sample was grinded in dark using 10 ml methanol and then it was filtrated by Whatman No1 paper and used as crude sample for estimation. The absorbance was taken at 480 nm, 645 nm and 663 nm against as described by Jenson (1978). Carotenoid content was estimated by the following formula

Total carotenoid = [A480 - (0.114 X A664) – (0.638 X A645)] µg/gm tissue

3.7. Molecular identification of *Pleurotus* species

3.7.1. Isolation of genomic DNA from fungi

Total genomic DNA from the fungi was isolated by N-cetyl-N,N,N-trimethyl-ammonium bromide (CTAB) method.

3.7.1.1. DNA isolation technique

0.5 g fungal mycelium was taken and grinded with 25 mg PVPP using mini grinder and then it was centrifuged at 10000 rpm 2 min. at 40^0C. The pellet was washed with sterile distilled water and again centrifuged at 10000 rpm 20 min. at 40^0C. Then 675 µl of extraction buffer was added and incubated at 37^0C for 30 min. After that 75µl of SDS (20%) was added and incubated at 65^0C for 2 hours and again centrifuged at 10000 rpm for 10 min at 4^0C.The supernatant was collected in a sterile micro centrifuge tube and equal volume of Phenol: chloroform: isoamyl alcohol (25:24:1) was added and shaken well. Then it was centrifuged at 10000 rpm for 10 min. at 4^0C and equal volume of chloroform: isoamyl alcohol (24:1) was added. It was again centrifuged at 10000 rpm for 10 min. at 4^0C. The aqueous phase was removed and taken in a sterile micro centrifuge tube. 0.6 volumes of isopropyl alcohol was added and incubated at room temperature for 1hour. Then it was centrifuged at 10000 rpm for 10 min. The pellet was washed using 500µl of 70% ethanol and centrifuged at 10000 rpm for 10 min at room temperature. Pellet was dried and dissolved in 20 µl sterile distilled water.

3.7.1.2. Purification of genomic DNA

Total genomic DNA from different isolates as per the above procedure was followed by RNAase treatment. Genomic DNA was again suspended in 100 µl 1X TE buffer and incubated at 37^0C for 30 min with RNAse (60µg). After incubation the sample was re-extracted with PCI (Phenol: Chloroform: Isoamyl alcohol 25:24:1) solution and RNA free DNA was precipitated with chilled ethanol as described earlier. The quality and quantity of DNA was analyzed both spectrophotometrically and in 0.8% agarose gel. The DNA from all isolates produced clear sharp bands, indicating the DNA quality.

3.7.2. Quantification of isolated DNA

The quantity of the isolated DNA was checked in UV-VIS spectrophotometer (Vivaspec Bio photometer, Germany). From the stock 1µl DNA was mixed with 49-µl

sterile distilled water to get 50 times dilution. For quantification, DNA absorbance at wave lengths of 260 nm and 280 nm were taken. Quantification was done as follows:

1 O.D. at 260 nm for double-stranded DNA = 50 ng/ul of dsDNA

1 O.D. at 260 nm for single-stranded DNA = 20-33 ng/ul of ssDNA

Pure preparations of DNA have OD_{260}/OD_{280} value 1.8. If there is contamination with protein or phenol, this ratio will be significantly less than the value given above, and accurate quantitation of the amount of nucleic acid will not be possible

3.7.3. Agarose gel electrophoresis

Gel electrophoresis is one of the most important molecular biology tool. Gel electrophoresis enables us to study DNA. It can be used to determine the sequence of nitrogen bases, the size of an insertion or deletion, or the presence of a point mutation; it can also be used to distinguish between variable sized alleles at a single locus and to assess the quality and quantity of DNA present in a sample.

3.7.3.1. Preparation of agarose gel

Agarose (0.8%) in 1X TBE buffer was melted, cooled and poured into the gel casting tray with ethidium bromide. Gels solidify in 15-20 min.

3.7.3.2. Gel electrophoresis of DNA sample

15µl of sample and 5µl of DNA loading dye mixed properly was loaded in each well of agarose gel (1%). The electrical head of the gel tank was attached firmly and electric supply was applied at constant current 90 mA and voltage 75 volt (BioRAD Power Pac 3000) at least for 90 min. The DNA migrated from cathode to anode. Run was continued until the bromophenol blue had migrated an appropriate distance through the gel. Then electric current was turned off and gel was removed from the tank and examined on UV trans illuminator and photographed for analysis.

3.7.4. PCR Amplification

3.7.4.1. Reagents and the optimal PCR reaction mixture

PCR amplification of ITS region was done in 20 µl of reaction mixture containing PCR buffer, 1X (Kappa, SA); MgCl2, 3 mM; dNTP mix, 0.25 mM; Taq DNA polymerase, 0.05 U; primer, 1 picomol and template DNA, 50 ng. Sterile nuclease free water is used as negative control.

3.7.4.2. ITS PCR analysis

Different species of oyster mushrooms were taken up for ITS-PCR amplification. Genomic DNA was amplified by mixing the template DNA (50 ng), with the polymerase reaction buffer, dNTP mix, primers and Taq polymerase. Polymerase Chain Reaction was performed in a total volume of 100 µl, containing 78 µl deionized water, 10 µl 10 X Taq pol buffer, 1 µl of 1U Taq polymerase enzyme, 6 µl 2 mM dNTPs, 1.5 µl of 100 mM reverse and forward primers and 1 µl of 50 ng template DNA. PCR was programmed with an initial denaturing at 94^0C for 5 min. followed by 30 cycles of denaturation at 94^0C for 30 sec, annealing at 59^0C for 30 sec and extension at 70^0C for 2 min and the final extension at 72^0C for 7 min in a Primus 96 advanced gradient Thermocycler. PCR product (20 µl) was mixed with loading buffer (8µl) containing 0.25% bromophenol blue, 40 % w/v sucrose in water, and then loaded in 2% Agarose gel with 0.1 % ethidium bromide for examination with horizontal electrophoresis.

Table 7: PCR primers of ITS 4 and ITS6 used for sequencing

	Oligonucleotide Sequences (5'- 3')	GC %	Tm Value	Length	Product Size
ITS 4	TCCTCCGCTTATTGATATG	50	51.0^0C	19	700 bp
ITS 6	GAAGGTGAAGTCGTAACAAGG	60	56.0^0C	21	

3.7.4.3. Amplification conditions

Temperature profile, 94^0C for 2 min. followed by 30 cycles of denaturation at 94^0C for 50 sec, annealing at 48^0C for 30 sec and extension at 72^0C for 1min 30 sec and the final extension at 72^0C for 6 min in a Primus 96 advanced gradient Thermocycler.

3.7.4.4. Sequencing of rDNA gene

The rDNA was used for sequencing purpose. DNA sequencing was done bidirectionally using the ITS primer pairs by Credora Life Sciences, Bangalore.

3.7.5. Sequence analysis

DNA sequence information was analysed using bioinformatics algorithms tool MEGA 4, as well as the few online software.

3.7.5.1. Chromatogram of sequence

The chromatogram of the DNA sequence was analysed by the software Chromus Lite.

3.7.5.2. Multiple sequence alignment of sequence data

DNA sequences edited by using the software BioEdit and aligned with Clustral W algorithms.

3.7.5.3. BLAST analysis of the sequences

The DNA sequences were analysed using the alignment software of BLAST algorithm (http://ingene2.upm.edu.my/Blast, Altschul *et al.*., 1997) for the different characteristic of DNA sequence for the identification of microorganism. Identification of microorganism was done on the basis of homology of sequence.

3.7.5.4. Submission of rDNA gene to NCBI Genbank

The DNA sequences were deposited to NCBI Genbank through BankIit procedure and approved as the ITS sequence after complete annotation and given accession numbers.

3.8. Antioxidant activity of oyster mushroom

3.8.1. Preparation of ethanolic extract of *Pleurotus* sp.

Antioxidant compound is very important for human health. For the estimation of different antioxidant activity, ethanolic extract was prepared of *Pleurotus* species.1gm dried powder o *Pleurotus* sp was suspended in 100 ml 95% ethanol and stirred for 24 h at room temperature. The suspension was then filtered with whatman no 1. Then the filtrate was evaporated by rotary evaporator at 40^0C. The extraction was then resuspended in 95% ethanol and used for further use.

3.8.2. DPPH Scavenging activity

Free radical scavenging activity of mushroom extracts were estimated by DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity. 100 µl ethanolic extract of four different species of oyster mushroom (*Pleurotus ostreatus, P. sajor-caju, P. djamor* and *P. florida*) was taken and 5µl DPPH solution was added to it and incubated for 30 min in dark. After incubation absorbance was taken at 517nm against a control. DPPH scavenging activity was measured using the following formula

$$\text{Inhibition \%} = (A_{control} - A_{sample})/A_{control} \text{ X } 100$$

3.8.3. Ferric Reducing Antioxidant Power Assay

2.5ml of (0.2 M) phosphate buffer (pH6.6) was taken in a test tube and to it 2.5 ml of 1% potassium ferricyanide, 1 ml of distilled water and 1 ml of ethanolic extract of the test sample was taken and incubated at 50^0C for 20 min in water bath. Then 2.5ml of 10% trichloro acetic acid was added and mixed well. Then it was centrifuged at 10000 rpm for 15 min. the upper layer of the mixture was collected. 2.5ml of reaction mixture was taken and to it 2.5 ml of distilled water and 300µl of 1% ferric chloride was added and mixed well. Then the absorbance was taken at 700nm in spectrophotometer.

3.8.4. Estimation of flavonoid content

Flavonoid content of the mushroom extracts was done following the methodology of Barros *et al* (2008). 100µl of mushroom extract was taken and 500µl distilled water was added and to which 30µl of 5% sodium nitrate was added and incubated for 5min. Then 60µl of 10% aluminium chloride added and incubated at room temperature for 6 min. After the incubation, 200µl of 1M NaOH and 110 µl distilled water was mixed well and absorbance was taken at 510nm.

3.9. Antidiabetic activity of oyster mushroom

3.9.1. Induction of diabetes

Male swiss albino rats (120-150g) were used for the experimental purpose. The animals were kept into polypropylene cages and maintained in Antisera Reserves of Plant Pathogen, Immuno phytopathology laboratory, Department of Botany, NBU under laboratory conditions as suggested by Niyonzima and Vlientinck (1993). Animals were maintained by feeding bread and pea at 50 gm twice per day before starting the treatment.

Animals were allowed to fast for 15-18 hrs and freshly prepared solution of Streptozotocin (65mg/kg body weight) in 0.1M Sodium citrate buffer pH4.5 was injected intra-peritoneally in a volume of 1ml/kg body weight. After 48 hrs of injection, blood sugar level was observed and rats with more than 200 mg/dl glucose level was considered as diabetic rat and used for further treatment.

3.9.1.1. Chemical used

Streptozotocin (N-Methyl nitrosocarbamoyl-α-D-glucosamine) was used for induction of blood sugar in swiss albino rats. The chemical procured from the HiMedia Laboratory Mumbai (Product code- CMS-1758-250MG).

3.9.2. Experimental Design

In this experiment, 35 rats were used and these were divided into four groups of 5 each.

- **Group-I** untreated rats receiving distilled water designated as Normal control
- **Group-II** rats were induced with Streptozotocin (65mg/kg body weight) was considered as Diabetic control or Negative control
- **Group-III** was induced with Streptozotocin and treated with a commercially available standard drug Metformin tablets and was designated as positive control
- **Group-IV** was Streptozotocin induced rats treated with aqueous suspension of *P. ostreatus* powder administered orally was designated as Sample Treated. Streptozotocin treated rats were fed with aqueous suspension (250mg/kg body weight) of *P. ostreatus* powder for 15 days.
- **Group-V** was Streptozotocin induced rats treated with aqueous suspension of *P. sajor-caju* powder administered orally was designated as Sample Treated. Streptozotocin treated rats were fed with aqueous suspension (250mg/kg body weight) of *P. sajor-caju* powder for 15 days.
- **Group-VI** was Streptozotocin induced rats treated with aqueous suspension of *P. djamor* powder administered orally was designated as Sample Treated. Streptozotocin treated rats were fed with aqueous suspension (250mg/kg body weight) of *P. djamor* powder for 15 days.
- **Group-VII** was Streptozotocin induced rats treated with aqueous suspension of *P. florida* powder administered orally was designated as Sample Treated. Streptozotocin treated rats were fed with aqueous suspension (250mg/kg body weight) of *P. florida* powder for 15 days.

3.9.3. Determination of body weight of experimental rats

The body weight of rats was measured in regular intervals of all rats of each group using the pan balance.

3.9.4. Determination of blood glucose level

Blood sample was collected from the tail vein of two hours fasted rats and glucose level was estimated using the Accucheck Blood Sugar testing kit.

3.9.5. Estimation of blood creatinine

Blood creatinine level was determined using the ERBA-CRE creatinine kit (Product code 120246). 100 µl sample or standard solution was added to 1000µl of working reagent and mixed well. Absorbance was taken after 20th second and 80th second at 505 nm as described by Young *et al* (1975).

Blood creatinine = ΔA of test sample X Conc. of Standard/ ΔA of standard (mg/dl)

3.9.6. Estimation of blood urea

Blood urea was determined using the ERBA-UREA (BUN) kit (Product code 120214). 20µl standard or the taste sample added to 1000µl working reagent and mixed well. Then the absorbance was taken at 340 nm and urea content calculated by following formula

Blood urea = ΔA of test sample X Conc. of Standard/ ΔA of standard (mg/dl)

3.9.7. Estimation of triglyceride

Lipid profile of blood includes Triglyceride which is an important parameter effected by blood sugar. Triglyceride was determined following the methodology of Fossati, (1969) using the ERBA-Triglyceride DES kit (Product code 120211). 1000µl of working reagent was taken and to it 10 µl dist. water, 10µl standard solution and 10µl of test sample was added and incubated at 37^0C for 10 min. after that, absorbance was taken at 505nm and calculated using the following formula

Triglyceride (mg/dL) = Abs. of test X Conc. of standard/Abs of Standard

3.9.8. Estimation of cholesterol

Cholesterol is an important compound that affects the human health. Blood cholesterol level was estimated according the methodology proposed by Roeschlau's *et al*. (1974). This test was performed using the ERBA Cholesterol DES kit (Product code 120194). 1000µl of working reagent was taken and to it 20 µl of test sample (serum) or standard

sample was added. Then it was mixed well and incubated for 10 min at 37^0C. Then the absorbance was taken at 505nm and calculated the concentration according to the following formula

Cholesterol (mg/dL) = Abs. of test X Conc. of standard/ Abs. of standard

3.9.9. Estimation of serum glutathione pyruvate transaminase (SGPT) activity

Serum glutamic pyruvate transaminase is an important enzyme secreted due to abnormalities in blood. SGPT was analysed following the methodology proposed by International Federation of Clinical Chemistry (IFCC) using the ERBA-SGPT kit (Product code120206). 1000µl of working reagent was taken and to it 100µl of test sample were added and aspirated for 10-15 min. Then the absorbance was taken at 340nm. Mean absorbance per min was calculated for each sample and the SGPT activity was calculated using the following formula

International unit (IU/L) =(ΔAbs./min)XTotal vol. X 10^3 /sample vol.XAbsorptivity X P

(Here P= Cuvette light path)

3.9.10. Estimation of serum glutathione oxaloacetate transaminase (SGOT) activity

Serum glutathione oxaloacetate transaminase (SGOT) is one of the major enzyme associated with the liver chronic diseases. SGOT activity was estimated following the methodology proposed by the International federation of clinical chemistry (IFCC; 1980) using the ERBA-SGOT testing kit (Product code- 120204). 1000µl of working reagent was taken in a test tube and to it 100µl of test sample was added, mixed well and aspirated at 37^0 C for 1-2 min. and then the absorbance was taken at 340nm in spectrophotometer. The activity was calculated according to the following formula

International unit (IU/L)=(ΔAbs./min)X Total Vol.X10^3/ sample vol. X AbsorptivityXP
(Here P= Cuvette light path)

3.10. Post-harvest processing of oyster mushroom

3.10.1. Long term processing

3.10.1.1. Drying process

Drying is perhaps the oldest technique known to the mankind for preservation of food commodities for long duration. It is the process of removal of moisture from the product to such a low level that microbial and biochemical activities are checked due to reduced water activity, which makes the products suitable for safe storage and protection against the attack by microorganisms during the storage. Mushroom contains about 90% moisture at the time of harvesting dried to a moisture level down below 10-12%. At a drying temperature of 55-60°C, the insects and microbes on the mushrooms will be killed in few hours, which give us the dehydrated final product of lower moisture content with longer shelf-life. The temperature, moisture of the mushroom and humidity of the air affect the colour of the dried product. Dehydrated mushrooms are used as an important ingredient in several food formulations including instant soup, pasta, snack seasonings, casseroles, and meat and rice dishes. Mushroom dried at higher temperature loose texture, flavour, and colour along with reduced rehydrability. Recently with advances in drying technologies, various drying methods such as solar drying, fluidized bed drying, dehumidified air- cabinet drying, osmo-air drying, freeze-drying, cabinet drying and microwave drying are efficiently used for almost all types of mushrooms. Fruiting body of *Pleurotus* sp were separated according to their size and then dried. Sundry, oven dry and freeze drying process was adopted for drying. In case of sundry, fruiting body of *Pleurotus* sp were kept in a tray covered by a thin cloth for 4 hrs and it took 3-4 days for complete drying. Oven drying was practiced using the hot air oven in which mushroom fruiting body were kept for 1-2 hrs at $35\text{-}40^0$ C which took 3-4days. In case of freeze drying, mushroom fruiting body were stored at 4^0 C for 5-6 days for complete drying.

3.10.1.2. Preparation of mushroom powder

Preparation of mushroom powder is also an effective step for long term storage of mushroom. Mushroom fruit body was dried in following different drying methods such as direct and indirect sun dry, oven dry and freeze dry. After drying, the dried fruitbody were grinded using the mixed grinder and then the powder was sieved. Sieving was done to get the fine grinded powder. Then the powder was kept in airtight container for further use.

3.10.1.3. Preparation of Mushroom biscuits

Delicious and crunchy mushroom biscuits were prepared by using the oyster mushroom powder and various ingredients viz., maida, sugar, butter, mushroom powder, crushed cashew nut, backing soda, and milk powder. Dough was prepared by mixing all the ingredients for 15-20 minutes. After that dough was kept for 10 minutes. Thereafter, thin sheets of dough was made and cut into different shapes of biscuits. These raw cut biscuits were kept in the trays in systematic manner and then these trays were shifted to micro weave oven (600^0C) for baking purpose for 10-15 minutes. After baking, trays were removed from the micro weave oven and cooled; the biscuits were ready for packaging and or for serving. The ingredient like sugar gives desired sweetness; butter gives smoothness to the biscuits. The various ingredients required for preparation of mushroom biscuits are as follows

Ingredients	Amounts
Maida	1000gm
Mushroom powder	150gm
Butter	250gm
Crushed Sugar	To taste
Milk powder	250ml
Cashew nut (crushed)	200gm
Baking Powder	5gm
Water	As required

3.10.2. Short term processing

3.10.2.1. Mushroom soup

Different mushroom dishes were prepared as one of the method of short term processing of oyster mushroom. Oyster mushroom soup was prepared by using the freshly harvested healthy fruiting body of different species of oyster mushroom. Mushroom fruiting body were chopped into small pieces and then dipped into boiling water and kept for 10 min for the dispersal of spores and dirt particles. Then the mushroom was washed and fried with the grinded onion, ginger, salt, and garlic cloves for 15-20 mins. Then the fried paste mixture was boiled for 10-15 min. Then black peeper was added to the soup and served with soup-sticks. The following ingredients were used for the preparation

Ingredients	Amount
Fresh Mushroom	500gm
Onion	100gm
Ginger	50gm
Garlic	10-15cloves
Salt	To taste
Black peeper	To taste
Butter	200gm
Water	1.5ltr.

3.9.2.2. Mushroom pakora

Mushroom pakora was also prepared using the freshly harvested oyster mushroom. The fruit body was washed with warm water and chopped into suitable pieces. Onion, chilli and capsicum were chopped into small pieces. Gram flour paste was prepared and to it onion, chillies, salt, turmeric powder was added and mixed thoroughly. The chopped mushroom was the dipped in the paste and fried in hot edible oil. Then the fried pakora was served with the sauce. Following ratio of ingredients were used to prepare mushroom pakora

Ingredients	Amount
Mushroom	1 kg
Onion	300gm
Capsicum	2pcs
Chili	50gm
Salt	To taste
Turmeric	½ teaspoon
Gram flour	350gm
Oil	As required

3.9.2.3. Mushroom curry

Mushroom curry is also one of the tasty and healthy dish and it was also prepared. Mushroom, tomato, pea and capsicum was washed and chopped, separately. Chopped

onion, grind garlic, and ginger was fried with the cumin seeds until it turns to brown and then the mushroom, tomato and capsicum was added and again fried well. All spices and salt added and again boiled for five minutes.

Ingredients	Amount
Mushroom	500gm
Tomato	200gm
Onion	100gm
Capsicum	2pcs
Garlic	30gm
Ginger	50gm
Oil	As required
Salt	To taste
Turmeric	½ teaspoon
Black peeper	To taste
Chili	20gm

3.11. Promotion and marketing of oyster mushroom

Oyster mushroom cultivation is one of the major earning sources of unemployed youth, women self-help groups. Hands on training of oyster mushroom cultivation, preparation of mushroom spawn management of contaminants as well as post-harvest processing of oyster mushroom was conducted for the easy and efficient training to the economically weaker people. In house training Immuno-phytopathology Laboratory, department of Botany was conducted throughout the year. Some workshop regarding the mushroom technology was also organized by the West Bengal Voluntary Health Association. A stall for the promotion of mushroom technology was set up during the Krishi Mela organized by the Department of Agriculture, Govt. of West Bengal which was very helpful for the rural people even the mushroom growers for better cultivation of oyster mushroom.

3.12. Determination of cost benefit ratio

Pleurotus species is very commonly cultivated in North Bengal region and a large number of mushroom growers cultivating different species of *Pleurotus*. Production

cost and marketing rate is very important factor for economic upliftment. A number of people trained and they are now growing mushrooms successfully. Cost benefit ratio was estimated depending upon the market rates of *Pleurotus* species, total production cost as well as total return. Different markets of Darjeeling and Jalpaiguri districts such as champasari bazar, naxalbari hut, batasi hut, bagdogra hut, shibmandir bazar, fulbari hut, Jalpaiguri hut, barivasa bazar and gate bazar was taken into consideration for market survey data. Different farm visited of the growers who have trained by the Immuno-phytopathology laboratory, department of Botany, NBU for the production cost as well as total cost return survey. Depending upon these field survey data, total cost benefit ratio was calculated using the following formula

Benefit cost ratio= Gross return/ total cost of production

CHAPTER 4
RESULTS

4.1. Study on mycelial growth pattern of oyster mushroom

Different media has been used to evaluate the growth rate of the fungal mycelia and it was observed that all the four fungal species grow fast in Malt extract agar when incubated at 25-27^0 C in BOD incubator. Increased growth rate was also observed in case of Potato dextrose agar but very slow growth was observed in case of water agar (Table 6). At first, large amount of hyaline areal mycelium was observed which become whitish after some time (Figure 4and 5). In case of *Pleurotus djamor*, the colour of mycelia was white in early stage but it becomes light pink at maturity. The mycelia cover the media with regular wavy mat with distinct margins. On the other hand, the mycelia of *P. florida* growing irregular with thin margin. Growth of *P. sajor-caju* and *P. ostreatus* was white cottony mat growing in concentric manner. Optimum temperature for mycelial growth was recorded at 25^0 C. With maturity distinct scanted smell was observed in all the culture plates (Table 8).

Table 8: Mycelial growth pattern of *Pleurotus* species in different media

Species	Media	Growth initiation (h.)	Growth rate (cm/day)	Colour	Texture
P. ostreatus	Water agar	36	0.50±0.023	White	Cottony
	Potato Dextrose agar	24	1.35±0.020		
	Malt extract agar	24	1.60±0.078		
P. sajor-caju	Water agar	38	0.40±0.040	White	Cottony with distinct weave
	Potato Dextrose agar	22.	1.25±0.026		
	Malt extract agar	20	1.40±0.012		
P. djamor	Water agar	40	0.30±0.008	Initial white become light pink after maturity	Regular with distinct weave
	Potato Dextrose agar	25.	1.45±0.032		
	Malt extract agar	22	1.70±0.052		
P. florida	Water agar	39	0.30±0.008	White	Irregular with thin margins
	Potato Dextrose agar	23	1.20±0.020		
	Malt extract agar	20	1.85±0.064		

(±) means Standard Error, calculated using 5 replicates of each plates

Figure 4: Mycelial growth pattern of *Pleurotus* species on potato dextrose agar (A) *P ostreatus*, (B) *P sajor-caju*, (C) *P djamor* and (D) *P florida*

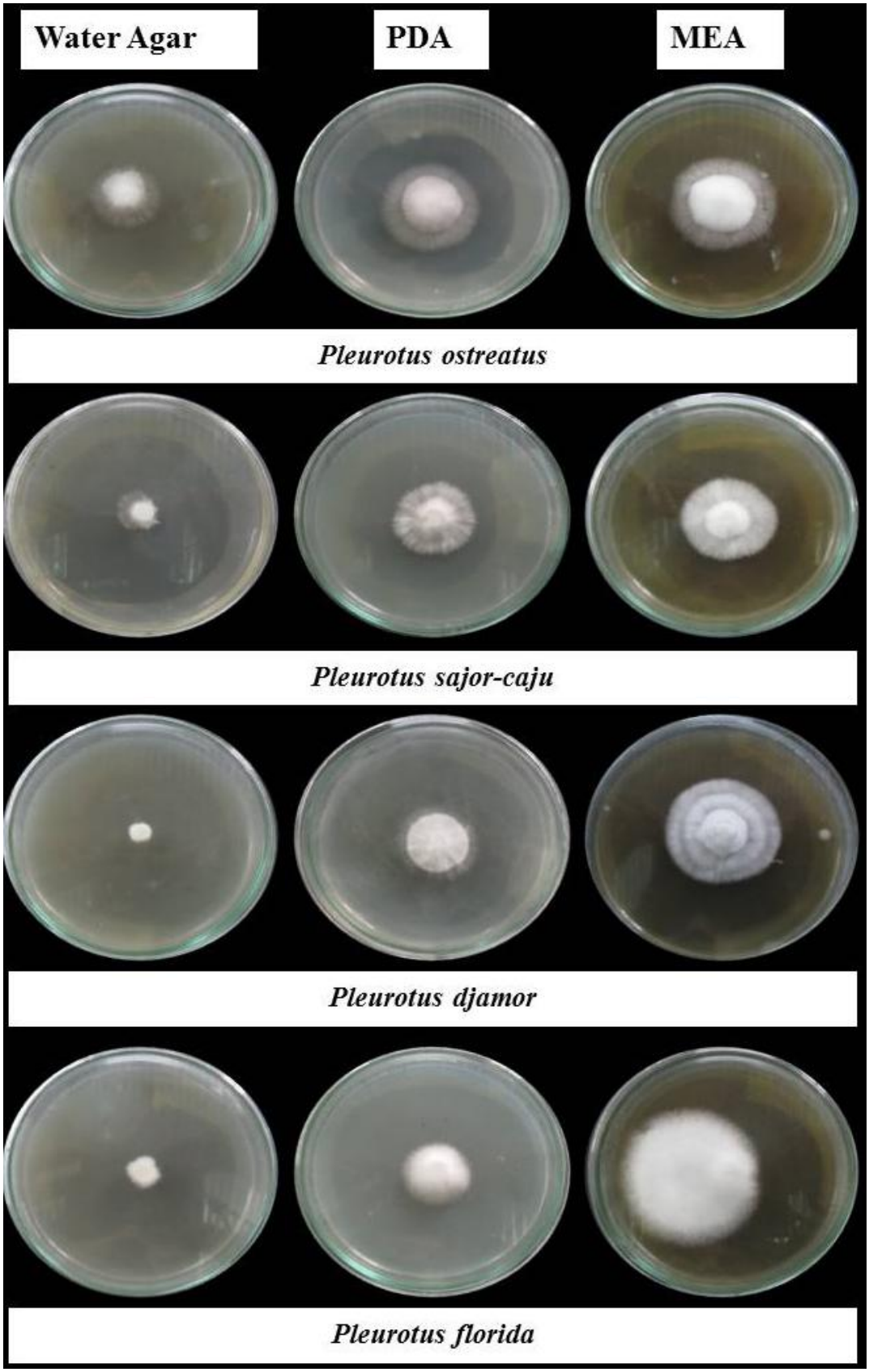


Figure 5: Mycelial growth rate of *Pleurotus* species on different media (incubation period 48h., temperature 28 $\pm 2^0$ C)

Table 9: Morphological Characteristics of different species of oyster mushroom

Organism	Shape	Size	Colour	Optimum Temperature	Characteristics	Spore characteristics
Pleurotus ostreatus (Jacq.Fr.) (Black Oyster)	The fruit body looks like a horse shoe	Diameter of the pileus is about 2-3 inches and the whole fruit body is about 4-5 inches	Dark black at young stage but become lighter at maturity	18-22^0 C is favourable but can grow up to 28^0 C	The mushroom grows in acropetal bunches. It is very popular for its fleshy velvety texture with distinct aroma. Gills are decurrent on lower side.	Spores are long, oval to kidney shaped, spores attached with basidium at middle portion of the base, about 2-4µm in length
Pleurotus djamor (Pink Oyster)	Fruiting body wide, wavy outer edge with no particular shape	3-4 cm diameter. Stipe is absent or present in very little length. Thickness is about 3-4mm outer edge.	Dark pink in young stage but become light pink on mature stage	Primordia initiated at lower temperature about 18-20^0 C.	Primordia are thin, leathery in texture with distinct aroma. Gills are also pink in lower side. Limited amount of water is required during fruiting.	Spores are oval shaped short, often kidney shaped and attached at the base with basidium. Spore length 1.8-3.5 µm
Pleurotus sajor-caju Fr. (Grey Oyster)	Fruiting body is fan shaped	Pileus diameter upto 4-5 inches	Grey at young stage and at maturity the colour become light grey	It grows at higher temperature about 25-30^0 C	Thick wide fruiting body With long stipe.	Spores are long, oval shaped with sharp edges and attached with basidium in one corner of the base, size about 2.5-5 µm
Pleurotus florida (Eger) (Milky white Oyster)	Pileus is like a disc on a long stipe	3-5 inch pileus with 2-3 inch of stipe	Bright white colour at pinhead as well as in mature stage	18-20^0 C is favourable but it can be grown in 28^0 C also.	Pileus is graciously white with delicate flesh which is turgid in texture with decurrent gills. Stipe is thick and extended upto the base of the pileus	Spores are short, single chambered attached in a corner of the base with basidium, size 2.6-4 µm

4.2. Morphological and histopathological study of oyster mushroom

Pleurotus ostreatus is one of the oyster mushroom widely cultivated in North Bengal for its shape, texture, taste and the environmental condition is very much favourable for its cultivation (Figure 6). Fruiting body initiated in 18-22^0 C with 80-85% relative humidity which is very common in this region. Pileus is thick, fleshy with a very short stipe. Pinhead appears light blackish thus it is popularly known as Black oyster mushroom (Table 9). Diameter of the pileus is about 2-3cm and the whole part of the fruiting body edible. Basidiospores are long, oval to kidney shaped about 2-4µm long and four spores attached with the basidium. On the other hand, *Pleurotus sajor-caju* is one of the major oyster mushroom cultivated in North Bengal in a very large scale. This mushroom is very popular for its large size which increases the production rate. This species can be cultivated throughout the year for its wide range of environmental requirement (Table 9). The fan shaped pileus diameter is very large (4-5cm) with a ling stipe (Figure 7). The pileus is fleshy, prominent edges with distinguish grey colour. Anatomical study reveals that the basidiospores are long, oval shaped with quite prominent edges. Spores are attached with the basidium by the corner of the basal part of the spores. Cultivation of oyster mushroom is very common practice in North Bengal and the environmental condition is very much suitable for the cultivation. There are two species mainly cultivated in North Bengal. Pink oyster mushroom is very commonly cultivated in the north western part of India. *Pleurotus djamor* is a new introduction of oyster mushroom in North Bengal. It looks very gracious on bed; pileus is pink in colour with very small stipe. Sometimes stipes is absent. Pileus diameter is about 2-3.5 cm, fleshy with light aroma, gills are decurrent (Figure 8). *P. djamor* had been cultivated during the autumn to spring as it requires lower temperature (18-20^0 C) with 75-85% relative humidity. Basidiospores are oval to kidney shaped and four spores attached with the basidium. *Pleurotus florida* also cultivated widely in this area and about 18-20^0 C requires for fruiting initiation. Pileus is bright white, fleshy, thick and about 2-3cm diameter. Stipe is long with decurrent gills. Fruiting initiation occurs in a cluster (Figure 9). Basidiospores are long oval shaped attached with basidium. Spores were about 2-3.5µm attached with basidium. *P. florida* is being cultivated very commonly during the winter season as it requires very low temperature for its fruiting body initiation with very low amount of relative humidity.

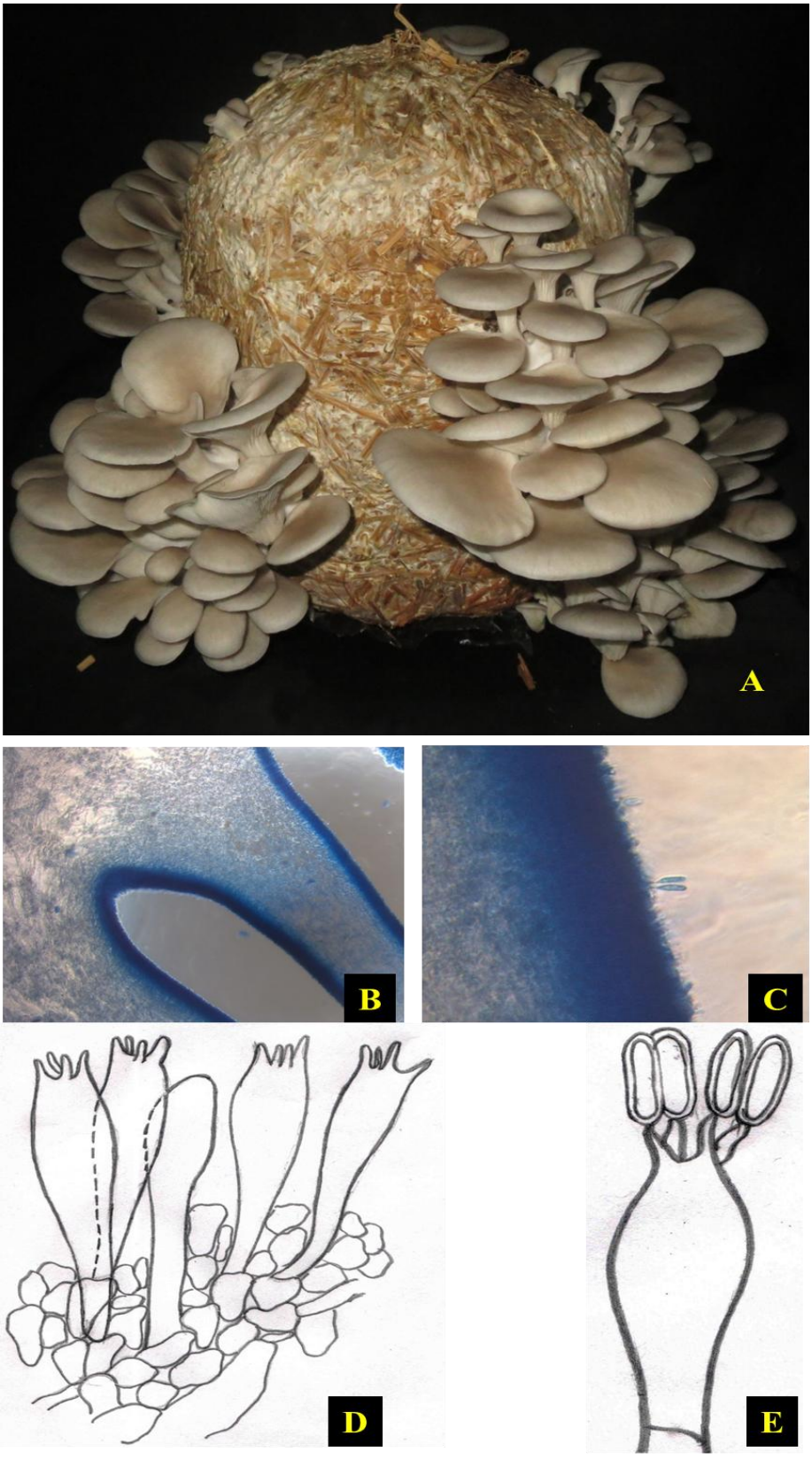


Figure 6: Morphological and anatomical features of *P ostreatus* (A) showing the fruiting body, (B) T.S of gill (10X); (C) basidiospore attached with basidium at 40X; (D) basidium attached with the hymenophore and (E) Basidiospore attached to basidium.

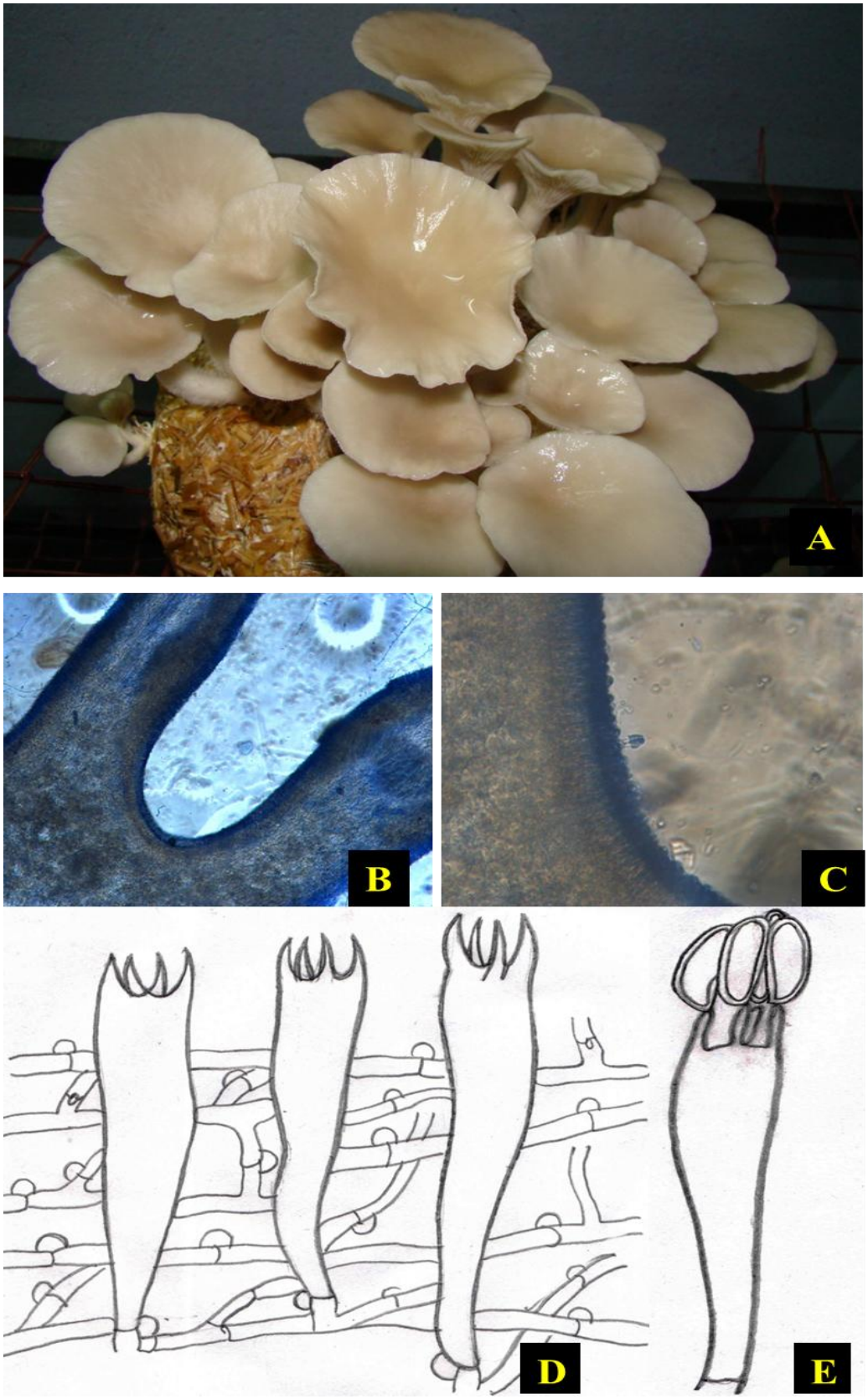


Figure 7: Morphological and anatomical features of *P sajor-caju* (A) showing the fruiting body, (B) T.S of gill (10X); (C) basidiospore attached with basidium at 40X; (D) basidium attached with the hymenophore and (E) Basidiospore attached to basidium.

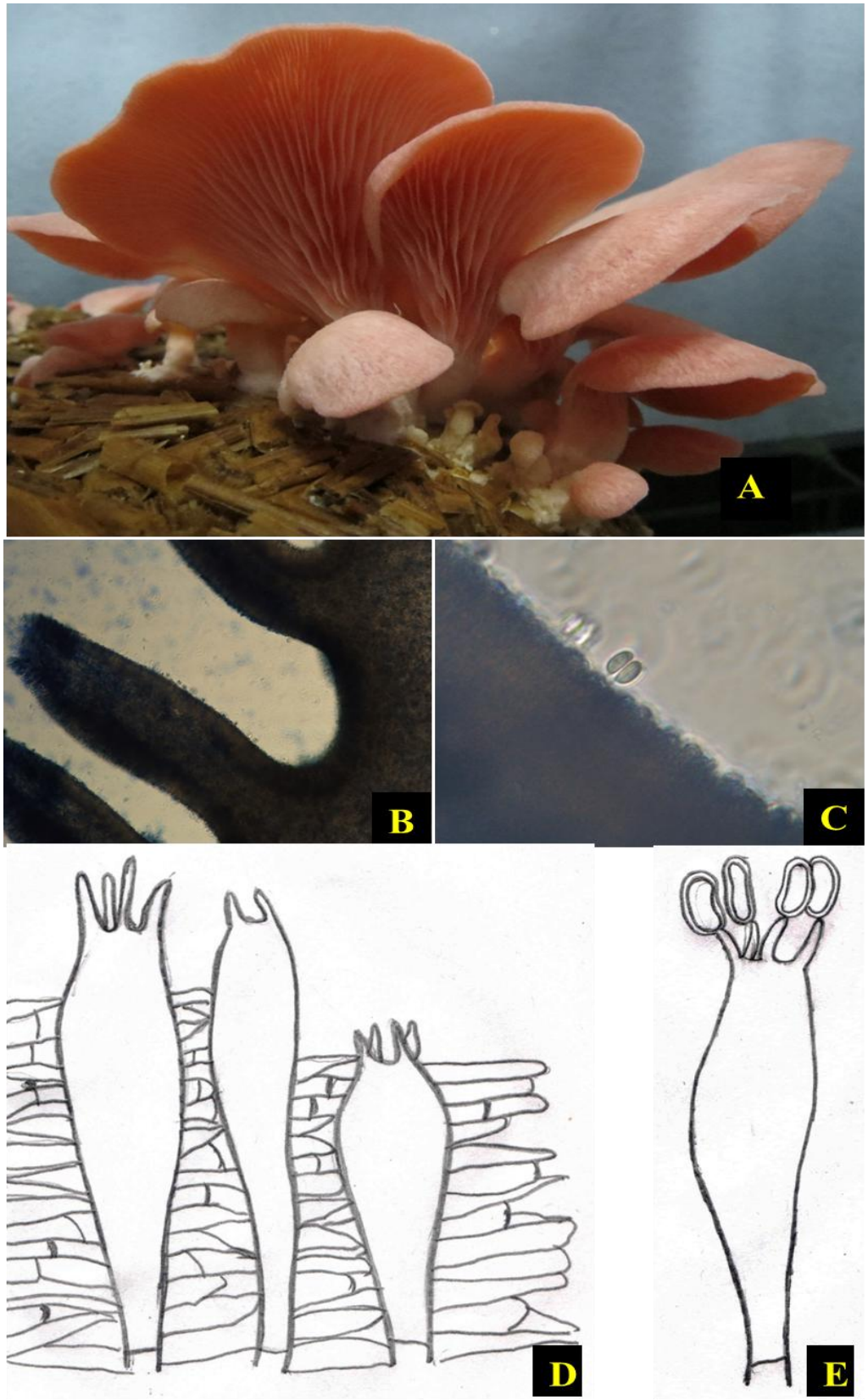


Figure 8: Morphological and anatomical features of *P. djamor* (A) showing the fruiting body, (B) T.S of gill 10X; (C) basidiospore attached with basidium at 40X; (D) basidium attached with the hymenophore and (E) Basidiospore attached to basidium.

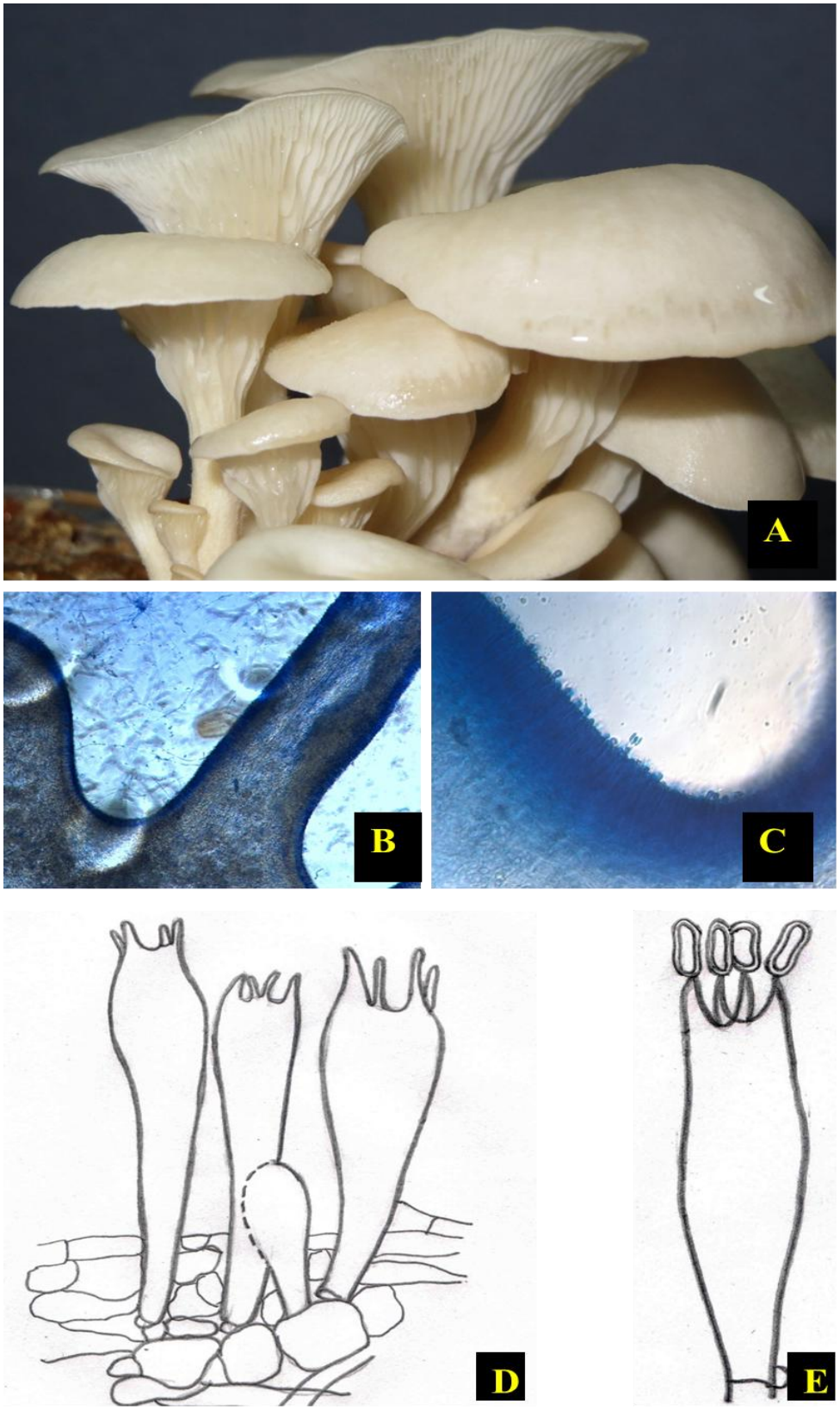


Figure 9: Morphological and anatomical features of *P. florida* (A) showing the fruiting body, (B) T.S of gill 10X; (C) basidiospore attached with basidium at 40X; (D) basidium attached with the hymenophore and (E) Basidiospore attached to basidium.

4.3. Molecular Characterization of *Pleurotus ostreatus*

4.3.1. 18S rDNA sequence and phylogenetic analysis of *P.ostreatus*

Genomic DNA of *Pleurotus ostreatus* (IPL/MC/PO-1) was suspended in 100µl 1X TE buffer treated with RNAse (60µg) until further use. Agarose gel electrophoresis of genomic DNA revealed that they were RNA free. Purity of DNA evaluated in terms of the ratio between absorbance of A_{260} and A_{280} showed that genomic DNA was ~1.8. ITS region of rDNA was amplified using genus specific T/ITS1 and T/ITS4 primers where the amplified product of 700 base pair size was produced by both the primers.

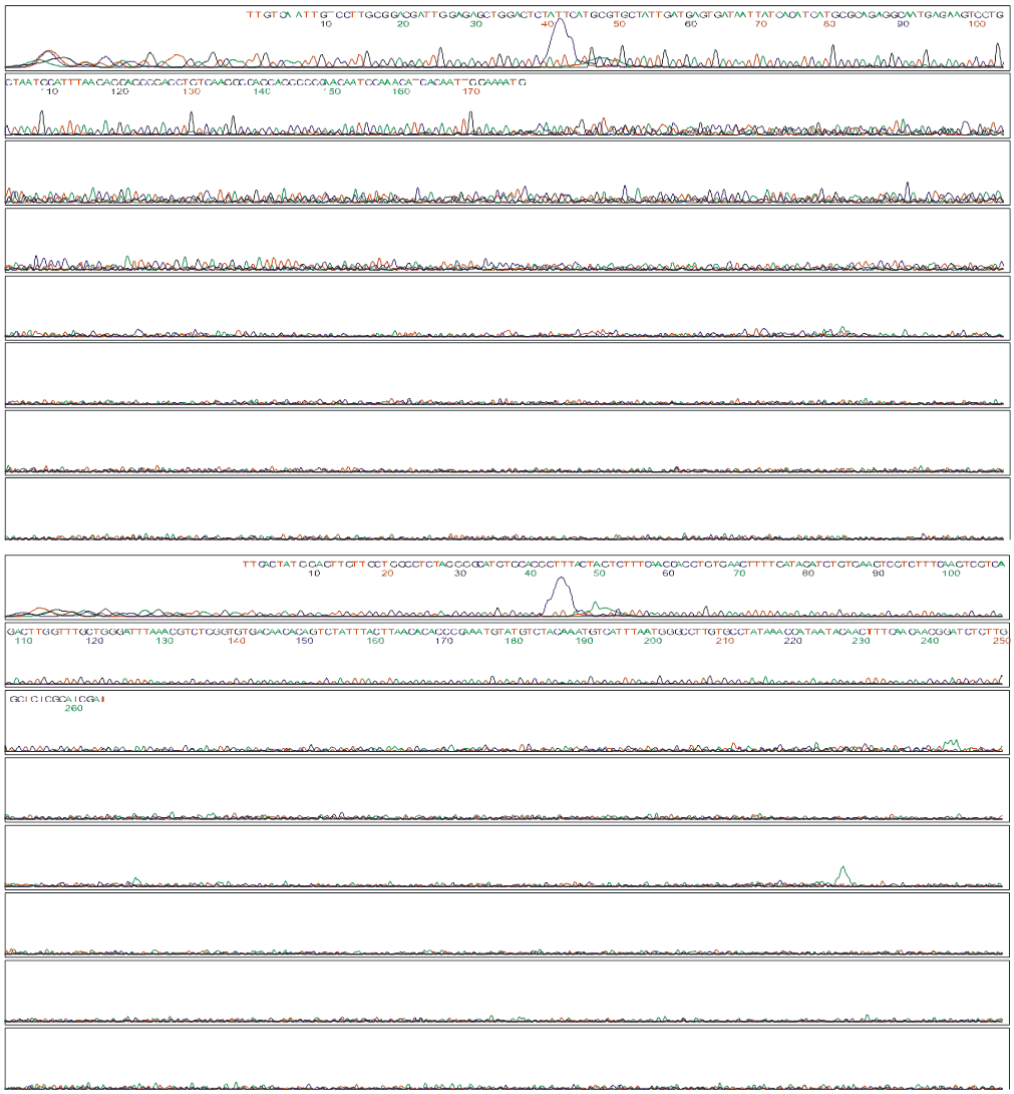
4.3.2. Chromatogram

The BLAST query of the 18S rDNA sequence of T/ITS1 and T/ITS4 (for *Pleurotus ostreatus*) against Genbank database confirmed their identity. The sequences have been deposited in NCBI, Genbank database under the accession no. KT768095. The sequence chromatograms have been represented in Figure 10.

4.3.3. Multiple sequence alignment

A multiple sequence alignment of ITS gene sequences of *Pleurotus ostreatus* was conducted. Sequences of other strains obtained from NCBI Genbank database showing maximum homology with our strain was conducted using CLUSTAL-W algorithm which is a general purpose multiple sequence alignment program for DNA of MEGA-4.1software. The use of CLUSTAL-W determines that, once a gap is inserted, it can only be removed by editing. Therefore, final alignment adjustments were made manually in order to remove artificial gaps. There were quite a number of gaps that were introduced in the multiple sequence alignment program within the region that were closely related and similar sequence indicated the relationship among the isolates. The differences in these highly conserved regions are shown in different colours (Figure 11). Phylogenetic analyses were completed using the MEGA package (version 4.01; Institute of Molecular Evolutionary Genetics, University Park, PA). Phylogenetic analysis was carried out with Ex-type strain sequences obtained from NCBI Genbank database which showed maximum homology with *P. ostreatus* (KT768095) (Table 10).

Chromatogram



Sequence Deposited NCBI

Accession No. KT768095
DNA Linear 470 BP
Strain No: IPL/MC/PO-1

Title: *Pleurotus ostreatus* strain (IPL/MC/PO-1) internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.

Partial sequence of 18S ribosomal RNA gene

GAGGACGGACATCTACCTGATTGAGGTCAATTGTCAATTGTCCTTGCGGACGATTGGAG
AGCTGGACTCTATTCATGCGTGCTATTGATGAGTGATAATTATCACATCATGCGCAGAGG
CAATGAGAAGTCCTGCTAATGCATTTAAGAGGAGCCGACCTGTCAAGGCCAGCAGCCC
CCAACAATCCAAACATCACAATTGGAAAATGGCAAAGGGCGTTTGTGTCTTCTACCCCC
TCTGCTGCGCAAGTCCCTCATATTACAACAAAGCTCATCTAGAATACTATGACCTGATCA
TCCAGCTCCTTATTGTGTATTCATCCTGACTTTTCATCCAGGAATCCACCATCACGGCAA
TTGAATCAAACGCCTTCCGCCCGCAGATTATGCTCTGCAAGGTGACCATCTCCCCGCAC
ATACCCCCCCATCACAAGATTTCCTGATGTACTGCATATTTTTTTGCAGAACATT

Figure 10: Chromatogram and sequence of 18S rDNA region *P ostreatus* (IPL/MC/PO-1) deposited in NCBI Genbank

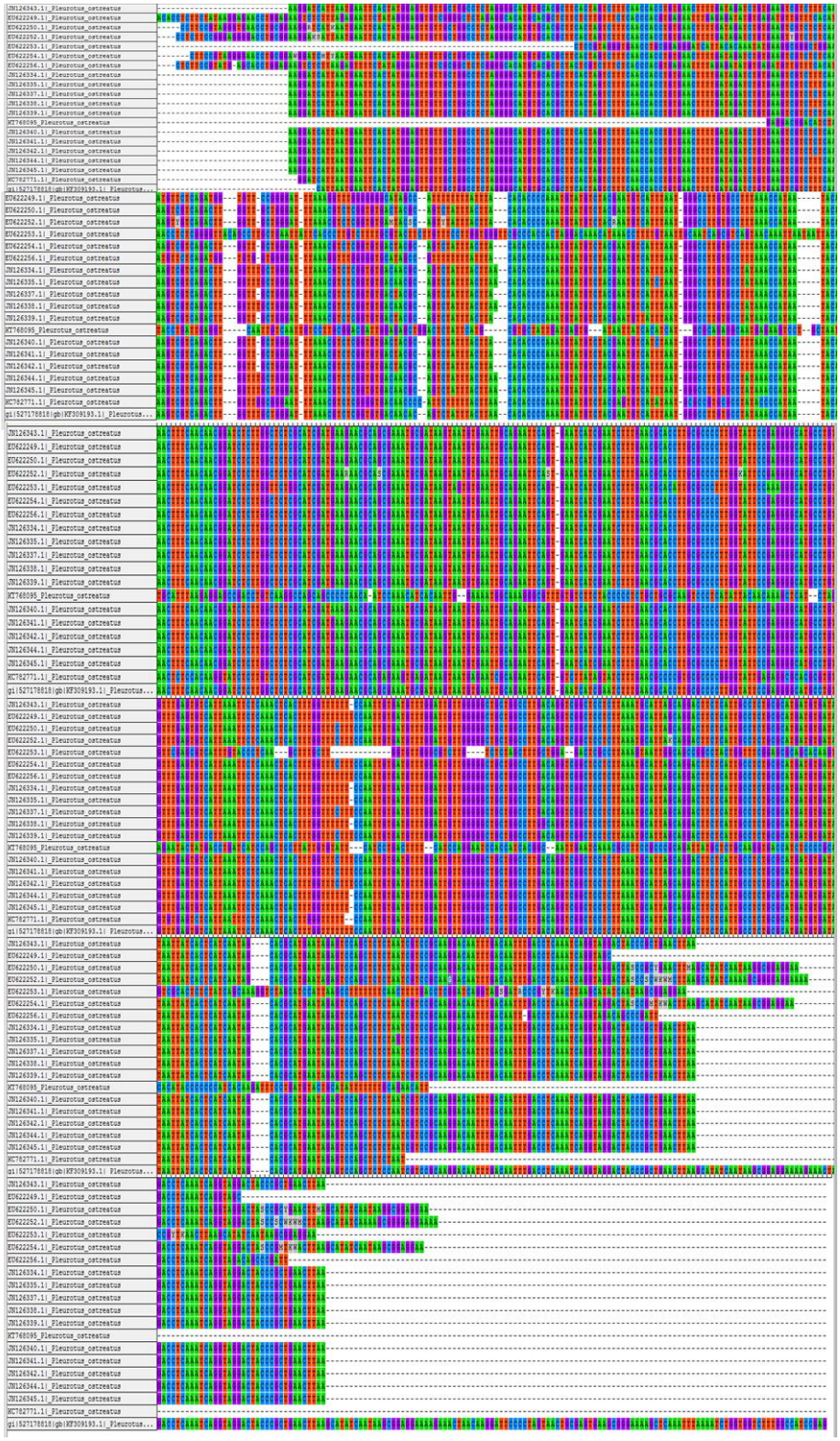


Figure 11: 18S r DNA sequence alignments of *P ostreatus* (IPL/MC/PO-1) with other ex-type isolates. The conserved regions of the gene are demonstrated in different colours

Table 10: Genbank accession numbers of the Ex-Type strains of *Pleurotus ostreatus* that showed the homology with the isolate.

Sl No	GenBank Accession No	Strain or Isolate	rDNA sequence (bp)	Origin
1	DQ077888	PHZAU2	639	China
2	HM998809	LGMACC 850404	630	Hungary
3	AY450345	6689	1551	Austria
4	EU622256	NW446	652	China
5	EU622249	NW423	648	China
6	GQ249947	PU001	563	India
7	HM067973	COIR PTK	635	India
8	HM138675	PAK1	635	India
9	KC782771	PLO6	575	Brazil
10	KT968336	PoVF8	677	Korea
11	KT968340	PoVF18	677	Korea
12	KJ020935	ST	632	Italy
13	KT818506	IPL/MC/PS-1	656	India
14	KT956122	EB1001	698	Thailand
15	KT768095	IPL/MC/PO-1	204	India

4.3.4. Phylogenetic analysis of *P. ostreatus*

The evolutionary history was inferred using the UPGMA method (Sneath and Sokal; 1973). The optimal tree with the sum of branch length = 55.72288571 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein; 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura, Nei and Kumar; 2004) and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 203 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura, Dudley, Nei and Kumar; 2007) (Figure 12).

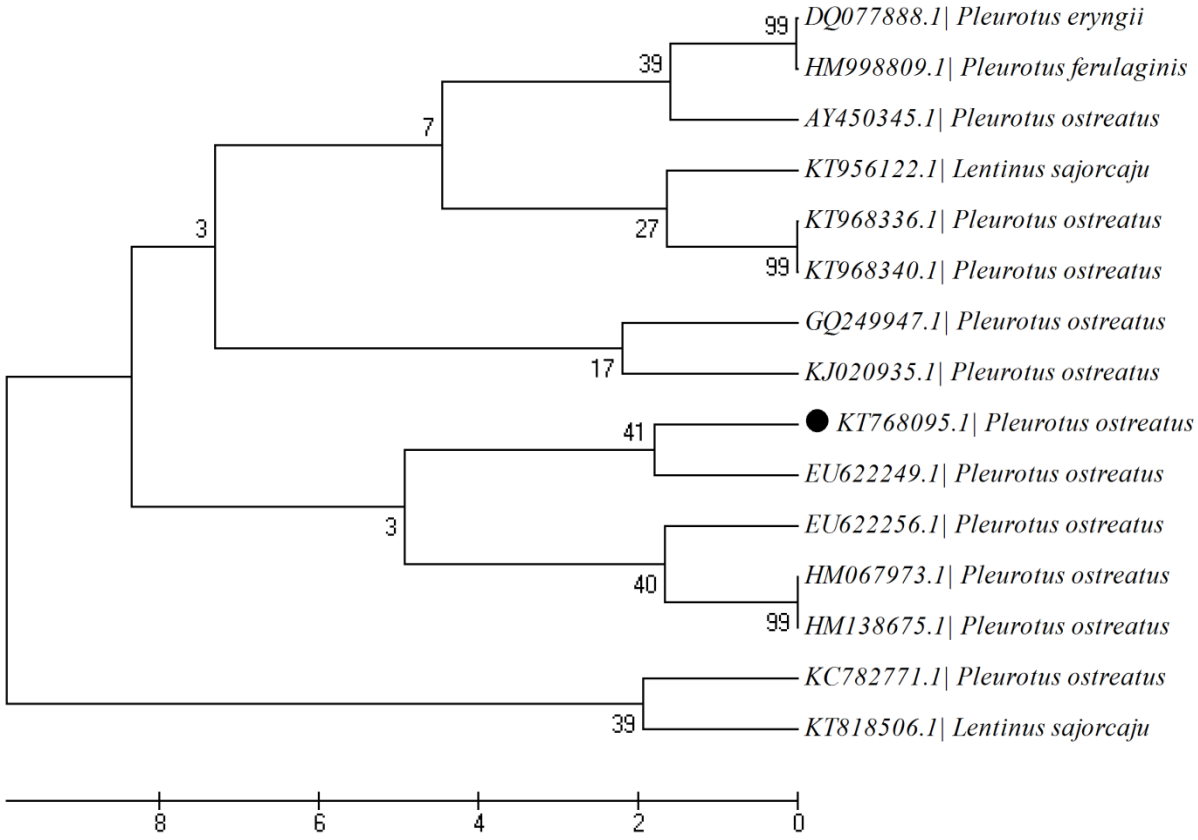


Figure 12: Phylogenetic placement of *P ostreatus* (IPL/MC/PO-1) with other ex-type strain sequences obtained from NCBI Genbank Database

4.4. Molecular characterization of *Pleurotus sajor-caju*

4.4.1. 18S rDNA sequence and phylogenetic analysis of *P. sajor-caju*

Genomic DNA of *Pleurotus sajor-caju* (IPL/MC/PS-1) was suspended in 100µl 1X TE buffer treated with RNAse (60µg) until further use. Agarose gel electrophoresis of genomic DNA revealed that they were RNA free. Purity of DNA evaluated in terms of the ratio between absorbance of A_{260} and A_{280} showed that genomic DNA was ~1.8. ITS region of rDNA was amplified using genus specific T/ITS4 and T/ITS6 primers where the amplified product of 700 base pair size was produced by both the primers.

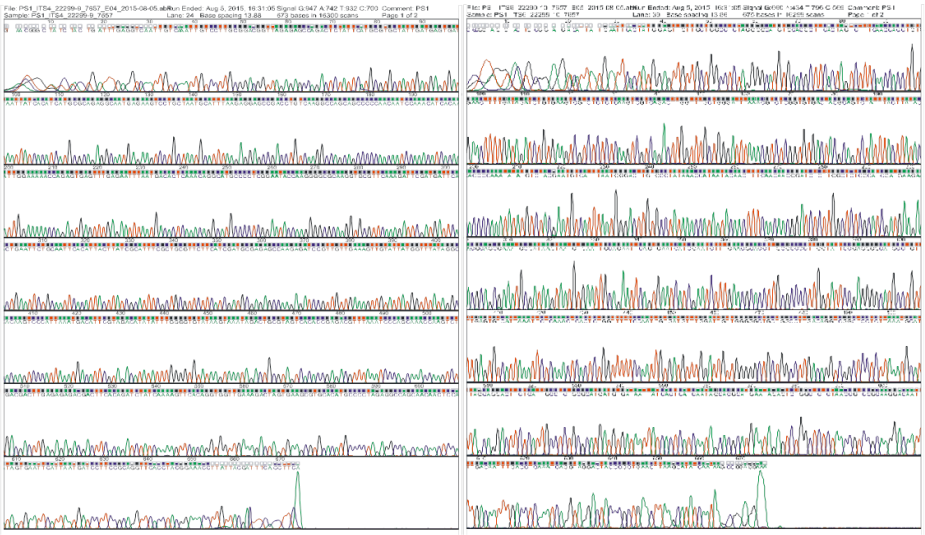
4.4.2. Chromatogram

The BLAST query of the 18S rDNA sequence of T/ITS4 and T/ITS6 (for *Pleurotus sajor-caju*) against Genbank database confirmed their identity. The sequences have been deposited in NCBI, Genbank database under the accession no. KT818506. The sequence chromatograms have been represented in figure13.

4.4.3. Multiple sequence alignment

A multiple sequence alignment of ITS gene sequences of *P. sajor-caju* was conducted. Sequences of other strains obtained from NCBI Genbank database showing maximum homology with our strain was conducted using CLUSTAL-W algorithm which is a general purpose multiple sequence alignment program for DNA of MEGA-4.1 software. There were quite a number of gaps that were introduced in the multiple sequence alignment program within the region that were closely related and similar sequence indicated the relationship among the isolates. The differences in these highly conserved regions are shown in different colours (Figure 13 and 14). Phylogenetic analysis was carried out with ex-type strain sequences obtained from NCBI Genbank database which showed maximum homology with *P. sajor-caju* (KT818506) (Table 11).

Chromatogram



Sequence Deposited NCBI

Accession No. KT818506
DNA Linear 656BP
Strain No: IPL/MC/PS-1

Title: *Lentinus sajor-caju* (*Pleurotus sajor-caju*) isolate IPL/MC/PS1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Partial sequence of 18S ribosomal RNA gene

CTGCGGAAGGATCATTAATGAATTCACTATGGAGTTGTTGCTGGCCTCTAGGGGCA
GTGCACGCTTCACTAGTCTTTCAACCACCTGTGAACTTTTGATAGATCTGTGAAGT
CGTCCTTCAAGTCGTCAGACTTGGTTTGCTGGGATTTAAACGTCTCGGTGTGACA
ACGCAGTCTATTTACTTAACACACCCCAAATGTATGTCTACGAATGTCATTTAATGG
GCCTTGTGCCTATAAACCATAATACAACTTTCAACAACGGATCTCTTGGCTCTCGC
ATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTG
AATCATCGAATCTTTGAACGCACCTTGCGCCCCTTGGTATTCCGAGGGGCATGCCT
GTTTGAGTGTCATTAAATTCTCAAACTCACATTTATTTGTGATGTTTGGATTGTTGG
GGGTTGCTGGCTGTAACAAGTCGGCTCCTCTTAAATGCATTAGCAGGACTTCTCAT
TGCCTCTGCGCATGATGTGATAATTATCACTCATCAATAGCACGCATGAATAGAGTC
CAGCTCTCTAATCGTCCGCAAGGACAATTTGACAATTTGACCTCAAATCAGGTAGG
ACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA

Figure 13: Chromatogram and sequence of 18S rDNA region *P sajorcaju* (IPL/MC/PS-1) deposited in NCBI Genbank

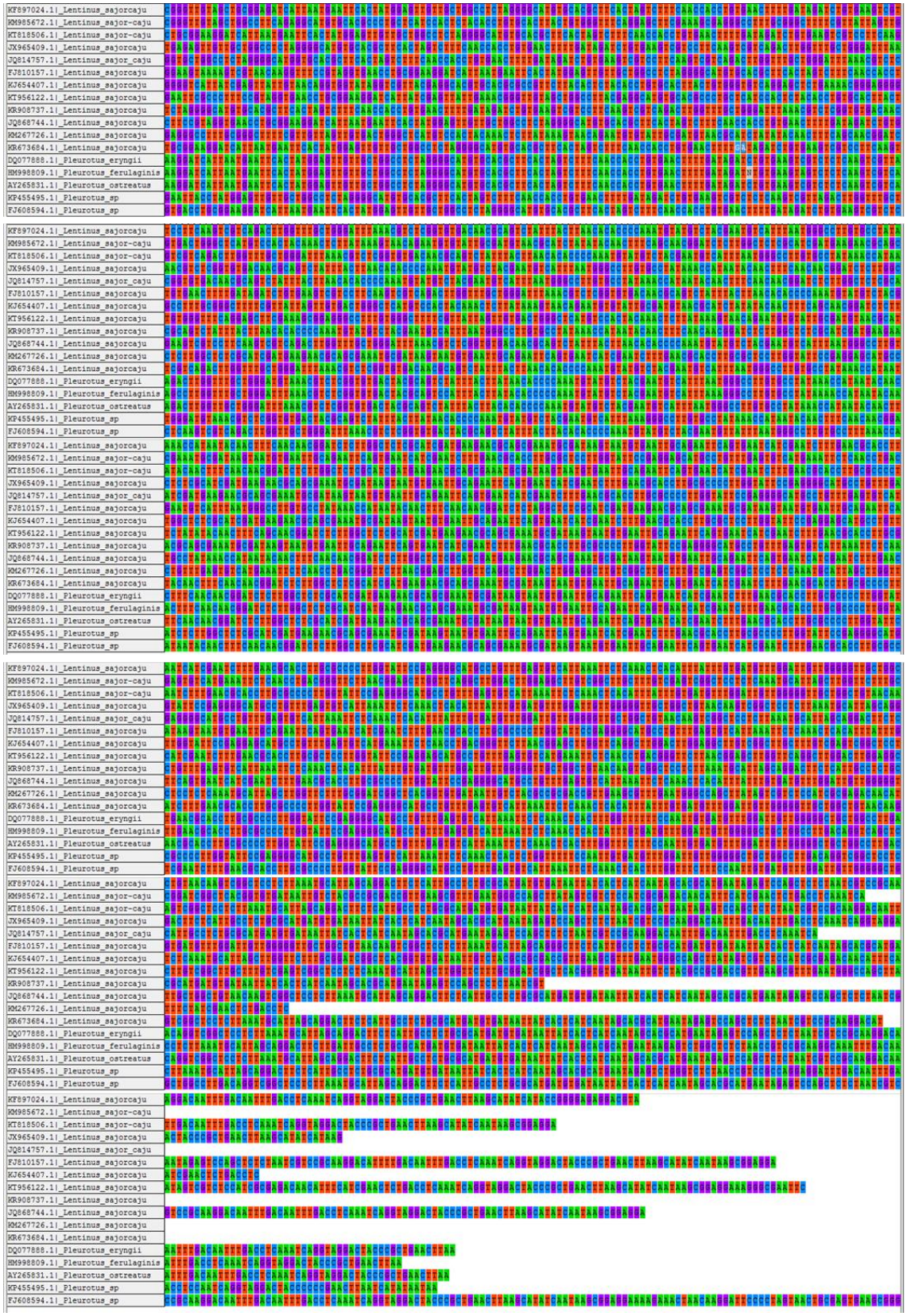


Figure 14. 18S r DNA sequence alignments of *P. sajor-caju* (IPL/MC/PS-1) with other extype isolates. The conserved regions of the gene are demonstrated in different colours

Table 11: Genbank accession numbers of the Ex-Type strains of *Pleurotus sajor-caju* that showed the homology with the isolate.

Sl No	GenBank Accession No	Strain or Isolate	rDNA sequence (bp)	Origin
1	KM985672	BPSM35	585	India
2	JX965409	pau3	620	India
3	JQ814757	CS-32	577	Russia
4	KJ654407	E882B	606	Australia
5	KT956122	EB1001	698	Thailand
6	KR908737	NCIM 1133"	531	India
7	KM267726	JMH36	488	Tanzania
8	KR673684	KA13-1213	588	South Korea
9	DQ077888	PHZAU2	639	China
10	HM998809	LGMACC 850404	630	Hungary
11	AY265831	ASI 2016	638	Korea
12	KP455495	DL501	636	India
13	FJ608594	AG X	848	Czech Republic
14	KT818506	IPL/MC/PS-1	656	India

4.4.4. Phylogenetic analysis of *P. sajor-caju*

The evolutionary history was inferred using the UPGMA method (Sneath and Sokal; 1973). The optimal tree with the sum of branch length = 31.00795241 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches (Felsenstein J; 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura, Nei and Kumar; 2004) and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 487 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura, Dudley, Nei and Kumar; 2007) (Figure 15).

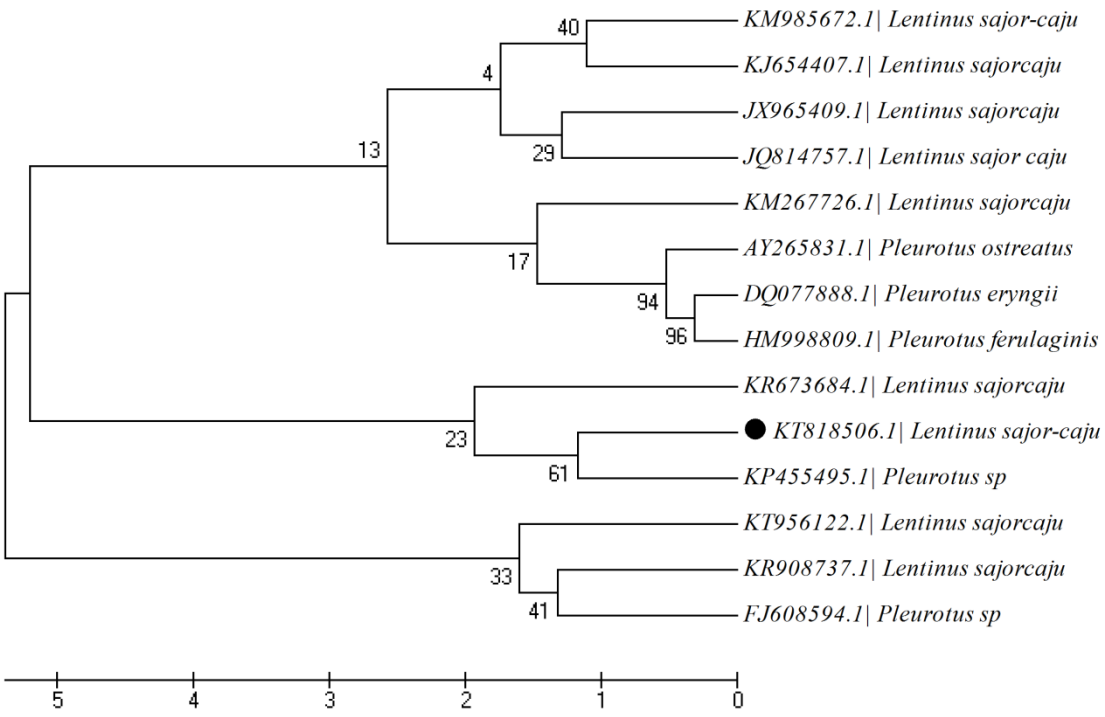


Figure 15: Phylogenetic placement of *P sajor-caju* (IPL/MC/PS-1) with other ex-type strain sequences obtained from NCBI Genbank Database

4.5. Molecular Characterization of *Pleurotus djamor*

4.5.1. 18S rDNA sequence and phylogenetic analysis of *P. djamor*

Genomic DNA of *Pleurotus djamor* (IPL/MC/PD-1) was suspended in 100µl 1X TE buffer treated with RNAse (60µg) until further use. Purity of DNA evaluated in terms of the ratio between absorbance of A_{260} and A_{280} showed that genomic DNA was ~1.8. ITS region of rDNA was amplified using genus specific T/ITS4 and T/ITS6 primers where the amplified product of 700 base pair size was produced by both the primers.

4.5.2. Chromatogram

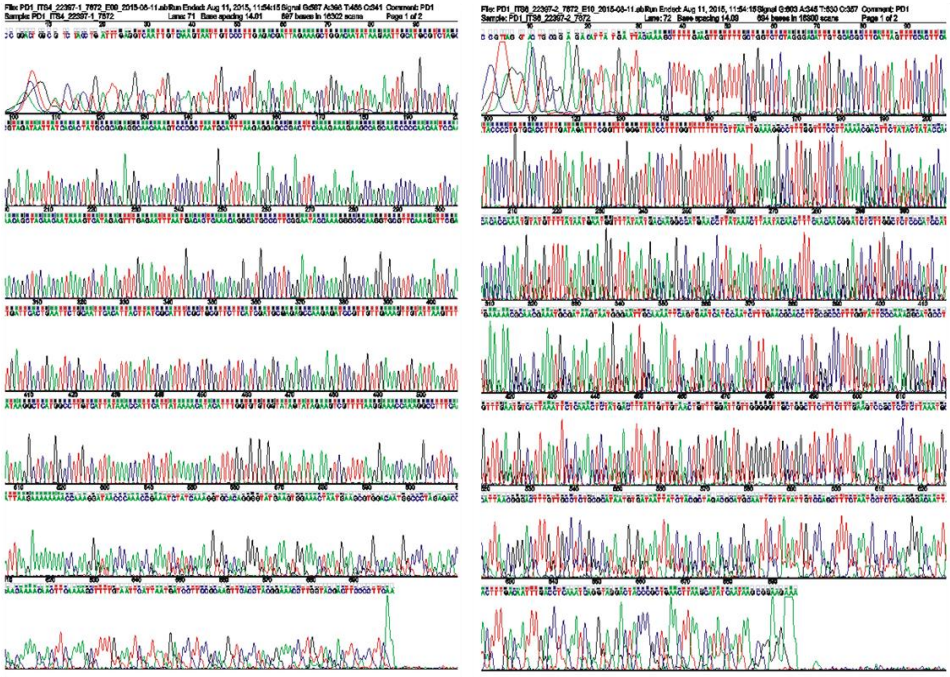
The BLAST query of the 18S rDNA sequence of T/ITS4 and T/ITS6 (for *P. djamor*) against GenBank database confirmed their identity. The sequences have been deposited in NCBI, GenBank database under the accession no. KT768094. The sequence chromatograms have been represented in figure 16 and 17.

4.5.3. Multiple sequence alignment

A multiple sequence alignment of ITS gene sequences of *P. djamor* was conducted. Sequences of other strains obtained from NCBI Genbank database showing maximum homology with our strain was conducted using CLUSTAL-W algorithm which is a

general purpose multiple sequence alignment program for DNA of MEGA4 software. There were quite a number of gaps that were introduced in the multiple sequence alignment program within the region that were closely related and similar sequence indicated the relationship among the isolates. The differences in these highly conserved regions are shown in different colours (Figure 16 and 17). Phylogenetic analysis was carried out with Ex-type strain sequences obtained from NCBI Genbank database which showed maximum homology with *P. djamor* (KT768094) (Table12).

Chromatogram



Sequence Deposited NCBI

Accession No. KT768094
DNA Linear 655BP
Strain No: IPL/MC/PD-1

Title: *Pleurotus djamor* isolate IPL/MC/PD1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Partial sequence of 18S ribosomal RNA gene

CTGCGAAGGATCATTAATAATTACAAAAGCTTTTGAAGTTGTTTTGCTGGTCTCTA
GGGACATTGTGCACGCTTCATTAGTTTCCACTTCATACCCCTGTGCACCTTTGATA
GATTTCGGTTTGGGTTATCCTTTGGTTTTTTTTTCTTAATTGAAAGGCCTTTGGTTT
CCTTAAAACGACTTCTATACTATACCACACACCAAATGTATGTTTTATAATGAATGG
TTTATAATGACAAGGCCATGACCTTATAAACTTAATACAACTTTCAACAACGGATCT
CTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTG
CAGAATTCAGTGAATCATCGATCTTTGAACGCACCTTGCGCCCTTTGGTATTCCGA
AGGCATGCCTGTTTGAGTGTCATTAAATTCTCAAACTCTATGACTTTATTGTTGTAG
CTGTTTGGATTGTTGGGGGTTGCTGGCTTCTTTCTTTGAAGTCGGCTCCTCTTAAA
TGCATTAGCGGGACTTTGTTGCCTCTGCGCATAGTGTGATAATTATCTACGCTAGAC
GCATGCAATTCTTATATTGTCCAGCTTTCTAATCGTCTCAAGGGACAATTACTTTGA
CAATTTGACCTCAAATCAGGTAGGACTACCCGCT

Figure 16: Chromatogram and sequence of 18S rDNA region *P djamor* (IPL/MC/PD-1) deposited in NCBI Genbank

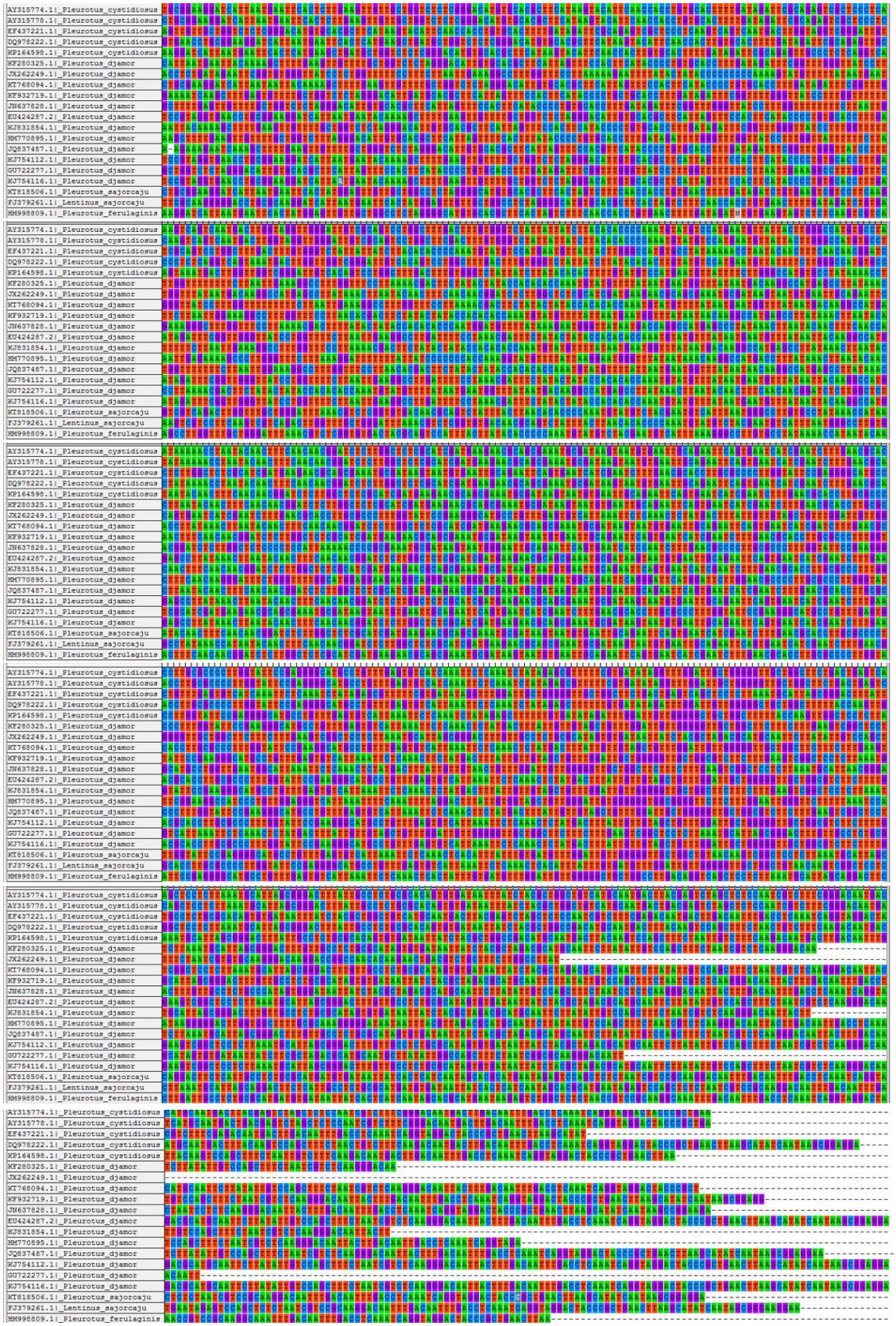


Figure 17: 18S r DNA sequence alignments of *P djamor* (IPL/MC/PD-1) with other ex-type isolates. The conserved regions of the gene are demonstrated in different colours

Table 12: Genbank accession numbers of the Ex-Type strains of *P. djamor* that showed the homology with the isolate.

Sl No	GenBank Accession No	Strain or Isolate	rDNA sequence (bp)	Origin
1	EF437221	P-19"	636	India
2	DQ978222	X 652	682	India
3	KP164598	ZYB 2013	651	China
4	KF280325	MBsn	604	Brazil
5	JX262249	CBE 11	560	India
6	KF932719	1526	666	Russia
7	JN637828	B-36	657	Cuba
8	EU424287	CBS 100134	687	China
9	KJ831854	IB36	603	Peru
10	HM770895	IUM1794	625	South Korea
11	JQ837487	Z1	675	Russia
12	KJ754112	7	687	Kenya
13	GU722277	ECS-01130	571	Mexico
14	KT818506	IPL/MC/PS-1	656	India
15	HM998809	LGMACC 850404	630	Hungary
16	KT768094	IPL/MC/PD1	655	India

4.5.4. Phylogenetic analysis of *P. djamor*

The evolutionary history was inferred using the UPGMA method (Sneath and Sokal; 1973). The optimal tree with the sum of branch length = 97.46225950 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein J; 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura, Nei and Kumar; 2004)and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 559 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura, Dudley, Nei and Kumar; 2007) (Figure 18).

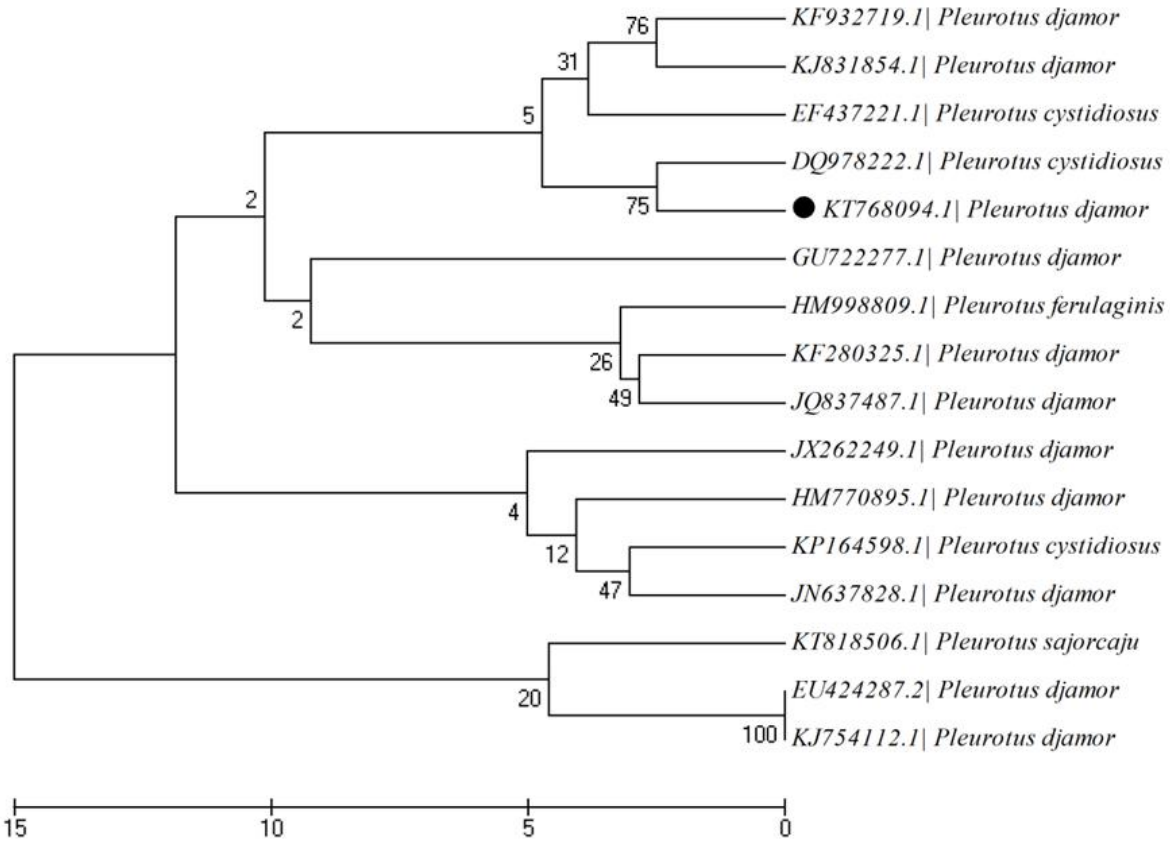


Figure 18: Phylogenetic placement of *P. djamor* (IPL/MC/PD-1) with other ex-type strain sequences obtained from NCBI Genbank Database

4.6. Molecular Characterization of *Pleurotus florida*

4.6.1. 18S rDNA sequence and phylogenetic analysis of *P. florida*

Genomic DNA of *P. florida* (IPL/MC/PF-1) was suspended in 100µl 1X TE buffer treated with RNAse (60µg) until further use. Purity of DNA evaluated in terms of the ratio between absorbance of A_{260} and A_{280} showed that genomic DNA was ~1.8. ITS region of rDNA was amplified using genus specific T/ITS4 and T/ITS6 primers where the amplified product of 665 base pair size was produced by both the primers.

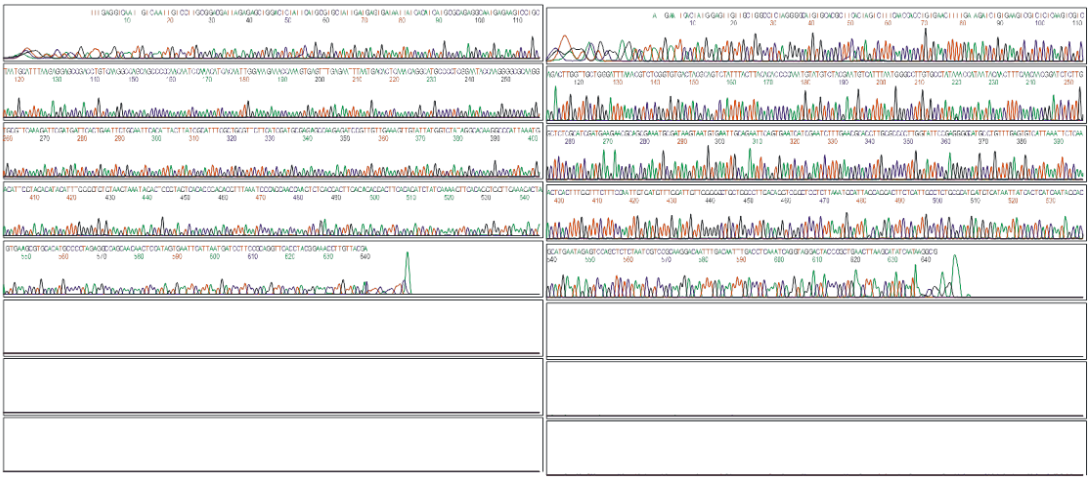
4.13.2. Chromatogram

The BLAST query of the 18S rDNA sequence of T/ITS4 and T/ITS6 (for *P. florida*) against Genbank database confirmed their identity. The sequences have been deposited in NCBI, Genbank database under the accession no. KT826605. The sequence chromatograms have been represented in figure 19.

4.6.3. Multiple sequence alignment

A multiple sequence alignment of ITS gene sequences of *P. florida* was conducted. Sequences of other strains obtained from NCBI Genbank database showing maximum homology with our strain was conducted using CLUSTAL-W algorithm which is a general purpose multiple sequence alignment program for DNA of MEGA4 software. There were quite a number of gaps that were introduced in the multiple sequence alignment program within the region that were closely related and similar sequence indicated the relationship among the isolates. The differences in these highly conserved regions are shown in different colours (figure 20). Phylogenetic analysis was carried out with Ex-type strain sequences obtained from NCBI Genbank database which showed maximum homology with *P. florida* (KT826605) (Table 13).

Chromatogram



Sequence Deposited NCBI
Accession No. KT826605
DNA Linear 665BP
Strain No: IPL/MC/PF-1

Title: *Pleurotus floridanus* isolate IPL/MC/PF 1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Partial sequence of 18S ribosomal RNA gene

CTGCGGAAGGATCATTATGAATTCACTATGGAGTTGTTGCTGGCCTCTAGGGGCATGTGCAC
GCTTCACTAGTCTTTCAACCACCTGTGAACTTTTGATAGATCTGTGAAGTCGTCTCTCAAGTC
GTCAGACTTGGTTGCTGGGATTTAAACGTCTCGGTGTGACTACGCAGTCTATTTACTTACAC
ACCCCAAATGTATGTCTACGAATGTCATTTAATGGGCCTTGTGCCTTTAAACCATAATACAAC
TTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAA
TGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCCTTGGTATTC
CGAGGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAAACTCACTTTGGTTTCTTTCCAATTG
TGATGTTTGGATTGTTGGGGGCTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATTAGC
AGGACTTCTCATTGCCTCTGCGCATGATGTGATAATTATCACTCATCAATAGCACGCATGAAT
AGAGTCCAGCTCTCTAATCGTCCGCAAGGACAATTTGACAATTTGACCTCAAATCAGGTAGG
ACTACCCGCTGAACTTAAGCATATCAATAAGGCGGAGGAA

Figure 19: Chromatogram and sequence of 18S rDNA region *P. florida* (IPL/MC/PF-1) deposited in NCBI Genbank

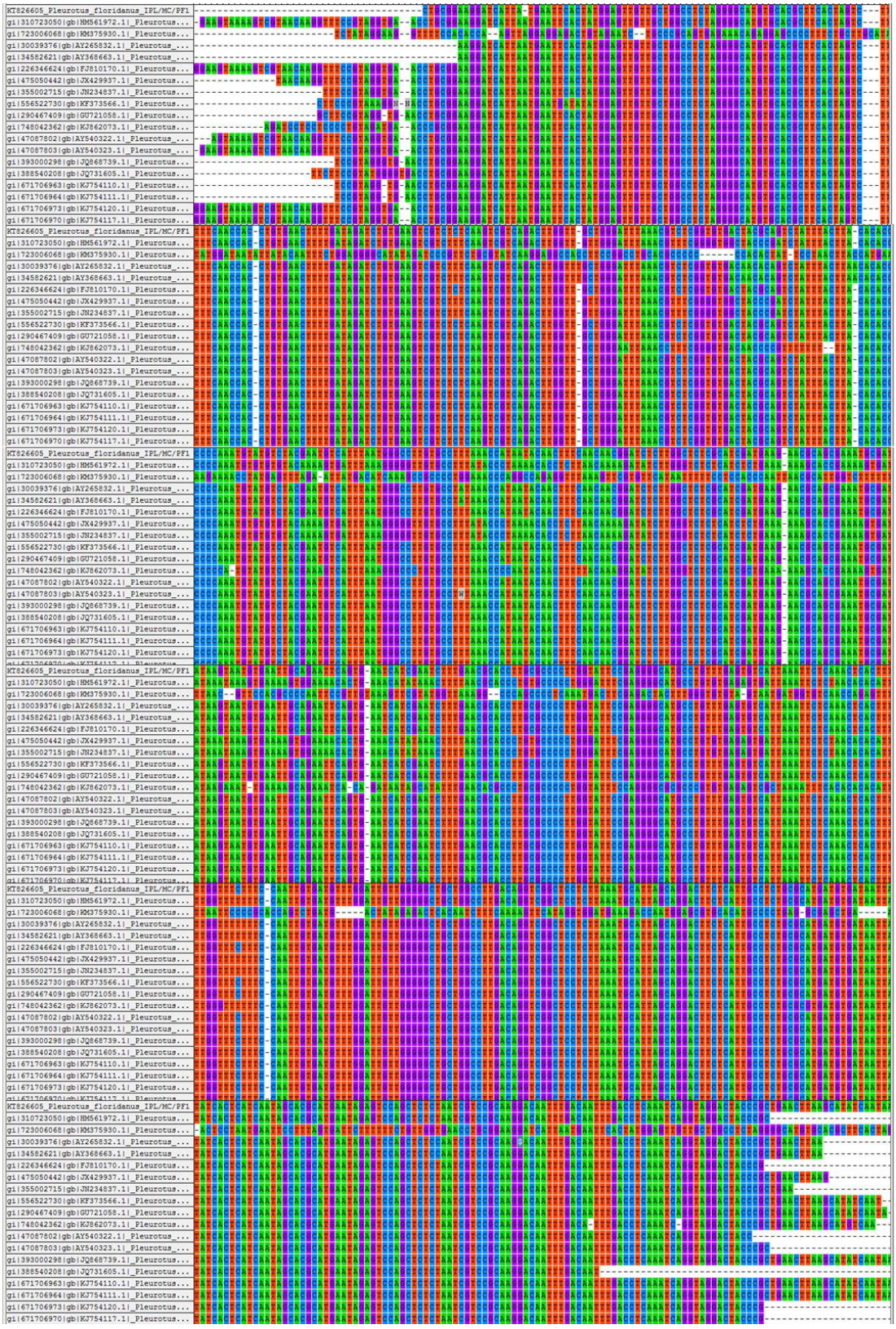


Figure 20: 18S r DNA sequence alignments of *P florida* (IPL/MC/PF-1) with other ex-type isolates. The conserved regions of the gene are demonstrated in different colours

Table 13: Genbank accession numbers of the Ex-Type strains of *P. florida* that showed the homology with the isolate.

Sl No	GenBank Accession No	Strain or Isolate	rDNA sequence (bp)	Origin
1	JQ731605	VKESR1	625	India
2	JX429937	FPFMK	668	Malaysia
3	JN234837	FTCW1 (PFW1)	654	Malaysia
4	KF373566	LCJ 155	683	India
5	GU721058	PF101	671	India
6	HM998809	LGMACC 850404	630	Hungary
7	DQ978222	X 652	682	India
8	KM375930	AAU-SAP	1835	India
9	KT968336	FLO-01	652	Korea
10	KT968340	Flo-01	667	Korea
11	KJ020935	Pfu-652	432	China
12	KT826605	IPL/MC/PF-1	665	India

4.6.4. Phylogenetic analysis of *P. florida*

The evolutionary history was inferred using the UPGMA method (Sneath PHA and Sokal; 1973). The optimal tree with the sum of branch length = 1886.85763040 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein J; 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamur, Nei and Kumar; 2004)and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 622 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura, Dudley, Nei and Kumar; 2007) (Figure 21).

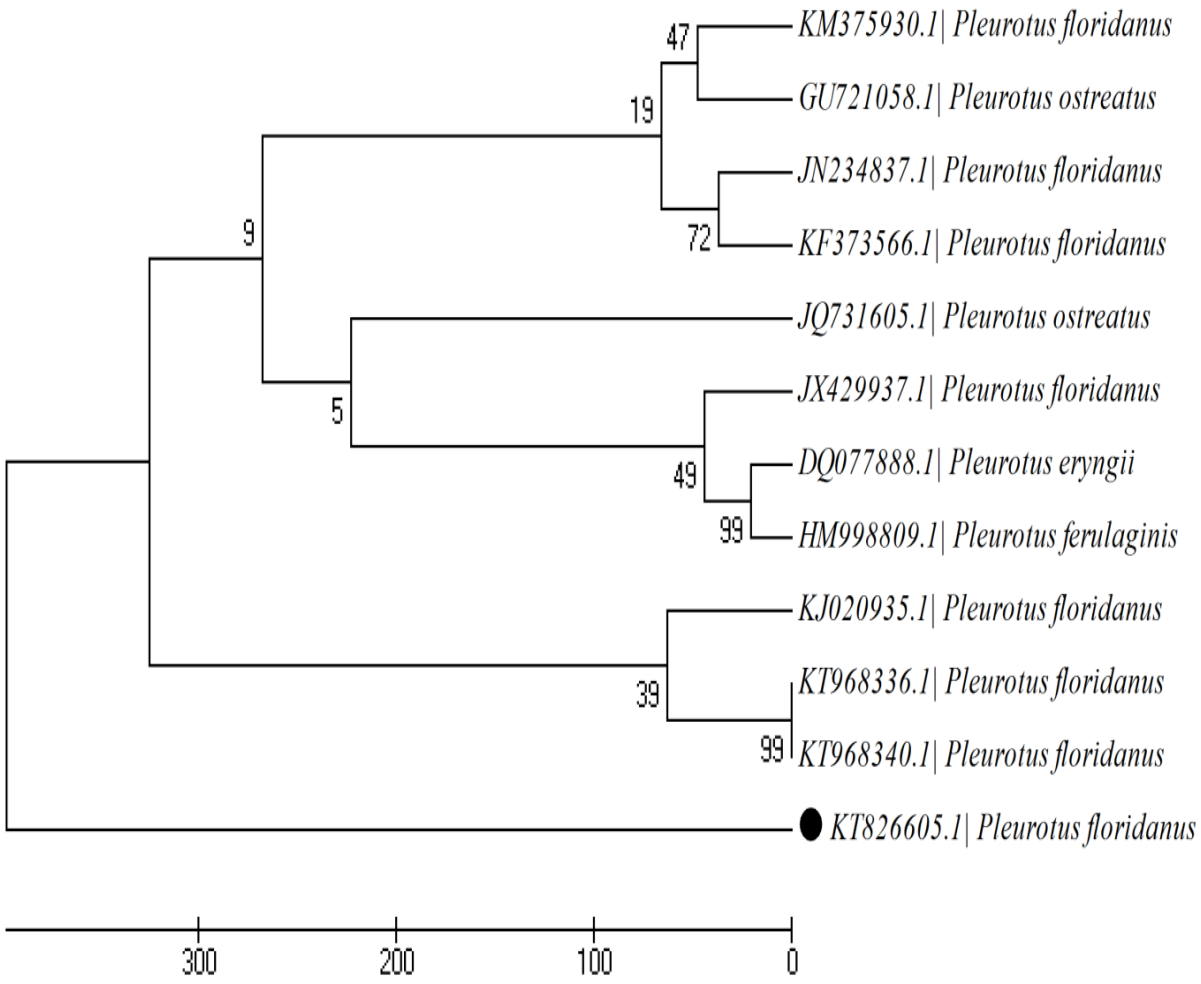


Figure 21: Phylogenetic analysis of *P florida* (IPL/MC/PF-1) with other ex-type strain sequences obtained from NCBI Genbank Database

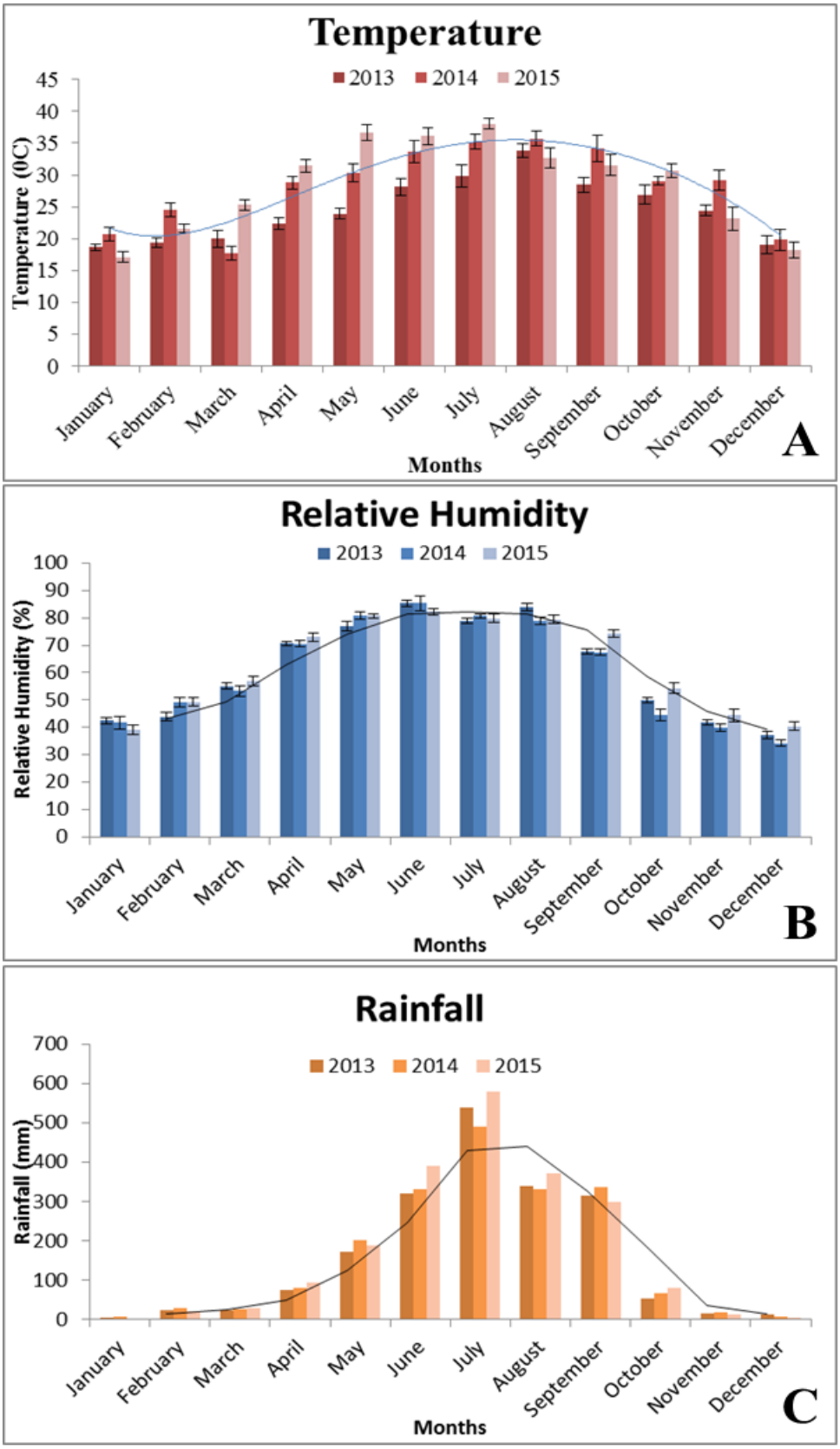


Figure 22: Weather condition of North Bengal during 2013-2015 (A) temperature, (B) relative humidity and (C) rainfall throughout the year

4.7. Cultivation of *P. ostreatus*

4.7.1. Growth in different substrates

Cultivation of *P. ostreatus* is very common in North Bengal. Paddy straw is one of the major substrate commonly used in this region. Paddy straw is largely used in cultivation throughout the year. Cultivation of *P. ostreatus* was done throughout the year in different agro climatic conditions and it was observed that the production was increased during the summer and rainy season when the temperature was about 21-32^0 C with 50-87% relative humidity (Figure 22). It was also observed that the production rate was very high during June to July. The above study reveals that paddy straw can be used as a major substrate for cultivation of *P. ostreatus*. Wheat is one of the major cereals cultivated throughout the world and India is one of the large producers of wheat. The straw after harvesting of the wheat was used as substrate for mushroom cultivation. Efficacy of wheat straw for the production of *P ostreatus* was evaluated and the results showed that the use of paddy straw for *P. ostreatus* cultivation enhances the production during July to September as the temperature was in between (24-30^0 C) with relative humidity (78-90%). It was also observed that fruiting body initiation was delayed during the winter season but it grows faster during the rainy season. From the above results, it can be said that the use of wheat straw as the substrate for mushroom cultivation showed very promising result (Figure 23 24). Evaluation of saw dust as a substrate has been done and it was observed that the growth of mycelia over the substrate has been increased and also the period of fruiting body initiation also decreased when the substrate was supplemented with saw dust (figure 25).

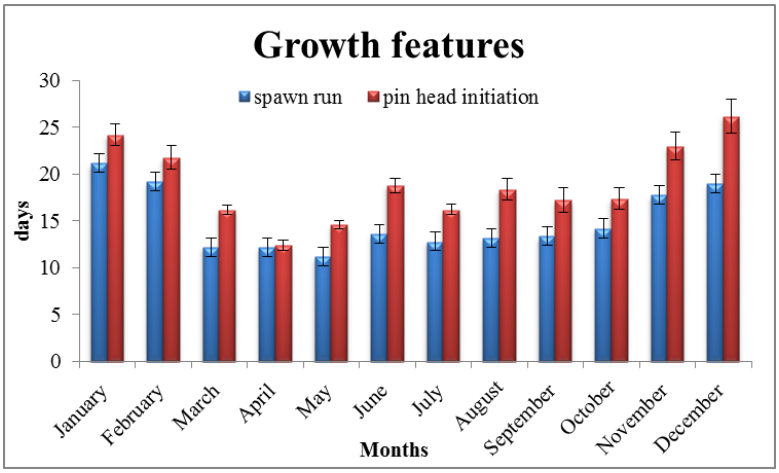


Figure 23: Assessment of spawn run period for pin head initiation during cultivation of *P. ostreatus* throughout the year

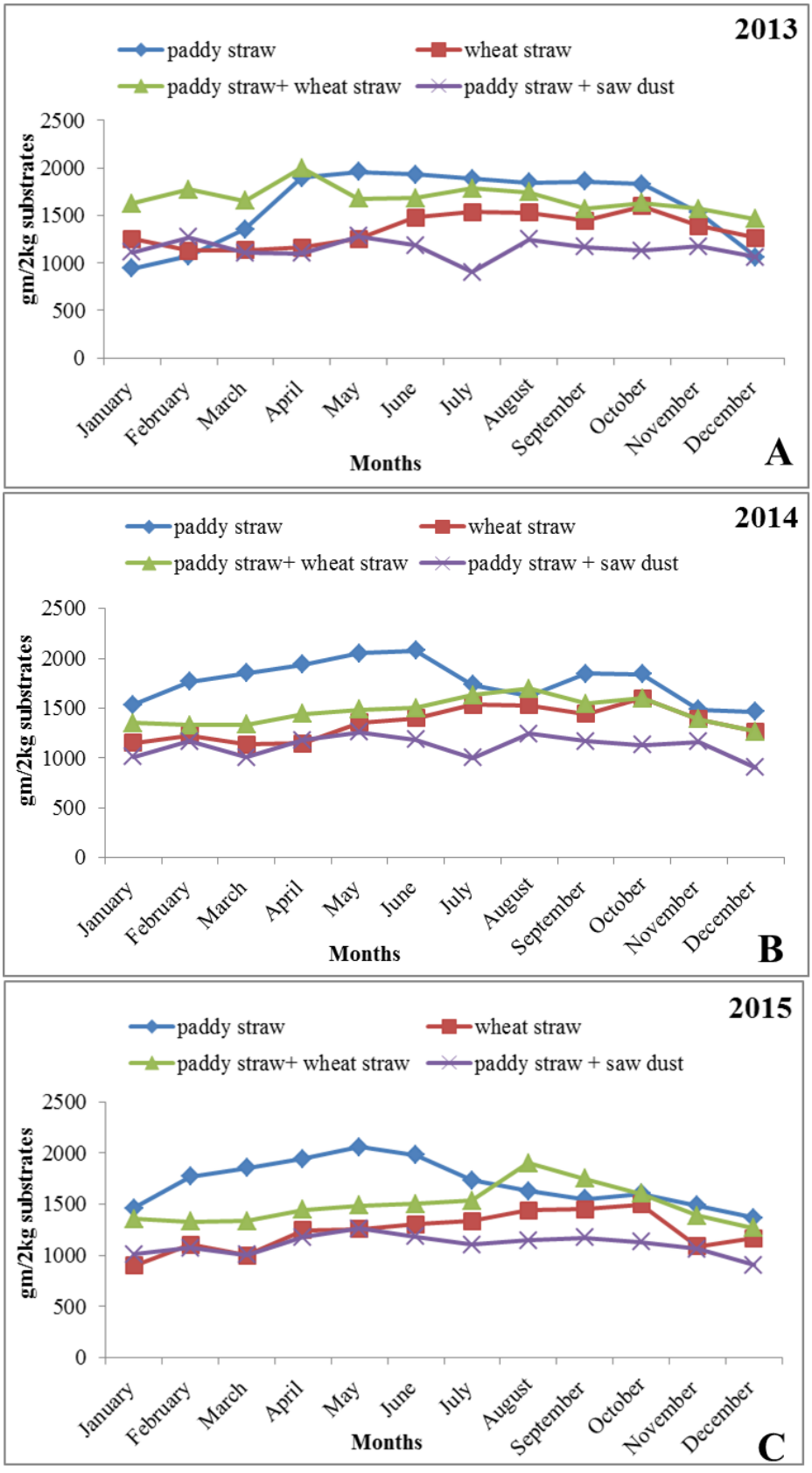


Figure 24: Comparative study on yield of *P. ostreatus* grown in paddy straw, wheat straw and saw dust substrates during 2013 (A), 2014 (B) and 2015 (C)

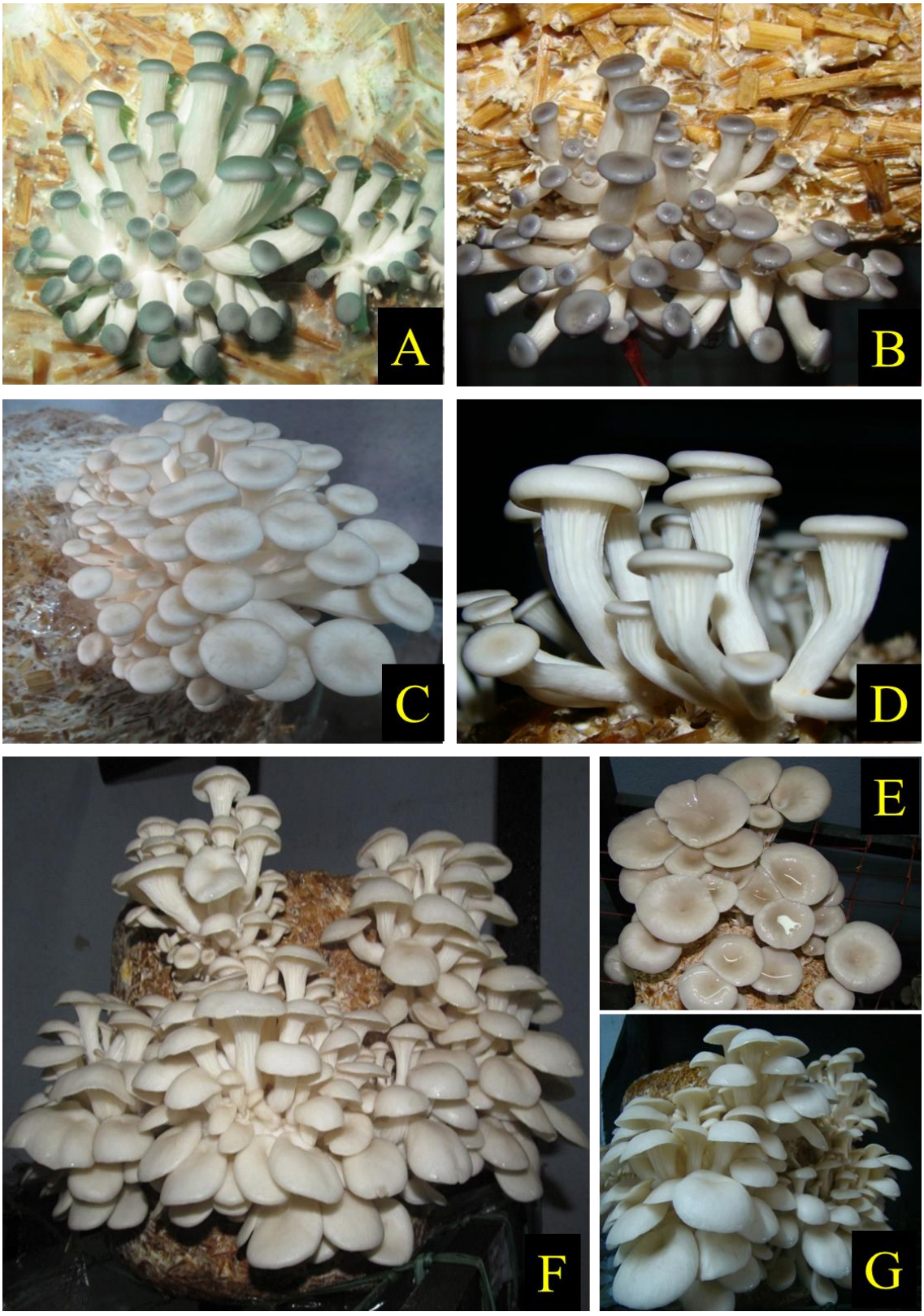


Figure 25: (A-G) Cultivation of *P ostreatus* in polypropylene bags.
(A-D) pinhead stage, (E) early mature stage and (F & G) mature stage.

Production of *P. ostreatus* also enhanced during September and October when the temperature was about 21-24^0 C with 40-42% relative humidity and with an average rainfall and it was also observed that during early summer, growth of mycelia and initiation of fruiting body period decreased. Paddy straw and wheat straw was singly used as substrate for the cultivation of *P. ostreatus*. To evaluate the efficacy of both the substrate in combination, the above results revealed that the period of mycelial run was very much less during August to October when cultivated in paddy straw wheat straw combined substrate (1:1). It was also observed that the rate of production was higher during the rainy season and during September the production per bag seems to be highest than the other months.

Table 14: Cultivation of *P. ostreatus* in different types of containers in compare polypropylene bags

Season	Type of Containers	Size of container (cm)	Average Days of colonization	Fruiting Initiation	Production (g/2kg substrate)
Summer	Polypropylene bags	45X30	16	18	1250
	Bottles	35X5(dia.)	12	15	575
	Box	80X40X30	13	16	545
Rainy	Polypropylene bags	45X30	15	21	590
	Bottles	35X5(dia.)	10	13	615
	Box	80X40X30	14	17	585
Winter	Polypropylene bags	45X30	15	20	425
	Bottles	35X5(dia.)	12	16	430
	Box	80X40X30	14	19	365

Mean value of three replicates of each case

4.7.2. Growth in different containers

Mushroom cultivation practiced in polypropylene bags is one of the most used traditional methods of cultivation. Cultivation of *P. ostreatus* was also practiced in different types of containers to evaluate their potentiality in cultivation. Polypropylene bags were also compared with the other containers like bottles and paper box (Figure 26). Experiment was conducted using the paddy straw as substrate and equal amount of substrate was used and the results revealed that the waste bottles were very much efficient in cultivation. During the summer and rainy season, *P. ostreatus* can be cultivated and it was observed that the mycelia run over substrate and initiation of

fruiting body also enhances. Production was also differs from polypropylene bags and in bottles and boxes yield was quite higher than the polypropylene bags. Bottles and boxes also reduce the full cropping period (Table 14) than that of the bags.

Figure 26: (A-H) Cultivation of *P. ostreatus* in different containers.
(A-B): Polypropylene bags; (C-E) plastic bottles (F) broken glass goods; and (G-H) box made up of aluminium sheet.

4.8. Cultivation of *Pleurotus sajor-caju*

4.8.1. Growth in different substrates

Oyster mushroom is one of the major mushrooms cultivated in North Bengal and in this region *Pleurotus sajor-caju* is widely cultivated out of other species for its large fruiting body and wide range of favourable cultivation period. Paddy straw mostly used a substrate as it is commonly found in this region. It was observed that the period of initiation of fruiting body is very less during the rainy season. Above results showed that the production increase during April to November and the cropping time also very lesser during this period (Figure 27). It was observed that the production increased during the August to October (about 510-560gm/kg substrate) when the temperature lies between 21-27^{0}C. Different lignocellulosic components are used to cultivate the oyster mushroom and it was observed that the cultivation period and spawn run period very less than the paddy straw. Results revealed that the *Pleurotus sajor-caju* can be cultivated throughout the year but the production rate high during march to November and the production increased around 610 gm/kg straw when the temperature lies between 21-30^{0}C with a relative humidity about 42-88. Saw dust is one of the very popular lignocellulosic components found in this region. Saw dust was used as supplement to increase the productivity and it was observed that during September and October, production ratio was very high (620-635 g/ kg substrate) (Figure 28). It also enhances the growth rate of the mycelia resulted in shortening the cropping period. It was observed that *P. sajor-caju* grows faster in wheat straw combined with paddy straw and fruiting body initiation and yield per kg substrate rate was also higher than the single substrates.

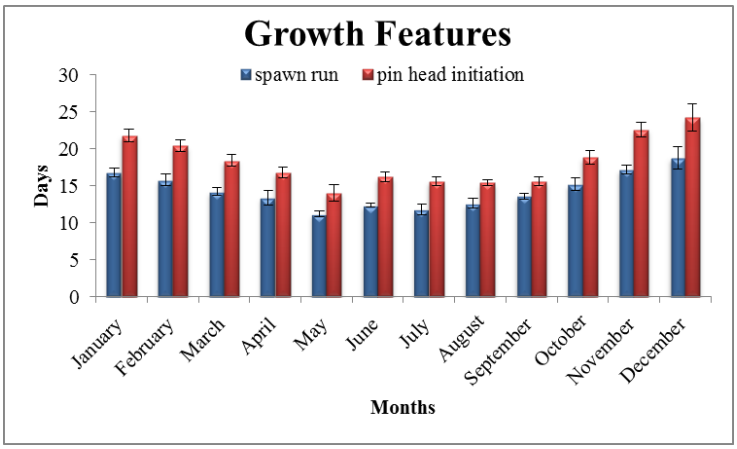


Figure 27: Assessment of spawn run period for pin head initiation during cultivation of *P. sajor-caju* throughout the year

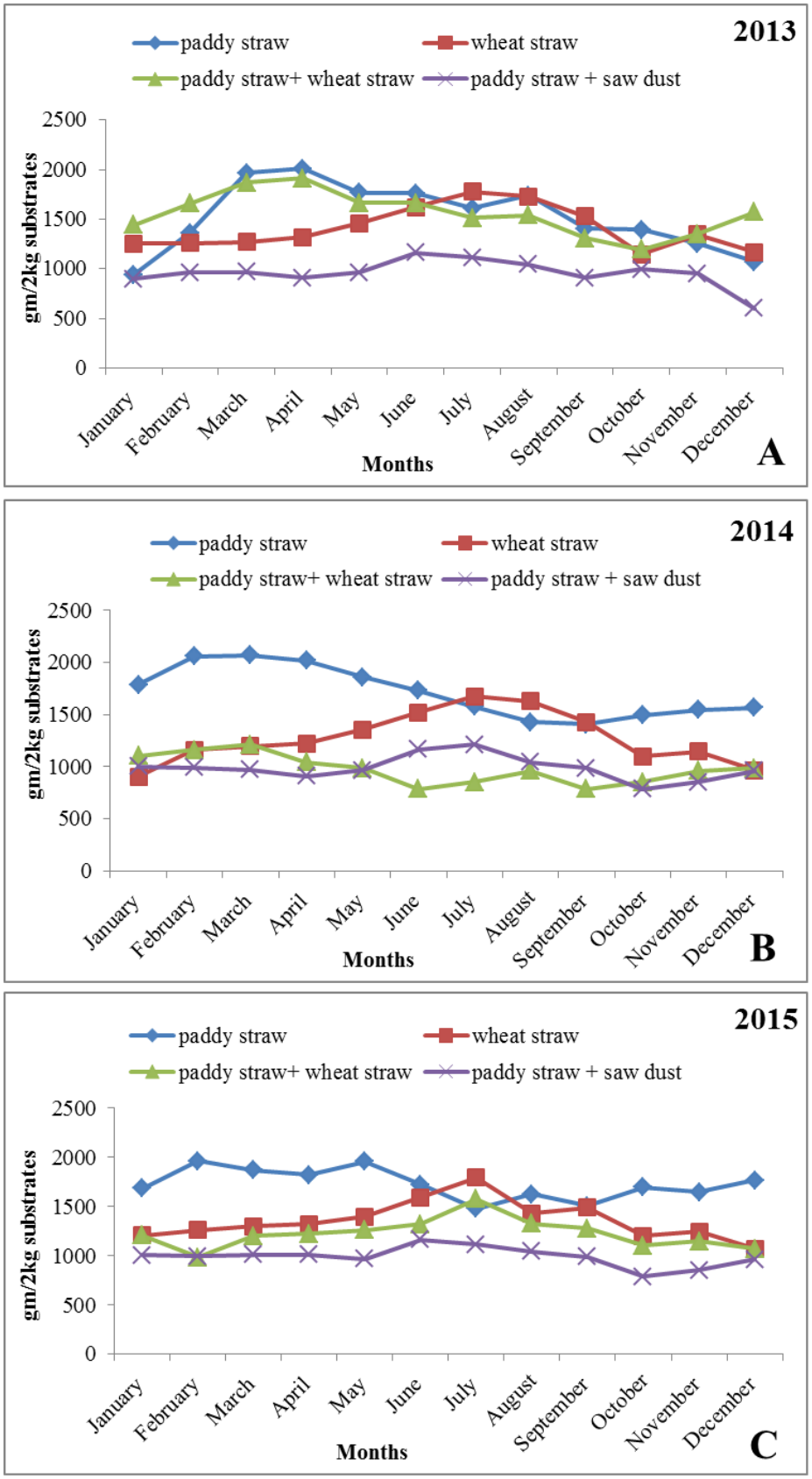


Figure 28: Comparative study on yield of *P. sajor-caju* grownin paddy straw, wheat straw and saw dust substrates during 2013 (A), 2014 (B) and 2015 (C)

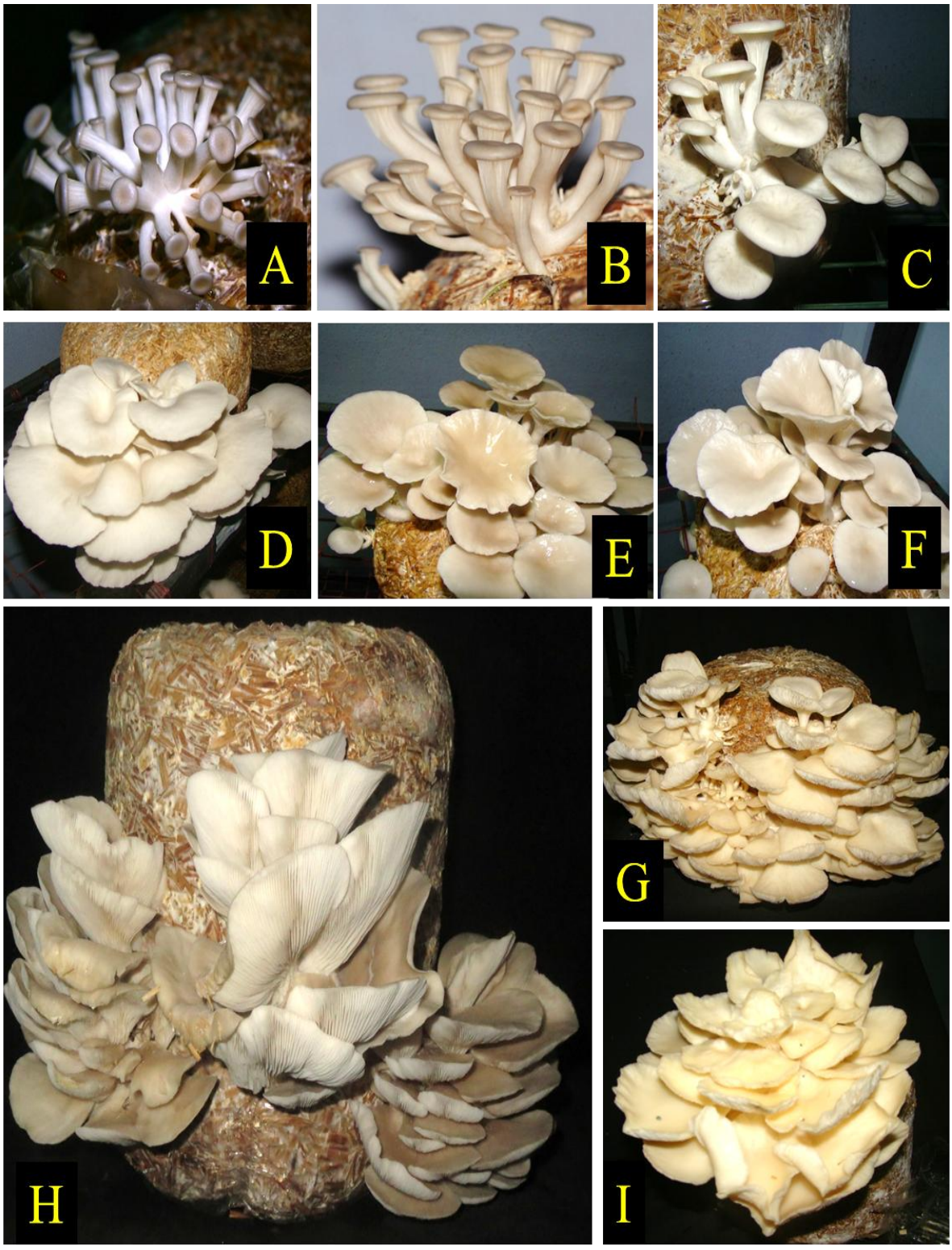


Figure 29: (A-I) Cultivation of *P sajor-caju*
(A-C) pinhead, (D-F) early mature stage and (G-I) showing the mature fruiting body.

Table 15: Cultivation of *P. sajor-caju* in different types of containers in compare polypropylene bags

Season	Type of Containers	Size of container (cm)	Days of colonization	Fruiting Initiation	Production (g/kg substrate)
Summer	Polypropylene bags	45X30	15	20	426.6±27.28
	Bottles	35X5(dia.)	11	16	491.6±10.13
	Box	80X40X30	15	20	411.6±11.66
Rainy	Polypropylene bags	45X30	14	17	545±25.65
	Bottles	35X5(dia.)	13	14	600±13.22
	Box	80X40X30	15	20	561.6±10.13
Winter	Polypropylene bags	45X30	15	23	366.6±18.55
	Bottles	35X5(dia.)	15	19	366.6±24.55
	Box	80X40X30	18	23	301.6±13.64

(±) standard error, mean value of 3 replicates

4.8.2. Growth in different containers

Pleurotus sajor-caju is widely cultivated in this region for its large fruiting body, ability to grow throughout the year and taste. Large number of people cultivating the species but due to proper cultivation technology knowhow most of the farmers are unable to cultivate successfully. Containers play an important role in the mycelial growth as well as production of oyster mushrooms. Different containers were used to evaluate their ability to grow as well as yield potential bottles and boxes were used. It was observed that mycelial matt covers the substrate much more early than in case of bottle cultivation which results in reduction in the full cropping period. On the other hand, it was also observed that the yield potential was higher in boxes than the polypropylene bags (Table 15). Waste plastic bottles results in higher yield and lower cropping period as well as recycling the containers. Using the plastic bottles also does not require large production unit (Figure 30)

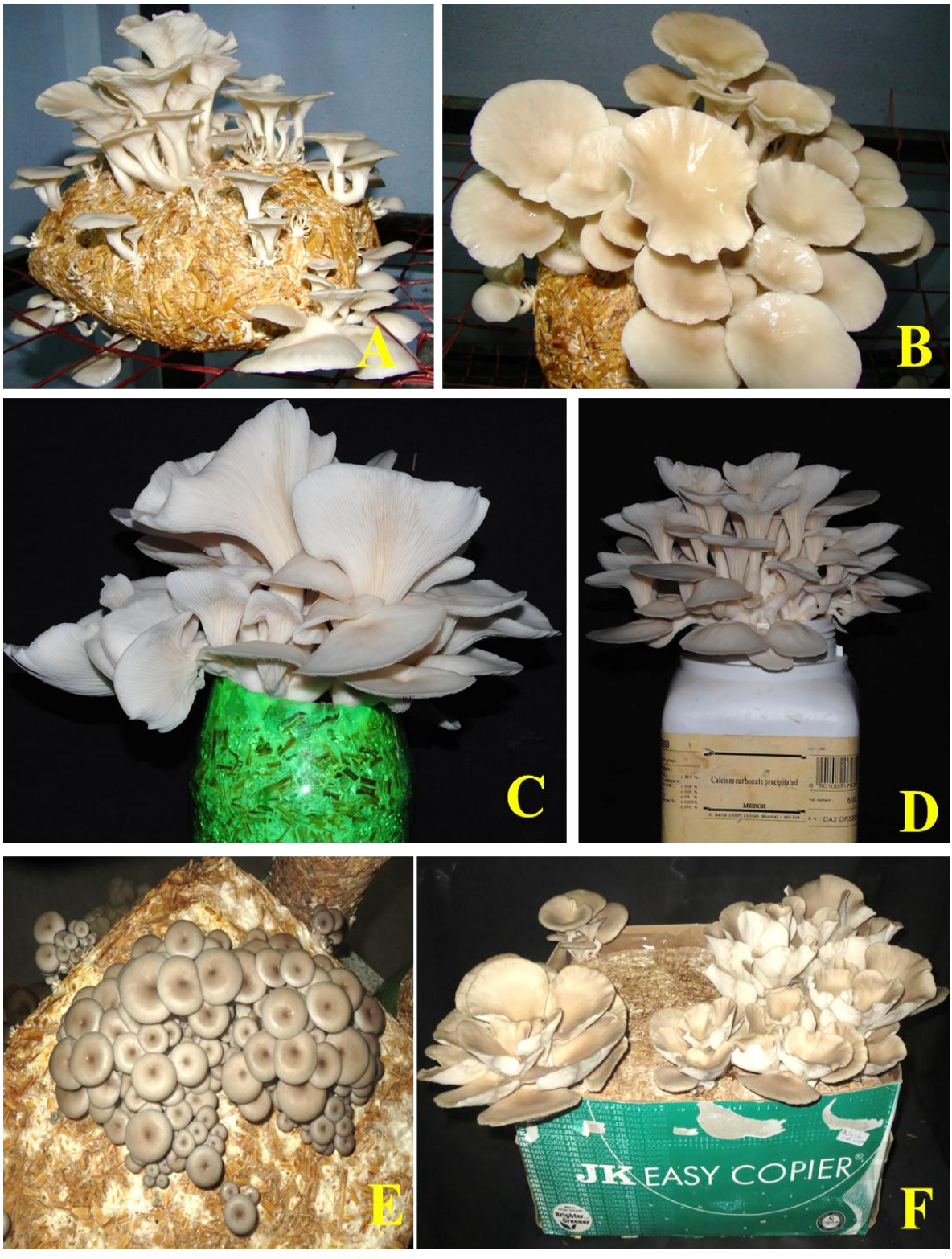


Figure 30: (A-F) Cultivation practices of *P. sajor-caju* using different containers, (A-B) polypropylene bags; (C) plastic bottles (D) waste bottles and (E-F) paper box.

4.9. Cultivation of *Pleurotus djamor*

4.9.1. Growth in different substrates

Different species of oyster mushroom cultivated in North Bengal. But *P. djamor* pink oyster mushroom is a new introduction in this region. Cultivation of pink oyster mushroom was carried out using different substrate. Paddy straw was used to evaluate the cultivation efficiency and it was observed that *Pleurotus djamor* gives better yield during winter season when the temperature lies between 18-22^{0}C with a very low range of relative humidity (50-80%). The results also revealed that the period of mycelial run as well as fruiting body initiation was very less during the winter (Figure 31). *Pleurotus djamor* commonly known as pink oyster mushroom can be cultivated a wide range of lignocellulosic substrate and it requires optimum temperature for its better growth and yield. Wheat straw is one of the major crop widely cultivated in India. *P. djamor* was cultivated using wheat straw as substrate and the result showed that the mycelial run period much lower during December to March when the temperature was quite lower than the summer. Final yield of *P. djamor* was also increased during this period which proves that the cultivation period of *P djamor* is much more favourable during the December to March in this region. It was also observed that fruiting body initiation require very lower amount of relative humidity (40-70%). Different substrate was used to evaluate their efficacy in cultivation of different species of oyster mushroom and *P djamor* was also cultivated using the paddy straw in combined with wheat straw (1:1). It was observed that the mycelial run period reduces in during the winter season (December to February) and also during the early summer season (Figure 32). The results revealed that using the paddy straw and wheat straw in combined form was more efficient and it was also extends the cropping time up to early summer season.

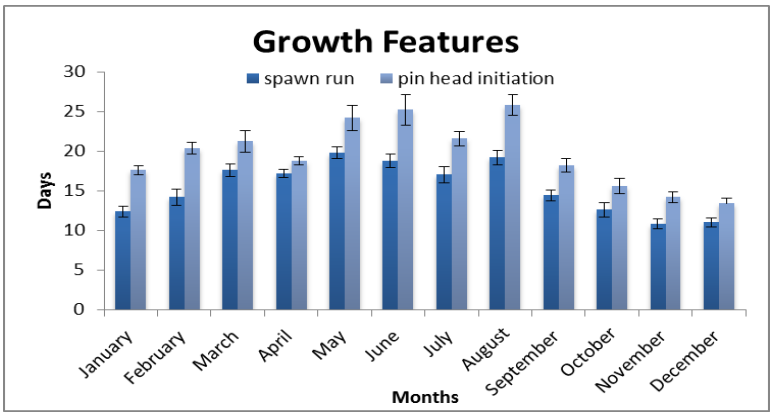


Figure 31: Assessment of spawn run period for pin head initiation during cultivation of *P. djamor* throughout the year

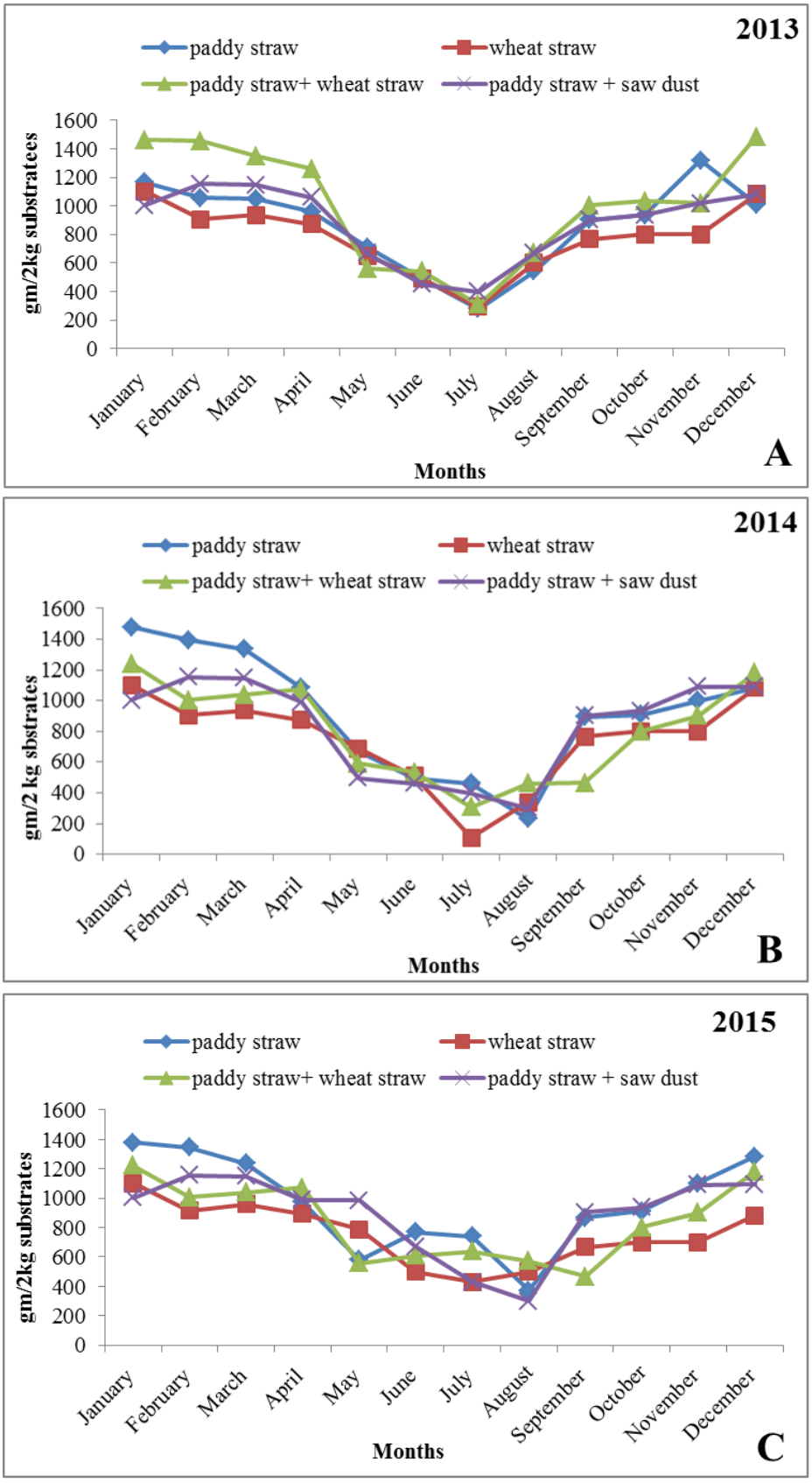


Figure 32: Comparative study on yield of *P. djamor* grown in paddy straw, wheat straw and saw dust substrates during 2013 (A), 2014 (B) and 2015 (C)

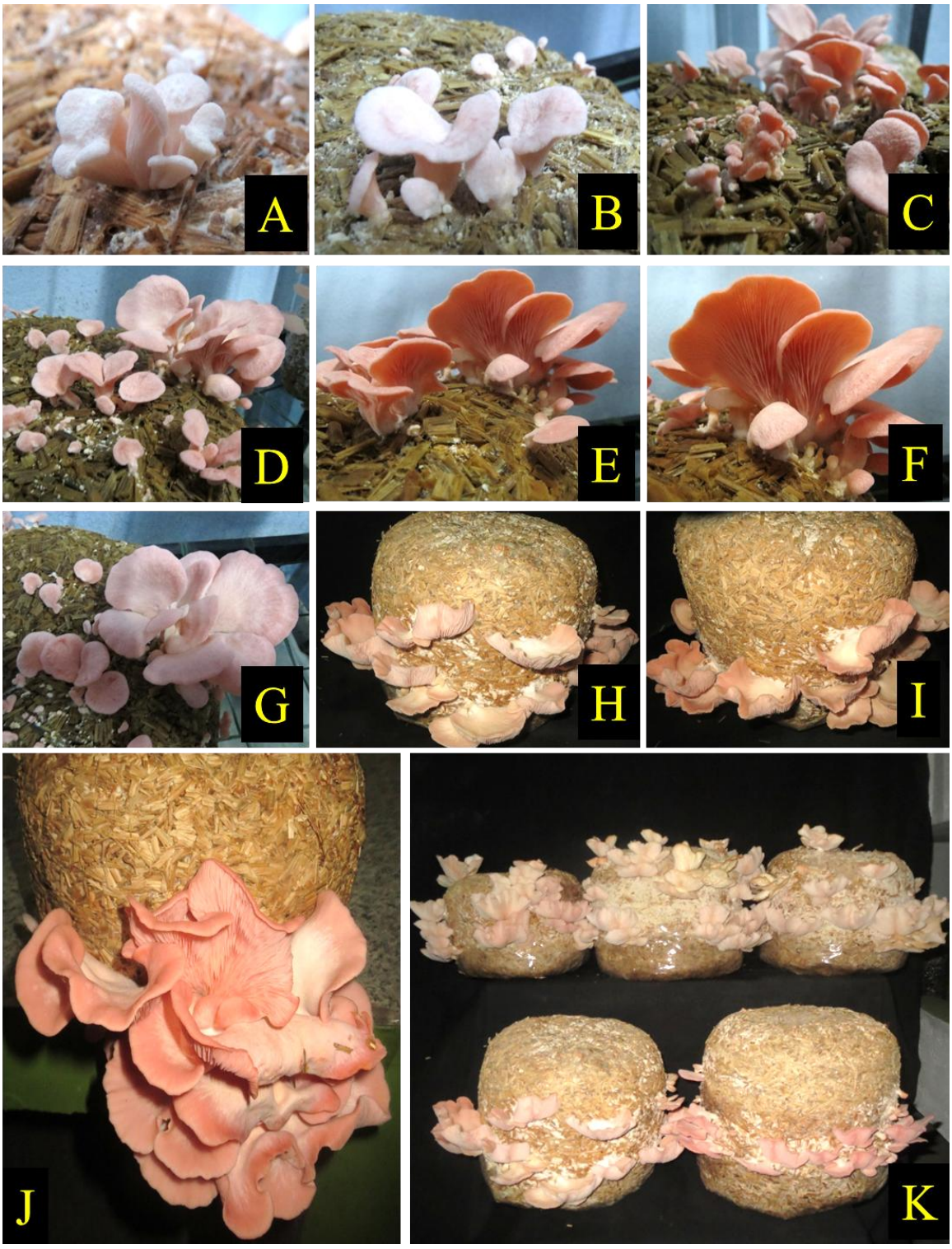


Figure 33: (A-K) Cultivation of *P djamor* (pink oyster mushroom); (A-C) pinhead stage, (D-G) early mature stage and (H-K) mature stage ready for harvesting.

Table 16: Cultivation of *Pleurotus djamor* in different types of containers in compare polypropylene bags

Season	Type of Containers	Size of containers	Days of colonization	Fruiting Initiation	Production (g/kg substrate)
Summer	Polypropylene bags	45X30	19	23	408.3±8.81
	Bottles	35X5(dia.)	18	22	410±13.22
Rainy	Polypropylene bags	45X30	21	25	308.3±15.89
	Bottles	35X5(dia.)	16	19	335±12.58
Winter	Polypropylene bags	45X30	13	18	591.6±14.24
	Bottles	35X5(dia.)	12	16	613.3±17.40

Mean value of five replicate containers, (±) standard error

4.9.1. Growth in different containers

Pleurotus djamor popularly known as pink oyster mushroom was cultivated in this region. Various substrates were tested for better cultivation and it was observed that paddy straw supplemented with saw dust and paddy straw mixed with wheat straw showed better result for its cultivation. Pink oyster mushroom is very much popular for its colour, texture as well as taste. Pink oyster mushroom very recently introduced in this region and the cultivation of this mushroom is now very popular. Pink oyster mushroom was cultivated using the plastic waste bottles in compare to polypropylene bags. Results revealed that the plastic bottles were very much useful to cultivate. Mycelial run period was very less than the polypropylene bags as the structure was much more compact that the bags (Table 14). It was also observed that yield was also increased. Plastic bottles were recycled to cultivate and for the large scale production, waste plastic bottles were much more efficient. The above result showed that the use of waste bottles can also be used for the cultivation as the colonization period, full cropping time reduces and production increases (Figure 34).

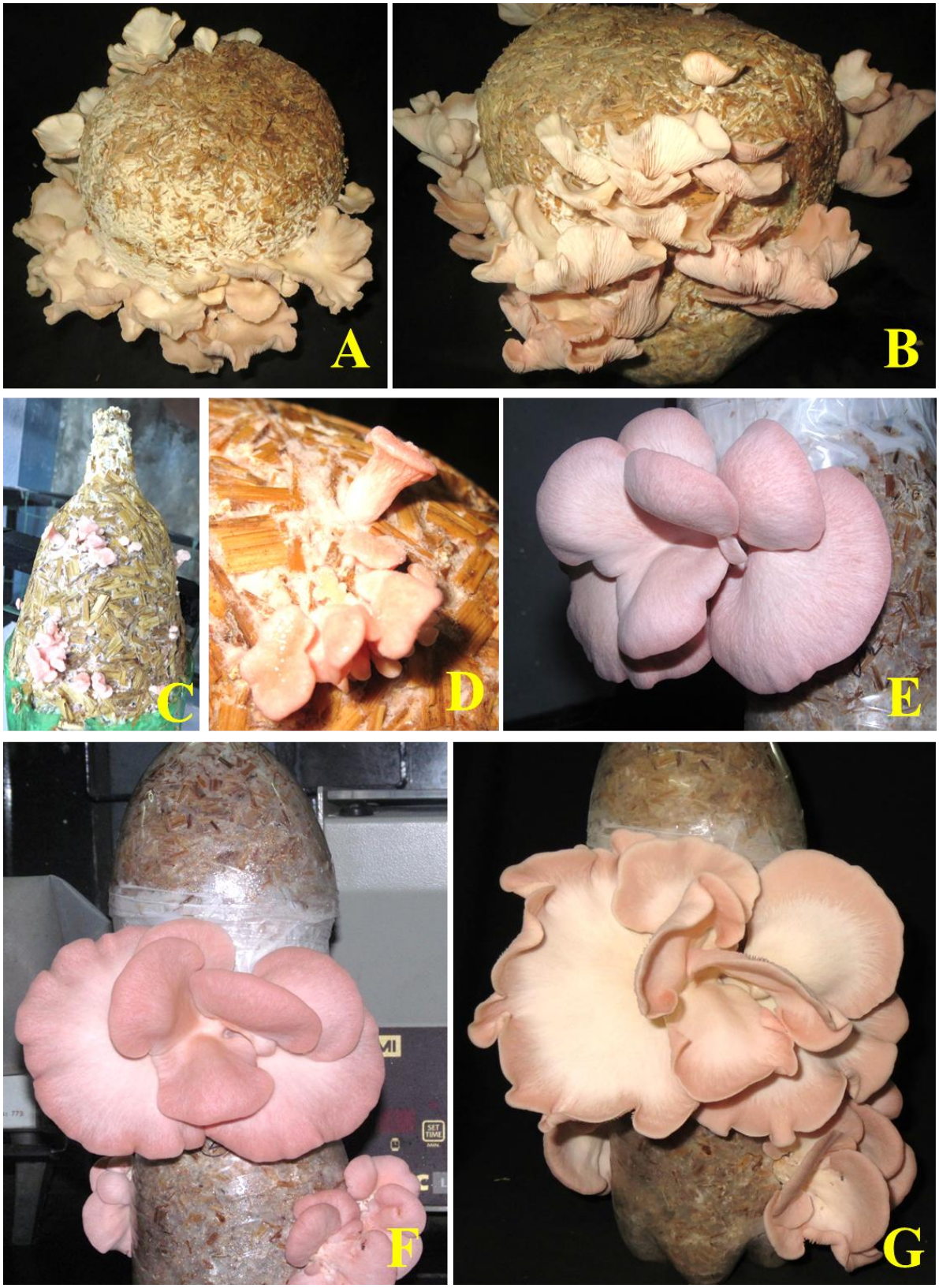


Figure 34: (A-G) Growth and development of fruitbody during cultivation of *P. djamor* in different containers; (A-B) polypropylene bags; (C-G) using waste plastic bottles

4.10. Cultivation of *Pleurotus florida*

4.10.1. Growth in different substrates

Oyster mushroom is one of the major mushrooms cultivating widely in North Bengal and different species of oyster mushroom are now being cultivated by a large number of farmers in this region. *P. florida* commonly known as white oyster mushroom largely cultivated for its bright white fruiting body, texture and great taste. In North Bengal, paddy straw is commonly used for the cultivation as it is the most available substrate in this region. The results showed that the mycelial run period was about 14 days during the early winter season to early summer (Figure 35). It was observed that the spawn run period reduces during November to April. Cultivation also depends on the environmental conditions and the results the production increased during December to April and the complete cropping period also decreased during this period. Wheat straw was used for the cultivation of *P. florida* and it was observed that the spawn run period decreased during the winter (January and February). Production rate was also high during this period (Figure 36). Results also revealed that for fruiting body initiation, lower temperature required with very low relative humidity. Wheat straw was successfully used for the cultivation and it requires less time period for full cropping as well as increase amount of production. *P florida* was also cultivated using the paddy straw in combined with wheat straw (1:1). It was observed that the mycelial run period reduces in during the winter season (December to March) and also during the early summer season. The results revealed that using the paddy straw and wheat straw in combined form was more efficient and it was also extends the cropping time up to early summer season. A large number of industries are producing timber and thus north Bengal is a very renowned source of saw dust. Saw dust is very hard and thus it was used as supplement with paddy straw (Figure 37).

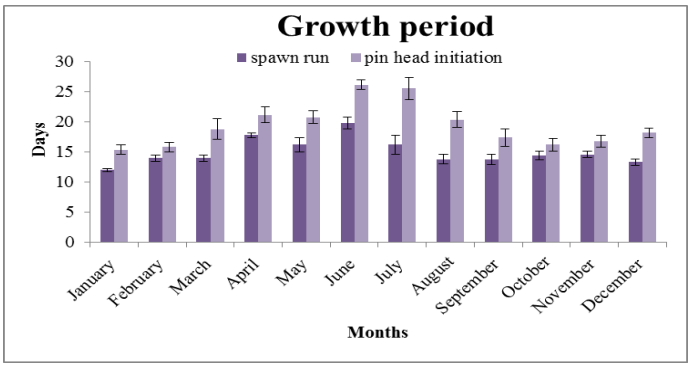


Figure 35: Assessment of spawn run period for pin head initiation during cultivation of *P. florida* throughout the year

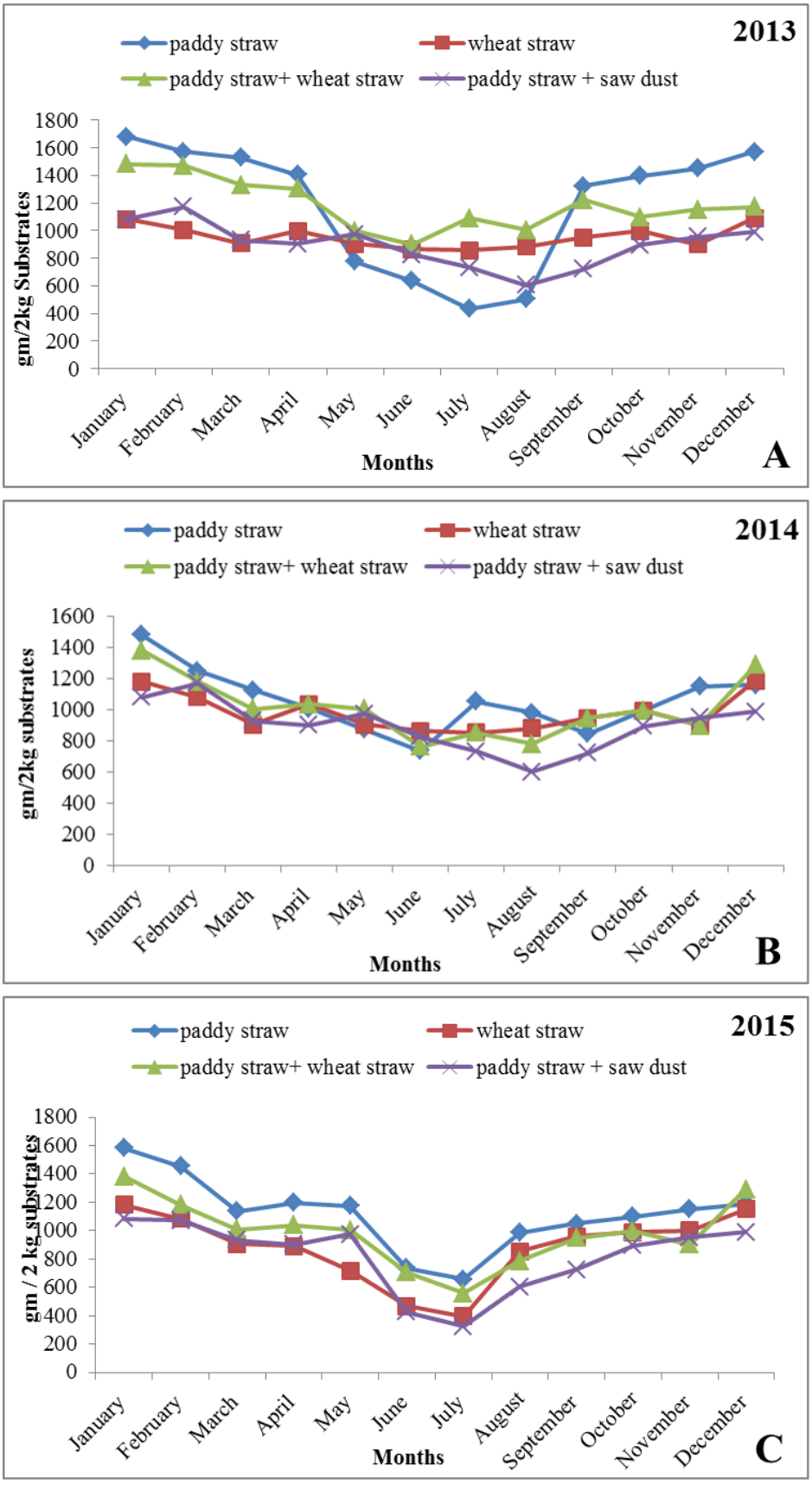


Figure 36: Comparative study on yield of *P. djamor* grown in paddy straw, wheat straw and saw dust substrates during 2013 (A), 2014 (B) and 2015 (C)

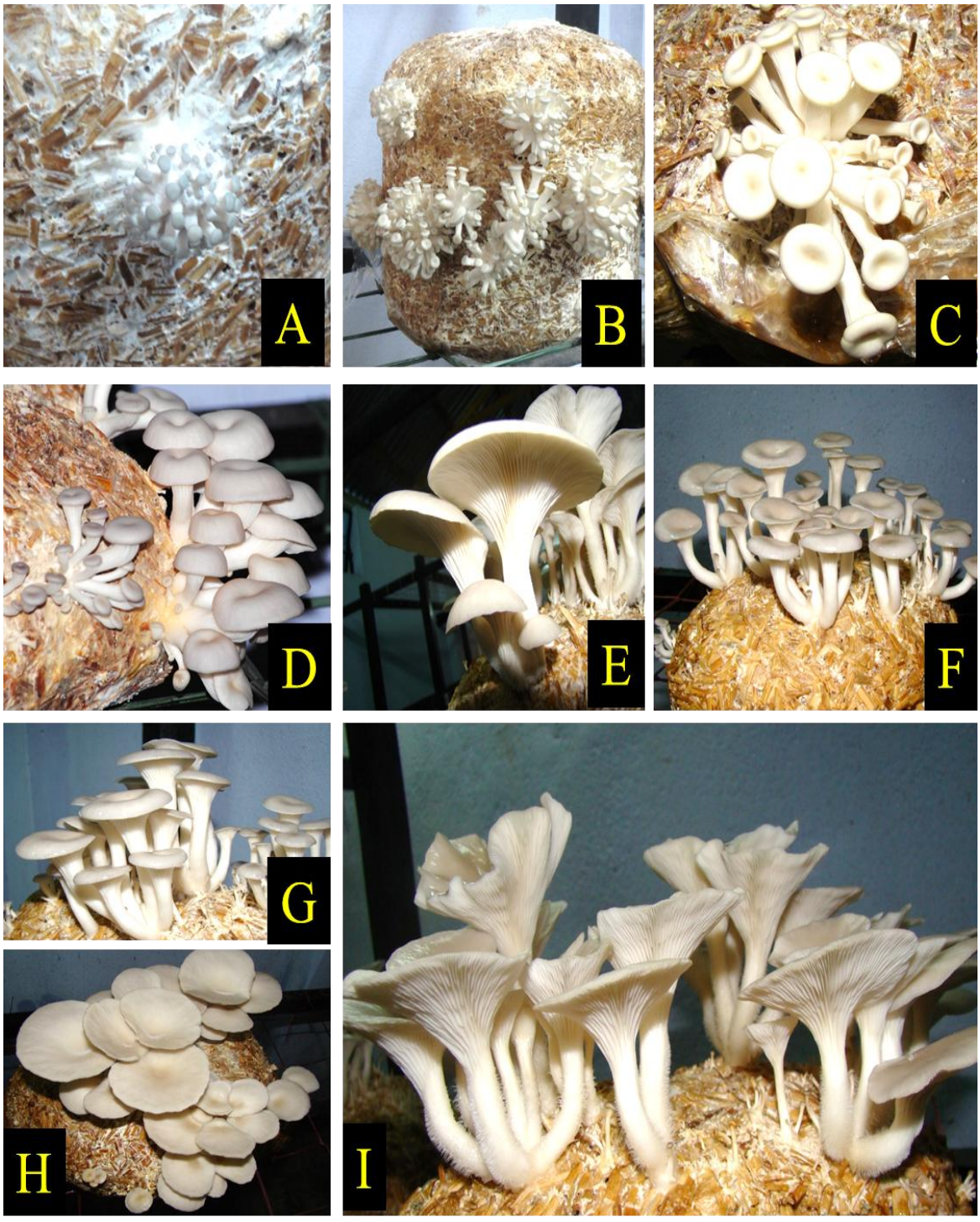


Figure 37: (A-I) Cultivation of *P. florida* (A-C) pinhead stage, (D-G) early mature stage and (H&I) showing the mature stage.

Table 17: Cultivation of *P. florida* in different types of containers in compare polypropylene bags

Season	Type of Containers	Size of container (cm)	Days of colonization	Fruiting Initiation	Production (g/kg substrate)
Summer	Polypropylene bags	45X30	25	26.33±1.45	480±8.66
	Bottles	35X5(dia.)	20	22	495±14.43
	Box	80X40X30	23	27	465±16.07
Rainy	Polypropylene bags	45X30	24	27	390±11.54
	Bottles	35X5(dia.)	20	23	406.6±9.27
	Box	80X40X30	25	27	363.3±14.8
Winter	Polypropylene bags	45X30	18	22	558.3±10.13
	Bottles	35X5(dia.)	14	17	575±16.07
	Box	80X40X30	18	23	530±8.66

Mean value of three replicates, (±) standard error

4.10.2. Growth in different containers

Pleurotus florida is one of the major oyster species cultivated in this region for its large fruiting body, ability to grow throughout the year and taste. Large number of growers of North Bengal region cultivating *P. florida*. For the cultivation different containers were used to evaluate their ability to grow as well as yield potential. Results revealed that the mycelial run period is quite lower in case of bottles as well as polypropylene bags while it took more time in case of boxes for colonization (Table 17). On the other hand, it was also observed that the yield potential was higher in boxes than the polypropylene bags. Waste plastic bottles results in yield, lower cropping period as well as recycling the containers (Figure 38). Using plastic bottles helps in lowering the cropping time as well as it was also observed that bottle cultivation dose not required such a large production unit.

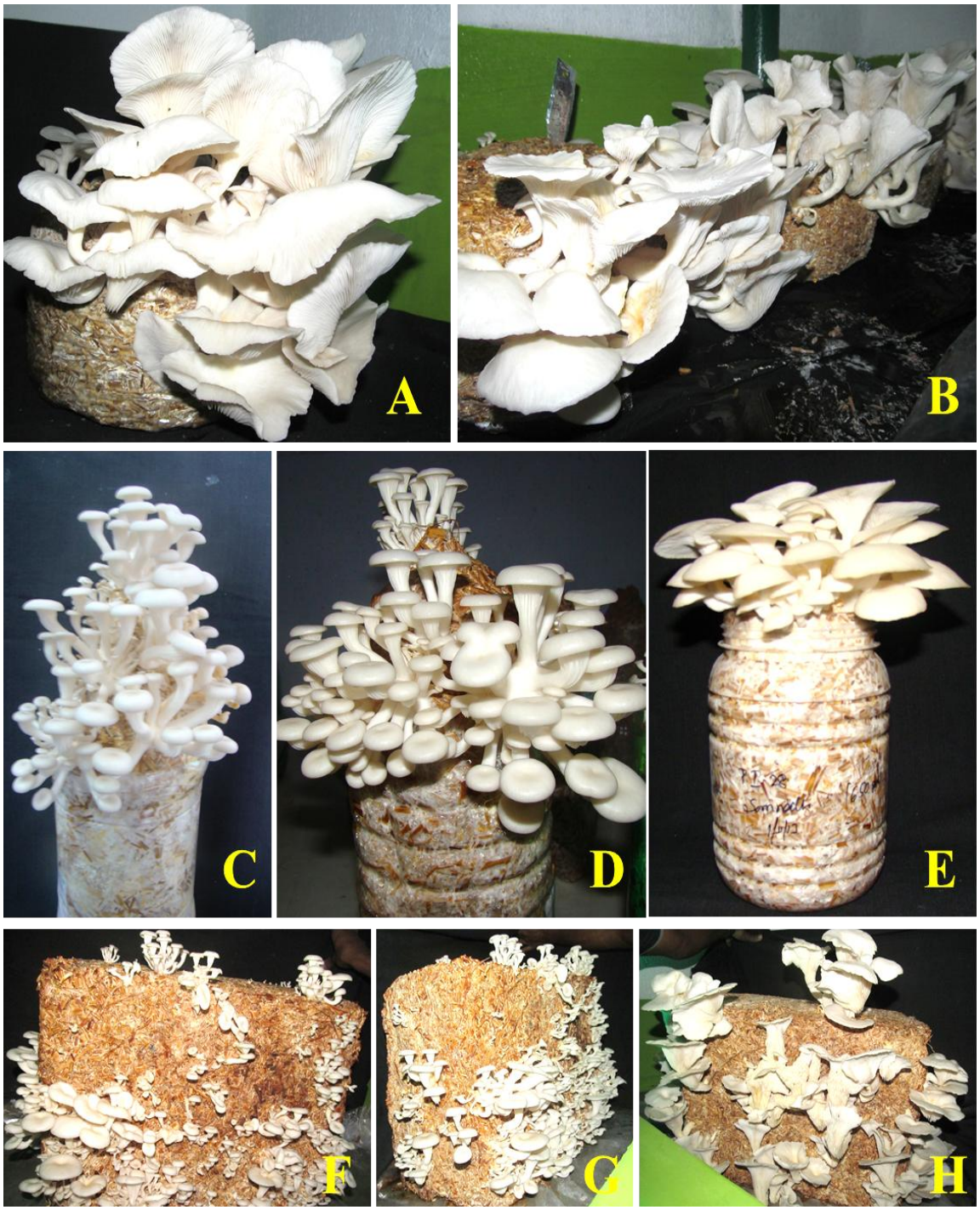


Figure 38: (A-H) Cultivation practice of *P. florida* using different types of containers (A-B) polypropylene bags; (C-E) waste plastic bottles and (F-H) in paper box.

4.11. Determination of moisture content of cultivated oyster mushroom (*P. ostreatus, P sajor-caju, P djamor* and *P florida*)

Different species of oyster mushroom was cultivated using different substrates such as paddy straw, wheat straw and saw dust in single as well as in combination with others. Mushroom fruiting body consists a high amount of water content and it was observed that substrate effects on moisture content. Results revealed that in case of *Pleurotus ostreatus* cultivated on paddy straw and wheat straw single as well as combination showed highest moisture content than the other substrates (Figure 39A). The moisture content of *Pleurotus sajor-caju* was found to be about 45-90% cultivated on different substrates (Figure 39B) highest moisture content was found in mature pileus cultivated on paddy straw followed by paddy straw combined with saw dust. It was also observed that the moisture content of *P. djamor* was higher in both young pileus as well as in mature pileus and pileus of paddy straw and wheat straw showed highest (80-90%) amount of moisture content (Figure 39C). In case of *P. florida,* moisture content was found to be higher in both young pileus as well as in mature pileus. Mature pileus cultivated on paddy straw and wheat straw showed very high (80-93%) amount of moisture content (Figure 39D).

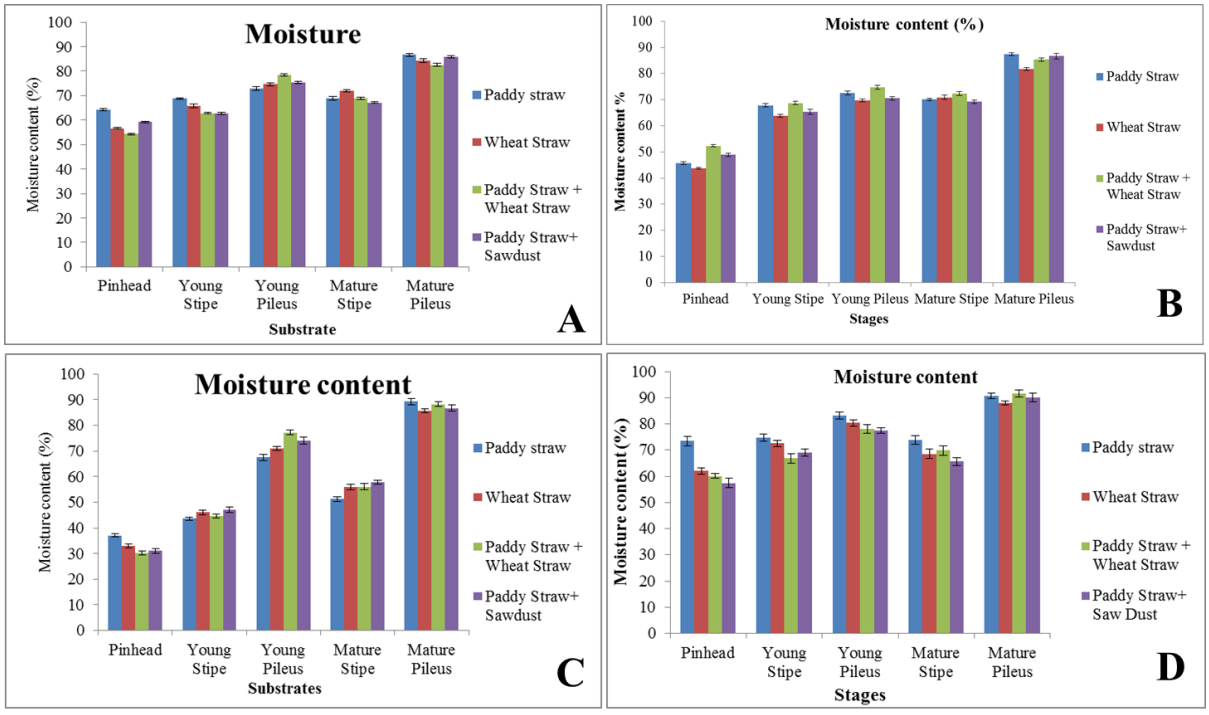


Figure 39: Comperative study of moisture content of (A) *P. ostreatus,* (B) *P. sajor-caju,* (C) *P djamor* and (D) *P florida*

4.12. Estimation of protein, sugar and lipid content of oyster mushroom grown on different substrates

4.12.1. *Pleurotus ostreatus*

Major compounds of mushroom are protein and carbohydrates. *Pleurotus ostreatus* was cultivated using different substrates which also effects on the nutritional composition. Total sugar and reducing sugar estimation were performed and it was found that mature pileus consists about 280-310 mg/gm tissue total sugar and 42-50 mg/gm tissue reducing sugar (Figure 40 A&B). The results also revealed that the reducing sugar significantly higher in case of mature pileus as well as young pileus cultivated in paddy straw. Different stages were taken into consideration for the analysis of protein, sugar and it was found that the substrates also affect the protein and carbohydrate content. In case of paddy straw and wheat straw mature pileus found to be consisting high amount of total sugar content as well as reducing sugar content in compare to other two substrates. Oyster mushroom is known to be a very good source of protein and such proteins was due to the presence of total free amino acid. Protein content was found to be very high in different stages of growth. In compare to carbohydrates, protein content was very high (Figure 41 A). The results showed that *P. ostreatus* containing about 250-400 mg/gm tissue protein. Oyster mushroom is also very popular as it contains lower amount of lipid content and it was observed that about 6-8mg/gm tissue lipid content (figure 41B)was found and highest lipid content was observed in case of paddy straw and wheat straw combined substrate.

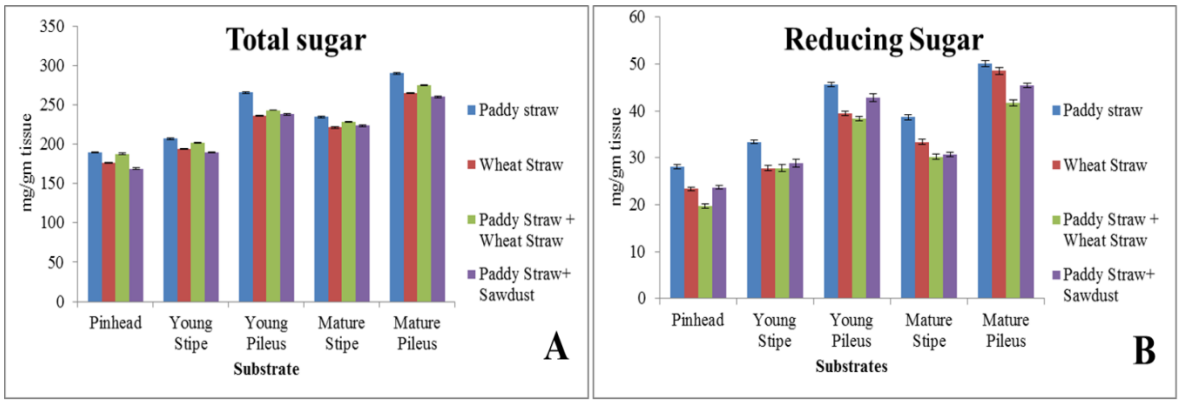


Figure 40: Total sugar (A), reducing sugar (B) of *P. ostreatus* in different stages of its growth

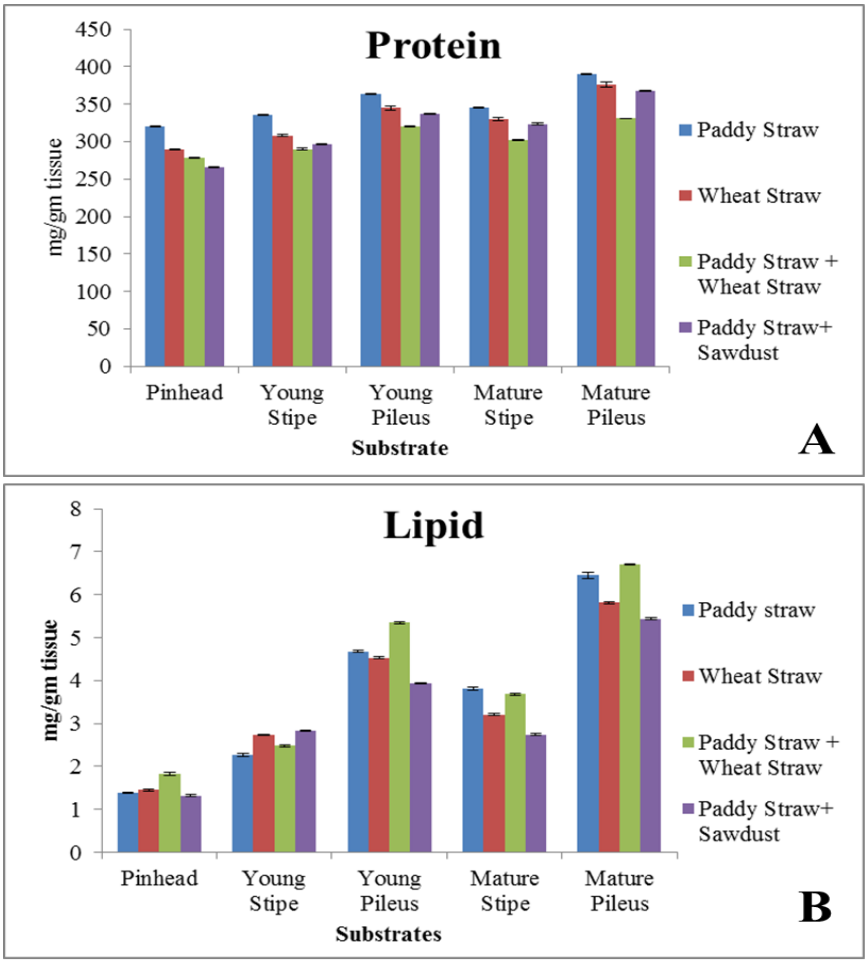


Figure 41: Total soluble protein (A) and total lipid content (B) of *P. ostreatus* in different stages of its growth

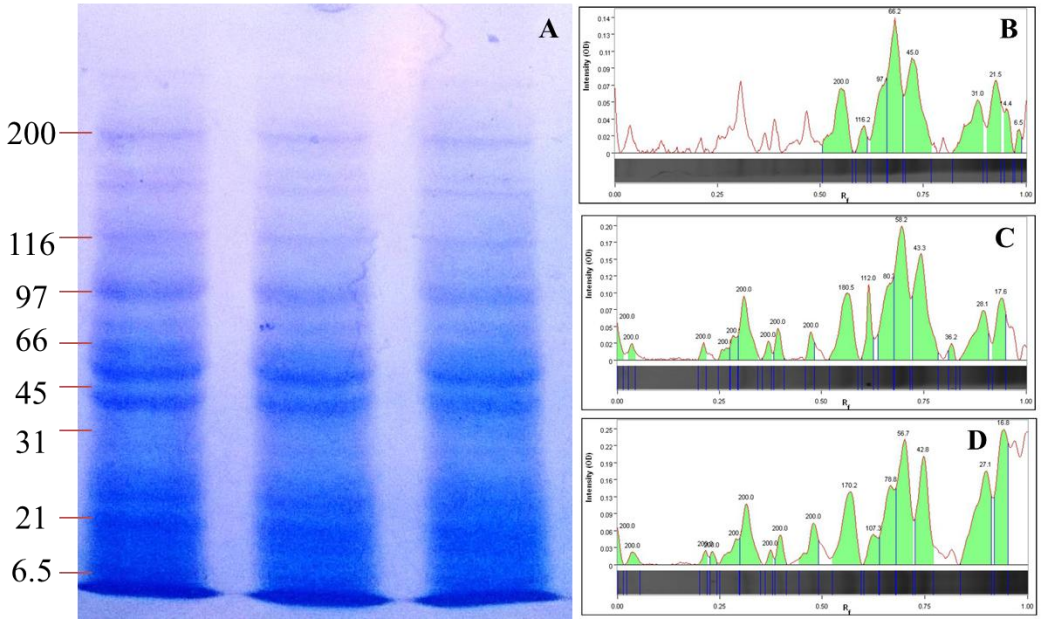


Figure 42: SDS PAGE analysis of soluble protein of *P. ostreatus* showing the bands on the gel (A) and band intensity analysed using image lab software (B-D).

Table 18: Study of band profileof SDS PAGE analysis of *P. ostreatus*

Lane	Band No.	Mol. Wt. (KDa)	Relative Front	Volume (OD)	Band %	Lane %
Lane 1 (Fig 42 A)	1	200.00	0.013189	12.44674	0.4008	0.337419
	2	200.00	0.081535	20.85496	0.671555	0.565358
	3	96.74	0.184652	97.44839	3.137954	2.641734
	4	69.61	0.302158	15.64255	0.503709	0.424055
	5	47.99	0.338129	64.47263	2.076096	1.747792
	6	32.61	0.364508	62.60395	2.015922	1.697134
	7	19.82	0.468825	92.97852	2.994019	2.52056
	8	16.41	0.546763	267.3797	8.609945	7.248411
	9	13.66	0.622302	500.562	16.11869	13.56976
	10	12.74	0.651079	264.3306	8.511762	7.165754
	11	11.88	0.679856	102.6751	3.306259	2.783424
	12	6.50	0.973621	992.6242	31.96369	26.90911
Lane 2 (Fig 42 B)	1	200.00	0.019185	11.02235	0.35936	0.302164
	2	166.91	0.148681	64.38208	2.099036	1.764952
	3	101.80	0.176259	12.69237	0.413807	0.347945
	4	93.86	0.195444	90.98459	2.966352	2.494226
	5	68.92	0.305755	52.19265	1.701627	1.430795
	6	40.65	0.348921	77.52654	2.527582	2.125291
	7	30.00	0.374101	74.18512	2.418643	2.03369
	8	19.82	0.468825	31.41851	1.024331	0.861298
	9	14.44	0.59952	124.1616	4.048015	3.40373
	10	13.34	0.631894	418.7857	13.65359	11.48048
	11	11.53	0.691847	91.66617	2.988574	2.512911
	12	6.50	0.983213	903.6602	29.46185	24.77269
Lane3 (Fig 42 C)	1	200.00	0.121103	28.72748	1.051931	0.828372
	2	133.13	0.154676	47.49951	1.739317	1.369674
	3	93.23	0.197842	171.5727	6.282577	4.947389
	4	69.38	0.303357	36.91237	1.351642	1.064388
	5	36.10	0.357314	60.86674	2.228793	1.755125
	6	28.47	0.383693	140.6357	5.149741	4.055306
	7	20.76	0.44964	22.42603	0.821187	0.646666
	8	18.86	0.489209	52.44514	1.920414	1.512283
	11	13.15	0.63789	508.7912	18.6307	14.67126
	12	12.16	0.670264	247.3634	9.057849	7.132854
	13	11.40	0.696643	135.378	4.957216	3.903697
	16	6.50	0.990408	593.5545	21.73453	17.11546

4.12.2. *Pleurotus sajor-caju*

Nutritional composition of *Pleurotus sajor-caju* was evaluated of its different stages of growth. Total sugar and reducing sugar also estimated and it was found that mature pileus of paddy straw consists about 310 mg/gm tissue total sugar while mature pileus of paddy straw combined with wheat straw consists about 270 mg/gm tissue total sugar. Reducing sugar was also estimated and mature pileus of paddy straw combined with saw dust possess highest amount (58 mg/gm tissue) among the other substrates (Figure 43 A&B). *Pleurotus sajor-caju* is also known to possess high amount of protein content and it was observed that protein content of *P. sajor-caju* ranges from 320-370mg/gm tissue. Paddy straw showed significantly high amount of protein content followed by paddy straw combined with wheat straw (Figure 44A). The results showed that *P. sajor-caju* contains lower amount of lipid content and it was observed that about 6-8 mg/gm tissue lipid content was found and highest lipid content was wheat straw substrate (figure 44B).

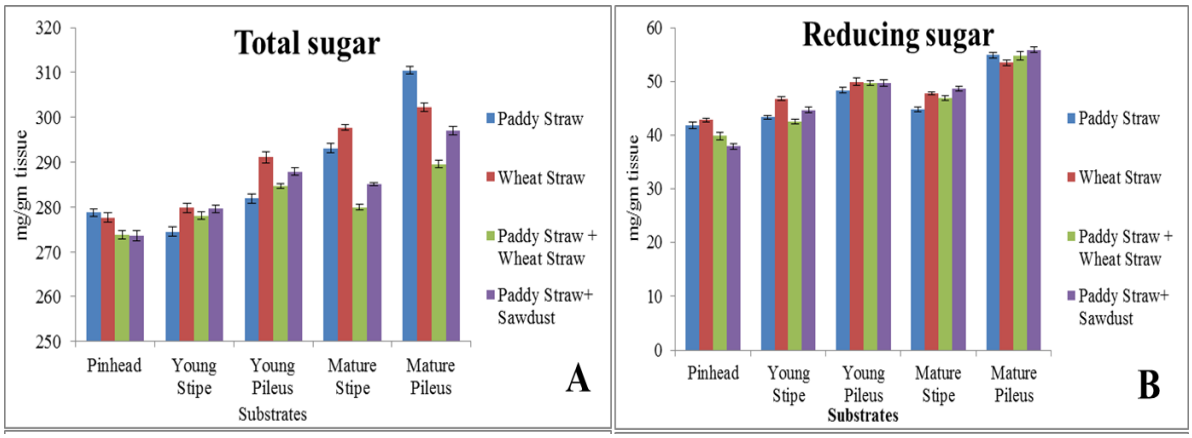


Figure 43: Total sugar (A) and reducing sugar (B) of *P. sajor-caju* in different stages of its growth

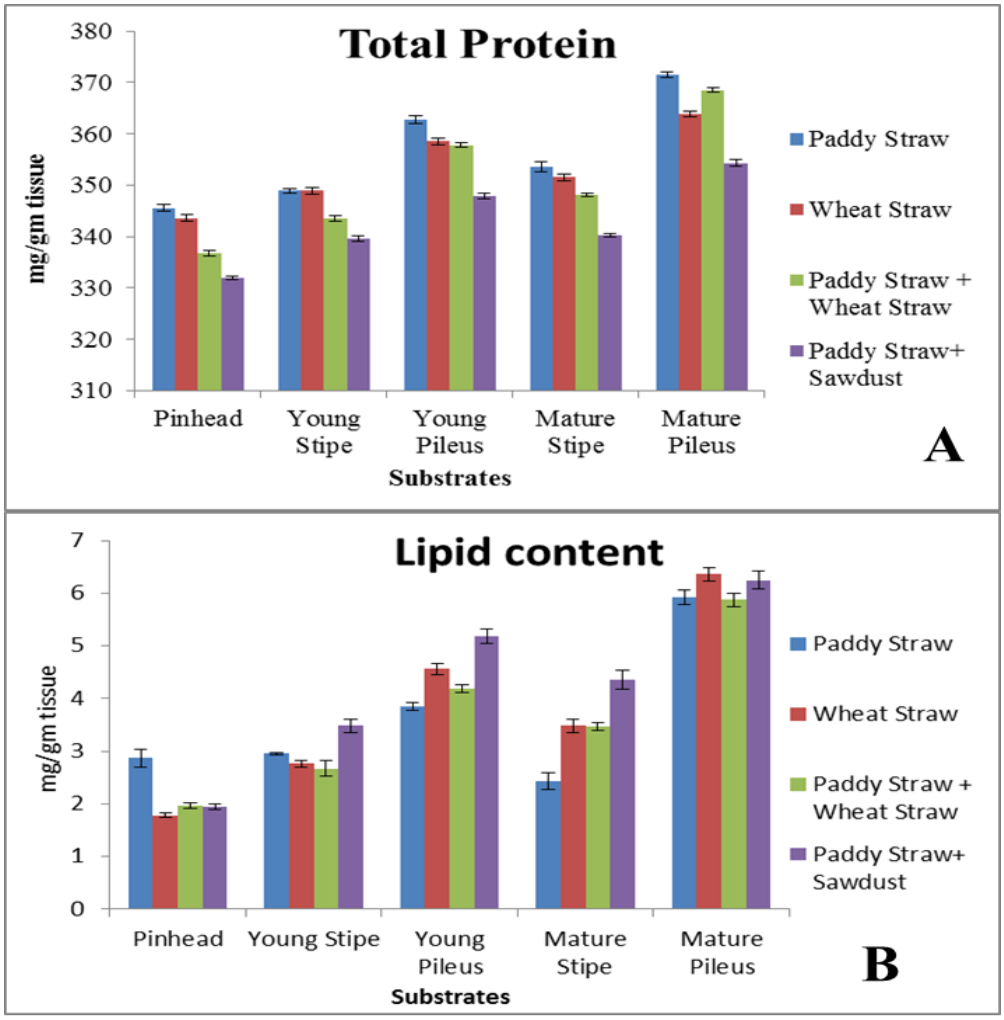


Figure 44: Total soluble protein (A) and lipid content (B) of *P. sajor-caju* in different stages of its growth

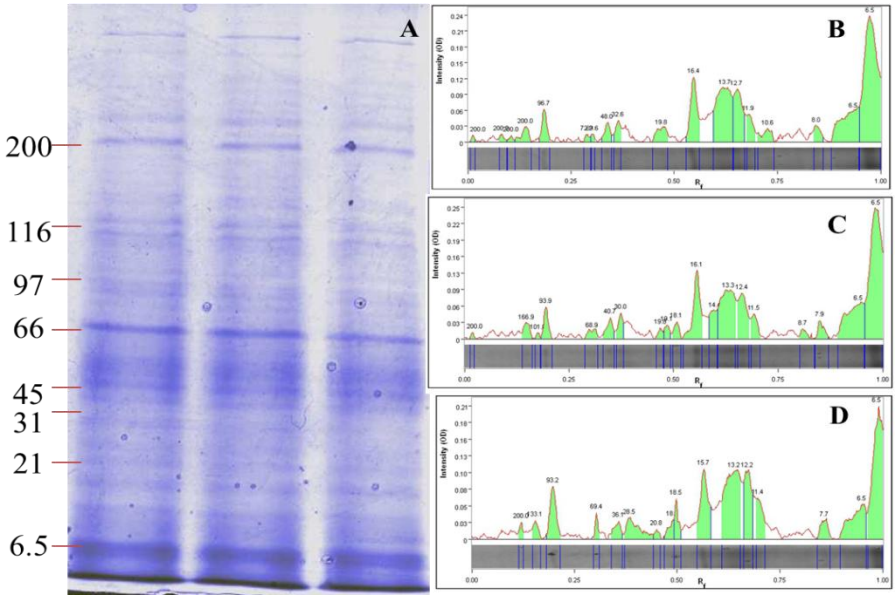


Figure 45: SDS PAGE analysis of soluble protein of *P. sajor-caju* showing the bands on the gel (A) and band intensity analysed using image lab software (B-D)

Table 19: Study of band profile of SDS PAGE analysis of *P. sajor-caju*

Sample	Band No.	Mol. Wt. (KDa)	Relative Front	Volume (OD)	Band %	Lane %
Lane 1 (Fig.45 A)	1	200	0.098321	91.5938	0.90804	0.859197
	2	200	0.129496	321.1632	3.18394	3.012675
	3	114.2114	0.160671	368.7475	3.65568	3.45904
	4	91.38015	0.205036	341.6145	3.386689	3.204518
	55	79.89527	0.252998	522.7445	5.182371	4.903611
	6	67.09514	0.315348	683.6541	6.777592	6.413024
	7	29.42106	0.377698	1,193.62	11.83331	11.1968
	8	22.80235	0.42446	2,222.84	22.03675	20.85139
	9	15.75675	0.563549	642.4049	6.368657	6.026086
	10	12.85324	0.647482	1,073.29	10.64036	10.06802
	11	7.809665	0.852518	126.2965	1.252075	1.184725
	12	6.614631	0.920863	454.0175	4.501027	4.258916
Lane 2 (Fig.45 B)	1	200	0.105516	111.6134	0.880936	0.727138
	2	200	0.134293	330.7872	2.610818	2.15501
	3	108.3077	0.167866	428.4581	3.38171	2.791316
	4	20.70332	0.450839	980.2002	7.736468	6.385802
	5	15.52957	0.569544	798.7853	6.304607	5.203922
	6	12.96608	0.643885	1,334.20	10.53048	8.692023
	7	9.410589	0.775779	437.7055	3.454697	2.851561
	8	8.673335	0.809353	291.9919	2.304617	1.902267
	9	7.832452	0.851319	157.1769	1.240557	1.023975
	10	7.389076	0.8753	71.73798	0.566209	0.467358
	11	6.5	0.979616	161.836	1.27733	1.054328
Lane 3 (Fig.45 C)	1	200	0.001199	45.44377	0.466087	0.419898
	2	200	0.134293	264.4839	2.712635	2.443818
	3	108.3077	0.167866	480.7018	4.930239	4.441661
	4	89.25734	0.213429	381.3492	3.911246	3.523648
	5	78.30174	0.260192	602.678	6.181268	5.568715
	6	67.09514	0.315348	418.6321	4.293631	3.86814
	7	20.88451	0.447242	735.9051	7.54769	6.799727
	8	15.71105	0.564748	692.2629	7.100081	6.396475
	9	12.92836	0.645084	1,252.72	12.84837	11.57512
	10	11.5061	0.693046	990.3778	10.15765	9.151043
	11	10.54306	0.729017	104.1724	1.068428	0.962548
	12	9.548689	0.769784	340.0886	3.488063	3.142402
	13	6.770624	0.911271	566.3581	5.808758	5.233121
	14	6.5	0.970024	108.0794	1.108499	0.998648

4.12.3. *Pleurotus djamor*

Pleurotus djamor is very commonly cultivated in north western part of India and very recently this species is introduced in North Bengal. Nutritional composition of was evaluated of its different stages of growth. Morphologically *P. djamor* possess very short stipe and thus the total sugar and reducing sugar was higher in pileus than that of stipe. Results revealed that young pileus and mature pileus possess highest amount of total sugar (180-230 mg/gm tissue) and maximum activity found in case of wheat straw than the other substrates. Reducing sugar was also estimated and it was observed that reducing sugar high in case of young pileus cultivated on paddy straw (45mg/gm tissue) supplemented by saw dust while in case of mature pileus, paddy straw showed highest (60mg/gm tissue) activity of reducing sugar (Figure 46). *Pleurotus djamor* is very well known to possess higher amount of protein content and it was observed that protein content ranges from 200-290 mg/gm tissue. In case of young pileus of all substrates showed significantly similar amount of protein content while in case of mature pileus, paddy straw showed highest amount of protein content followed by paddy straw supplemented with saw dust. The results showed that *P. djamor* possess low amount of lipid content (5-7 mg/gm tissue) it was also found that young pileus of paddy straw combined with wheat straw and mature pileus of wheat straw showed highest lipid content (Figure47B).

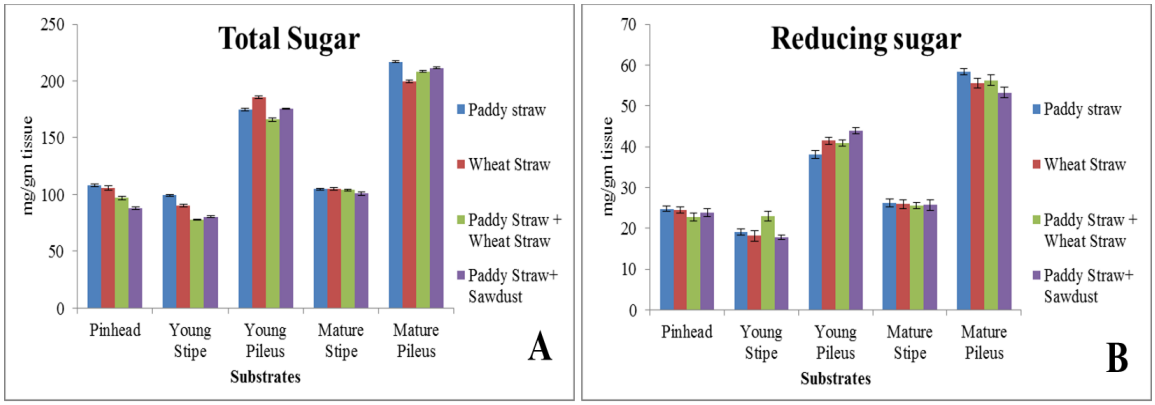


Figure 46: Total sugar (A) and reducing sugar (B) of *P. djamor* in different stages of its growth

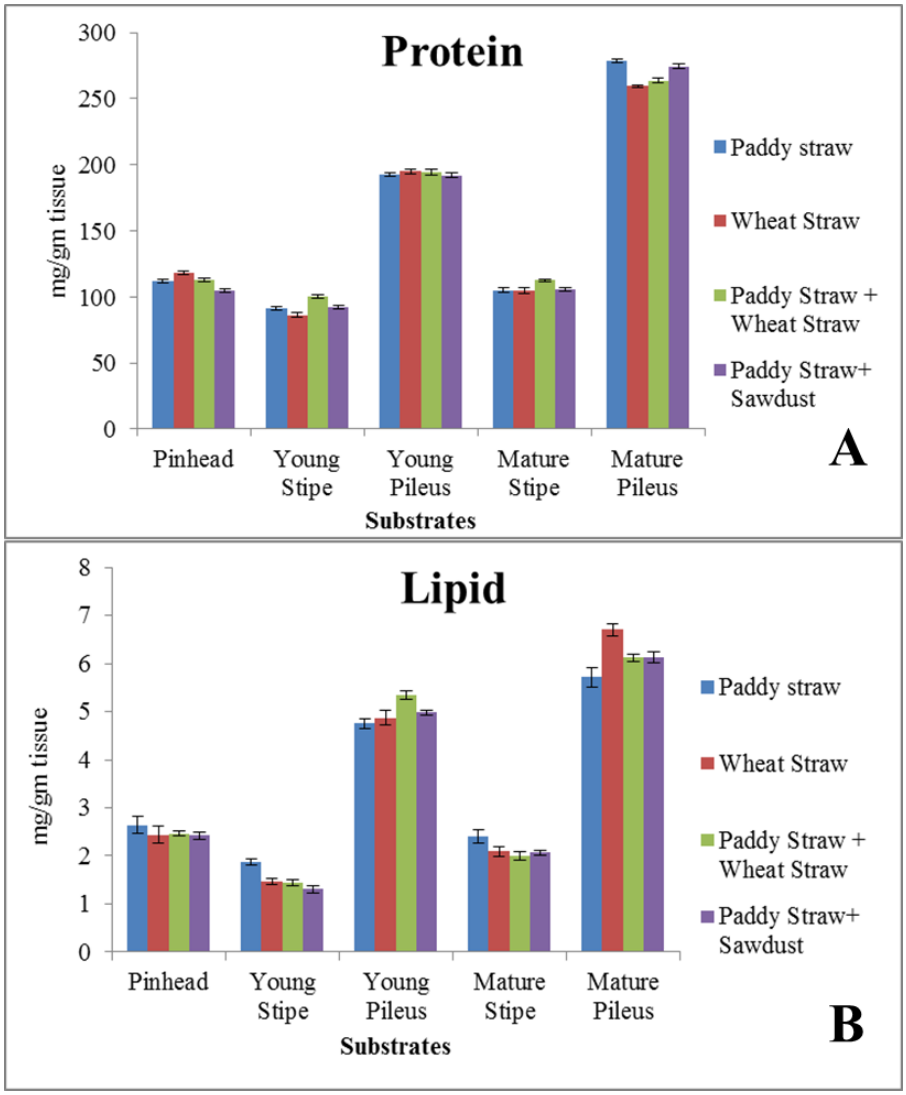


Figure 47: Total soluble protein (A) and lipid content (B) of *P. djamor* in different stages of its growth

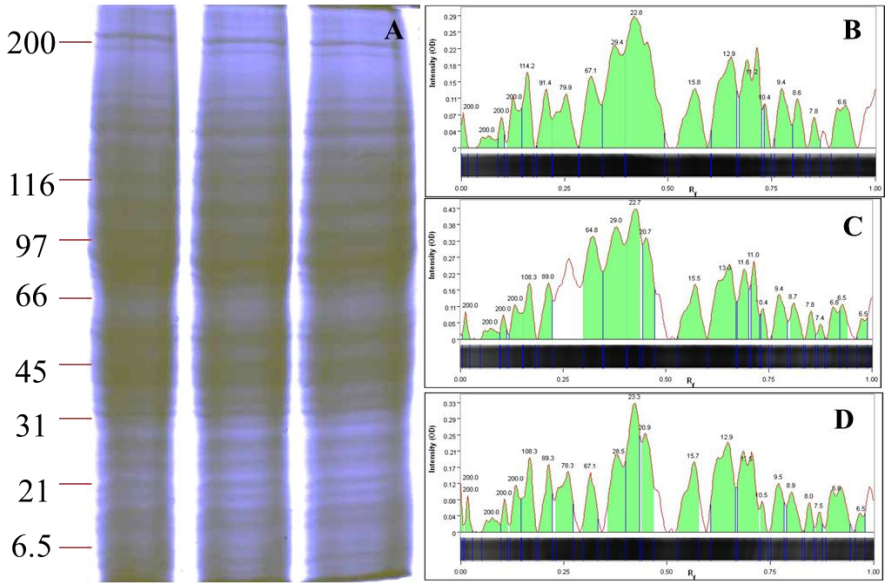


Figure 48: SDS PAGE analysis of the protein of *P. djamor* showing the bands on the gel (A) and band intensity analysed using image lab software (B-D)

Table 20: Study of band profile of SDS PAGE analysis of *P. djamor*

Lane	Band No.	Mol. Wt. (KDa)	Relative Front	Volume (OD)	Band %	Lane %
Lane 1 (Fig.48 A)	1	200	0.044386	279.82	3.62	3.18
	2	200	0.087467	43.52	0.56	0.49
	3	149.74	0.562663	449.21	5.82	5.10
	4	106.73	0.617493	140.88	1.82	1.60
	5	97.4	0.656658	458.32	5.94	5.21
	6	73.92	0.682768	640.69	8.30	7.28
	7	60.59	0.725849	525.85	6.81	5.97
	8	35.38	0.861619	683.04	8.85	7.76
	9	21.20	0.902089	656.49	8.50	7.46
	10	16.05	0.955614	473.84	6.14	5.38
Lane 2 (Fig 48 B)	1	200	0.056136	335.1687	3.598444	3.402808
	2	200	0.099217	61.59143	0.661259	0.625308
	3	200	0.25718	72.8148	0.781756	0.739254
	4	136.7993	0.569191	630.7203	6.77155	6.403402
	5	105.4391	0.622715	184.32	1.9789	1.871313
	6	93.45247	0.660574	515.0943	5.530164	5.229506
	7	69.95445	0.68799	729.0304	7.827028	7.401497
	8	60.16323	0.72846	586.4365	6.296109	5.953809
	9	35.38283	0.861619	654.2344	7.024003	6.64213
	10	20.36268	0.909922	1,378.88	14.80399	13.99914
	11	14.4	0.993473	3,179.31	34.13377	32.27802
Lane 3 (Fig 48 C)	1	200	0.074413	320.5739	4.08886	3.856258
	2	200	0.266319	26.06545	0.33246	0.313547
	3	200	0.5	141.1019	1.799728	1.697347
	4	122.7317	0.577023	376.3368	4.800105	4.527043
	5	103.527	0.630548	80.68259	1.029091	0.97055
	6	86.031	0.668407	414.2325	5.283457	4.982899
	7	65.96597	0.694517	610.1875	7.782826	7.340087
	8	59.52741	0.732376	517.3527	6.598736	6.223356
	9	32.92052	0.869452	725.2917	9.250958	8.724702
	10	19.68261	0.916449	1,321.33	16.85332	15.89459
	11	14.4	0.992167	2,999.93	38.26359	36.0869

4.12.4. *Pleurotus florida*

Pleurotus florida commonly known as white oyster mushroom is very rich in nutritional constituents. Reducing sugar was higher in pileus than that of stipe. Results revealed that young pileus and mature pileus possess highest amount of total sugar (210-300 mg/gm tissue) and maximum activity was found in case of paddy straw and wheat straw combined substrates than the other substrates. Reducing sugar was also estimated and it was observed that reducing sugar very much high in case of mature pileus than that of the young pileus (60-65 mg/gm tissue) (Figure 49). *Pleurotus florida* is also very popular to possess higher amount of protein content and it was observed that protein content ranges from 160-280 mg/gm tissue (Figure 50 A and 51). In case of young pileus and mature pileus of all substrates showed significantly similar amount of protein content. The results showed that *P. florida* possess low amount of lipid content (2-6.5 mg/gm tissue) it was also found that mature pileus of wheat straw showed highest lipid content (figure 50B).

Figure 49: Total sugar (A) and reducing sugar (B) of *P. florida* in different stages of its growth

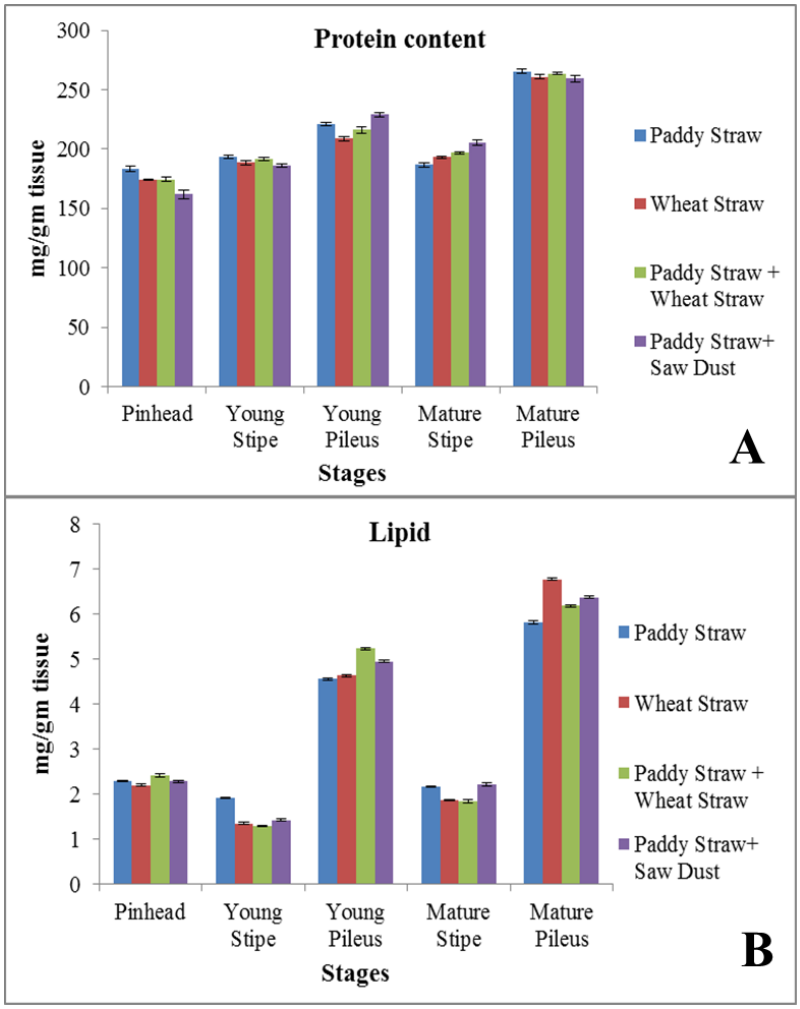


Figure 50: Total soluble protein (A) and lipid content (B) of *P. florida* in different stages of its growth

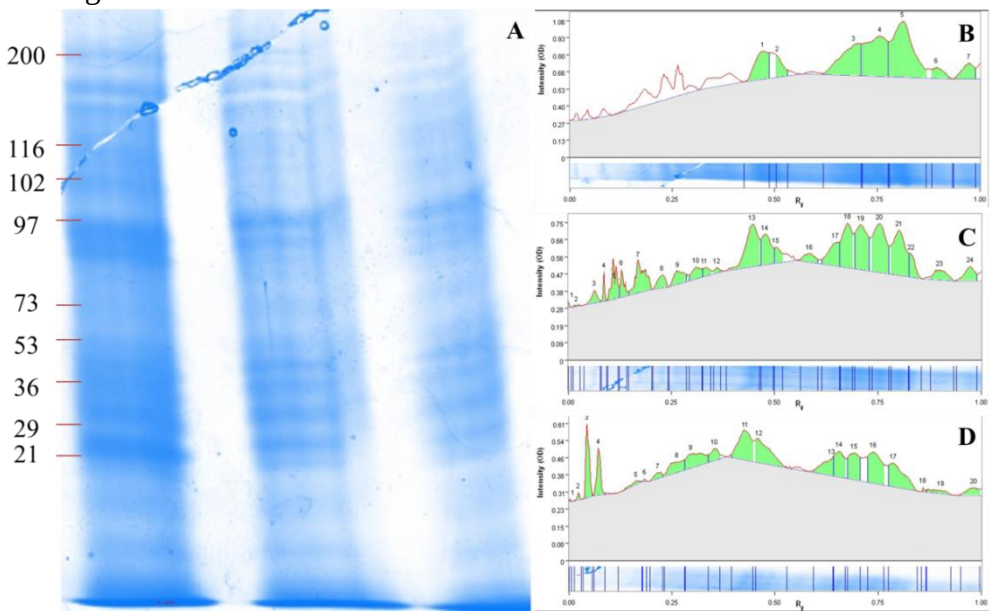


Figure 51: SDS PAGE analysis of the protein of *P. florida* showing the bands on the gel (A) and band intensity analysed using image lab software (B-D)

Table 21: Study of band profile of SDS PAGE analysis of *P. florida*

	Band No.	Mol. Wt. (KDa)	Relative Front	Volume (OD)	Band %	Lane %
Lane 1 (Fig. 51A)	1	200	0.360265	34.653	0.520023	0.486758
	2	200	0.446358	709.9122	10.65336	9.97188
	3	183.9832	0.476821	376.6394	5.652069	5.290517
	4	121.2045	0.503311	137.7503	2.067161	1.934928
	5	107.955	0.584106	131.539	1.97395	1.84768
	6	101.6451	0.647682	346.7105	5.20294	4.870117
	7	98.87763	0.676821	569.8318	8.551227	8.00422
	8	88.43693	0.708609	692.2633	10.38851	9.723972
	9	67.27373	0.753642	831.5951	12.4794	11.68111
	10	48.06567	0.801325	742.4813	11.14211	10.42937
	11	41.17908	0.830464	188.7193	2.832031	2.650871
	12	30.25289	0.899338	180.8631	2.714137	2.540519
	13	21.5	0.97351	192.1468	2.883467	2.699017
Lane 2 (Fig. 51B)	1	200	0.429139	426.8258	11.42745	10.35526
	2	200	0.460927	383.0376	10.2551	9.292911
	3	102.6705	0.637086	208.8628	5.591905	5.067241
	4	100.8827	0.655629	266.2467	7.128248	6.459436
	5	97.4	0.692715	313.3303	8.388823	7.601737
	6	73.49824	0.739073	442.685	11.85205	10.74002
	7	53.31053	0.786755	400.7581	10.72954	9.722831
	8	36.36858	0.858278	10.42855	0.279205	0.253008
	9	29.88611	0.901987	64.00752	1.71368	1.552893
	10	21.5	0.981457	101.1085	2.706987	2.453002
Lane 3 (Fig. 51C)	1	200	0.074413	320.5739	4.08886	3.856258
	2	200	0.266319	26.06545	0.33246	0.313547
	3	200	0.5	141.1019	1.799728	1.697347
	4	122.7317	0.577023	376.3368	4.800105	4.527043
	5	103.527	0.630548	80.68259	1.029091	0.97055
	6	86.031	0.668407	414.2325	5.283457	4.982899
	7	65.96597	0.694517	610.1875	7.782826	7.340087
	8	59.52741	0.732376	517.3527	6.598736	6.223356
	9	32.92052	0.869452	725.2917	9.250958	8.724702
	10	19.68261	0.916449	1,321.33	16.85332	15.89459
	11	14.4	0.992167	2,999.93	38.26359	36.0869

4.13. Utilization of dry tea leaves following pruning practice in tea estates in North Bengal as alternative substrate for cultivation of *P. ostreatus* and *P. sajor-caju*

Tea is one of the major economic crops in North Bengal and Darjeeling is world famous for tea. Large numbers of tea gardens are situated in this region and every year pruning is commonly practiced in this region. After pruning, generally tealeaves are used as fuel for the common people. Efficacy of tea leaves in cultivation was done and it was observed that tea leaves were very much efficient in cultivating the *P. ostreatus*. Tea leaves were collected and the dried for 7-10 days. Spawning was done in two different combinations such as (A) tea leaves in combination with paddy straw (1:1) and (B) tea leaves alone and it was observed that the mycelia colonize rapidly over the tea leaves. Similar growth was also observed in case of combined substrate. Results also revealed that tea leaves in combined with paddy straw helps to colonize mycelia rapidly (table 22). Number of pinhead was higher in case of combined substrate than that of the single substrate. Development of fruiting body was rapid in case of tea leaves alone substrate. Production of *P. ostreatus* was also very high in case of combined substrate and tea leaves single substrate. It was observed that the production rate was higher in case of combined substrate than that of the single tea leaves substrates (figure 52&53). In compare to *P. ostreatus*, *P sajor-caju* was also cultivated using the tea waste as substrate (figure 54). Using pruned dry tea leaves helps is rapid growth and higher yield of *P sajor-caju*. The results revealed that the mycelial run period become very less in case of tea leaves in compare to combine substrate of paddy straw along with tea leaves. Along with the short growth period, it also helped in increase number of pinhead per bags. Yield of *P sajor-caju* also varies and the results revealed that the use of combined substrate helps in increasing the yield performance.

Table 22: Cultivation of *P. ostreatus* and *P sajor-caju* using pruned tea leaves along with paddy straw

Substrate	Initial wt. of bags (gm)	Days of colonization	No. of Pinhead	Yield (gms)			Total production (gm)
				1st flush	2nd flush	3rd flush	
Pleurotus ostreatus							
Tea leaves + Paddy straw (1:1)	450	18	115	200	120	90	410
	500	19	135	350	120	50	520
	1200	21	143	500	210	115	825
	1000	21	136	350	100	60	510
	850	21	157	250	100	45	395
	1200	20	123	375	120	70	570
Tea Leaves	350	17	30	280	135	65	480
	950	20	94	390	145	80	615
	900	18	102	300	160	95	555
	650	21	110	265	105	55	425
	350	20	98	130	75	20	225
	400	19	84	210	105	45	360
Pleurotus sajor-caju							
Tea leaves + paddy straw	350	17	75	290	100	35	425
	490	19	87	200	120	50	370
	450	19	98	170	90	30	290
	600	21	117	310	100	40	450
	650	16	93	180	75	30	285
	500	18	107	275	100	90	465
Tea leaves	350	17	83	210	90	45	345
	390	19	76	185	80	50	315
	490	15	89	240	110	45	395
	560	21	94	275	90	25	390

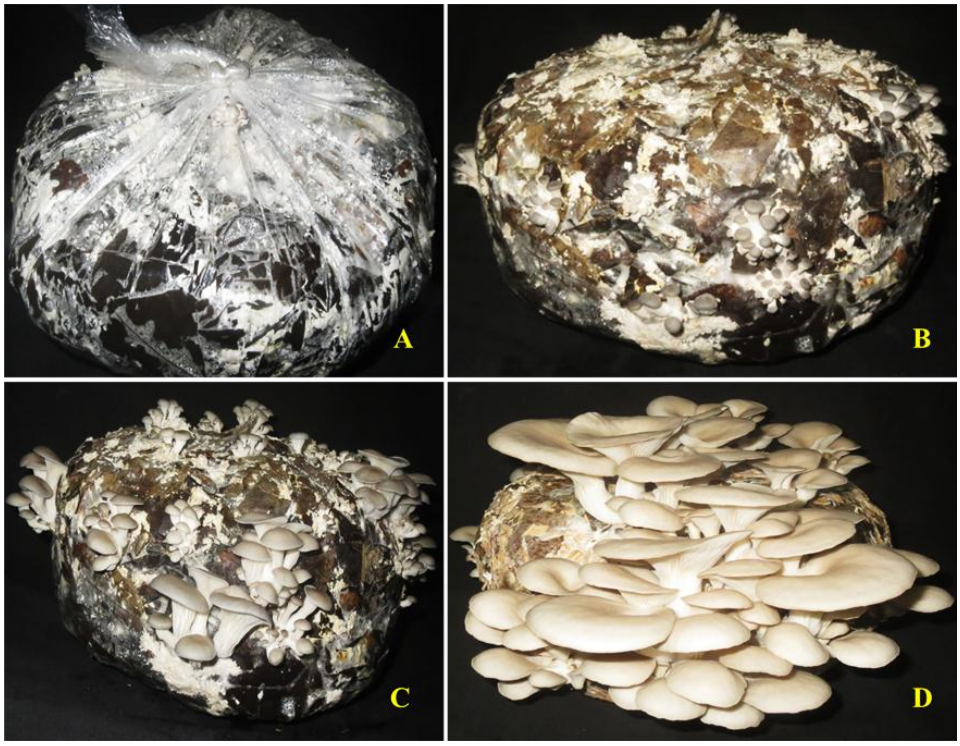


Figure 52: Cultivation of P. ostreatus using pruned tea leaves; (A)fully colonized bag, (B) pinhead formation, (C) young stage and (D) mature fruiting body

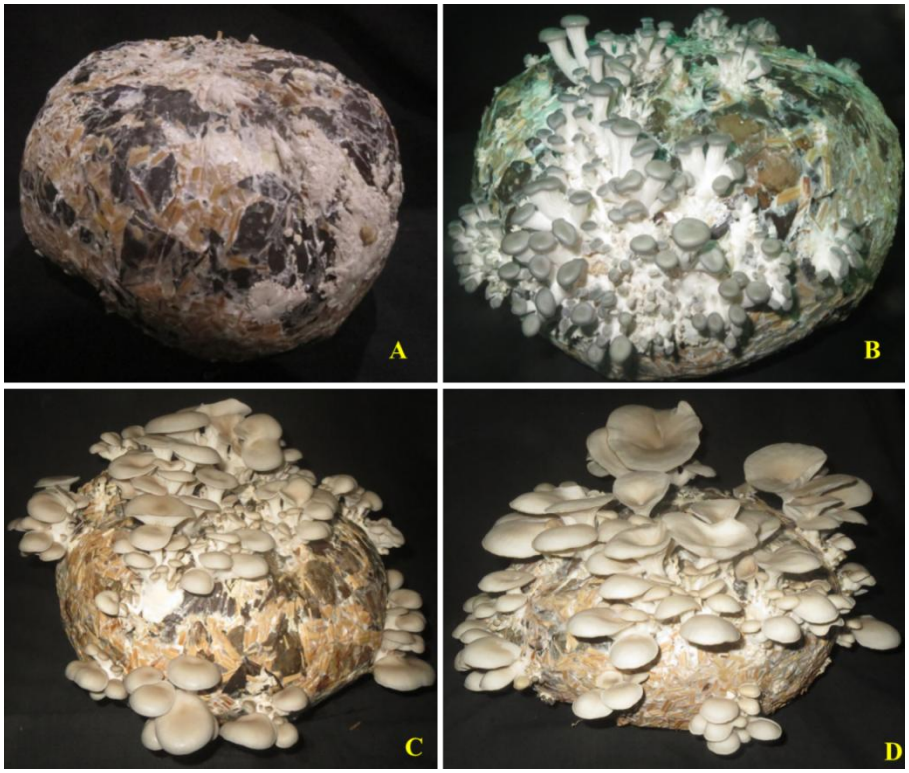


Figure 53: (A-D) Cultivation of *P. ostreatus* on tea waste substrate in combination with paddy straw (A) fully colonized, (B) pinhead stage, (C) young fruiting body and (D) mature fruiting body

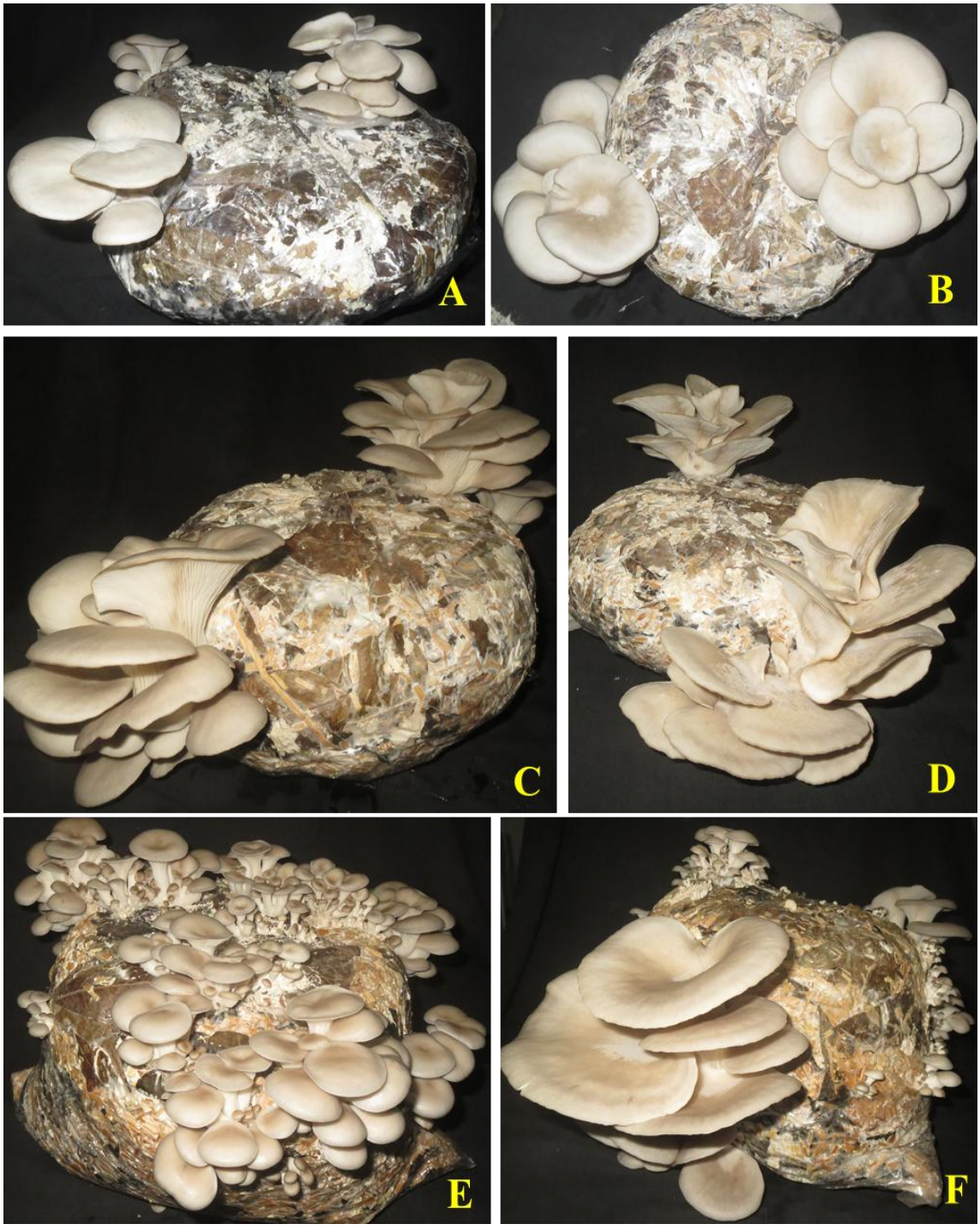


Figure 54: Cultivation of *Pleurotus sajor-caju* on pruned tea leaves (A-D) and in combine with paddy straw (E and F)

4.14. Biochemical characterization of fruiting body cultivated on tea waste

Waste tea leaves were used as substrates singly or in combination with paddy straw to evaluate the effect of waste tea leaves as an alternative substrate for the cultivation of oyster mushroom. The growth and production was significantly higher in case of the waste tea leaves used singly. Further the biochemical characterization of the harvested fruiting body was estimated to evaluate the effect of tea leaves on their food value. Results revealed that the moisture content was higher in case of tea waste in compare to combined substrates (figure 55). Mature pileus of tea waste substrates showed highest moisture content. Protein content of the fruiting body cultivated on tea waste was estimate and it was observed that the mature pileus of tea waste was significantly high in compare to the combined substrates. Protein content of the young pileus was also high but pinhead stage and stipe was very much similar in compare to combined substrates (Figure 56C). Total sugar and reducing sugar content was also evaluated and it was also estimated and the results revealed that the total sugar as well as the reducing sugar was significantly enhanced in compare to the combined substrates (figure 56A&B). It was also observed that the total sugar in young pileus enhanced more and the results revealed that the total sugar more or less similar to the mature pileus.

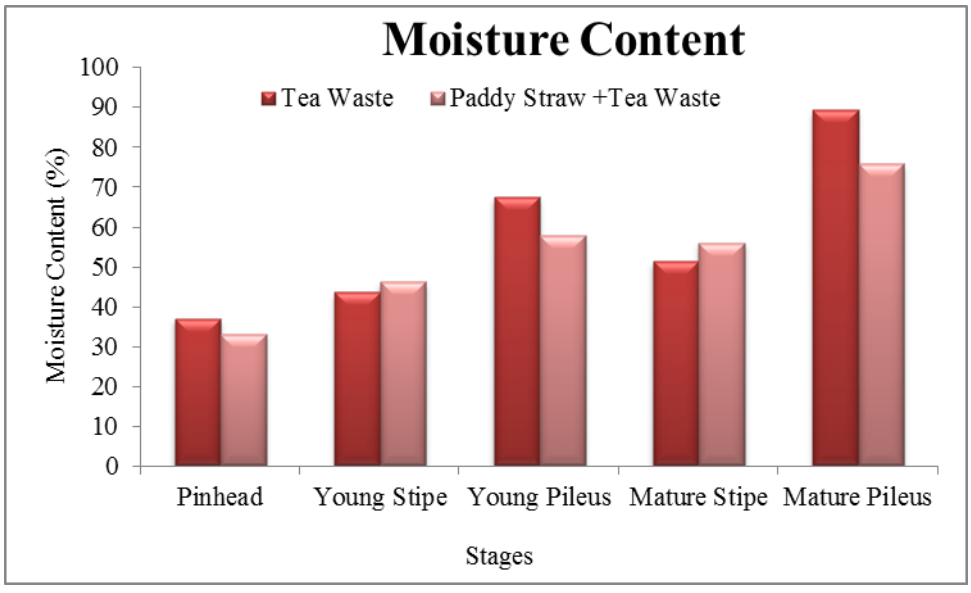


Figure 55: Moisture content of *P. ostreatus* grown on tea waste

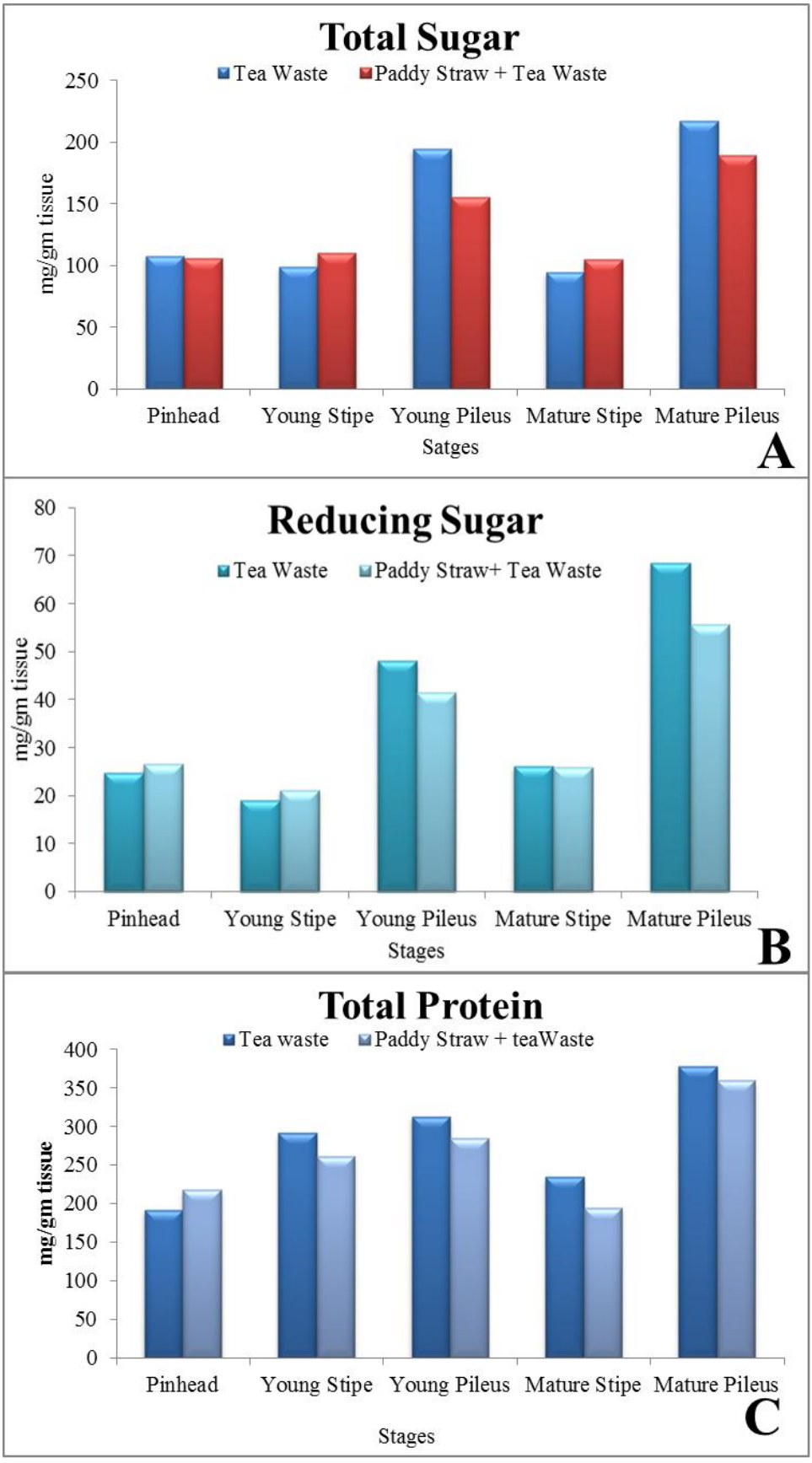


Figure 56: Total sugar (A) reducing sugar (B) and total protein (C) content of *P. ostreatus* grown on tea waste

4.15. Antioxidant activity of different species of oyster mushrooms

The extracts of different species of oyster mushroom showed positive antioxidant activity by fading the violet colour of DPPH solution to yellow to pale violet colour. Results revealed that the scavenging activity of DPPH were directly proportional with the concentration of the samples used. As the concentration of the sample was increased, the scavenging activity of towards DPPH radicles also elevated (Figure 57A). Different concentrations were used for the evaluation of DPPH scavenging activity and it was observed that *P. djamor* showed maximum activity among the other species in respect to all the concentrations. It was found that *Pleurotus djamor* showed about 88% DPPH scavenging activity in 20mg/ml concentration while *P. florida* showed lowest scavenging activity (77%) among the other species.

All the mushroom species showed appreciable reducing power activity in different concentrations (5-20mg/ml). Highest amount of reducing power ability was observed in case of *Pleurotus djamor* at 20 mg/ml concentration while *P. ostreatus* and *P. sajor-caju* showed lowest amount of reducing power activity at 20 mg/gm tissue concentration. Free reducing power activity was estimated using the different concentrations of four species of oyster mushroom and the highest activity was observed in case of *P. djamor* whereas in case of *P. ostreatus* and *P. sajor-caju* the activity is quite lower than that of the others (Figure 57B). Antioxidant is an important parameter and mushroom is one of the major sources of antioxidant compounds.

Total flavonoid content and carotenoid content is also an important compound showing antioxidant activity. Total flavonoid content of four different species was assessed and the results revealed that all the species were showing significant amount of flavonoid content. Different concentrations were taken into consideration and it was observed that *Pleurotus djamor* and *P. florida* showed highest amount of flavonoid in compare to other two species (Figure 57C). The results were also revealed that the higher concentration of the sample helps in increasing the flavonoid content and thus highest flavonoid content activity was found in case of *P. djamor* and *P florida* in 20mg/ml concentration in compare to 5gm/ml and 10mg/ml concentration.

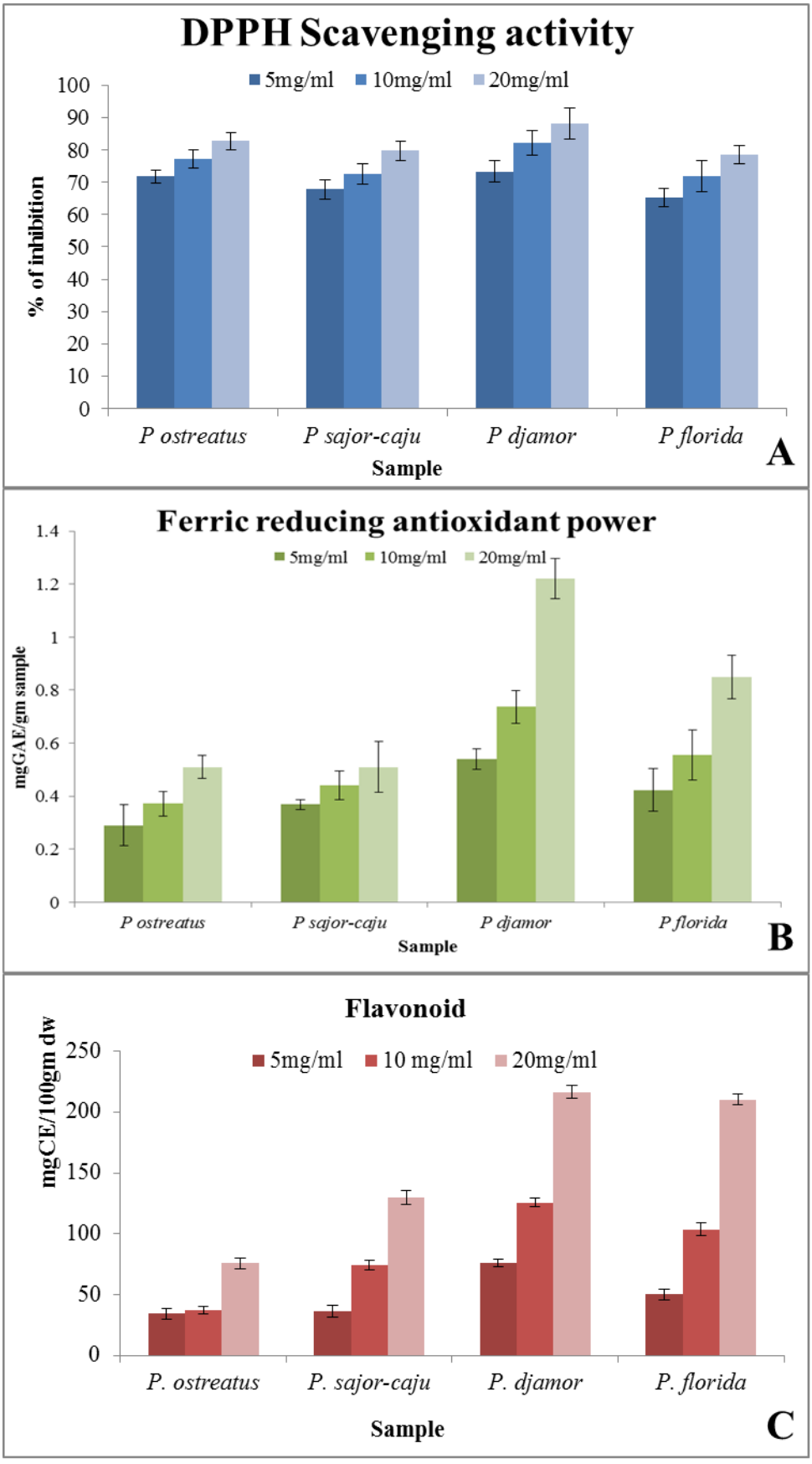


Figure 57: Antioxidant activity of *Pleurotus* species; (A) DPPH scavenging activity (B) Free radicle antioxidant power assay and (C) flavonoid activity

4.16. Antidiabetic activity of different species of oyster mushroom

Diabetes mellitus is a disease that affects millions of people throughout the world. A growing number of people each day are diagnosed with diabetes mellitus, due to the rise in obesity and sedentary lifestyle. In many places throughout the world, diabetes is kept under control by the use of different treatments. Healthy white albino rats were taken for the induction of blood sugar (Figure 58). After induction, body weight were measured and it was observed that in case of control groups, there was no significant changes in case of normal control group, but in case of negative control body weight drastically decreased and gradually the rats were very sick. On the other hand, initially the body weight of the positive control rats was decreased but gradually the rats were gained their body weight and become healthy again (Figure 59A). In case of mushroom treated rats, it was observed that initially after the induction the body weight decreases which generally happened in diabetic patients. But by the treatment, it was observed that the rats were able to regain their weight. The results revealed that the treatment of *P djamor* helps rapidly for gaining the body weight followed by *P. sajor-caju, P. florida* and *P. ostreatus*.

It was also observed that the blood glucose level increases after the induction of Streptozotocin. In case of normal control, blood glucose level was similar throughout the experiment. In case of negative control, the blood glucose level increases drastically and the experimental rats were become very sick while in positive control, initially the blood glucose level was high but after continuous treatment with metformin, the blood glucose level become normal. In case of sample treated groups, similar trends were observed that the blood glucose level initially increases after the induction of Streptozotocin and the sample treatment helps in lowering the blood glucose level (Figure 59B). *Pleurotus djamor* and *P. florida* showed high antidiabetic activity in compare to the other two species. The results revealed that the sample treatment significantly helps in controlling the blood glucose level in compare to control sets.

Figure 58: Induction of hyperglycaemia in white albino rats; (A &B) selected healthy rats of same size and weight kept in grouped, (C) induction of hyperglycaemia using STZ intraperitoneally, (D) oral treatment of mushroom, (E) treatment after taking blood from tail vein.

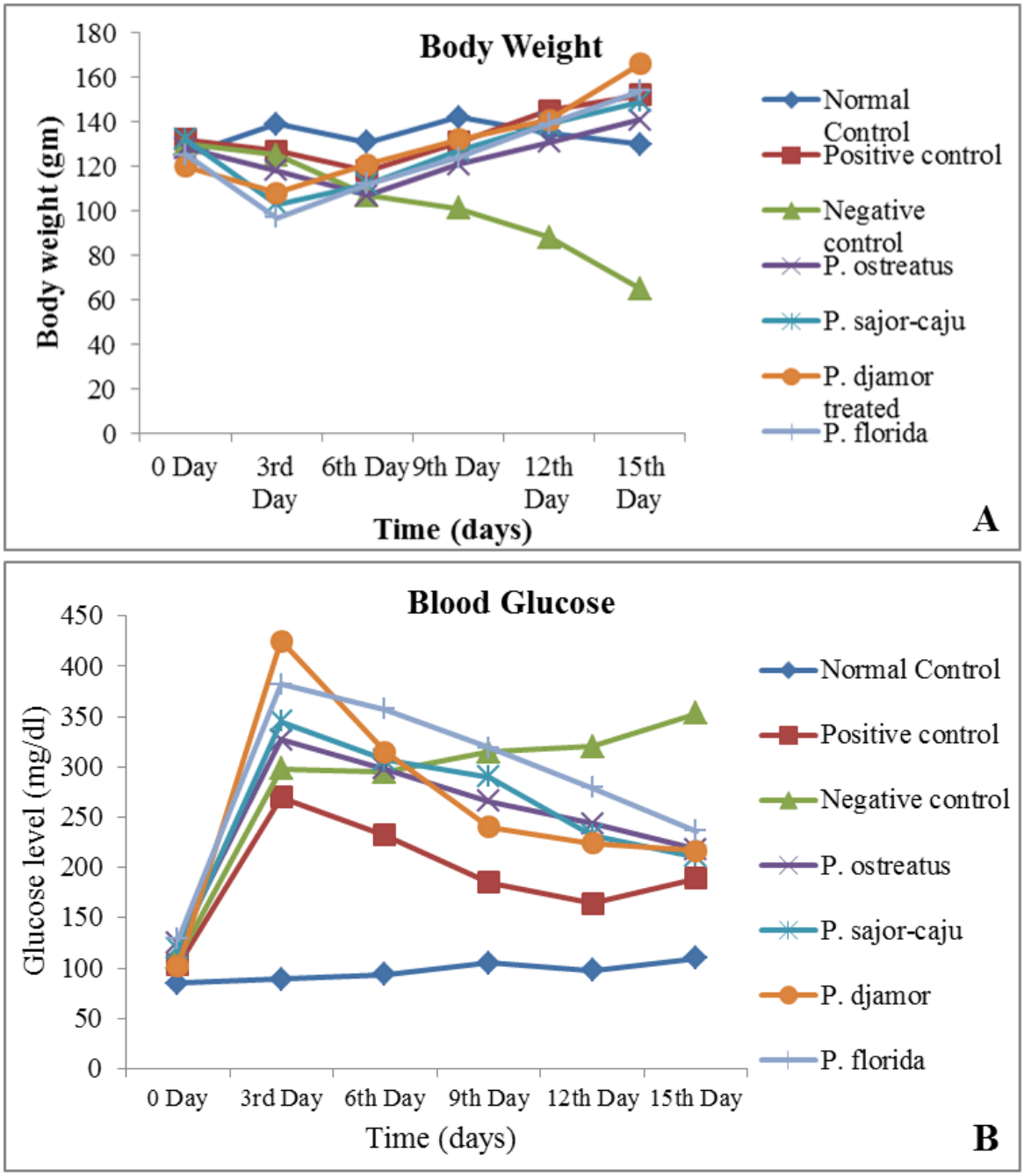


Figure 59: Evaluation of antihyperglycemic activity of *Pleurotus* species on induced rats (A) effect on body weight and (B) blood glucose level following induction

4.16.1. Effect of oyster mushroom on kidney function test of STZ induced diabetic rats

Creatinine is a waste product formed in the muscle from the high energy storage compound creatinine phosphate and it is an important indicator of renal function. Creatinine level is also increasing in proportionate to blood glucose level. The results revealed that the blood creatinine level was increased after induction of all the groups of animals. In case of negative control group, blood creatinine level was very high which affects the animal health but in case of positive control and mushroom treated rats, the creatinine level was less in compare to negative control (Figure 60). It was also observed that the effect was much less in case of *P. djamor* and *P. ostreatus* while compared with the positive control. *P. sajor-caju* and *P. florida* also successfully reduced the amount of creatinine level in compare to control sets.

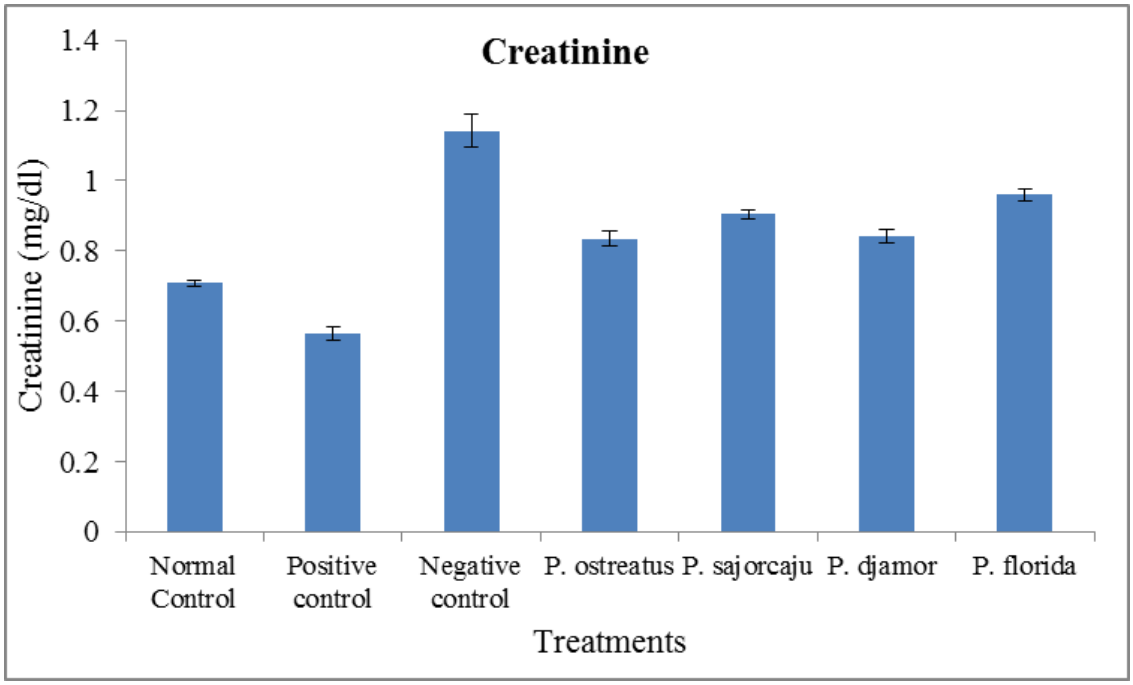


Figure 60: Changes in creatinine level following oral treatment of *Pleurotus* species in hyperglycaemic rats

On the other hand, blood urea level was also estimated which regulates the liver disease as well as kidney function. Urea level of diabetic patients increased which results in different renal diseases and also affects in liver function. The results showed that the induction of Streptozotocin treated negative control possesses highest amount of blood urea level but in case of positive control, it was observed that the medicine helps in reducing the blood urea level and become closer to normal range. Blood urea level of the mushroom powder treated groups were also analysed and it was observed that *P. djamor* showed highest activity in lowering the blood urea level in followed by *P. ostreatus, P florida* and *P. sajor-caju* (Figure 61B). Results also prove that the use of mushroom powder of different species of oyster mushroom significantly reduces the blood urea level in compare to positive control group as well as negative control groups.

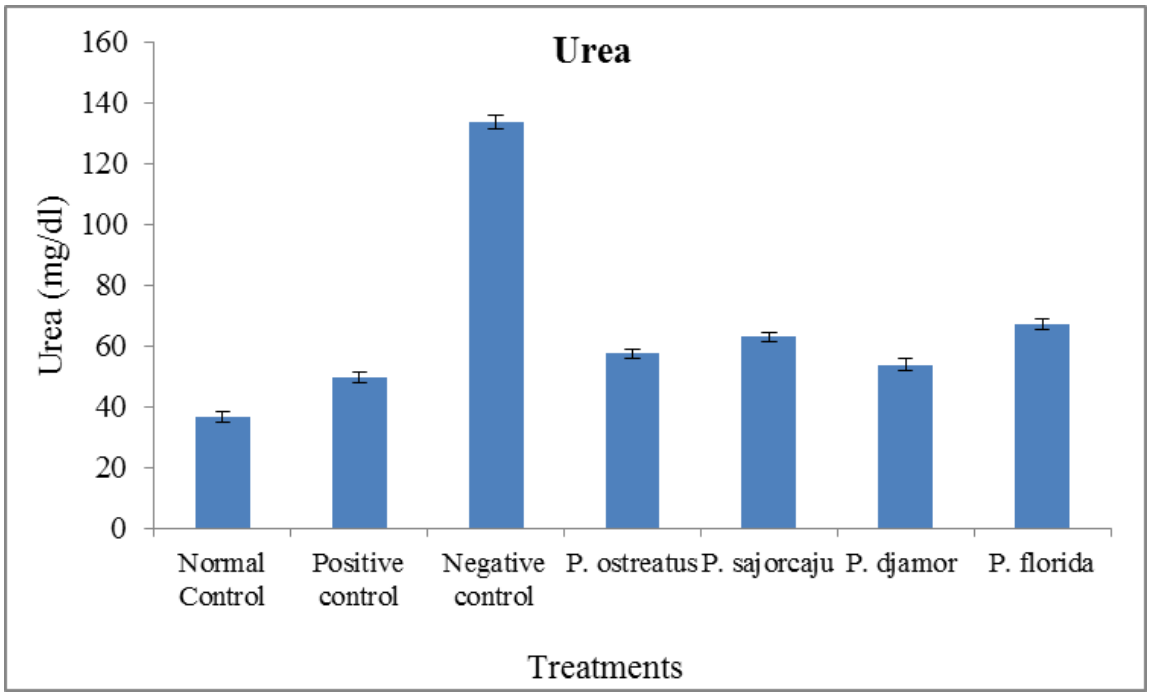


Figure 61: Changes in blood urea level following oral treatment of *Pleurotus* species in hyperglycaemic rats

4.16.2. Effect of oyster mushroom on liver enzymes of STZ induced diabetic rats

Diabetes is one of the major diseases spread throughout the world. Different age groups are now suffering from diabetes. Diabetes also effects on the liver function and thus liver induced to secrete some liver enzymes. Serum Glutamic Pyruvate Transaminase (SGPT) is one of the important enzymes secreted by the liver. The results revealed that SGPT enzyme was controlled by the normal control about 52 IU/L. similar results was found in case standard drug treated rats but in case of negative control, it increased (Figure 62). Significant result found in case of sample treated rats where it reduced to normal. Among the sample treated rats, it was clearly observed that *Pleurotus djamor* showed highest activity in lowering the SGPT level followed by *P. florida, P. ostreatus* and *P. sajor-caju*.

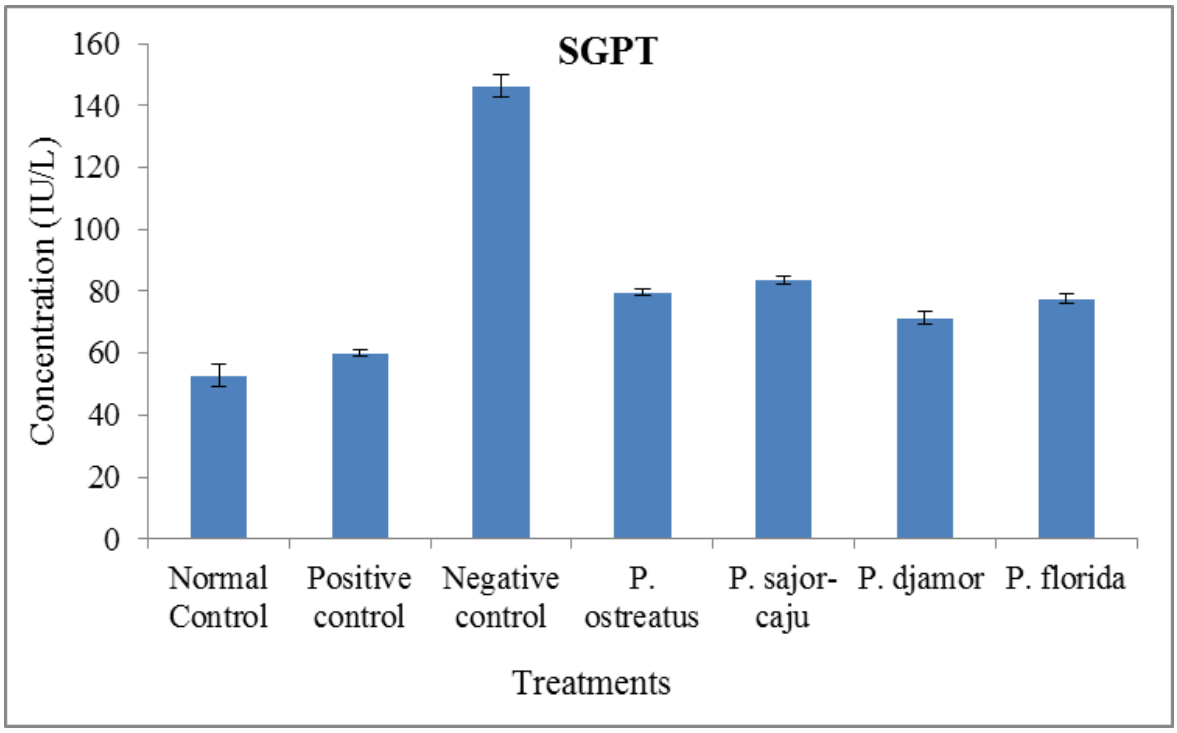


Figure 62: Liver enzyme serum glutamic pyruvate transaminase (SGPT) activity of STZ treated diabetic rats following oral treatment of *Pleurotus* species in relation to control

Serum glutamic oxaloacetic transaminase is also another liver enzyme secreted by the liver due to high diabetic level. Results revealed that SGOT level increase in case of negative control but in case of standard drug treated rats, the SGOT level decreases to normal level. It was also observed that the sample treatment significantly reduced the SGOT activity. *Pleurotus ostreatus* and *P. djamor* decreases more than the *P. sajor-caju* and *P. florida* (Figure 63). Results revealed that the samples were capable of lowering the SGOT secretion significantly in compare to the control sets.

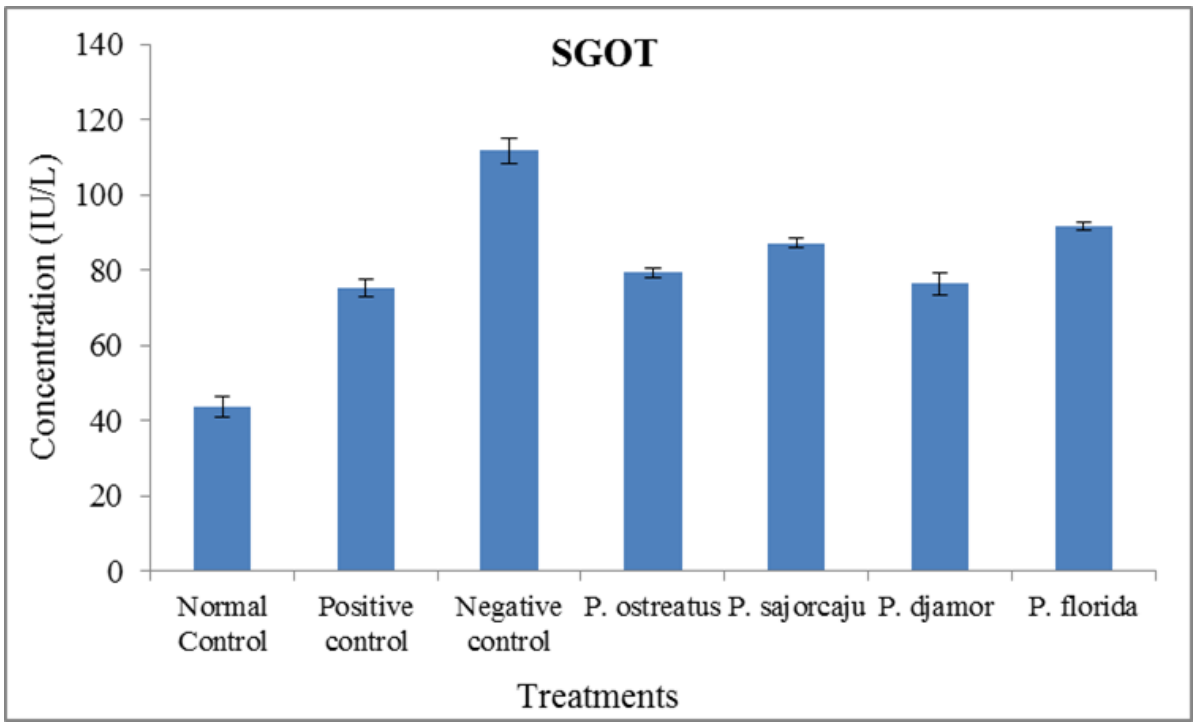


Figure 63: Liver enzyme serum glutamic oxaloacetic transaminase (SGOT) activity of STZ treated diabetic rats following oral treatment of *Pleurotus* species in relation to control

4.16.3. Effect of oyster mushroom on serum cholesterol level of STZ induced diabetic rats

Cholesterol is one of the major biochemical constituents which regulate the coronary arterial occlusion, myocardial infraction, liver function, thyroid function and adrenal disease. Diabetes severely affects the cholesterol level of serum. Results revealed that the cholesterol level higher in case of negative control but in case of positive control rats, lower cholesterol activity indicates that the drug able to control the cholesterol and triglyceride level. Treatment of mushroom samples also showed significant result in

lowering the blood cholesterol and triglyceride level (Table 23). All the four mushroom species showed lowering activity but *P. djamor* and *P. ostreatus* showed more activity than the *P. florida* and *P. sajor-caju*.

Table 23: Effect of oral treatment of *Pleurotus* species on cholesterol and triglyceride levels of diabetic rats

Groups	Triglycerides	Cholesterol
Normal Control	80.89±2.69	122.09±1.74
Positive control	170.40±1.37	146.30±1.00
Negative control	226.83±3.23	206.13±1.15
P. ostreatus	193.64±1.83	168.13±0.82
P. sajor-caju	201.96±1.21	183.26±1.14
P. djamor	184.9±1.31	156.63±1.38
P. florida	200.95±1.34	190.98±0.98

'±' standard error of three replicates of each group

4.17. Effect of spent mushroom substrate on plant growth and yield

4.17.1. *Capsicum chinense*

Spent mushroom substrate of oyster mushroom was tested of their effect on growth promotion of *C. chinense* in potted condition. Spent mushroom were used directly in the soil at 250gm/kg soil ratio and the growth promotion in terms of height, number of leaves, leaf size and yield were evaluated. The results revealed that all the treated plants showed significant increase in height in comparison to control after 7 days of planting (Figure 64). After 49 days of interval, the height, number of leaves was showed significant result. It was observed that flowering started after 25 days in case of treated plants while it started 35 days in case of control plants. Size of the leaves also showed very wide variation in comparison to the control plants. In case of treated plants, leaf length ranges from 17 - 20cm while it is ranges from 7-12.5cm in case of control plants. Leaf diameter also increased in case of treated plants (7.5-9.5cm) while it is lower in control plants (2.5-4.5cm). Final yield also was determined and it was found that both the size and number of fruits was higher in the treated plants (Table 24).

Table 24: Comparison of growth of *C. chinense* grown in spent substrate of *P. ostreatus* and in untreated soil.

Treatment	Height (cm)	No of leaf	Average Leaf size (cm)		Flowering (days)	Yield/plant (gm)
			Length	Diameter		
Paddy straw	64.0	35	18.0	8.8	48	30
Paddy straw + Saw dust	64.5	37	20.0	9.0	45	37
Wheat straw	56.0	30	17.5	9.0	54	45
Paddy straw + wheat straw	60.0	35	17.0	7.5	51	42
Control (soil)	21.0	12	7.0	4.5	57	20

Average of 5 replicate plants of each treatment

Figure 64: Effect of spent substrate of oyster mushroom on growth of *Capsicum chinense*;(A) control plants and (B) plants grown in spent substrate amended soil [after 15 days]; (C) untreated control and (D) plants grown spent substrates amended soil [after 45 days]; (E) flower, (F) developmental stages of fruit.

4.17.2. *Capsicum annuum*

Spent mushroom substrate of oyster mushroom and compost of button mushroom were tested for their effect on growth promotion of *C. annuum* L.in potted conditions. Spent mushroom substrates were used directly as well as in leached form and weathered compost was also applied either singly or in combination. After this growth promotion in terms of height, number of branches and root-shoot biomass were evaluated at several intervals. Final yield was also estimated by harvesting the capsicum according to their treatment. The results revealed that all the treated plants showed significant increase of height after 35days out of which, those treated with spent substrate of fresh oyster mushroom, button mushroom leachate and weathered compost of button mushroom showed highest increment in growth (Figure 65). On the other hand, it was observed that the number of branches significantly increased in oyster mushroom leachate, button mushroom weathered and button mushroom fresh compost (Table 26). In case of yield highest yield was obtained by treatment with SMS of oyster mushroom leachate followed by oyster mushroom weathered SMS. It has been reported that the PGPR also stimulate the beneficial plant fungal symbiosis involving both AM fungi and ectomycorrhizae. Results revealed that the spent oyster mushroom leachate, fresh oyster mushroom substrate and button mushroom leachate showed better yield. It was also reported that the ectomycorrhizal treatment influences the growth of plants.

Table 25: Effect of spent mushroom substrate on growth of *C. annum*

Treatment	Height of Plants (cm)				
	7days	14days	21days	28days	35days
Control	18.7±0.3	19.3±0.3	28.33±0.2	37.67±0.3	41.17±0.4
Fresh oyster SMS	21.9±0.4	24.33±0.4	30.66±0.2	49.3±0.6	64.3±0.3
Oyster SMS leachate	20.5±0.4	25.1±0.6	31.0±0.1	45.7±0.3	60.3±0.7
Oyster Weathered SMS	19.5±0.1	22.3±0.2	27.75±0.1	39.4±0.1	53.5±0.6
Button SMC leachate	22.3±0.5	25.3±0.2	33.67±0.6	55.8±1.1	60.9±0.6
Button weathered Compost	22.57±0.4	27.7±0.4	39.7±0.4	63.3±0.6	72.1±0.5
Fresh SMC of Button	19.7±0.3	23.7±0.4	30.3±0.5	64.03±0.6	71.9±0.3
Oyster SMS + Button SMC	19.3±0.4	21.0±0.2	32.0±0.6	56.73±0.2	69.16±0.3

'±' standard error of 5 replicate plants of each treatments;

Table 26: Effect of spent mushroom substrate on number of branches

Treatment	21 days	28 days	35 days
Control	4.28±0.1	6.27±0.1	7.55±0.1
Fresh oyster SMS	6.39±0.2	8.39±0.2	11.39±0.5
Oyster SMS leachate	7.39±0.2	11.16±0.6	12.33±0.6
Oyster Weathered SMS	8.68±0.4	9.39±0.2	10.72±0.1
Button SMC leachate	4.68±0.0	7.61±0.2	11.22 ±0.3
Button weathered Compost	14.04±0.1	7.52±0.0	12.61±0.4
Fresh SMC of Button	4.63±0.1	7.5±0.28	11.53±0.5
Oyster SMS + Button SMC	4.53±0.3	7.3±0.1	9.56±0.1

'±' standard error of 5 replicate plants of each treatments;

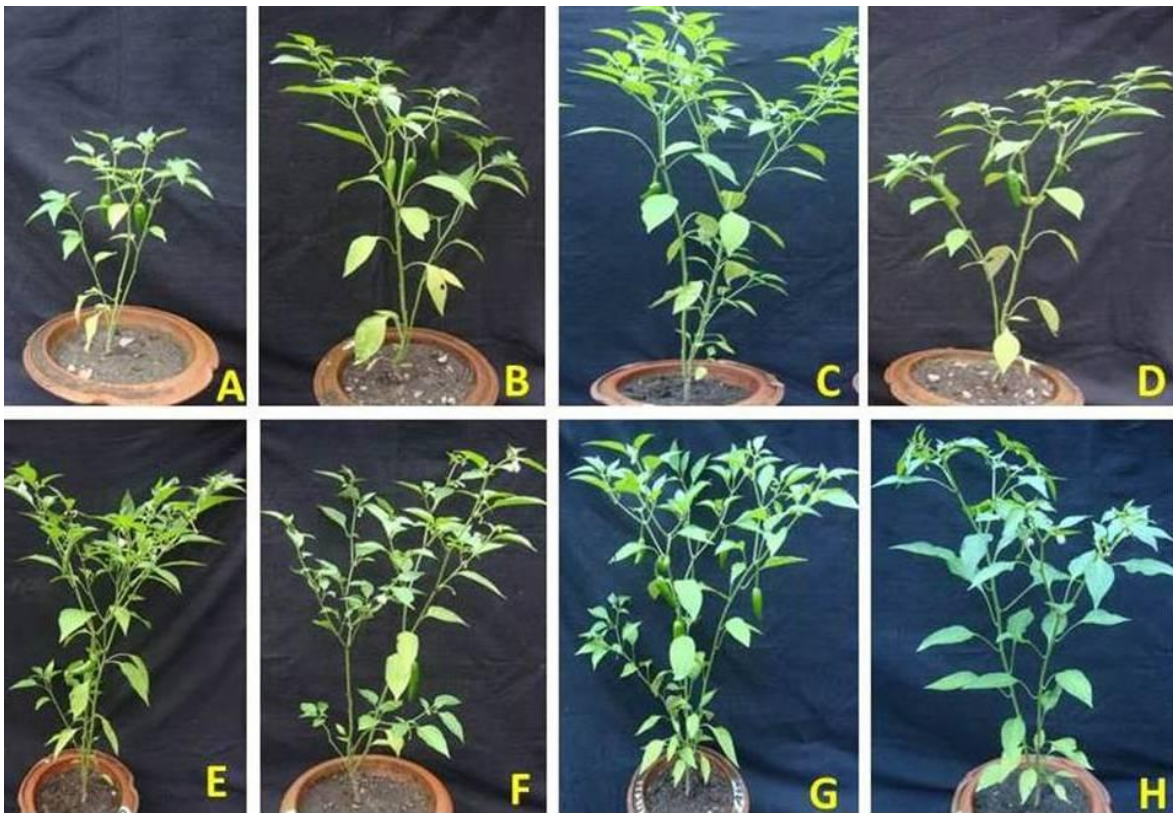


Figure 65: Effect of spent mushroom substrates on the growth of *Capsicum annuum* L. (A) control; (B) fresh spent oyster mushroom substrate; (C) spent oyster mushroom substrate leachate; (D) spent weathered oyster mushroom substrate; (E) fresh button mushroom compost; (F) spent button mushroom compost leachate; (G) spent weathered button mushroom compost; (H) combined treatment of spent oyster mushroom and button mushroom substrate

4.17.3. *Solanum lycopersicum* and *Amaranthus* sp

Tomato and *Amaranthus* are very important vegetable widely cultivated and consumed by the world. Spent mushroom substrate of Oyster mushroom was tested for their effect on growth promotion of Tomato and *Amaranthus* in field condition (Figure 66). 10 days old seedlings of tomato were sowed in pre-treated plots and seed of *Amaranthus* was also sowed. After this growth promotion in terms of height, number of branches was evaluated. The results revealed that all the treated plants showed significant increase of height after 35 days out of in treated plants in compare to untreated control plants (Table 30). Germination period was also lesser in case of *Amaranthus* plant SMS treated in compare to control plots. On the other hand, it was observed that the number of branches significantly increased in SMS treated plants than the untreated plots. In case of yield highest yield was obtained by treatment with SMS of oyster mushroom followed by the control plants (Table 31).

Table 27: Effect of spent mushroom substrate on height of tomato *and Amaranthus* in different intervals

Plant	Treatment	Height of plants (cm)			
		7days	14days	21days	28days
Solanum lycopersicum	Control	12.7±0.3	15.3±0.3	23.33±0.2	27.67±0.3
	SMS	17.9±0.4	21.33±0.4	29.66±0.2	39.3±0.6
Amaranthus sp.	Control	2.5±0.4	5.1±0.6	12.0±0.1	19.7±0.3
	SMS	9.5±0.1	16.3±0.2	21.75±0.1	33.4±0.1

'±' standard error;

Table 28: Effect of spent mushroom substrate on number of branches of tomato *and Amaranthus* in different intervals

Plant	Treatment	Branches (cm)		
		21 days	28 days	35 days
Solanum lycopersicum	Control	7.28±0.1	9.53±0.1	13.55±0.1
	SMS	12.39±0.2	18.39±0.2	21.39±0.5
Amaranthus sp.	Control	3.43±0.2	7.16±0.6	15.33±0.6
	SMS	8.68±0.4	17.32±0.2	19.72±0.1

'±' standard error;

Figure 66: Effect of spent mushroom substrates of oyster mushroom on *Solanum lycopersicum* (A& D) untreated control and (B &C) SMS treated and *Amaranthus* (C) Untreated control and (D) SMS treated

4.18.1. Effect of spent mushroom substrates on biochemical changes of crop plants

4.18.1. *Capsicum chinense*

Mobilization of soil phosphate after treatment were evaluated in terms of total phosphate content in soil, roots and leaves of the treated plants in comparison to the control sets. The uptake of phosphate content was significantly increased in spent mushroom substrate treated plants while it shows lower uptake of soil phosphate to the root and leaf in case of control plants (Table 25). Spent mushroom substrate improves the soil quality by having a direct influence on the uptake of phosphate content and thus, aeration and water movements in addition to increasing availability of insoluble sources of phosphorus. Chlorophyll is the main photosynthetic pigment and it was observed that the use of these spent mushroom substrates affects in the total chlorophyll content of the plant leaves. Chlorophyll content (mg/g tissue) includes the chlorophyll a and chlorophyll b which significantly increased in the plants treated with spent mushroom substrate of oyster mushroom (Figure 67A).

Table 29: Effect of spent mushroom substrate on total phosphate content of soil, root and leaf of *C. chinense* after 15 days of seedling transfer.

Treatment	Total Phosphate Content (µg/gm)		
	Soil	Root	Leaf
Control	51.30±2.3	9.12±1.3	7.67±0.8
Paddy straw	44.51±1.3	11.53± 0.7	8.47± 1.2
Paddy straw + Saw dust	42.50±3.4	15.45±1.3	13.35±1.4
Wheat straw	45.65 ± 1.1	14.25± 1.4	10.44± 1.2
Paddy straw + wheat straw	40.35± 0.8	11.75± 1.2	9.33± 1.1

± Standard error,

Carotenoid is one of the most important compounds which show antioxidant activity and this was also estimated to evaluate the effect of spent oyster mushroom substrate on the enhancement of antioxidant compound. The results revealed that the application of spent substrate enhances the carotenoid as the leaf and fruit of the treated plants showed significant higher amount of carotenoid in comparison to the control plants. The results also shows that the effect different substrates influence the level of carotenoid compound and it was observed that the combined treatment of spent mushroom substrate of saw dust and paddy straw showed maximum enhancement of carotenoid compound followed by the spent mushroom substrate of paddy straw (Figure 67B).

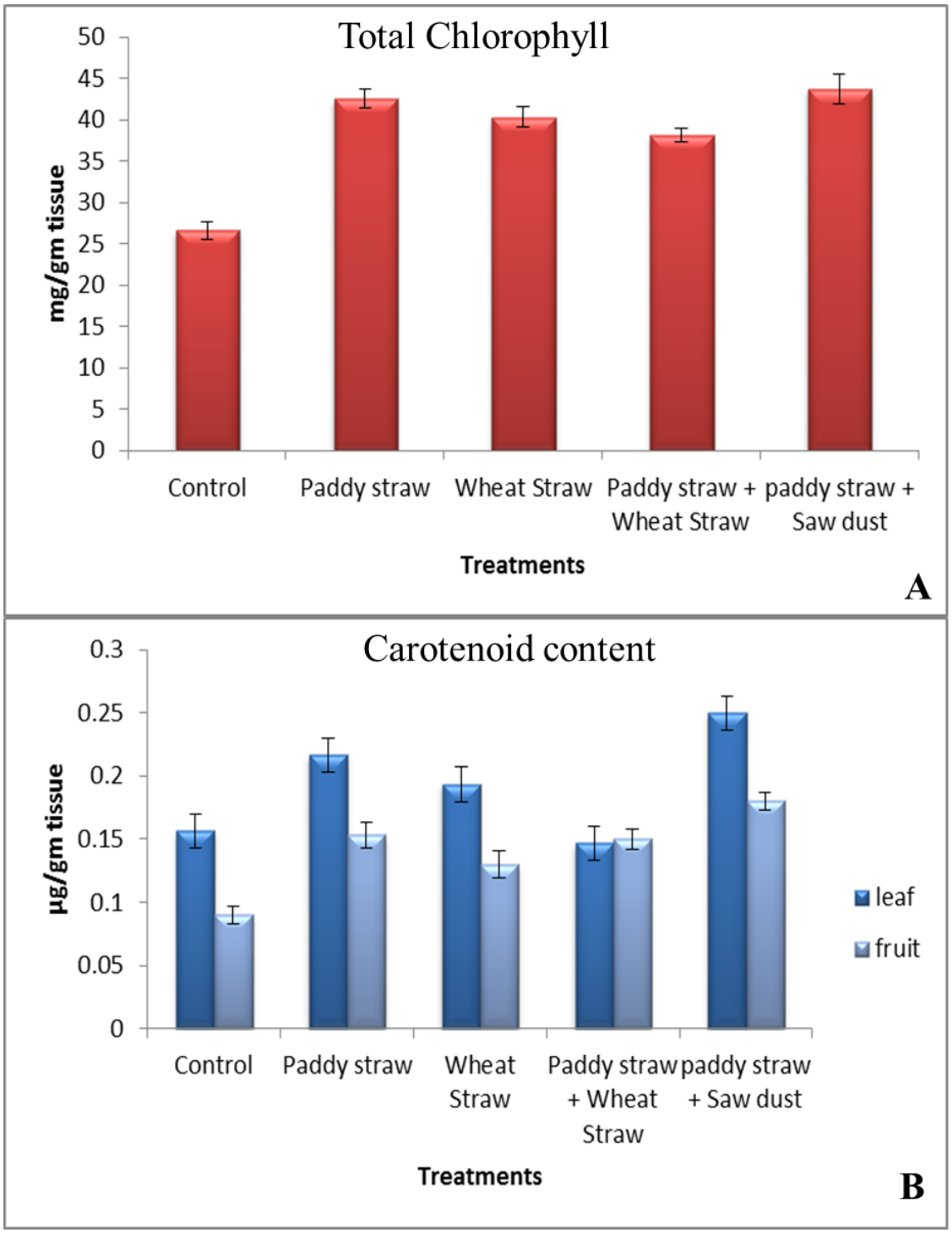


Figure 67: Effect of spent mushroom substrate on total chlorophyll (A) and carotenoid content (B) of *C. chinense*.

4.18.2. *Capsicum annuum*

Apart from this, mobilization of soil phosphate by these treatments were evaluated in terms of total phosphate content in soil, roots and leaves of the treated plants in comparison to the untreated control sets. The uptake of phosphate content was

significantly increased in fresh oyster mushroom substrate and in button mushroom weathered compost (Table 28). SMS improved soil quality by having a direct influence on soil aggregation and thus, aeration and water movements in addition to increasing availability of insoluble sources of phosphorus.

Table 30: Effect on phosphate content of soil, root and leaf after 15days of seedling transfer

Treatment	Total Phosphate Content (µg/gm)		
	Soil	Root	Leaf
Control	53.47±4.73	11.42±1.73	7.67±0.83
Fresh SMS of oyster	43.50±3.45	17.45±1.44	10.33±1.76
Oyster mushroom SMS leachate	47.51±3.33	13.3±1.12	10.65±1.32
Weathered SMS of oyster	39.50±3.21	11.42±1.39	9.05±1.11
SMC leachate of Button	41.1±3.02	12.51±1.12	9.65±0.91
SMC weathered of Button	48.39±4.12	13.70±1.83	10.25±0.93
Fresh SMC of Button	44.56±3.31	12.15±1.19	10.70±1.12
SMS + SMC	41.35±4.17	11.95±1.90	10.25±0.93

'±' standard error; ($P<0.5$);

Effect of spent substrates on chlorophyll content of *C. annuum*

Chlorophyll is the main photosynthetic pigment and it was observed that the use of these spent mushroom substrates enhanced the total chlorophyll content of the leaves of treated plants. Chlorophyll content including chlorophyll a and chlorophyll b was significantly increased in both oyster mushroom and button mushroom leachate, and in fresh oyster mushroom substrate. The dual treatment of both the substrate also showed significant amount of chlorophyll content (Table 29).

Table 31: Effect of Spent mushroom substrate on chlorophyll content of *C. annuum*

Treatments	Chlotophyll a (µg/gm tissue)	Chlorophyll b (µg/gm tissue)	Total Chlorophyll (µg/gm tissue)
Control	8.38	6.38	14.76
Fresh SMS of oyster	12.92	4.72	17.64
Oyster mushroom SMS leachate	13.51	5.30	18.81
Weathered SMS of oyster	11.40	3.5	14.90
SMC leachate of Button	12.76	4.81	17.57
SMC weathered of Button	11.24	4.35	15.59
Fresh SMC of Button	10.32	3.37	13.69
SMS + SMC	12.40	3.41	15.81

Effect of spent mushroom substrate on total protein and carotenoid activity of *C. annuum*

Total protein content was also evaluated in leaves and fruits. The results revealed that the total protein content was maximum in the fruits of fresh oyster mushroom substrate and fresh button mushroom compost treated plants ranging between 200-250 µg/gm tissue while it was lower in case of treatment with oyster mushroom weathered substrate and the dual treatment of button mushroom and oyster mushroom substrate. Higher leaf protein was also observed in oyster mushroom leachate, button mushroom weathered compost treatment as well as dual application of both the substrates (Figure 68A). Among the pigments, carotenoid, being an antioxidant compound is also important and hence carotenoid content was estimated in the study. Carotenoid was estimated in the leaf as well as in fruits of *Capsicum annuum* L. and button mushroom leachate treated plants showed a high range of carotenoid compound (0.25-0.30 µg/gm tissue) followed by the oyster mushroom fresh substrate and button mushroom weathered compost treatment (0.15-0.20 µg/gm tissue). Mature pepper fruits are also rich in carotenoids, compounds with antioxidant and anti-carcinogenic capacity; furthermore, either immature or mature fruits contain a high concentration of antioxidant phenolic compounds (Figure 68B).

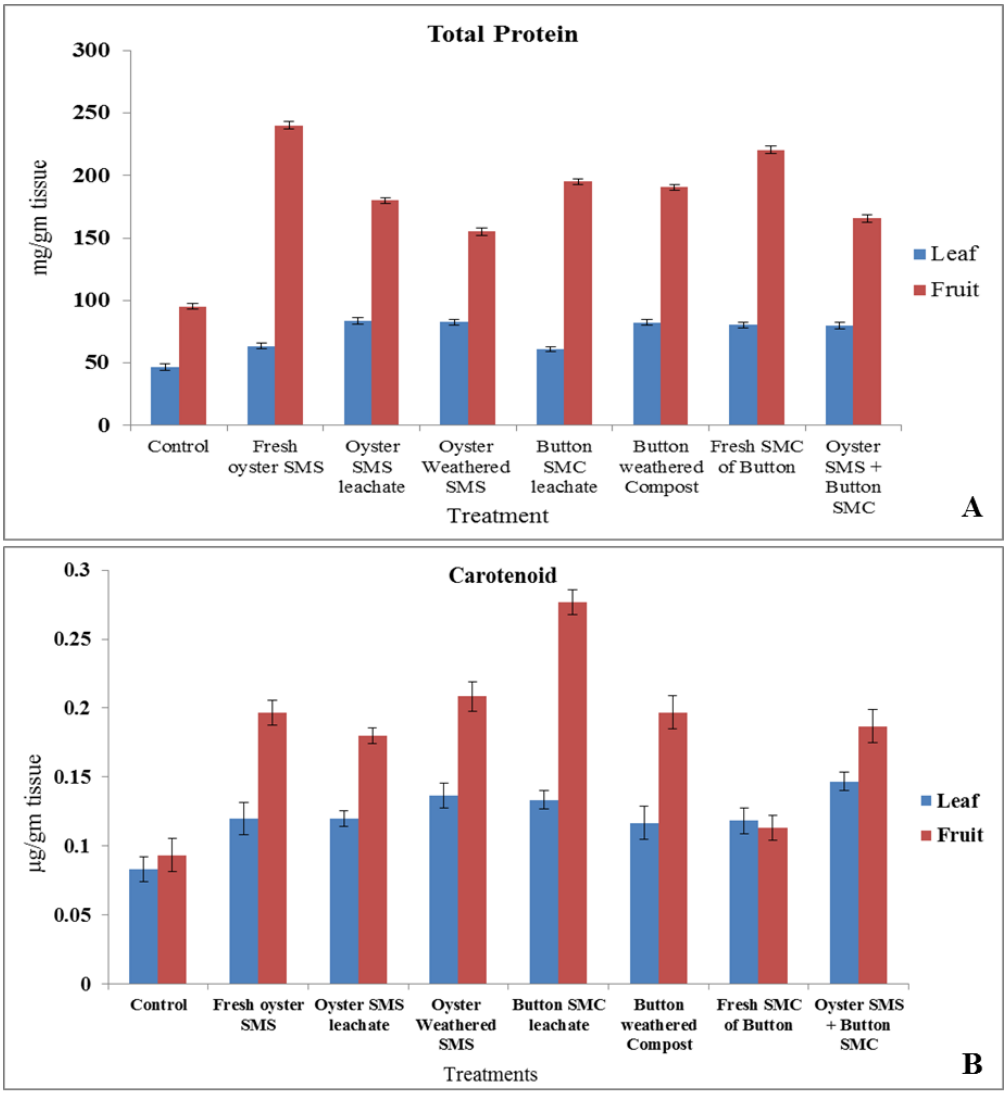


Figure 68: Effect of spent mushroom substrate on soluble protein (A) and carotenoid contents (B) of *Capsicum annuum*

4.18.3. Effect of spent substrates on chlorophyll content of *Solanum lycopersicum* and *Amaranthus* sp.

Photosynthesis is the most important physiological phenomenon then required for all plant kingdom and chlorophyll is the most important photosynthetic pigment responsible for photosynthesis. Results revealed that the use of spent mushroom substrates as potential biofertilizer helps in increasing the photosynthetic pigment in leaf in compare to the control plants. Chlorophyll content including chlorophyll a and chlorophyll b was significantly increased in both plant ie tomato and *Amaranthus* when treated with the spent mushroom substrates (Table 32).

Table 32: Effect of Spent mushroom substrate on chlorophyll content of tomato and *Amaranthus* sp.

Treatments	Chlorophyll a (µg/gm tissue)	Chlorophyll b (µg/gm tissue)	Total Chlorophyll (µg/gm tissue)
Untreated control	5.3067	2.188	7.4947
Treated *S. lycopersicum*	9.1791	6.5455	15.710
Untreated *Amaranthus*	4.329	1.874	6.203
Treated *Amaranthus*	6.743	2.397	9.14

4.19. Post-harvest processing of oyster mushroom

Processing of oyster mushroom is one of the major steps to utilize the cultivated mushroom. Shelf-life of oyster mushroom is very less in compare to milky mushroom and button mushroom. But it was very difficult to keep the cultivated mushroom fresh for 3-4 days. Thus processing is the most important step for the management of oyster mushroom. Processing technology of oyster mushroom was practiced to avoid the loss of cultivated mushroom (Figure 69). Fresh mushroom was harvested and was processed using different techniques. Following are the some of the processing techniques.

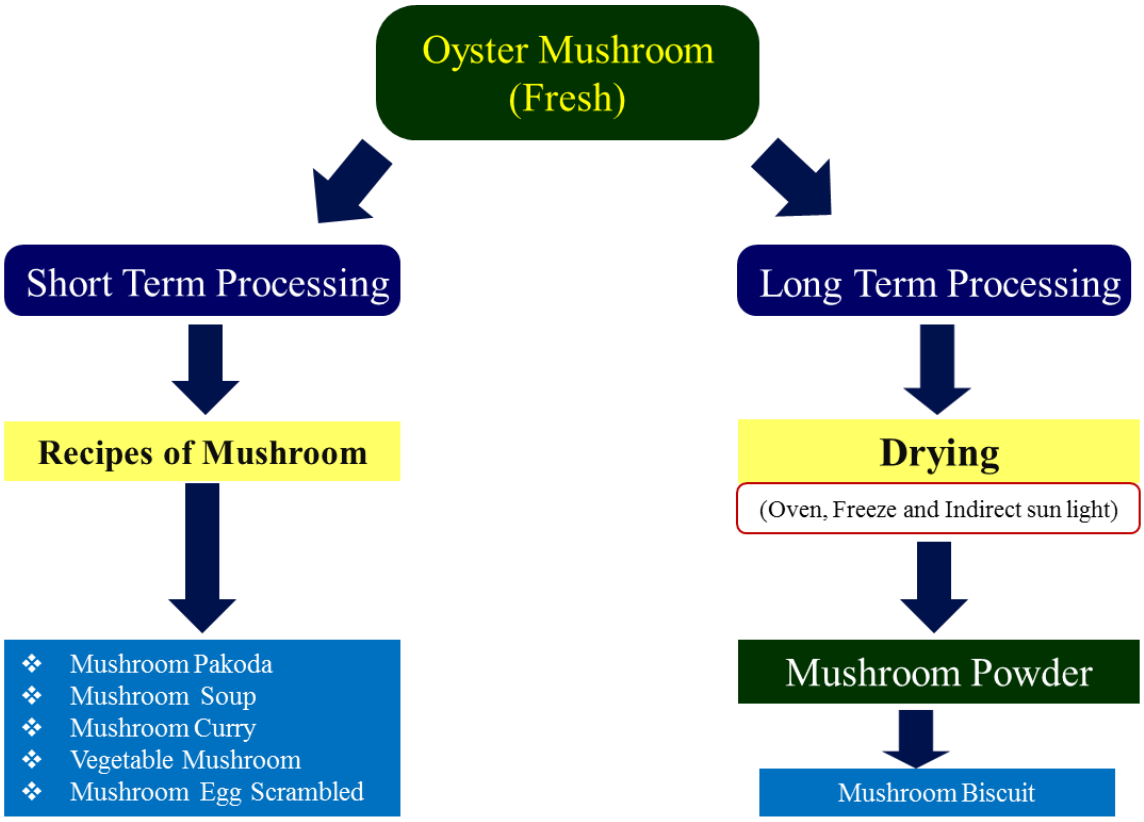


Figure 69: Schematic diagram of post-harvest processing of *Pleurotus* species

4.19.1. Short term processing

Shelf life is one of the major factors which affected the processing of oyster mushroom. Short term processing leads to packaging of mushroom for better preservation followed by making different recipes. Selection of fruiting body is one of the important steps for good quality packaging. Freshly harvested fruiting body was selected for packaging and sampling was done according to their size (Figure 70). The selected fruiting body was then cleaned and the basal part of the stipe was removed. Then the mushroom fruiting body was packaged in polypropylene bags according to their size, colour and species. Then the bags were sealed and weighted. The packaged mushrooms were then kept at 4^0 C for 3-4 days preservation.

Figure 70: Harvesting and packaging of oyster mushroom

Short term processing of oyster mushroom also includes the preparation of different recipes. A variety of delicious food items were prepared with oyster mushroom depending on personal choice and tested in the laboratory among the lab members for the evaluation of their taste and flavour. Mushroom curry, Vegetable mushroom with tomato, mushroom Pakoda and mushroom soup was prepared (Figure 71). These recipes were prepared using the cultivated different species of oyster mushroom. The mushroom soup was prepared following two different procedures like crushed mushroom soup and chopped mushroom soup and both the soups were delicious and tastes were completely different.

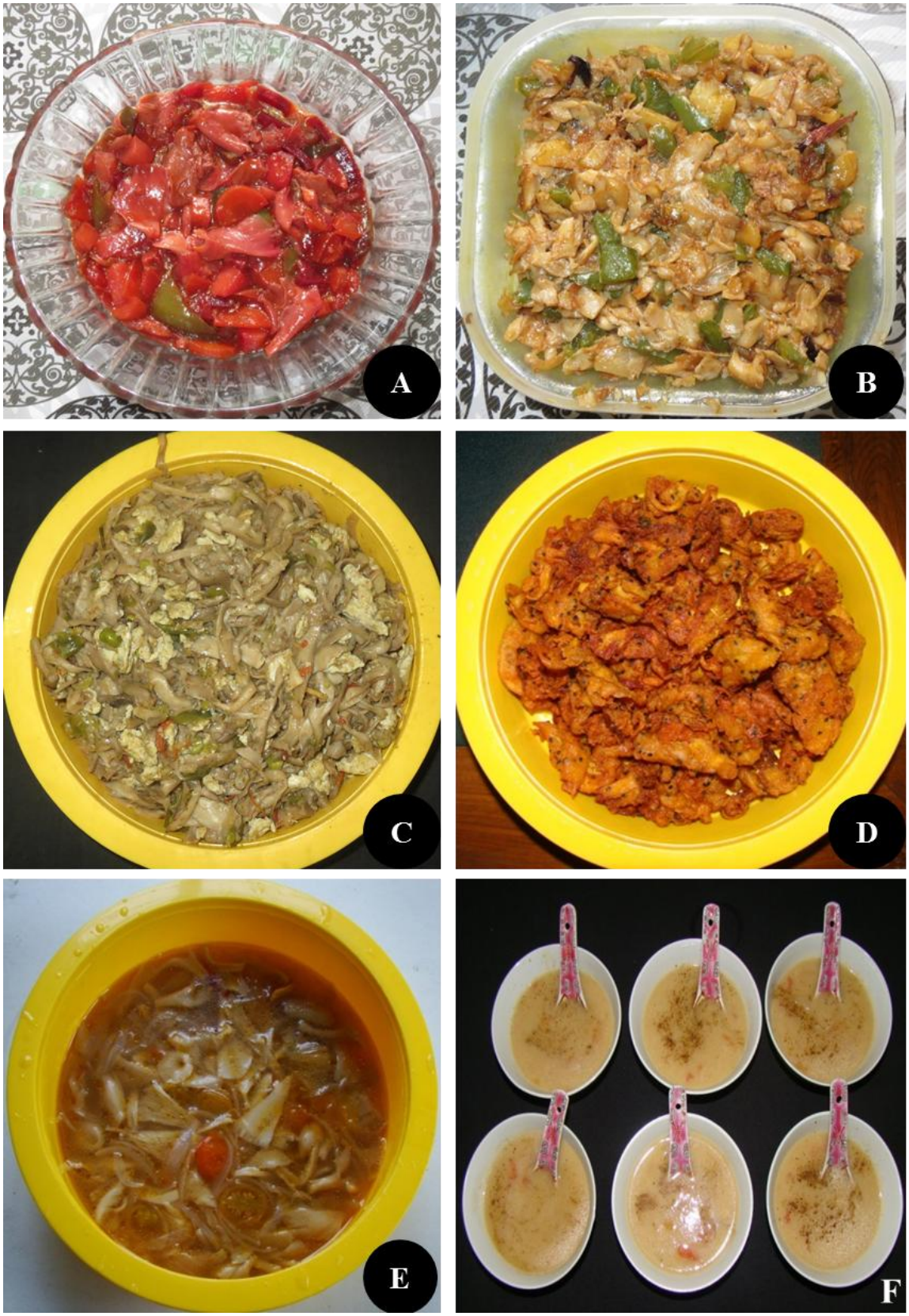


Figure 71: Different recipes of oyster mushroom (A) mushroom tomato curry, (B) vegetable mushroom, (C) egg mushroom scramble (D) onion mushroom pakoda, and (E-F) mushroom soup.

4.19.2. Long term Processing

Most of the mushrooms, being high in moisture and delicate in texture, it cannot be stored for more than 48 hours at the ambient conditions prevailing in the tropics. The spoilage of mushroom might be caused by the action of bacteria on the mushroom tissue and browning of mushrooms was due to a combination of auto-enzymatic and microbial action on the tissue. Postharvest practices have since been developed to extend the shelf life of fresh mushrooms. As far as processing technologies are concerned, sun drying of mushrooms is one of the simplest and oldest methods followed by the growers from the time immemorial. During the recent years, there has been an increased emphasis on the quality of fresh vegetables including mushrooms, which is reflected in the price of the produce. Effective processing techniques will not only prevent the post-harvest losses but also result in greater remuneration to the growers as well as to the processors. Value can be added to the mushrooms at various levels, right from grading to the readymade snacks. Technologies for production of some other products like mushroom based biscuits have been developed but are yet to be popularized. Attractive packaging of the value-added products is yet another area, which may be called the secondary value-addition.

Drying is perhaps the oldest technique known to the mankind for preservation of food commodities for long duration. It is the process of removal of moisture from the product to such a low level that microbial and biochemical activities are checked due to reduced water activity, which makes the products suitable for safe storage and protection against the attack by microorganisms during the storage. Mushroom contains about 90% moisture at the time of harvesting dried to a moisture level down below 10-12%. At a drying temperature of 55-60^{0}C, the insects and microbes on the mushrooms will be killed in few hours, which give us the dehydrated final product of lower moisture content with longer shelf-life (Figure 72 and 73). The temperature, moisture of the mushroom and humidity of the air affect the colour of the dried product. Dried mushrooms can be easily powdered and used in soups, bakery products, etc. Mushroom dried at higher temperature loose texture, flavour, and colour.

Figure 72: Dry mushroom of different *Pleurotus* species

Figure 73: Mushroom powder prepared from dried *Pleurotus* species

Figure 74: Mushroom biscuit prepared using dried oyster mushroom powder

Delicious and crunchy mushroom biscuits (Figure 74) were prepared by using the button/ oyster mushroom powder and various ingredients viz., maida, sugar, ghee (bakery fats), mushroom powder, backing soda, cashew nut crushed and milk powder. Biscuits were prepared to evaluate the quality of mushroom powder for long term processing in bakery industry. The biscuits were tasted among the several people and it was observed that the taste was very good along with the flavour of mushroom powder. Preparation of mushroom biscuit was successfully done and it was proven that the preparation was a good was to management of mushroom powder into a delicious health food material.

4.19.3. Biochemical characterization of powdered fruiting body of *Pleurotus* species

Processing practice is one of the major steps for the preservation of mushroom fruiting body. Various methods were adopted for preservation of the mushroom fruiting body. Dehydration of the fruiting body helps in long term preservation up to 6 months for *Pleurotus* species. Mushroom fruit body were harvested and dried under indirect sunlight and then it was powdered. Mushroom powder is also very nutritious for human health. Various biochemical constituents were analysed of the four cultivated species of *Pleurotus*. In case of total sugar content, it was found that the amount was higher in case of *P. djamor* followed by *P sajor-caju*, *P ostreatus* and *P. florida*. While in case of reducing sugar, *P ostreatus* and *P djamor* showed higher activity than *P. sajor-caju* and *P. florida* (Figure 75). Total protein content of the *Pleurotus* species was also evaluated

and it was found that *P djamor* and *P ostreatus* powder contains high amount of protein in compare to *P sajor-caju* and *P florida*. Evaluation of total lipid contend was also done and the results suggested that all the four species of *Pleurotus* consists very low amount of lipid content and among them *P florida* showed highest lipid content than that of the other species. Dietary fibre of mushroom is also an important biochemical constituent good for human health (Figure 76). The results revealed that *P. djamor* showed highest amount of dietary fibre followed by *P ostreatus, P florida* and *P. sajor-caju*.

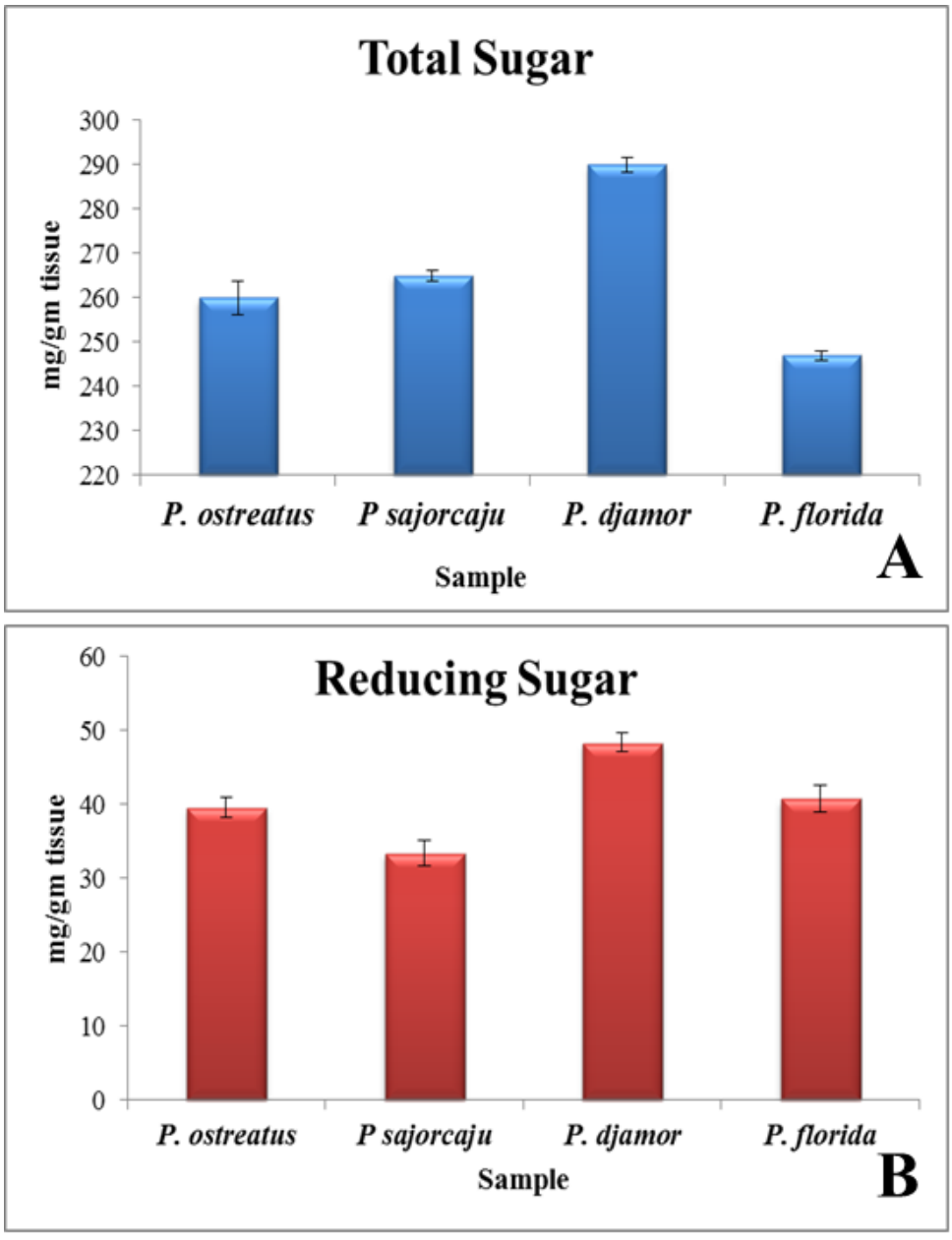


Figure 75: Evaluation of biochemical constituents of oyster mushroom powder (A) total sugar, (B) reducing sugar

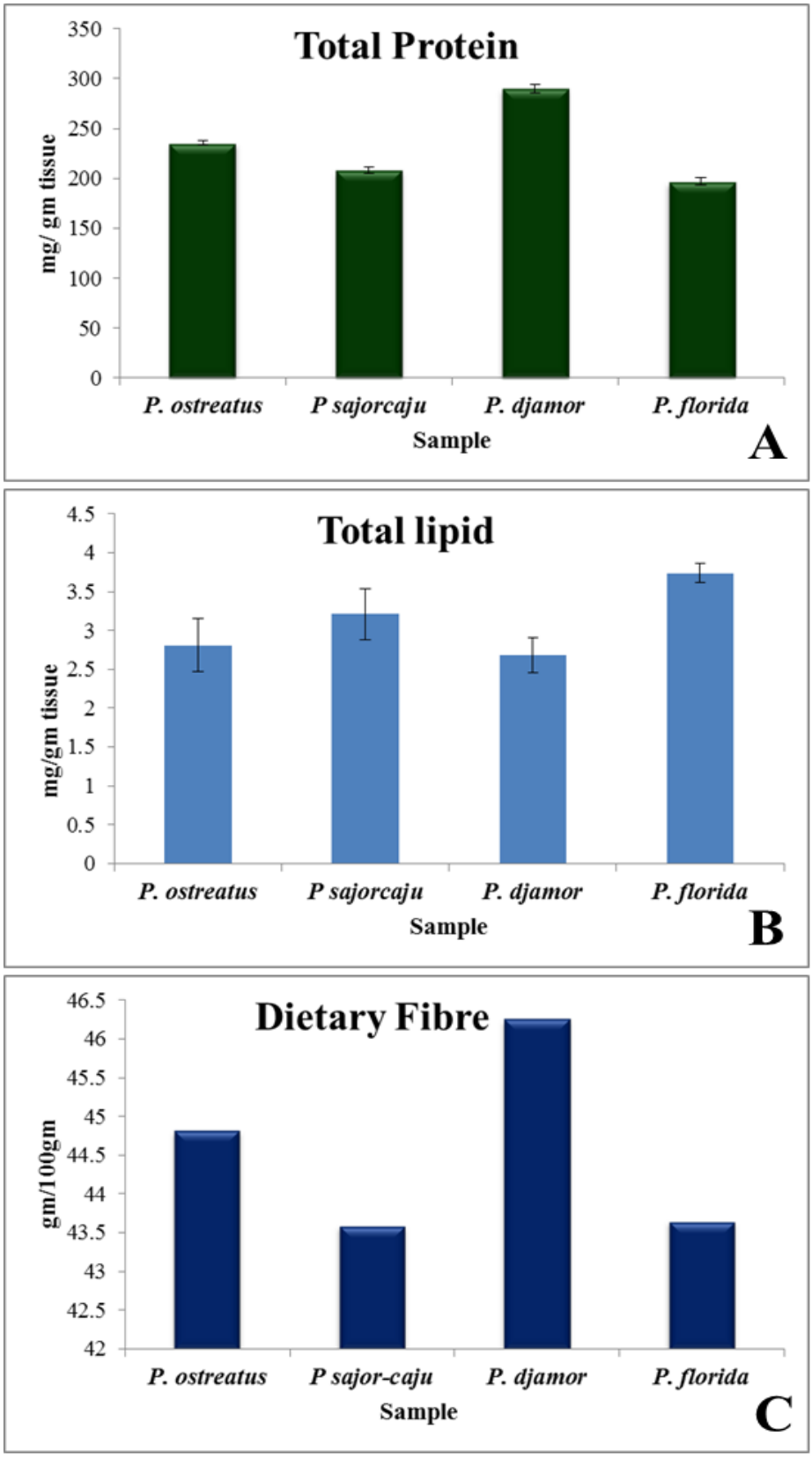


Figure 76: Evaluation of biochemical constituents of oyster mushroom powder (A) total protein, (B) total lipid and (C) dietary fibre

4.20. Management of Contaminants of oyster mushroom

Like other crops, mushrooms are also very much affected by different types of contaminants. Both biotic and abiotic agents are involved directly or indirectly which damages the mushroom results in reduction of production. Among biotic agents, it includes bacteria, fungi, viruses and even pests. Many of these organisms, acts as a competitor moulds which affects in the spawn run where as other attacks the fruiting body in different stages of development. The different types of mushroom contaminants found during the cultivation and their management are discussed as follows.

4.20.1. Ink Cap Disease

Ink cap disease is one of the major disease severely affected the cultivation of oyster mushroom caused by the attack of *Coprinus cometus, C. logopus.* Ink caps were appeared during the spawn run. They were slender, bell-shaped mushrooms. Initially they were cream coloured but later it turns into bluish black usually covered with scales (Figure 77). It was observed that the fungus grows in cluster on beds having a long stipe immerged deeply into the substrate.

Figure 77: Ink Cap disease caused by *Coprinus* sp.

Disease Management

Ink cap disease was one of the most severe diseases causing great loss in the production. Pretreatment of substrate was done by proper pasteurization; fresh substrate was used during cultivation; watering was limited during the colonization of the mycelia over the substrates; young pileus of ink caps were removed immediately; the contaminated bags were removed immediately from the room and was completely burnt to avoid further spread to another bags.

4.20.2. Cob Web Disease

Cob web is also commonly known as mildew or soft decay disease often caused by the fungus *Cladobotryum dendroides*. It was observed that the fungus extensively damages the substrate colonization as well as the fruiting body. A floccose white mycelium covers the substrate and gradually it affects the pileus and stipe and eventually results in decomposing the fruiting body (Figure 78). It was severely attacks with a white dense mould developed over the substrate first and finally results in degradation of the fruiting body. The white mycelia became turns into pinkish red. It was also observed that the higher humidity and temperature increase the chances of the disease.

Figure 78: Cobweb disease during cultivation of oyster mushroom

Disease Management

Cobweb disease was severely affects the production of oyster mushroom and it was observed that the disease caused by using the old substrates. Pasteurization was done properly so that the chance of contamination was reduced. Sanitation of the cultivation unit was done regularly using citronella oil and neem oil and other commercially available disinfectants to avoid the contaminants. Water spray to the mushroom bags was reduced at the time of summer and room temperature maintained by spraying water on the floor. The infected bag was removed immediately and burnt to avoid further contamination.

4.20.3. Green Mould Disease

Green mould disease is also a very common fungal disease caused by different species *Trichoderma*, *Penicillium* and *Aspergillus* and this disease is very well known Trichoderma blotch or green mould. Among the moulds, *Trichoderma* causes a great loss in the quality and quantity of mushroom production. Different species of *Trichoderma* affected the growth of mycelia of oyster mushroom and it was observed that the green mould grows faster than the mushroom mycelia and gradually it covers the whole substrate rapidly (Figure 79).

Figure 79: Green Mould disease caused by *Trichoderma* sp.

Disease Management

Green mould is a major competitor mould that grows very rapidly from one bag to another as the fungal spore spread through air. Very good hygiene was maintained in the production unit and proper pasteurization was done before spawning. Substrates were collected fresh and they were treated properly before spawning. 2% Formaldehyde was used to completely avoid the contamination. Moreover it was observed that sometimes the bag was contaminated by the green mould; so the contaminated bags were removed from the production unit to avoid further spread of the organisms.

4.20.4. Yellow Mould Disease

Yellow mould is also an important fungal disease commonly affects the growth of oyster mushroom often caused by the fungi *Myceliophthora lutea* and *Chrysosporium luteum*. It was observed that yellow mould developed on the substrate with dark yellow circular colonies and gradually it distributed throughout the substrate. Rapidly the substrate becomes dark brown or turns into black (Figure 80). Like the green mould, this organism is a competitor mould that grows before the mushroom mycelia covers the substrates and thus mushroom production was severely inhibited.

Figure 80: Yellow mould disease during cultivation of *Pleurotus* species

Disease Management

Unlike green mould disease, yellow mould is one common disease that affected rapidly over the substrate. Proper pasteurization was very much effective in controlling the disease. It was observed that the increased pasteurization time helps in reducing the disease severity of yellow moulds. Application of formalin (2%) was also found to be very effective in reducing the chances of yellow mould. Moreover yellow mould contaminated bags were discarded from the production unit and it was then burnt to stop further spreading of the fungal spores.

4.20.5. Bacterial Blotch of oyster mushroom

Oyster mushroom was cultivated throughout the year and during cultivation, several other organisms affected the production. Bacterial blotch was one of the major contaminants severely damages the mycelia and gradually reduces the production. *Pseudomonas* was one of the bacterial genus destructively affected the production. It was characterize by the brown spots or blotches over the substrates (Figure 81). Under favourable conditions, circular or irregular spots were observed on the surface of the substrate and the spot initially light and became dark within 2-3 days. The substrates become loose and very strong pungent smell was one major characteristic feature of the bacterial blotch. The enlargement of the spot was dependent on the environmental condition.

Figure 81: Bacterial blotch disease

Disease Management

It was observed that high relative humidity enhances the disease severity. Continuous persistence of water on the surface of the substrate and also under the mushroom bags helps in bacterial contamination and thus to avoid bacterial disease, proper ventilation along with the sanitation of the production unit was done regularly. Spraying of water was controlled during the summer and winter to avoid water logging on the bed.

4.20.6. Pest Disease of Oyster Mushroom

4.20.6.1. Sciarid fly

Sciarid fly, *Lycoriella auripila* causes severe damage to subtropical mushroom *Pleurotus* sp. tunnelling in the stalk and the cap by the maggot is the characteristic visible symptom. Growth may be often arrested when in the pin-head stage the maggot attack. Even when the infected pin-head develops into button, they become small brown

and leathery. Sciarids, the small fungal gnats, are mosquito type flies. Colour of flies varies from brown black to black. Body length varies from 1.5 to 3.5 mm depending upon the species. Antennae are long (14 annuli) which are held characteristically erect. Larvae feed on substrates, mycelium and mushrooms. Through on consumption of substrate and mycelia by the larvae, pH of the substrate changes and thus the growth of mushroom mycelium become slow down. As the infestation by the larvae is often in groups, bare patches without mushrooms can be seen on the beds (Figure 82A). When larval attack occurs at pin head stage, it was observed that further development of pinheads completely stops and pin heads eventually die. Adult Sciarids consume minute quantity of water and other liquids but do not feed on mushrooms. Flies also transport spores of the pathogenic fungi, virus infected fungi, nematodes and mites. Sciarid infestation can cause up to 50% reduction in crop. It was also observed that the larvae prefer to feed in moist areas and tend to move away from dry areas. Fully grown larvae are dirty white with visible longitudinal black streaks. Larvae are 5-8 mm in length. The larval period is of 16 days. They then go in to resting or developmental stage called pupation. In this stage larvae may appear dead but in fact they are undergoing changes within the larval skin. Just after pupation, colour of pupa changes to yellowish brown. Male pupa is comparatively smaller than the female pupa. These flies have been found to stay in the cropping rooms throughout the year. Temperature affects the duration of life cycle to great extent.

4.20.6.2. Phorid Fly

These are small hump backed black or light to dark brown flies measuring 1.9-2.0 mm in size. These flies are diminutive of house flies. Antennae are inconspicuous. Wing venation is reduced. The infested mushroom turns brown along the tunnel in the stipe. Attack at pinning stage restricts the further development of pin heads. Larvae also feed on mushroom mycelium in compost and casing soil. In case of button mushroom phorids can cause up to 46% loss in yield. Phorid flies are less harmful than sciarid flies if we compare damage per larva. In oyster mushrooms, particularly during rainy season 100% loss in yield has been reported. These are small hump backed black or light to dark brown flies measuring 1.9-2.0 mm in size (Figure 82B). They move rapidly with jerky movements. Adult phorids are most common in early summer and are attracted to light and swarm near windows and doors of the cropping rooms. Unspawned compost and fully grown mature compost are not so attractive to oviposition phorid flies. The

eggs are whitish, slightly curved, 0.3 mm long. The newly emerged larvae are nearly transparent. The mature larva is dirty white and measures 3.3-4 mm in length with pointed head and blunt rear end. Larvae feed on mushroom tissue and move upward into the cap forming tunnels in stipe. The infested mushroom turns brown along the tunnel in the stipe. Attack at pinning stage restricts the further development of pin heads. Larvae also feed on mushroom mycelium in compost and casing soil. In case of button mushroom phorids can cause up to 46% loss in yield. Phorid flies are less harmful.

Figure 82: Pest of mushroom Sciarid fly (A), phorid fly (B) and beetle fly (C&D)

4.20.6.3. Beetle fly

Beetle fly is one of the major pest devastatingly attacks the oyster mushroom. These were commonly found during the winter season which feed upon the fruiting body of oyster mushroom. It was observed that this pest affect mostly *P. florida* as it was cultivated during the winter. *Sphaerius* sp is commonly known as Beetle fly about 1-1.5cm in length, mostly black coloured with red spot on it (Figure 82C). Beetle fly

generally lay their egg on the substrates and they feed the mature fruiting body which results in decreasing the production rate of mushrooms.

4.20.6.4. Pest Disease Management

Oyster mushroom very severely affected by different pests during its colonization and fruiting body initiation. Several control measure was practiced to avoid the attack of mushroom pests. Hygiene is the primary method of pest control in mushroom farming. It is the foundation upon which success of all other control techniques depends. The objectives of any hygiene programme include exclusion of pests and diseases from production cycle, elimination of pest and pathogens and destruction of pest and disease present in a crop at its termination. Sanitation focuses on elimination or killing a pest. Routinely removing stumpage from the production unit, is a sound sanitary practice. Sanitary practices are designed not only to remove mushroom pests but to kill significant crop threats.

Screening of doors and ventilators mushroom flies can easily pass through ordinary wire screen and enter the mushroom house to breed on spawned compost and mushroom beds. Screening of doors and ventilators with nylon net of 35 meshes or more can effectively check the entry of flies in the cropping rooms (Figure 83).

Figure 83: Protective measures against insects and pests during mushroom cultivation practice inside the production unit

Polythene sheets coated with sticky material and attached to a fluorescent tube light in each cropping room help in controlling adult flies. Insects are attracted to white light

above 15ºC and to yellow light at lower temperature. Use of light trap (15 W yellow bulb and polythene sheet coated with mustard oil) is very effective for monitoring as well as for the management of the flies.

4.21. Promotion of mushroom cultivation to the rural people of North Bengal

Oyster mushroom is one of the most popular mushrooms cultivated throughout the world and India is one of the largest producers of oyster mushroom. Large number of people are now cultivating mushroom in commercial level throughout India. North Bengal is also popular for producing Oyster mushroom. Awareness of mushroom production is one of the major problems of North Bengal. Large number of unemployed youth are very much interest in mushroom cultivation. For the promotion of oyster mushroom cultivation, unemployed youth of Darjeeling district and Jalpaiguri district were selected and training was given to those persons who are interested in cultivation in large scale as well as in small scale. The hands-on training was based on the oyster mushroom which includes the cultivation of oyster mushroom with special reference to seasonal productivity of different species, promotion of pink oyster mushroom production and bottle and box cultivation for small scale production, preparation of mushroom spawn, post-harvest processing for long time preservation of oyster mushroom with special reference to different techniques drying, powder, biscuit preparation, management of spent mushroom substrates for crop improvement. A large number of unemployed youth are now cultivating different species of oyster mushroom and following are the list of some of the mushroom growers who are cultivating mushroom successfully and producing a great amount of mushroom every year.

Growers are now cultivating the oyster mushroom in a large scale production unit and they are very much known to the technical defaults. It was also observed that the growers are no longer limited their business into the mushroom cultivation but they are now trying to process the extra mushroom left after fresh selling. Growers are now drying the mushroom for long term preservation. In North Bengal, growers are very much interested in selling the fresh mushrooms. But now-a-days, growers are drying the mushroom and packaging quality was also modernized by them.

Table 33: List of trained mushroom growers successfully cultivating *P. ostreatus, P. sajor-caju* and *P. florida* in different part of Darjeeling and Jalpaiguri district.

Sl No	Name of the Growers	Area/ Location	GPS Location	Farm Size (sq.ft)	Farm Capacity (Bags)	Annual Production (kg)
District– Darjeeling						
1	Supok Singha	Naxalbari	26.6823^0E 88.1998^0N	400	1050	16,800
2	Sushanta Das			360	1000	16,000
3	Biswanath Barman			290	650	10,400
4	Jagannath Baroi	Bagdogra	26.7006^0E 88.3433^0N	540	800	12,800
5	Promod Thakur			750	1550	24,800
6	Iswar ch. Rajbanshi			200	350	6.000
7	Rebika Roy			180	300	5,500
8	Ujjal Biswas			350	550	8,500
9	Nugumanandu Barman			400	650	9,600
10	Hitendra Nath Roy			280	350	7,500
11	Nirmal singha	Batasi	26.7308^0E 88.1958^0N	240	450	7,500
12	Ashok Prashad Bhagat			320	600	10,000
District- Jalpaiguri						
13	Baburam Sarkar	Maynaguri	26.5738^0E 88.8214^0N	425	1000	16,000
14	Gopal Biswas			230	650	10,400
15	Shivshankar Roy	Balakoba,	26.5860^0E 83.5993^0N	400	900	14,400
16	Dinabandhu Barman			450	1200	19,200
17	Subhash Roy			320	750	12,000
18	Tapu Baramn			250	600	9,600
19	Chitramohan Biswas			300	550	8,000
20	Jay Kr. Mahanta			350	650	8,500
21	Utpal Biswas			300	750	9,000
22	Noresh Sarkar	Dhupguri,	26.5782^0E 89.0161^0N	360	850	13,600
23	Biashwjit Sarkar			440	1350	21,000
24	Amit kr. Sarkar	Ambari	26.6445^0E 88.5057^0N	360	950	15,200
25	Babun Sarkar			350	800	12,800
26	Sukallyan basu	Jalpaiguri	88.4853^0E 26.1627^0N	300	600	9,500
27	Bappa mandal			280	450	6,500
28	Ranjan Barman			350	850	10,500
29	Jay kumar Mahanta			500	1050	12,500
30	Narayan mandal			250	750	9,800
31	Subal mazumdar			280	600	8,500
32	Mani Bhusan Roy			320	725	11,300

Data collected from field survey of the trained mushroom growers

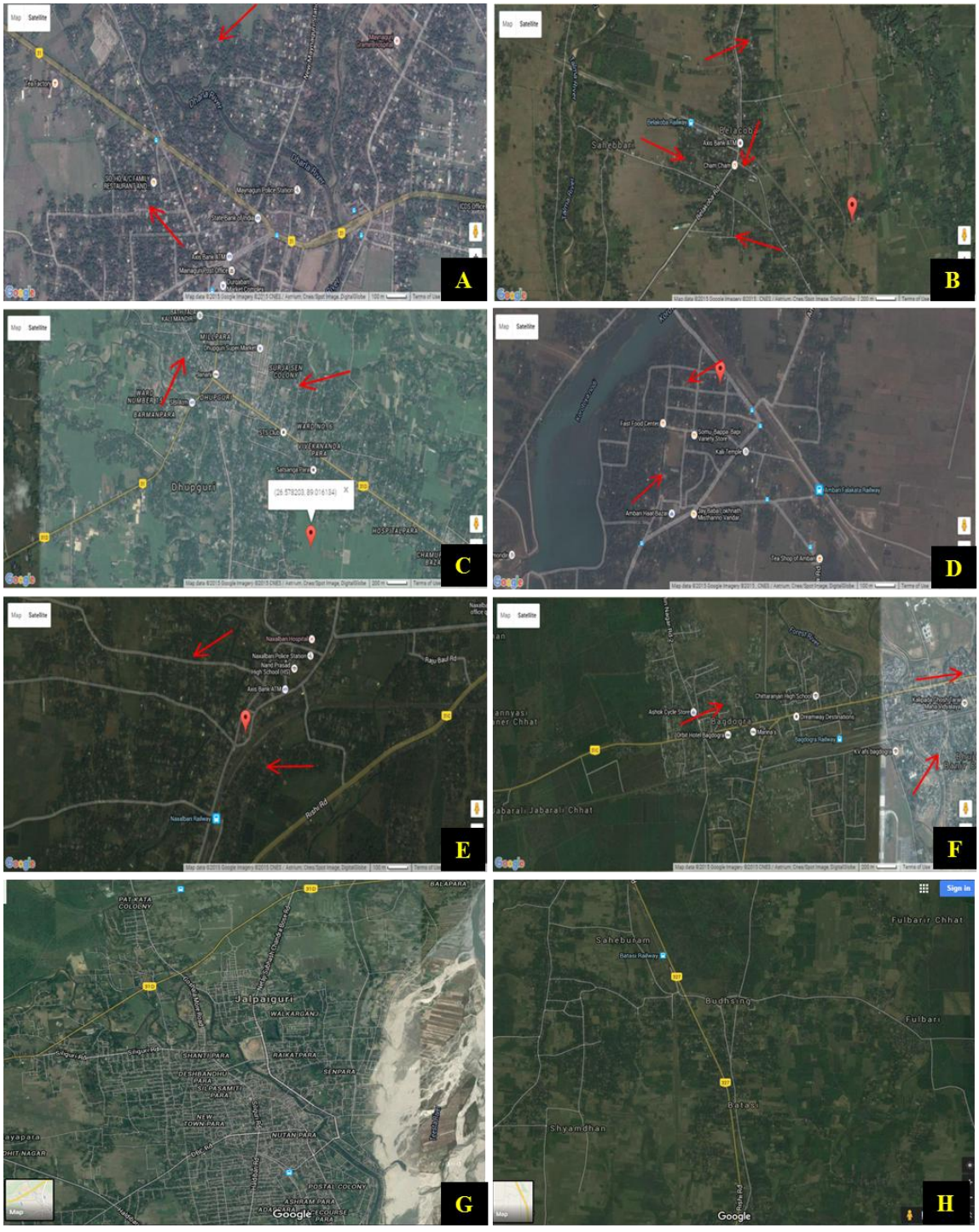


Figure 84: GPS location of mushroom farms established by the rural people after proper training in different districts of North Bengal (A) Mainaguri; (B) Belakoba; (C) Dhupguri; (D) Ambari; (E) Naxalbari, (F) Bagdogra, (G) Jalpaiguri and (H) Batasi

Figure 85: Promotion of oyster mushroom cultivation in different parts of North Bengal (A) Supak Singha Naxalbari, (B) Baburam Sarkar, Maynaguri, (C) Topu Barman, Belakoba, (D) Amit Kumar Sarkar, Aambari and (E) Jagonnath Baroi, Bagdogra

Figure 86: Demonstration of oyster mushroom cultivation practices during Krishi Mela organized by the Department of Agriculture, Govt. of West Bengal at Atharakhai Gram Panchayet, Shivmandir

Figure 87: Hands on training of cultivation process of oyster mushroom to the post-graduate students and unemployed youth (A) training for the unemployed youth, (B) students of Ananda Chandra College, Jalpaiguri, (C-D) Post-graduate students of NBU.

4.22. Cost benefit ratio of mushroom production in Darjeeling and Jalpaiguri district

Pleurotus is very common mushroom generally cultivated in North Bengal region. Different species of *Pleurotus* such as *Pleurotus ostreatus*, *Pleurotus sajor-caju P florida* and *P djamor* is being cultivated in this region. A large number of people is now involved in production and selling of *Pleurotus*. Unemployed youth, women self-help groups and retired persons are very active in production of oyster mushroom. Thus, a very good amount of mushroom is being produced every year in North Bengal. Darjeeling and Jalpaiguri district is very common in growing *Pleurotus* as the available raw material as well as the pleasant weather is very much favourable for the cultivation of *Pleurotus*. Depending upon the production capacity, growers are of three types, such as small, medium and large growers (Table 34). In this area, there are very good number of small mushroom growers in compare to large growers. On the other hand, depending upon the selling patterns, mushroom marketing is being done in four different channels which sequentially involves the growers, wholesalers, retailers and the consumers. In this region, most of the mushroom marketed through growers-wholesalers-retailer-consumer channel. On the other hand, small growers sell mushrooms directly to the market. To calculate the cost benefit ratio of mushroom marketing, market survey was done such as, champasari bazar, bidhan market, naxalbari hat, batasi hat, panitanki of Darjeeling district and fulbari, gatebazar, Jalpaiguri bazar and barivasa hat of Jalpaiguri district(Figure 88).

Table 34: Different types of mushroom selling pattern of mushroom in Darjeeling and Jalpaiguri district

Type of marketing	Small growers		Medium growers		Large growers	
	No of growers	Average qty. sold (kg)	No of growers	Average qty. sold (kg)	No of growers	Average qty. sold (kg)
Darjeeling District						
Grower-wholesaler-retailer-consumer	12	1,025	9	1885	3	1500
Grower- wholesaler-consumer	7	315	3	460	1	450
Grower-retailer-consumer	3	60	4	620	-	-
Grower-consumer	5	125	2	245	-	-
overall	27	1,525	18	3,210	4	1,950
Jalpaiguri District						
Grower-wholesaler-retailer-consumer	10	1450	7	1135	2	900
Grower- wholesaler-consumer	6	440	2	310	1	350
Grower-retailer-consumer	4	185	3	390	-	-
Grower-consumer	8	245	5	125	-	-
overall	28	2320	18	1960	4	1250

Data based on the market survey as well as the farm survey of different trained farmers in Darjeeling and Jalpaiguri districts

Table 35: Cost and returns of mushrooms in different categories of Darjeeling district

Particulars	Categories of farms			
	Small	Medium	Large	Average
District Darjeeling				
Mushroom production (kg)	1525	3210	1950	2228.5
Cost of production (Rs)	29737	60187	39975	43299.6
Average market rate (Rs/kg)	90	110	120	106.6
Gross returns (Rs)	137250	353100	234000	241450
Benefit cost ratio	**4.615**	**5.866**	**5.853**	**5.576**
District Jalpaiguri				
Mushroom production (kg)	2320	1960	1250	1843.3
Cost of production (Rs)	45240	38220	24375	28631.6
Average market rate (Rs/kg)	95	100	120	105
Gross returns (Rs)	220400	196000	150000	188800
Benefit cost ratio	**4.871**	**5.128**	**6.153**	**5.384**

Cost benefit ratio on the market survey data of selected markets of Darjeeling and Jalpaiguri district of North Bengal region

Figure 88: Mushroom sell at local markets; (A) fulbari haat, (B) Naxalbari, (C) Batasi haat, (D) Barivasa bazar and (E &F) departmental stores

It was observed that the cost of mushroom per kg ranges between Rs. 90-120. Market survey results also revealed that the cost benefit ratio much higher in case of large scale growers than that of the small growers. It was also observed that in all markets only fresh mushroom are being sold by the sellers. There are no such processed mushroom products such as canned of dried mushroom sold in the market. The use of compost has a positive relationship with the farm size. On the whole, in total variable cost, the average share of compost is maximum followed by labour charges and spawn. There is a positive relationship between mushroom production and farm-size. The income of mushroom growers goes up with the increase in farm size. The large growers adopt the better management practices, resulting into higher net income, that is followed by medium and small farmers. This demonstrates the applicability of "economies of scale" in mushroom cultivation. Of various channels, channel-I (Producer- Wholesaler-Retailer-Consumer) is the most common channel amongst different categories of mushroom growers, followed by the channel-II (Producer-Wholesaler-Consumer) in small and medium size farms, while channel-III only in case of large growers.

CHAPTER 5
DISCUSSION

Oyster mushrooms are one of the most popular edible mushrooms and belong to the genus *Pleurotus* and the family Pleurotaceae. Like oyster mushroom (*Pleurotus ostreatus*), many of *Pleurotus* mushrooms are primary decomposers of hardwood trees and are found worldwide. The type species of the genus *Pleurotus* (Fr.) Quel.is *P. ostreatus* (Jacq. et Fr.) Kummer. This mushroom has basidium with four basidiospores and a tetra polar mating system. Its hyphae have clamp connections and most members of the genus, excepting a small minority, have ammonitic hyphal system. Approximately 70 species of *Pleurotus* have been recorded and out of which about 30 species are now being cultivated in different part of India. Determination of a species is difficult because of the morphological similarities and possible environmental effects. Some reports indicate partial compatibility between them, implying the possibility for the creation of another species.

Oyster mushroom is one of the most popular mushrooms in North Bengal and a large number of growers are now cultivating oyster mushroom throughout the year. Three growing media were used to evaluate the mycelial growth characters of four species of oyster mushrooms (*Pleurotus ostreatus, P. sajor-caju, P. djamor* and *P. florida*) such as potato dextrose agar (PDA) and malt extract agar (MEA) and water agar (WA). Their mycelial growth completed on the 8th day at 25-27^{0}C. In the present study, results revealed an increase in the growth rate as the incubation temperature was increased from 24 to 28^{0}C which was also reported by Thulasi *et al* (2010). Besides malt extract agar showed highest growth rate followed by the potato dextrose agar and water agar was lowest. Initiation of mycelial growth was also analysed and it was found that the growth of *P. florida* initiated earliest (20hrs of incubation) in malt extract agar while in case of potato dextrose agar it took 23 h but in water agar it took longest period to initiate the colonization. Asghar *et al* (2007) also mentioned the average maximum growth was obtained on malt extract agar (MEA) than on potato dextrose agar (PDA) medium at 25 ºC under humid (65 – 80% RH) conditions.

In the present study, four different species were taken into consideration and they were separated depending upon their morphological as well as molecular characterizations. *Pleurotus ostreatus* commonly known as black oyster mushroom was characterized depending upon their morphological structure such as light blackish fruiting body, smaller pileus structure as well as the basidium consisting four of basidiospore. Spores were very small and oval shaped. On the other hand, *Pleurotus sajor-caju*, commonly called as grey oyster mushroom characterized based on its greyish fan shaped large fruiting body with small oval or kidney shaped basidiospore attached with tetrasporic basidium. Besides this, the white oyster mushroom *P. florida* characterized by the bright white fruiting body along with its decurrent gills and kidney shaped small spore attached to the basal part with the basidium. Pink oyster mushroom, *P. djamor* is a new introduction in North Bengal was also characterized with its distinct pink fruiting body with very small stipe. It was also observed that sometimes, the stipe absent or very small in size. The results revealed that spore of all the species were very small (1.8-5µm) and spores of *P. djamor* was found to be smallest among the investigated Pleurotus species. In this present investigation, all the species were identified using 18S rDNA sequencing and they all were identified by as *P. ostreatus*, *P. sajor-caju*, *P florida* and *P. djamor* and the sequence deposited in the NCBI Genbank.

Different substrates were investigated to determine the growth and yield of different species of oyster mushroom. Kang (2004) suggested that environmental factors include temperature; relative humidity affects the production of oyster mushroom. He also stated that increased growing room temperature decreases the relative humidity. A higher temperature promotes fruiting body metabolism, which results in high carbon dioxide production. Three species of oyster mushroom (*Pleurotus ostreatus*, *P. sajor-caju*, and *P. florida*) which were being cultivated in this region. Out of these three mushrooms, *P. ostreatus* and *P. sajor-caju* are generally cultivated in summer as it grows in a temperature between 25-33^{0}C while *P. florida* is generally cultivated during winter as it requires 15-20^{0}C for its growth. The present study revealed that optimum temperature for the cultivation of *Pleurotus sajor-caju* is 20-28^0 C, *P. ostreatus* 20-25^0 C and *P. florida* was 18-20^0 C as reported by Roy *et. al* (2014). *Pleurotus ostreatus* was cultivated using four different substrates throughout the year. Paddy straw, wheat straw and saw dust was used singly or in combine form. Growth of mycelia, initiation of fruiting body as well as yield and biological efficiency was evaluated throughout the

year in different substrates. Seasonal productivity of *Pleurotus ostreatus* was observed and the results indicated that the cultivation of *Pleurotus sajor-caju* and *P. ostreatus* possible during summer and rainy season while the cultivation of *Pleurotus florida* suitable for winter season and can grow up to early summer. Different available substrates used for the cultivation of *Pleurotus* species and the results revealed that the paddy straw and wheat straw is much more suitable for the cultivation singly as well as in combination. Saw dust also showed a very good amount of production in case of *Pleurotus sajor-caju* while paddy straw in combination with wheat straw showed better yield in case of *P. ostreatus* and *P. florida*. Pathmashini *et al* (2008) studied on the three different substrates using3 strains of *P. eryngii* such as Pe-1(native to Bangladesh), Pe-2 (germplasm collected strain from China) andPe-3 (germplasm collected strain from Japan). Results revealed that on saw dust and rice straw and their growth and yield parameters were investigated. Pe-1 on saw dust showed the highest biological yield and efficiency (73.5%) than other strains. Also the mycelium run rate and number of fruiting bodies were higher in Pe-1 than other two strains. The quality of mushroom strains was near about similar. On saw dust, the yield and efficiency were better than those cultivated on rice straw, however, on straw; the mushroom fruiting bodies were larger in size. Moonmoon *et al (*2010) cultivated *Pleurotus ostreatus* on different substrates such as rice straw, rice straw + wheat straw, rice straw+paper, sugarcane bagasse and sawdust and all the substrates except rice straw were supplemented with 10% rice bran. The substrate without supplement was considered as control. The effects of various substrates on mycelial growth, colonization time, primordial appearance time, mushroom yield, biological efficiency (BE), and size of the mushroom and chemical composition were analyzed. Among all aspects, rice straw(control) was found as a best substrate with yield (381.85 gm) and BE (95.46%) followed by rice plus wheat straw, rice straw plus paper waste for the production of mushroom (Sharma *et al* 2013). Ashraf *et al* (2013) compared the effect of different agricultural wastes on growth and yield of mushroom production, three species of *Pleurotus* viz. *P. sajor-caju* (V1), *P. ostreatus*, and *P. djamor* were grown on three different substrates cotton waste (T1), wheat straw and paddy straw (T3). The fastest spawn running, primordial initiation, harvesting stage, maximum number of fruiting bodies and maximum yield was observed in T1 took minimum number of days T3 showed maximum yield in 1st flush showing no significant differences with treatment T1 whereas T1 took maximum yield in 2nd flush and 3rd flush. Islam *et al* (2009)

revealed that the use of different sawdust of different plant also affects the production of oyster mushroom when cultivated on seven different types of saw dust source for growing mushroom. Seven different type of substrates like Mango, Jackfruit, Coconut, Jam, Kadom, Mahogony, Shiris sawdust supplemented with wheat bran and $CaCO_3$ were evaluated which revealed that the maximum biological yield per packet was obtained with Mango sawdust followed by Mahogony, Shiris, Jackfruit and Coconut sawdust. Bhatti (1987) also suggested that the variation of biological efficiency and incubation period of oyster Mushroom on different substrates may be due to their different composition. The cultivation of edible fungi is a controlled bioconservation of agro industrial lingo-cellulosic waste and residues. Mushroom cultivation fits in very well with sustainable farming and has several advantages. It uses agricultural waste products. A high production per surface area can be obtained, after picking the spent substrate is still a good soil conditioner. The Mushrooms are good cash crop. The development of oyster mushroom (Grey and pink) production methodologies on agricultural waste like Paddy straw and wheat straw gives very high yield as proposed by Randiv (2012).In an another study, Siddhant *et al* (2013) reported that among various component of wheat straw, pieces of stem (1.0 and 1.7cm) were proved best with 341 gm, 68.2% and 336 gm, 67.2% yield and biological efficiency, respectively than wheat straw as a whole. Nallathambi and Marimuthu (1993)cultivated *Pleurotus* species on different agro wastes like paddy straw, wheat straw etc. and they reported maximum yield with paddy straw. Dinesh Babu (2010) revealed that paddy straw was the most suitable substrate for the cultivation of *P. platypus* and *P. eous.* Sharma *et al* (2013) cultivated of *Pleurotus ostreatus* on different substrates such as rice straw, wheat straw and sawdust and all the substrates except rice straw were supplemented with 10% rice bran. The results revealed that different substrates affected mycelial growth, colonization time, primordial appearance time, mushroom yield as well as biological efficiency (BE). They found that among all, rice straw was found as a best substrate with yield (381.85 gm) and BE (95.46%) %) followed by rice plus wheat straw, rice straw plus paper waste for the production of mushroom which significantly similar. Moreover, Yildiz *et al*. (2002) explained that the woods are natural substrates which contains very small amount of nitrogen and they are very much efficient in producing the fruiting body of different species of oyster mushroom. *Pleurotus sajor-caju* was also cultivated using different substrates and it was observed that the paddy straw supplemented with saw dust and wheat straw was very much efficient in mycelial

run as well as in production and biological efficiency was high and Pokhrel *et al* (2013) also reported that the biological efficiency was about 78-84% when cultivated in paddy straw supplemented with maize stalk .Chakraborty *et al* (2015) also reported that the effect of paddy straw in combine with wheat straw increases the production of *P. ostreatus*. Baysal *et al.,* (2003) also reported that the supplementation of 20% rice husk initiate the fastest mycelial growth, pin head formation and fruit body formation which proves that the supplementation is an advantage in the production of oyster mushroom. Pala *et al* (2013) also reported that the growth of *Pleurotus sajor-caju* was significantly rapid in case of paddy straw followed by wheat straw and the production was higher in paddy straw than that of wheat straw. Zhng *et al* (2002) also reported that the yield of *P. sajor-caju* was increased at least 10% more when cultivated in paddy straw. Ingale and Ramteke (2010) also stated that the cultivation of *P. ostreatus, P. sajor-caju* and *P. djamor* was worldwide practiced industrially and it was observed that the production was high using the different agro wastes like paddy straw, wheat straw and saw dust. *Pleurotus djamor* is a new introduction in North Bengal has been reported by Roy *et al* (2015) which showed significant growth and yield in plastic bag as well as in bottles cultivated in paddy straw. In the present investigation, the results revealed that the cultivation of *P. djamor* grown in different substrates gives higher yield during the winter season in compare to summer season. It was also observed that during the fruiting initiation, *P. djamor* requires very less amount of water. The results also revealed that the amount of production increases in wheat straw and combination of wheat straw and paddy straw while it was less in saw dust.

Mushrooms are rich in proteins, vitamins, and minerals and popularly called as the vegetarian's meat. Mushroom proteins are considered to be intermediate between that of animals and vegetables. Moisture content of the cultivated *Pleurotus* species were also evaluated in this present investigation and results revealed that fruiting body possess high amount of moisture content. It was also observed that the pinhead stage contains lower moisture content than the mature stage. Among the *Pleurotus* species, *P. djamor* contains lower amount of moisture content while *P sajor-caju* and *P ostreatus* possess high moisture content. Ahmed *et al* (2009) explained that the maximum moisture (92.45 %) of fruiting bodies was recorded on paddy straw cultivation. The high moisture content of fruiting bodies of *P. djamor* is agreeable to earlier reports worked on different species (Manzi *et al.,* 1999; Alam *et al.,* 2008). The combination of

wheat straw and paddy straw showed significantly highest lipid content, protein content as well as sugar content. The carbohydrate content found in our study was 50.4 %; the results are in conformity with the values that were stated by Patil and Telang, (2010); Garcha *et al.*, (1993), Regula and Siwulski, (2007). Paddy straw, wheat straw was used as substrates for growing the four different species of oyster mushroom i.e. *P. ostreatus, P. sajor-caju, P djamor* and *P florida*. Growth was observed and it was found that the effect of wheat straw was higher in different species than that of the other species. It was also found that the substrate variation differs in various biochemical constituents like protein, reducing sugar, total sugar and lipid content of selected species. Sinha and Mehta (2014) used wheat straw, banana leaves cotton waste, and paddy straw as substrates and compared the biochemical parameters of the fruiting body and results revealed that the fruiting body of wheat straw showed higher biochemical constituents in compare to other substrates. Ashraf *et al* (2013) also reported that *P. djamor* showed the highest percentage of dry matter and moisture content was found high in *P. sajor-caju*. *Pleurotus djamor, P. ostreatus* and *P. sajor-caju* showed the maximum protein and fiber contents. The ash contents were found maximum *P. sajor-caju*. The highest fat and carbohydrate contents were found in *Pleurotus sajor-caju* and *P. ostreatus*. Yang *et al* (2001) reported that the fibre content in *P. djamor* was much higher than those in white and yellow winter mushrooms (*Flammulina velutipes*). Dundar (2008) found that the carbohydrate values of *P. sajor-caju*, *P. ostreatus* and *P. eryngii* more or less similar in all the species. Total protein content of all mushroom species was investigated. In the present investigation, different stages of cultivated species were taken for the study of protein content. Results revealed that all the mushroom species contains a very high amount of protein content. Study of protein during its growth were also investigated which revealed that the pinhead stage as well as the mature stage possess high amount of total protein content. SDS-PAGE of all *Pleurotus* species showed a good amount of protein bands and band pattern analysis clearly indicated that all *Pleurotus* species possess a good amount of high as well as low molecular weight proteins. Alam *et al* (2008) referred that *Pleurotus florida, P. sajor-caju, P. ostreatus* and *Calocybe indica* were rich in proteins and fibres and contained a lower amount of lipid. In this investigation, the total sugar and educing sugar content was estimated and it was observed that *P. ostreatus* and *P florida* contains higher total sugar and reducing sugar content in compare to other two species. The total lipid content of *P. florida* was grown on wheat and paddy straw being the highest followed by paddy straw alone. The

content of protein and fat content were similar (Patil *et al.*, 2008; and Patil and Dakore, 2007). Lipid content of all four species was estimated in the present investigation and it was also observed that the mushroom fruiting body possess very low amount of lipid content. Lipid content of different stages also revealed that at pinhead and young stage, the lipid content found to very low in compare to mature stages. Shin *et al.* (2007) also explained that the lipid content ranges between 4.3-4.9 g per 100 g in dry matter of cultivated *Pleurotus* species. Dietary fibre of mushroom powder was also estimated in this present study and the results revealed that *P. djamor* consists about 46.5% dietary fibre while *P. ostreatus, P. sajor-caju* and *P. florida* possess lower amount of dietary fibre. The results also revealed that *Pleurotus* sp. consists 34.8% dietary fibre which was significantly similar as suggested by Justo *et al.* (1999). They also reported that the pileus and gills were protein and lipid rich and stripe was carbohydrate and fibre-rich.

Natural anti-oxidants are the good source for neutralizing free radicals generated in the body after oxidative stress. The present study was aimed at *in vitro* evaluation of anti-oxidant properties of edible mushrooms such as *Pleurotus ostreatus, P. djamor, P. sajor-caju* and *P florida* widely consumed in North Bengal region. Ethanolic extracts of *Pleurotus* sp. were investigated for the antioxidant activity. Different concentrations such as 5mg/ml, 10mg/ml and 20mg/ml were estimated and it was found that *P djamor* and *P ostreatus* showed higher DPPH scavenging activity in compare to other two species. Results also indicated that DPPH scavenging activity increases directly proportional to the concentration. The present investigation also includes the ferric reducing antioxidant power and the results revealed that *P. djamor* and *P florida* showed higher FRAP activity and *P djamor* showed highest activity in 20mg/ml. concentration. Adebayo *et al* (2012) explained that the antioxidant activity of evaluated mushroom extracts gave positive results with free radical scavenging activity found to be higher in all used in vitro methods and also shown the potential of mushroom extract as a potent therapeutic agent and a food supplement. The anti-oxidant studies included DPPH radical scavenging activities, free radical antioxidant power activity total flavonoid activity as well as carotenoid activity. Chaturvedi *et al* (2011) stated that oyster mushrooms are a potential source of antioxidant compounds and the antioxidant activity were concentration dependent. Dubost *et al*., (2007) explained that the methanol extract of fruiting bodies of *P. ostreatus* showed reducing power and high antioxidant properties. Menaga *et al* (2013) reported that the methanolic extract of

P. florida showed the most potent radical-scavenging activity at a maximum concentration of 100 µg/ml and the scavenging effects on DPPH radicals. Md. Rahman *et al* (2013) also reported that the ethanol extract of *P. florida* possesses appreciable antioxidant activity, as indicated by the polyphenol contents, DPPH scavenging activity, reducing power effect. The extract manifested significant reducing power which exceeded even that of ascorbic acid at a concentration of 500 µg/ml. The radical scavenging activity of *P. ostreatus* mushroom is reported to be higher (6 mg/ml) than those of other mushrooms like *Agaricus bisporus, Volvariella volvaceae, Calocybe indica* and *Hybsizus ulmarius* reported by Ramkumar *et al* (2010). When compared to *P. ostreatus* mushroom the methanolic extract of *P. florida* has higher chelating activity against ferrous ion was also reported by Md. Imran *et al* (2011). Finimundy *et al.* (2013) reported that IC_{50} value of DPPH scavenging ability of aqueous extract of *P. sajor-caju* showed 9.01 % and the EC_{50} values of *P. abalones* in DPPH radicals scavenging ability and reducing power were 8.68 and 4.68mg/ml respectively as suggested by Wang *et al* (2012). Deshmukh and Shinde (2014) compared the cold water and hot water extracts of *Pleurotus florida* and *P. sajor-caju* and found that the cold water extract of both the species showed higher antioxidant activity. The IC_{50} value of hot water extract of *P. squarrosulus* was found to be 340 µg/ml as resulted by Pal *et al* (2010). Total flavonoid content of *Pleurotus* species were also investigated and it was observed that *P djamor* and *P florida* possess high amount of total flavonoid activity and it was also observed that the activity increase depending upon the concentration. Rao *et al* (2013) also reported that the Ethanolic extracts of the button mushrooms, showed higher antioxidant activity such as DPPH free radical scavenging, carotenoid activity, total phenolic compounds and total flavonoid concentration revealed antioxidant activity in *Agaricus bisporus*. Sathyaprabha *et al* (2011) also reported that total flavonoid compound was higher in *Pleurotus platypus* which was cultivated in Teak leaves in compared to other substrates and *Pleurotus eous* shows highest amount in paddy straw. Khan *et al* (2011) also proved the antioxidant activity of *P florida* and *P sajor-caju* using the hypercholesteraemic rats and confirmed their activity in animal system.

In vivo antidiabetic activity of selected *Pleurotus* species were done to obtain the antidiabetic activity of oyster mushroom using Streptozotocin induced albino rats. Effect of Streptozotocin in albino rats was observed and it was found that stz helps in

enhancing the blood sugar level in compare to the normal control sets. In the present investigation, the results clearly showed that the extracts of *P. djamor, P ostreatus, P. florida* and *P. sajor-caju* exert significant anti-hyperglycaemic effects in Streptozotocin induced diabetic rats in compare to normal control, positive control and negative control. The results also revealed that the powder of *Pleurotus* sp. helps in regaining the body weight of the experimental rats which was significantly similar as positive control. *Pleurotus djamor* and *P. ostreatus* showed better results in case of blood glucose as well as body weight. Andrade and Wiedenfeld (2001) showed that Streptozotocin helps in lowering the blood glucose level. The results also revealed that the body weight of the treated rats decreased due to induction of blood sugar. Furuse *et al* (1993) also reported that the body weight of the sugar induced rats decreased and it was subjected to recover with proper treatment. Non-insulin dependent diabetes mellitus (NIDDM) condition, which is common amongst diabetic subjects, is characterized by reduced circulating concentration of insulin, poor insulin sensitivity or insulin resistant, poor glucose tolerance resulting in high sugar in plasma. Hyperglycaemia condition per se impairs insulin secretion (Davis and Granner, 1996). Ravi *et al* (2013) studied on the antidiabetic activity of *P. ostreatus* using alloxan-induced diabetic mice. He explained that *P. ostreatus* showed a significant hypoglycemic effect on diabetic mice which was capable of improving hyperlipidemia and also helps in improving the normal kidney functions. Sultana *et al* (2014) also applied extracts of *Pleurotus florida* on the alloxan induced mice along with metformin and reported that it helps in lowering the blood glucose level which was also found in case of our study. Kang *et al* (2001) also reported that the mycelial powder of *P. eryngii* helps in lowering the plasma glucose level and also effective in regaining the body weight of the Streptozotocin induced albino rats. Polysaccharides of *Pleurotus* species helps in lowering the plasma glucose level and also helps in regaining the body weight of the rats. Rushita *et al* (2013) also explained that the methanolic extract of *Pleurotus citrinopileatus* significantly helps in lowering the blood glucose level in alloxan induced albino rats. Kiho *et al* (2001) reported that the molecular mass of the polysaccharide of mushrooms was high and thus it showed higher antidiabetic activity. Along with the blood glucose level, other blood parameters such as urea, creatinine, triglyceride, cholesterol and liver enzymes like serum glutamic pyruvate transaminase (SGPT) and serum glutamic oxaloacetate transaminase were also investigated in the present study. The results clearly revealed that the *Pleurotus* mushroom powder had positive effect on controlling the other blood parameters.

Diabetic induced rats suffers from different disorders which caused by different blood parameters. High urea and creatinine indicates the impurity in blood which results in various kidney disorders. Results revealed that suspension feeding helps in lowering the urea and creatinine level in compare to control set. It was also observed that the application of Streptozotocin drastically increased in negative control but in case of positive control, it helps in lowering the urea, creatinine, triglyceride as well as serum glutamic pyruvate transaminase and serum glutamic oxaloacetic transaminase activity. In case of treatment of *P. ostreatus, P sajor-caju, P. djamor* and *P. florida* powder, they also helps in lowering the other blood parameters in compare to the control sets. Yamamoto *et al,* (1981) suggested that alloxan and beta cytotoxin, destroys beta cells of islets of Langerhans of pancreas and reducing the endogenous insulin secretion and paves ways for the decreased utilization of glucose by body. Johny and Okon (2013) also reported that ethanolic extract of *Pleurotus ostreatus* significantly lowers the plasma glucose level in alloxan induced albino mice and also compared with the standard drug metformin. Dhanabal *et al* (2007) also reported that the increased rate of plasma glucose decreases the protein content of the cell and rapidly increasing the plasma cholesterol, creatinine urea as well as plasma triglyceride level.

Mushroom industries generate discharges a bi-product called spent mushroom substrate (SMS). This is the unutilised substrate and the mushroom mycelium left after harvesting of mushrooms. As the mushroom industry is steadily growing, the volume of SMS generated annually is increasing. Spent mushroom substrates were applied in the field for the crop improvement and results revealed that the effect of spent substrates helps in the growth and development of the *Capsicum chinense, C. annumm, Solanum lycopersicum* and *Amaranthus* sp. It was also observed that the yield was increased in case of treated pants in compare to untreated plants. Results in this present investigation also revealed that the soluble phosphate content of soil increases and also leaf and root phosphate content which clearly indicates that application of spent mushroom substrate acts as soil conditioner which helps in phosphate solubilisation and also mobilization of phosphate from soil to leaf through root which helps in plant growth promotion. Application of the spent mushroom substrates as biofertilizer for crop improvement is the only way out of the problem (Phan and Sabaratnam; 2012). Royse *et al.* 2004 explained that approximately 600 ton of SMS is produced in South Korea annually, of which 58 % is from *Pleurotus ostreatus* cultivation. These spent mushroom substrates

were helps in growth and yield of the crop plants. The results revealed that spent mushroom substrates showed higher phosphatase activity in soil; root as well as in leaves. It was also reported that after harvesting of fruiting body, spent mushroom substrates possess some amount of extracellular enzymes secreted by the mushroom species and thus it helps in plant growth. Of all the enzymes, laccase is the most reserved and common in SMS from *A. bisporus* (Mayolo- Deloisa *et al.* 2009), *Pleurotus sajor-caju* (Singh *et al*. 2003), *P. ostreatus, L. edodes, and Hericium erinaceum* (Ko *et al*. 2005).

Processing is an important step for mushroom cultivation. Shelf-life of oyster mushroom is very limited and it as very difficult to keep it for very long time fresh. So processing technique was adopted for long term preservation of the oyster mushroom. Drying is an important step for processing of mushroom. Fruiting body of the *P. ostreatus, P sajor-caju, P djamor* and *P florida* was dried in various way like direct sundry, indirect sundry and freeze dry. Drying of oyster mushroom results in reducing the water content form fruiting body which results in long term preservation of the fruiting body. The cultivation of mushroom has a great potential for the production of protein rich quality food and for recycling of cellulose agro-residues and other wastes. The moisture content of freshly harvested mushroom is 70-90% based upon the variety. In view of their highly perishable nature and commercial value, the fresh mushrooms can be processed to extend their shelf life such as canning, drying, pickling, etc. Drying preserves mushrooms for very long periods of time with little or no deterioration in flavour or quality. Drying methods vary from sun/air drying to machine drying (dehydrators) to microwave drying as resulted by Balan and Mahendran (2014). Mushroom biscuits were prepared using the dried mushroom powder in the laboratory and it was tasted for its different quality such as taste, texture, its softness and it was fond that the biscuits were very good for health and it can be stored for long time. Desayi *et al* (2012) also prepared biscuits by adding mushroom powder to the biscuit recipe at 5, 10and 15 per cent concentrations along with addition of strawberry and vanilla flavours at each level, whereas biscuits prepared only with bakery recipe were kept as control. Among different treatments, 10 per cent mushroom powder along with 0.2 per cent vanilla flavour recorded highest scores for organoleptic parameters like colour and appearance, flavour, crispness, taste and overall acceptability even up to 30 days of storage. Mahamud *et al* (2012) the composition of mushroom powder and bread

prepared incorporating various levels of mushroom powder were analyzed to determine the effects of various levels of mushroom powder in nutritional constituents and consumers' acceptability. The mushroom powder showed significantly better result for texture and overall acceptability variables of consumer acceptability. Nutritional composition, of 5% mushroom powder consists content 10.07% protein, 9.20% fat, 1.82% ash and carbohydrate 62.87%) was better and good for human health. Rosli *et al* (2012) reposted that the application of powder of *P sajor-caju* in preparation of mushroom biscuits was rich in nutritional constituents like reducing sugar, protein as well as dietary fibre and lower lipid content in compare to the wheat flour made biscuits.

Cultivation of *Pleurotus* species practiced throughout the world and India is one of the large producer as well as transporter of *Pleurotus* species. Several species cultivated in India and in North Bengal about four species cultivated throughout the year namely *P. ostreatus, P sajor-caju, P djamor* and *P florida*. Cultivation of *Pleurotus* species facing some problems of contaminants which results in decreasing in the production and sometimes it turns into an epidemic that rapidly spread the entire cultivation unit and gradually contaminates all bags. Several bacterial, fungal contaminants affected the growth of *Pleurotus* sp. Fungus like *Trichoderma, Fusarium, Coprinus, Cladobotryum dendroides;* affects the mushroom substrates and grows before the mycelial run of *Pleurotus* species over the substrates. Some bacterial contaminants were also affected the mushroom substrates which resulted in limiting the spawn rung over the substrates and also reduction the rate of fruiting body initiation. Singh *et al* (2014) studied on the green mould disease of *P ostreatus* and *P florida* caused by *Trichoderma harzianum* which results in rapid decrease in spawn run rate on the substrates and lowering the yield substantially. Carbandazium was used against the green mould and it helps in lowering the spread of *T. harzianum* on the substrate was also supported by Singh *et al* (2014). *Pleurotus* mushroom is subject to many vagaries of nature like pests and diseases that adversely affect its production and productivity. Among the various moulds and competitors of *Pleurotus* spp. green moulds are reported to be devastating disease in the crop production of this mushroom. The main fungal species causing green mould have been identified as *Trichoderma viride* and *Trichoderma harzianum* (Sharma and Bahukhandi, 2003). Oh *et al.,* (2003) reported that *Trichoderma* spp. is most common antagonistic and mycoparasitic pathogen of *Pleurotus* crop causing green

mould disease. Park *et al.*, (2005) also supported that the pathogen inhibits the growth of mushrooms and in severe outbreaks; the fruiting bodies are not produced from contaminated beds. Shah *et al.*, (2013) also supported the use of carbendazim and it was found to be best fungicide, against the infection of green mould disease of mushrooms.

Training is a process of acquisition of new skills, attitude and knowledge in the context of preparing for entry into a vocation or improving ones productivity in an organization or enterprise. It plays an important role for imparting knowledge and updating skills of the farmers. To make training meaningful and effective, it is very much important to identify the training needs of the farmers based on which a suitable training module can be designed. It is essential to organize an appropriate training to the right people, in the right form, at the right time. Singh *et al* (2014) undertaken to know the relationship of selected personnel, socio-economic and psychological characteristics of the respondents with their training needs in relation to commercial mushroom cultivation. Mishra (2012) reported that the most important promoting factor for mushroom enterprise is simple technology, which farmwomen can handle very easily. The impact assessment of mushroom enterprise on growers indicates the improvement in their occupation followed by 'standard of living', 'saving, employment generation and 'knowledge and attitude'. The farmwomen faced much constraint in 'non-availability of spawn in locality' and least problem in 'mushroom treated as non-veg item'. Sixty percent of the respondents stated that "supply of quality spawn", 'thorough coverage of marketing aspects' and 'insurance' are the three major constraints for successful mushroom entrepreneurship. Sud *et al* (2013) reported about the training programme on cultivation and value addition of oyster mushroom to the growers which includes of 50 percent theory and 50 percent practical helped in improving the cultivation practice in Himachal Pradesh. Growers of North Bengal are very efficient in producing the *Pleurotus* species. Formerly there were only two species namely *P. ostreatus* and *P sajor-caju* largely cultivated in North Bengal but after proper training of mushroom cultivation and knowing about the seasonal productivity, growers are now efficiently producing all the four species of *Pleurotus* according to their season and a good amount is now produced by them. Post-harvest processing was also a very big issue for the growers in this region. Growers who have trained are now adopting various drying techniques, mushroom powder and mushroom biscuits for long term processing of *Pleurotus* species. Sud *et al* (2013) explained that the training programmes which involve both

theoretical and practical aspects of production and value addition proved to be effective as indicated by feedback of the trainees. Shirur *et al*., (2011) organization of such training programmes is beneficial in many ways as it promotes popularization of mushroom production and value addition among farming community in the region and acts as a mean to expose farmers with mushroom based industries such as instrumentation, mushroom processing and other units.

Chapter 6
CONCLUSION

- *Pleurotus* species is one of the most popular mushrooms cultivated in North Bengal. Four species namely *Pleurotus ostreatus, P sajor-caju, P. djamor* and *P. florida* were selected for cultivation in this environmental condition.
- Morphological and histopathological characteristics were studied with special reference to their fruiting body structure, structure of gill and also the spore characters. Mycelial growth pattern of *Pleurotus* species were also studied using various medium and it was found that the growth rate varies in different medium.
- Molecular detection of the four fungal isolates IPL/MC/PO-1, IPL/MC/PS-01, IPL/MC/PD-01 and IPL/MC/PF-01 were carried out using 18S rDNA sequencing using ITS1 and ITS 4 primers. BLAST query of 18S rDNA sequence were analysed and identified as *P. ostreatus, P. sajor-caju, P djamor* and *P. florida* respectively. The sequences of the identified species were submitted in NCBI GenBank under the accession number KT768095, KT818506, KT 768094 and KT826605 for *P. ostreatus, P sajor-caju, P. djamor* and *P florida* respectively.
- Cultivation of the four identified *Pleurotus* species were practiced in this area. Several lignocellulosic wastes were tasted for the cultivation of all the four species. Paddy straw, wheat straw and saw dust were used in singly or in combined form to check the effect of substrates on yield of *P. ostreatus, P. sajor-caju, P djamor* and *P florida*.
- In North Bengal, *P. ostreatus, P sajor-caju* and *P florida* commonly cultivated. A new species namely *P djamor*, the pink oyster mushroom introduced in this area and successfully cultivated in this area.
- Effect of substrate was evaluated and it was found that the combination of paddy straw with wheat straw and also the paddy straw with saw dust enhances the yield per kg substrate. In case of *P djamor*, wheat straw was found to be the appropriate substrate in compare to other three substrates. *P. ostreatus P sajor-caju*, and *P florida* showed higher yield in case of paddy straw and wheat straw but saw dust resulted lower yield per kg substrates.

- Along with the different substrates, the seasonal productivity of all the selected *Pleurotus* species was determined. The results clearly indicate the variable seasonal productivity. Depending upon the temperature, relative humidity and rainfall, the productivity was found to be different. In case of *P. ostreatus*, February to September was found to be suitable while in case of *P sajor-caju*, temperature and relative humidity was quite higher and thus it found to be suitable to cultivate during May to September.
- Seasonal productivity of *P florida* and *P djamor* was found to be quite similar depending upon the temperature, relative humidity and rainfall required for the growth and fruiting body initiation. It was observed that at higher temperature, the fruiting initiation required very long time and also the productivity decreased during May to September. It was also observed that the productivity increased during winter season in North Bengal.
- Polypropylene bags are very commonly used as container for the cultivation of *Pleurotus* species in this region. Various type containers were also tasted for the cultivation practice of *Pleurotus* species like waste bottles, laboratory glassware and also paper boxes. Using this type of containers was found to be very effective as the containers can be recycled for many times and it requires small area for the cultivation. It was observed that the bottles and boxes produces same amount of fruiting body of *Pleurotus* while it takes less time for fruiting initiation. In case of *P ostreatus* and *P djamor* waste bottles was found to be very effective and the productivity was also very high.
- Tea is one of the major plantation crop largely practiced throughout North Bengal especially in Darjeeling and Jalpaiguri. Pruning is commonly practiced for tea plantation and the pruned tea leaves commonly used for fuel in the tea gardens. Pruned tea leaves were dried and used as a substrate for cultivation of *Pleurotus ostreatus* as well as *P. sajor-caju* and tea leaves waste found to be very promising in the spawn run and also fruiting body initiation. Tea leaves along with paddy straw was also tasted and the yield was remarkably found very high in compare to other substrates.
- Biochemical constituents such as moisture, total sugar, reducing sugar, protein and lipid content were also estimated of cultivated *Pleurotus* species. Results revealed that the selected mushroom species possess a high amount of moisture content. All four species cultivated using paddy straw, wheat straw and saw dust

were found to be very high amount of protein, total sugar and reducing sugar content. Results also revealed that mushroom possess very low amount of lipid content and among the all four selected species *P djamor* and *P ostreatus* contains lower amount of lipid content.

- Antioxidant activity of selected *Pleurotus* species were also estimated and it was found that all four species showed very high amount of antioxidant activity such as DPPH scavenging activity, ferric reducing antioxidant power activity as well as flavonoid activity. Among the cultivated *Pleurotus* species, *P djamor* and *P ostreatus* showed comparatively higher antioxidant activity.
- *In vivo* antidiabetic activity of *Pleurotus* species were also tasted using Streptozotocin induced albino rats. Results found that the oral treatment helps in regaining the body weight as well lowering the plasma glucose level in compare to control. *P djamor, P sajor-caju* and *P ostreatus* found to be effective in lowering the plasma glucose level.
- Blood urea, creatinine, triglyceride, cholesterol as well as liver enzymes like serum glutamic pyruvate transaminase (SGPT) and serum glutamic oxaloacetic transaminase were also estimated and the oral treatment of *P. djamor* and *P. ostreatus* found to be very effective in recovering the other blood parameters.
- Spent mushroom substrate is an important by product of *Pleurotus* cultivation and this spent mushroom substrate were applied as fertilizer for crop improvement. *Capsicum chinense, C annuum, Solanum lycopersicum* and *Amaranthus* sp. were tasted using the spent mushroom substrates of *Pleurotus* species which was found to be very effective as a soil conditioner and also helps in increasing growth and yield of crop plants in compare to untreated plants.
- Processing of oyster mushroom is very important step in the cultivation practices. Short term processing of *Pleurotus* species was done which include Packaging of mushroom for sell as well as different recipes were tasted in laboratory like mushroom pakora, soup, curry, scrambled mushroom. Long term processing of *Pleurotus* was also practiced in the form of drying, powder and also preparing mushroom biscuits. Drying method includes sundry, freeze dry and oven dry and among them, indirect sundry and freeze drying was found to be very effective in long term preservation of *Pleurotus* species.
- Contaminants were found to be a very serious problem during the cultivation of *Pleurotus* species. Several fungal species like *Coprinus, Fusarium* as well as

Trichoderma; bacterial species like *Pseudomonas* and pastes like Sciarid, phorid and Beetle fly drastically affected the growth and yield of *Pleurotus*. Management strategies like application of bavistin, phenyl, carbandazium and formaldehyde were adopted which reduces the effect of the fungal as well as bacterial genera. Racks of the mushroom production unit were covered with nylon nets which helped in reducing the attack of flies on the substrates.

- Promotion of mushroom cultivation and marketing were also done. Several unemployed youth, post graduate students as well as mushroom growers on North Bengal were trained about the seasonal productivity, spawn preparation as well as post-harvest processing of *Pleurotus* species. Promotion of *Pleurotus* cultivation was also done at the Krishi Mela organised by the Department of Agriculture, Govt. of West Bengal.
- Several unemployed youth, retired persons as well as students of different colleges and women of self-help groups were trained successfully and established their own cultivation unit. They are also very much efficient in spawn production and thus the growers produce their own spawn for cultivation.
- Economic efficiency of mushroom production and marketing were also studied and it was found that the growers selling mushroom at very high price and the cost benefit ration become very high which results in economic upliftment of different economically weaker people.

Chapter 7
BIBLIOGRAPHY

Ahlawat OP, Gupta P, Kumar S, and Sharma DK. Bioremediation of fungicides by spent mushroom substrate and its associated microflora. *Ind. J. Microbiol.* **50(4):** 390-395, 2010.

Ahlawat OP, Indurani C and Sagar MP. Spent Mushroom Substrate properties and recycling for beneficial purposes. In: *Frontiers in Mushroom biotechnology.* (R.D. Rai, R.C. Upadhyay and S.R. Sharma eds.) National Research Centre for Mushroom, Solan, India **pp.**314-334. 2005.

Ahlawat OP, Raj D, Indurani C, Sagar MP, Gupta P and Vijay B. Effect of spent mushroom substrate recomposted by different methods and of different age on vegetative growth, yield and quality tomato. *Ind. Hort*., **66(2):** 208-214, 2009

Ahlawat OP, Sagar MP, Raj D, Gupta P and Vijay B. Effect of recomposted button mushroom spent substrate on growth and yield attributes of wheat (*Triticum aestivum* L.). *Mush. Res.* **16(1):** 41-46, 2007.

Ahmed S. Performance of different substrates on the growth and yield of Oyster Mushroom *Pleurotus sajor-caju* (Fr.) Sing. M.S. thesis, Department of Horticulture. Institute of postgraduate studies in Agriculture, Salna, Gazipur, Bangladesh, 1998.

Ahmed SA, Kadam JA, Mane VP, Patil SS and Baig MMV. Biological Efficiency and Nutritional Contents of *Pleurotus florida* (Mont.) Singer Cultivated on Different Agro-wastes. *Nat. Sci.,* **7(1):** 44-48; 2009.

Akyuz M, Onganer, AN, Erecevit P and Kirbag S. Antimicrobial activity of some edible mushrooms in the eastern and south east Anatolia region of Turkey. *GU J. Sci.,* **23(2):** 125-130, 2010.

Alam N, Hossain S, Khair A, Amin R and A K. Comparative effects of oyster mushrooms on plasma lipid profile of hypercholesterolaemic rats. *Bangladesh J. Mush.* **1:**15-22. 2007.

Alam, N, Amin R, Khan A, Ara I, Shim MJ, Lee MW and Lee TS. Nutritional Analysis of Cultivated Mushrooms in Bangladesh – *Pleurotus ostreatus, Pleurotus sajor-caju, Pleurotus florida* and *Calocybe indica*. *Mycobiol,* **36:** 228-232. 2008.

Alarcon-Aguilara FJ, Roman-Ramos R, Perez-Gutierrez S, Aguilara-Contreras A, Contreras-Weber CC and Flores-Sanez JL. Study of the antihyperglycemic effect of plants used as antidiabetics. *J. Ethnopharmacol.,* **61:**101-110, 1998**.**

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W and Lipman DJ. "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs." *Nucleic Acid Res.* **25:**3389-3402, 1997.

Ames BN, Shigenaga MK and Hagen TM. Mitochondrial decay in aging. *Biochimica et Biophysica Acta* **1271:** 165–170, 1995.

AOAC Method 991.43.Total, soluble and insoluble dietary fibre in foods. Enzymetic-gravimetric method. MES-TRIS buffer. Official methods of Analysis of the Association of Official Analytical Chemists. 15th ed., 3rd suppl. Association: Arlington, VA, 1992.

Asfors KE and Ley K. Sulfated polysaccharides in inflammation. *J. Lab. Clin. Med.,* **121:** 201-202, 1993.

Asghar R, Md. Tariq and Rehman T. Propagation of *Pleurotus sajor-caju* (Oyster Mushroom) through Tissue Culture. *Pak. J. Bot*., **39(4):** 1383-1386, 2007.

Ashraf J, Md. Ali A, Ahmad W, Md Ayyub C and Shafi J. Effect of Different Substrate Supplements on Oyster Mushroom (*Pleurotus* spp.) Production. *Food Sci. Technol.,* **1(3):** 44-51, 2013.

Badole SL, Patel NM, Thakurdesai PA and Bodhankar SL. Interaction of Aqueous Extract of *Pleurotus pulmonarius* (Fr.) Quel-Champ. with Glyburide in Alloxan Induced Diabetic Mice. *Evid Based Complement Alternate Med,* **5(2):**159–164, 2008.

Balan N and Mahendran R. Microwave drying of edible mushroom. *Mush. Res.,* **23(1):** 81-87, 2014.

Balon TW, Jasman AP and Zhu JS. A fermentation product of *Cordyceps sinensis* increase whole body insulin sensitivity in rats. *J. Altern. Complement. Med*. **8:** 315-323. 2002.

Bano Z, Rajarathnam S, Nagaraja N. Some important studies on *Pleurotus* mushroom technology. In: Kaul TN, Kapur BM (eds) *International conference on science and cultivation technology of edible fungi.* Jammu Tawi, India**, pp.** 53-64. 1987

Bano Z, Shasirekha MN and Rajarathnam S. Improvement of the bioconversion and biotransformation efficiencies of the oyster mushroom (*Pleurotus sajor-caju*) by supplementation of its rice straw with oil seed cakes. *Enz. Microb. Technol.*, **15:** 985-989, 1993.

Barros L, Cruz T, Baptista P, Estevinho LM and Ferreira IC. Wild and commercial mushrooms as source of nutrients and nutraceuticals. *Food Chem. Toxicol.* **46:** 2742-2747, 2008.

Bayer M. The impact of mushroom industry on environment. *Mush. News.* **44:** 6-13, 1996.

Beattie G, Lannom R, Lipsick J, Kaplan NO and Osler AG. Streptozotocin- induced diabetes in athymic and conventional BALB/c mice. *Diabetes* **29:** 146-150, 1980.

Berne S, Krizaj I, Pohleven F, Truk T, Macek P and Sepcic K. *Pleurotus* and *Agrocybe* haemolysins new protein hypothetically involve in fungal fruiting. *Biochem. Biophys Acta.*, **1570:** 153-159, 2002.

Bhatnagar D, Soran H and Durrington PN. Hypercholesterolemia and its management. *Bangladesh Mush. J.*, **337:** 993. 2008.

Bhatti MA. Effect of different bedding materials on relative yield of oyster Mushroom in the successive flushes. *Pak. J. Agric. Res.*, **8(3):** 256-259, 1987.

Biswas S, Dutta M and Ngachan SV. Mushroom cultivation a prosperous venture for improvement of livelihood of poor people of Tripura. *J. Mycopathol Res.* **53(1):** 31-39, 2015.

Bobek P, Ozdyn L and Kunik L. The effect of Oyster (*Pleurotus ostreatus*) ethanolic extracts and extraction residues on cholesterol levels in serum lipoproteins and liver in rats. *Nahrung*., **39:** 98-99, 1995.

Bobek P. and Galbavy S. Hypocholesterolemic and antiatherogenic effect of oyster mushroom (*Pleurotus ostreatus*) in rabbit. *Nahrung*. 43: 339-342. 1999.

Bobek, P, Ozdin L and Kuniak L. Effect of Oyster mushroom and on the activities of antioxidative enzymes in rats fed the cholesterol diet. *J. Ntrl. Biochem*., **8:** 469-471, 1997

Brandt CR and Piraino F. Mushroom Antivirals. *Recent Res. Dev. Antimicrob. Agents Chemother.* **4:**11-26, 2000.

Buah JN, Vander Puije GC, Bediako EA, Abole EA and Showemimo F. The Growth and Yield Performance of Oyster Mushroom (*Pleurotus ostreatus*) on Different Substrates. *Biotechnol.*, **9:** 338-342, 2010.

Chakraborty BN and Saha A. Detection and cellular location of cross-reactive antigens shared by *Camellia sinensis* and *Bipolaris carbonum*.*Physiol. Mol. Pl. Pathol.,* **44:**403–416, 1994.

Chakraborty BN, Basu P, Das R, Saha A and Chakraborty U. Detection of cross reactive antigens between *Pestalotiopsis theae* and tea leaves and their cellular location. *Ann. Appl. Biol.*, **127:**11–21, 1995.

Chakraborty BN, Chakraborty U, Barman S and Roy S. In: Production and Processing of edible mushrooms. University of North Bengal press, 2014.

Chang R. Functional properties of edible mushrooms. *Nutr. Rev.* **54:**91-93, 1996.

Chang S. Global impact of edible and medicinal mushrooms on human welfare in the 21st century: Nongreen revolution. *Int. J. Med. Mush.*, **1:**1-7, 1999.

Chang ST and Miles PG. Mushrooms: Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact (IInd edition), CRC Press, New York. 2004

Chang ST and Miles PG. Recent trends in World Production of Cultivated Edible Mushrooms. *Mush. J.*, **503:** 15-18, 1991.

Chang ST. Mushroom cultivation using the "ZERI" principle: potential for application in Brazil, *Micologia Aplicada Int.*, **19(2):** 33-34, 2007.

Chang ST. World production of cultivated and medicinal mushrooms in 1997 with emphasis on *Lentinula edodes* (Berk.) Sing, China. *Int. J. Med. Mush.* **1:** 291-300, 1999.

Chaturvedi P, Khare KB, Kwape TE and Moses M. Anti-oxidant properties of two edible mushrooms, *Agaricus bisporus* and *Pleurotus ostreatus* grown in Botswana. *Mush. Res.*, **20(1):** 14-19, 2011.

Chen J, Mao D, Yong Y, Li J, Wei H and Lu L. Hepatoprotective and hypolipidemic effects of water-soluble polysaccharidic extract of *Pleurotus eryngii*. *Food Chem*., **130:** 687-694, 2012.

Chenni A, Yahia DA, Boukortt FO, Prost J, Lacaille-Dubois MA and Bouchenak M. Effect of aqueous extract of *Ajuga iva* supplementation on plasma lipid profile and tissue antioxidant status in rats fed a high-cholesterol diet. *J. Ethnopharmacol*. **109(2):** 207-213. 2007

Cheung LM, Cheung PCK and Ooi VEC. Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chem.*, **81:** 249-255, 2003.

Cho EJ, Hwang HJ, Kim SW, Oh JY, Baek YM, Choi JW, Bae SH, Won J and Yun JW. Hypoglycemic effects of exopolysaccharides produced by mycelial cultures of two different mushrooms *Tremella fuciformis* and *Phellinus baumii* in ob/ob mice. *Appl. Microbiol. Biotechnol.*, **75:**1257–1265, 2007.

Chockchaisawasdee S, Namjaidee S, Pochana S and Stathopoulos CE. Development of fermented oyster mushroom sausage. *Asian J. Food Agro-Indust*. **3(1):** 35-43, 2010.

Choi KW. Oyster mushroom cultivation: Shelf or Bag? (http://www.mushworld. com). 2003.

Chorváthová V, Bobek P, Ginter E and Klvanová J. Effect of the oyster fungus on glycaemia and cholesterolaemia in rats with insulin-dependent diabetes. *Physiol. Res.*, **42:**175–179, 1993.

Chye FY, Wong JY and Lee JS. Nutritional quality and antioxidant activity of selected edible wild mushrooms. *Food Sci. Tech. Int.* **14:** 375-384, 2008.

Cohen R, Persky L and Hadar Y. Biotechnological applications and potential of wood degrading mushrooms of the genus *Pleurotus*. *App. Micro. Biotechnol*., **58:** 582, 2002.

Cui B, Han L, Qu J and Lv Y. Hypoglycaemic activity of *Grifola frondosa* rich in vanadium. *Biol. Trace Elem. Res.*, **131(2):** 186-191, 2009.

Daba AS and Ezeronye OU. Anti-cancer effect of polysaccharides isolated from higher Basidiomycetes mushrooms. *Afr. J. Biotech.*, **2:** 672-678, 2003.

Das AR, Das P, Bhattacharjee S and Saha AK. Chemical analysis of a wild edible mushroom *Pleurotus djamor* (Rmph. ex Fr.) Boedjin. *Mush Res.* **23(2):** 161-166, 2014.

Das N and Mukherjee M. Cultivation of *Pleurotus ostreatus* on weed plants. *Bioresour*. *Technol*. **98(14):** 2723-2726, 2007.

Davis SN and Granner DK. Insulin, oral hypoglycemic agents and the pharmacology of the endocrine pancreas. In: Goodman, L.S., Gilman, A.G. (Eds.), The Pharmacological Basis Of Therapeutics, 9th Ed.. Mcgraw Hill, New York, pp. 1487_/1511, 1996.

Desayi DB, Kukanoor LH, Patil CP, Shirur M and Shivakumar BB. Utilization of dried mushroom powder for development of mushroom fortified biscuits. *Mush. Res.*, **21 (1):** 67-71, 2012.

Deshmukh S and Shinde M. Comperative study for antioxidant activity of cold and hot water extract of *Pleurotus sajor-caju* and *Pleurotus florida. Int. J. Inov. Pharma. Sci. Res*. **2(4):** 894-909, 2014.

Dhar BL, Shrivastava, N, Himanshu, Kumar J, Tyagi S and Atrey P. Cultivated edible specialty mushrooms- scope in India and EU countries. Proceedings of the 7th International conference on Mushroom Biology and Mushroom products (ICMBMP), **pp.** 537-547, 2011.

Dinesh Babu P and Subhasree RS. Valuing the Suitable Agro-Industrial Wastes for Cultivation of *P. platypus* and *P. eous*. *Adv.Biol. Res.*, **4(4):** 207-210, 2010.

Domondon DL, He W, Kimpe ND, Hofte M and Poppe J. B- Adenosine, a bioactive compound in grass chaff stimulating mushroom production. *Phytochem*., **65:**181-187, 2004.

Dubost NJ, Ou B and Beelman RB. Quantification of polyphenols and ergothioneine in cultivated mushroom and correlation to total antioxidant capacity. *Food Chem.*, **105:** 727 -735, 2007.

Dundar A, Acay H and Yildiz A. Yield performances and nutritional contents of three oyster mushroom species cultivated on wheat stalk. *Afr. J. Biotechnol.*, **7(19):** 3497-3501, 2008.

Erkel I. Effects of Different Growing Medium on yield of *Pleurotus ostreatus* and *Pleurotus florida* Cultivation. *Fourth Congress of Edible Mushroom of Turkey*, Yalova, **pp:**1-8, 1992.

Eswaran A and Ramabadran R. Studies on some physiological, cultural and post-harvest aspects of oyster mushroom, *Pleurotus eous. Trop. Agri. Res.*, **12:** 360-374, 2000.

Fasidi IO, Kadiri M, Jonathan SG, Adenipekun CO and Kuforiji OO. Cultivation of tropical mushrooms. Ibadan: Ibadan University Press, 2008.

Finimundy TC, Gambato G, Fontanab R and Camassolab M. Aqueous extracts of *Lentinula edodes* and *Pleurotus sajor-caju* exhibit high antioxidant capability and promising in vitro antitumor activity. *Nutr. Res.*, **33(1):** 76-84, 2013.

Folch J, Lees M and Saloane-Stanely GH. A simple method for the isolation and purification of total lipids from animal tissue. *J. Biol. Chem.* **226:** 497-509, 1957**.**

Fritsch G and Jong N. Breeding work in Chang, ST. and Hays, WA. Eds. The biology and cultivation of edible mushrooms. *Academic press*, New York. p. 239- 248, 1978**.**

Fu HY, Shieh DE and Ho CT. Antioxidant and free radical scavenging activities of edible mushrooms. *J. Food Lipids*, **9:**35-43, 2009.

Garcha HS, Khanna PK and Soni GL. Nutritional Importance of Mushrooms. In: *Mushroom Biology and Mushroom Products* (S.T. Chang, J.A. Buswell and S.W. Chiu, eds.). Chinese University of Hong Kong Press, Shatin, 1993.

Gerasimenya VP, Efremenkova OV, Kamzolkina OV, Bogush TA, Tolstych IV and Zenkova VA. Antimicrobial and anti-toxical action of edible and

medicinal mushroom *Pleurotus ostreatus* (Jacq;Fr.) Kumm. extracts. *Int. J. Med. Mush.* **4:** 48-54, 2002.

Ghaly IS, Ahmed ES, Booles HF, Farag IM and Nada SA. Evaluation of antihyperglycemic action of oyster mushroom (*Pleurotus ostreatus*) and its effect on DNA damage, chromosome aberrations and sperm abnormalities in streptozotocin-induced diabetic rats. *Global Veter.*, **7(6):**532–544, 2011.

Goswami V, Sharma S and Sehgal SP. Possibilities of cultivation of *Pleurotus sajor-caju* (Fr) Singeron agricultural waste in Rajasthan. In: Kaul TN, Kapur BM (eds) *International conference on science and cultivation technology of edible fungi*. Jammu Tawi, India, **pp.** 75-77, 1987.

Gregory A, Svagelj M and Pohlevan J. Cultivation techniques and medicinal properties of *Pleurotus* spp. *Food Technol. Biotechnol.,* **45:**238-249, 2007.

Gupta P, Indurani C, Ahlawat OP Vijay B and Mediratta V. Physico Chemical Properties of SMS of *Agaricus bisporus* and its utilization in improving soil fertility. *Mush. Res.,* **13(2):** 84-91, 2004.

Gupta VK and Langar PN. *Pleurotus florida* for upgrading the nutritive value of wheat straw. *Biol. Wastes,* **23:** 57-64, 1988.

Halliwell B and Gutteridge JMC. Free radicals in biology and medicine 2nd Ed, Oxford: Clarendon Press. pp. 136-158, 1989.

Harbone JB. Phytochemical Methods. Champman and Hall Ltd. London. **pp.** 204, 1973.

Hasler CM. Functional food: the western prospective. *Nutri. Rev.,* **54:** 506-510, 1996.

Hassan S, Mohammad AY and Kiramat K. Cultivation of the oyster mushroom (*Pleurotus ostreatus* (Jacq.) P. Kumm.) in two different agroecological zones of Pakistan. *Afr. J. Biotechnol.,* **10:**183–188, 2011.

Hayes WA and Haddad SP. The nutritive value of mushrooms. *Mush. J.* **30:**204, 1976.

Holker U and Lenz J. Trickle-film processing: An alternative for producing fungal enzymes. *BIOforum Eur.,* **6:**55-57, 2004.

Hu SH, Wang JC, Lien JL, Liaw ET and Lee MY. Antihyperglycemic effect of polysaccharide from fermented broth of *Pleurotus citrinopileatus*. *Appl. Microbiol. Biotechnol.*, **70:** 107–113, 2006.

Hwang HJ, Kim SW, Lim JM, Joo JH, Kim HO, Kim HM and Yun JW. Hypoglycemic effect of crude exopolysaccharides produced by a medicinal mushroom *Phellinus baumii* in streptozotocin-induced diabetic rats. *Life Sci*, **76:** 3069–3080, 2005.

Ingale A and Ramteke A. Studies on cultivation and biological efficiency of mushrooms grown on different agro-residues. *Innov Romanian Food Biotechnol.,* **6:** 25-28, 2010.

Iqbal SM, Rauf CA and Sheik MI. Yield performance of oyster mushroom on different substrates. *Int. J. Agric. Biol*., **7:** 900–903, 2005.

Isıldak O, Turkekul I, Elmastas M, Tu M and Zen M. Analysis of heavy metals in some wild-grown edible mushrooms from the middle black sea region, Turkey. *Food Chem.,* **86:** 547–552, 2004.

Islam MZ, Rahman MH and Hafiz F. Cultivation of oyster mushroom (*Pleurotus flabellatus*) on different substrates. *Int. J. Sustain. Crop Prod.* **4(1):**45-48, 2009.

Iwalokun BA, Usen UA, Otunba AA and Olukoya DK. Comparative phytochemical evaluation, antimicrobial and antioxidant properties of *Pleurotus ostreatus*. *Afri. J. Biotechnol.,* **6:**1732-1739, 2007.

Jayakumar T, Thomas PA and Geraldine P. Protective effect of an extract of the oyster mushroom, *Pleurotus ostreatus*, on antioxidants of major organs of aged rats. *Exp. Gerontol.,* **42:** 183-191, 2007.

Jeong SC, Jeong YT, Yang BK, Islam R, Koyyalamudi SR, Pang G, Cho KY and Song CH. White button mushroom (*Agaricus bisporus*) lowers blood glucose and cholesterol levels in diabetic and hypercholesterolemic rats. *Nutr. Res.,* **30:** 49–56, 2010.

Johnny I and Okon J. Antidiabetic Effect of *Pleurotus ostreatus* (Jacq.ex Fr) kumm. Mushroom on Alloxan-induced Diabetic Rats. *Ind. J Pharma. Biol. Res.*, **1(1):** 31-36, 2013.

Jonathan SG, Muritala ML and Oyetunji JO. Effect of Spent Mushroom Compost of *Pleurotus pulmonarius* on Growth Performance of Four Nigerian Vegetables. *Mycobiol.,* **39(3):**164-169, 2011.

Jose N and Jananrdhanan KK. Antioxidant, antiinflammatory and antitumor activities of culinary-medicinal mushroom *Pleurotus pulmonarius* (Fr.) Quel. (Agaricomycetideae*). Int. J. Med. Mush.,* **4:** 329-335, 2002.

Jose N and Janardhanan KK. Antioxidant and antitumor activity of *Pleurotus florida. Curr. Sci.* **79:** 941-943, 2000.

Jose N, Ajith TA and Jananrdhanan KK. Methanol extract of the oyster mushroom, *Pleurotus florida*, inhibits inflammation and platelet aggregation. *Phytotherapy Res*., **18:** 43-46, 2004.

Jose N, Ajith TA and Janardhanan KK. Antioxidant, anti-inflammatory and antitumor activities of culinary-medicinal mushroom *Pleurotus pulmonarius* (Fr.) Quel. (Agaricomycetideae). *Int. J. Med. Mush.* **4:**59-66, 2002.

Kamilya D, Ghosh D, Bandopadhyay S, Mal BC and Maiti TK. *In Vitro* effects of bovine lactoferrin, mushroom glucans and *Abrus agglutinin* on Indian major carp catla (*Catla catla*) head kidney leukocytes. *Aquaculture.* **253:**130-139, 2006.

Kang W. Oyster mushroom cultivation. Mushroom Grower's Hand Book **pp.** 1-4, 2004.

Kasuga A, Aoyagi Y and Sugahara T. Antioxidant activity of fungus *Suillus bovinus, J. Food Sci.,* **60:** 1113-1115, 1995.

Kaul TN. Biology and Conservation of Mushrooms, Oxford and IHB Publishing Co. Ltd. New Delhi, India. **pp.** 117-145, 2001.

Khan MA, Tania M, Zhang DZ and Chen HC. Antioxidant enzymes and cancer. *Chin. J. Cancer Res.* **22(2):** 87-92, 2010.

Khan MA, Tania M, Zhang DZ and Chen HC. *Cordyceps* Mushoom: A potent anticancer nutraceutical. *Open Nutra. J.* **3:** 179-183, 2010.

Khan NA, Khaliq N, Haq MIU, Javed N, Gondal AS and Bhatty N. Carbohydrates and mineral content of oyster mushroom cultivated on different agricultural wastes. *Mush. Res.,* **23(1):**31-35, 2014.

Khan P and Garcha HS. *Pleurotus* mushroom, a source of food protein. *Mush. Newslett. Trop.* **4:** 9-14, 1984.

Khare KB, Mutuku JM, Achwania OS and Otaye DO. Production of two oyster mushrooms, *Pleurotus sajor-caju* and *P. florida* on supplemented and un-supplemented substrates, *Bots. J. Agric. Appl. Sci*., **6:** 4–11, 2010.

Kiho T, Hui J, Yamane A and Ukai S. Polysaccharide in fungi. XXXII. Hypoglycaemic activity and chemical properties of a polysaccharide from the cultural mycelium of *Cordyceps sinensis*. *Biol. Pharm. Bull.* **16:**1291-1293, 1993.

Kiho T, Tsujimura Y, Sakushima M and Usui S. Polysaccharides in fungi. XXXIII. Hypoglycaemic activity of an acidic polysaccharide (AC) from *Tremella fuciformis*. *Yakugaku Zasshi.* **114(5):** 308-315, 1994.

Kiho T, Yamane A, Hui J, Usui . and Ukai S. Polysaccharides in fungi XXXIV. Hypoglycaemic activity of a polysaccharide (CS-F30) from the cultural mycelium of *Cordyceps sinensis* and its effect on glucose metabolism in mouse liver. *Biol. Pharm. Bull*. **19:**294-296, 1996.

Kiho TK, Ookubo S, Usui S, Ukai S and Hirano K. Structural features an hyperglycaemic activity of polysaccharide (CS-F10) from the cultured mycelium of *Cordyceps sinensis*. *Biol. Pharm. Bull*. **22:**966-970, 1999.

Kim DH, Yang BK, Jeong SC, Hur NJ, Das S, Yun JW, Choi JW, Lee YS and Song CH. A preliminary study on the hypoglycaemic effect of the exo-polymers produced by five different medicinal mushrooms. *J Microbiol. Biotechnol.*, **11:**167–171, 2001.

Kim JI, Kang MJ, Im J, Seo YJ, Lee YM, Song JH, Lee JH and Kim ME. Effect of King Oyster Mushroom (*Pleurotus eryngii*) on Insulin Resistance and Dyslipidemia in db/db Mice. *Food Sci. biotechnol.*, **19:** 239–242, 2010.

Kim MY, Park MH and Kim GH. Effects of mushroom protein-bound polysaccharides on the blood glucose levels and energy metabolism in streptozotocin-induced diabetic rats. *J. Kor. Nutr.*, **30:** 743-750, 1997.

Kim YW, Kim KH, Choi HJ and Lee DS. Anti-diabetic activity of beta-glucans and their enzymatically hydrolysed oligosaccharides from *Agaricus blazei*. *Biotechnol. Lett.*, **27:** 483–487, 2005.

Kimenju JW, Odero GOM, Mutitu EW, Wachira PM, Narla RD and Muiru WM. Suitability of locally available substrates for oyster mushroom (*Pleurotus ostreatus*) cultivation in Kenya. *Asian J. Plant Sci*., **8:** 510–514, 2009.

Klein BP and Perry AK. Ascorbic acid and vitamin A activity in selected vegetables from different geographical areas of the United States. *J. Food Sci.* **47:** 941-945, 1982.

Knudsen D and Beegle D. Recommended phosphorous tests. In: *Recommended chemical soil tests procedures for the north central region*. WC Dahnke (ed) *Bull North Dakota Agric Exp Stn.* North Dakota, USA No. **499:** 122-15, 1988.

Ko HG, Park SH, Kim SH, Park HG, Park WM. Detection and recovery of hydrolytic enzymes from spent compost of four mushroom species. *Folia Microbiol.*, **50:**103−106, 2005.

Kobayashi T. Subtype of insulin dependent diabetes mellitus (IDDM) in Japan: Slowly progressive IDDM. The clinical characteristics and pathogenesis of the syndrome. *Diabetes Res. Clin. Pract.,* **24:** 95-99, 1994.

Kotra LP and Mobashery S. Lactam antibiotics, lactamases and bacterial resistance. *Bull. Inst. Pasteur.,* **96:** 139-150, 1998.

Kotwaliwale N, Bakane P and Verma A. Changes in textural and optical properties of oyster mushroom during hot air drying. *J. Food Eng.* **78:** 1207-1211, 2007.

Kubo K, Aoki H and Nanba H. Antidiabetic activity present in the fruit body of *Grifola frondosa* (Maitake). *Biol. Pharm. Bull.,* **17(8):**1106-1110, 1994.

Kurtzman RH. Nitration of *Pleurotus sapidus* effects of lipid. *Mycologia*. **68:**268-295, 1976.

Kuzua T, Nakagawa S, Satosh J, Kanazawa Y, Iwamoto Y and Kobayashi M. Report of the committee on the classification and diagnostic criteria of diabetes mellitus. *Diabetes Res. Clin. Pract.,* **55:** 65-85, 2002.

Laemmili UK. Cleavage of structural proteins during assembly of the head of bacteriophage T_4. *Nat.* **227:** 265-275, 1970.

Li SP, Zhang GH, Zeng Q, Huang ZG, Wang YT, Dong TT, Tsim KW. Hypoglycaemic activity of polysaccharide, with antioxidation, isolated from cultured *Cordyceps* mycelia. *Phytomed.*, **13(6):**428–433, 2006.

Li SP, Zhao KJ, Ji ZN, Song ZH, Dong TT, Lo CK, Cheung JK, Zhu SQ and Tsim KW. A polysaccharide isolated from *Cordyceps sinensis*, a traditional Chinese medicine, protects PC12 cells against hydrogen peroxide-induced injury. *Life Sci*, **73(19):** 2503–2513, 2003.

Li, YR, Liu QH, Wang HX and Ng TB. A novel lectin with potent antitumor, mitogenic and HIV-1 reverse transcriptase inhibitory activities from the edible mushroom *Pleurotus citrinopileatus Biochimica et Biophysica Acta* (BBA) – *General Subjects*, 1**780(1):** 51-57, 2008.

Lindequist U, Niedermeyer THJ and Julich WD. The pharmacological potential of mushrooms: Evidence based complement. *Altern. Med*., **2(3):**285-299, 2005.

Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. Protein measurement with folin phenol reagent. *J. Biol. Chem.*, **193:** 265-275, 1951.

Mahajan RG, Patil SI, Mohan, DRK and Shastry P. *Pleurotus eous* mushroom lectin (PEL) with mixed carbohydrate inhibition and antiproliferative activity on tumor cell lines. *J. Biochem. Mol. Biol. Biophys.* **6:**341-345, 2002.

Maiti S, Mallick SK, Bhutia SK, Behera B, Mandal M and Maiti TK. Antitumor effect of culinary-medicinal oyster mushroom, *Pleurotus ostreatus* (Jacq.: Fr.) P. Kumm., derived protein fraction on tumor-bearing mice models. *Intl. J. Med. Mush.*, **13:** 427-40, 2011.

Manpreet K, Giridhar S and Khanna PK. *In vitro* and *in vivo* antioxidant potentials of *Pleurotus florida* in experimental animals. *Mush. Res.***, 13:**21-26, 2004.

Manzi P, Aguzzi A and Pizzoferrato L. Nutritional value of mushrooms widely consumed in Italy. *Food Chem.*, **73:**321, 2001.

Manzi P, Gambelli L, Marconi S, Vivanti V and Pizzoferrato L. Nutrients in edible mushrooms: an interspecies comparative study. *Food Chem.,* **65:**477–482, 1999.

Mayolo-Deloisa K, Trejo-Hernández MDR and Rito-Palomares M. Recovery of laccase from the residual compost of *Agaricus bisporus* in aqueous two-phase systems. *Process Biochem.,* **44:**435-439, 2009.

Md. Khan A, Md Rahman M, Mousumi T, Md. Nazim U and Ahmed S. *Pleurotus sajor-caju* and *Pleurotus florida* Mushrooms Improve Some Extent of the Antioxidant Systems in the Liver of Hypercholesterolemic Rats. *The Open Nutraceutic. J.,* **4:**20-24, 2011.

Md. Mahedy M, Md. Shirshir RIand Md. Hasan R. Fortification of wheat bread using mushroom powder. *Bangladesh Res. Pub. J.,* **7(1):** 60-68, 2012.

Menaga D, Rajakumar S and Ayyasamy PM. Free Radical Scavenging Activity of Methanolic Extract of *Pleurotus florida* Mushroom. *Int. J. Pharm. Sci.,* **5(4):** 601-606, 2013.

Miles PG and Chang ST. Mushroom Biology: Concise basics and current developments. World Scientific, Singapore **pp:** 194**,** 1997.

Mittler R. Oxidative stress, antioxidants and stress tolerance. *Trends Pl. Sci.* **7:** 405-410, 2002

Mizuno T. Bioactive biomolecules and Mushrooms: food function and medicinal effects of mushroom fungi. *Food Rev. Int.,* **11:**7-21, 1995.

Mohamed Imran M, Mohamed Mahroop RM, Abdul BJ and Asarudeen A. Determination of total phenol, flavonoid and antioxidant activity of edible mushrooms *Pleurotus florida* and *Pleurotus eous. Int. Food Res. J.,* **18:** 574-577, 2011.

Mohammed A, Adelaiye AB, Abubakar MS and Abdurahman EM. Effects of aqueous extract of *Ganoderma lucidum* on blood glucose levels of normoglycemic and alloxan induced diabetic wistar rats. *J. Med. Plants. Res.,* **1(2):** 34-37, 2007.

Mohammed A, Adelaiye AB, Bakari AG and Mabrouk MA. Anti-diabetic and some haematological effects of ethylacetate and n-butanol fractions of *Ganoderma*

lucidum aqueous extract in alloxan-induced diabetic wistar rats. *Int. J. Med. Sci.,* **1(12):** 530-535, 2009.

Moonmoon M, Uddin MN, Ahmed S, Shelly NJ and Khan MA. Cultivation of Different Strains of King Oyster Mushroom (*Pleurotus eryngii*) on Saw Dust and Rice Straw in Bangladesh. *Eur. J. Biol. Sci.,* **2(1):** 13-18, 2010.

Mshandete AM and Cuff J. Cultivation of three types of indigenous wild edible mushrooms: *Coprinus cinereus*, *Pleurotus flabellatus* and *Volvariella volvocea* on composted sisal decortications residue in Tanzania. *Afr. J. Biotechnol*. **7(24):** 4551-4562, 2008.

Mushigeni KE and Chang TS. Mushrooms: Their biology, nutritional and medicinal properties, cultivation technologies and perspectives on mushroom research and development. *Proceedings of the Mushroom Farming Training Workshop* held in Lilongwe, Malawi, **pp.** 74, 2001.

Nayana J and Janardhanan KK. Antioxidant and antitumour activity of *Pleurotus florida*. *Curr. Sci.* **79:**941-943, 2000.

Ngai P and Ng T. A hemolysin from the mushroom *Pleurotus eryngii. Appl. Microiol. Biotechnol.,* **72:**1185-1191, 2006.

Nithya R and Ragunathan R. Synthesis of silver nanoparticle using *Pleurotus sajor-caju* and its antimicrobial study. Digest *J. Nanomater. Biostruc.,* **4(4):** 623-629, 2009.

Nosál'ová V, Bobek P, Cerná S, Galbavý S and Stvrtina S. Effects of pleuran ($-glucan isolated from *Pleurotus ostreatus*) on experimental colitis in rats. *Physiol. Res.*, **50(6):** 575-581, 2001.

Nunomura A, Castellani R, Zhu X, Moreira P, Perry G and Smith M. Involvement of oxidative stress in Alzheimer disease. *J. Neuropathol. Exp. Neurol*. **65(7):** 631-641, 2006.

Obodai M, Cleland-Okine J and Vowotor KA. Comparative study of the growth and yield of *Pleurotus ostreatus* on different lignocellulosic by-products. *J. Ind. Microbiol. Biotechnol.* **30:**146-149, 2003.

Oh SJ, Park JS, Lee DC and Shin PG. Studies on the effect of vinyl mulching on *Pleurotus* cultivation: Control of mushroom disease on *P. ostreatus* (II). *Microbiol.,* **31:**50-53, 2003.

Okamoto K, Narayama S, Katsuo A, Shigematsu I and Yanase H. Biosynthesis of p-anesaldehyde by the white rot basidiomycetes *Pleurotus ostreatus*. *J. Biosci. Bioeng.,* **93:** 207-210, 2002.

Onyango BO, Palapala VA, Arama PF, Wagai SO and Gichumu BM. Sustainability of selected supplemented substrates for cultivation of Kenyan native wood ear mushrooms (*Auricularia auricula*). *Am. J. Food. Technol.,* **6:** 395-403, 2011.

Osei P. Mushroom Cultivation with Special Emphasis on Appropriate Techniques for Developing Countries. 2nd Edn., Backhuys, Amsterdam, The Netherlands, **pp:** 111-122, 1996.

Oyetayo VO and Ariyo OO. Antimicrobial and antioxidant properties of *Pleurotus ostreatus* (Jacq:Fries) cultivated on different tropical woody substrates. *J. Waste Cons. Bipro. Biotechnol*., **1(2):**28-32, 2013.

Pal J, Ganguly S, Tahsin KS and Acharya K. *In vitro* free radical scavenging activity of wild edible mushroom *Pleurotus squarrosulus* (Mont.) Singer. *Indian J Exp. Biol* **47:**1210-1218, 2010.

Pala SA, Wani AH and Mir RA. Evaluation of yield performance of *Pleurotus sajor-caju* on different agro-based wastes. *Afr. J. Agric. Res.,* **8(23):**3025-3028, 2013.

Pandey RS and Ghosh SK. *A Handbook on Mushroom Cultivation*. Emkay publications, Delhi. **pp**. 134, 1996.

Park MS, Seo GS, Lee KH, Bae KS and Yu SH. Morphological and cultural characterization of *Trichoderma* sp. associated with green mold of oyster mushroom in Korea. *Plant Pathol. J.* **21:** 221-228, 2005.

Patil A, Ahmed SA, Telang B and Baig VMM. The nutritional value of *Pleurotus ostreatus* Kumm cultivated on different lignocellulosic agro-wastes. *Innovat. Rom. Food Biotechnol.* **7:**66-76, 2010

Patil SS and Dakore HG. Comparative study on yield performance and Nutritive value of oyster mushroom on soybean straw. *Bioinfolet*, **4(1):** 57-59, 2007.

Patill SS, Kadam RM, Shinde SL and Deshmukh SA. Effect of different substrate on productivity and proximate composition of *P. florida*. *Int. J. Plant Sci.* **3(1):** 151-153, 2008.

Pavlik M. Growing of *Pleurotus ostreatus* on woods of various deciduous trees. *Acta. Edulis Fungi*, **12:**306-312, 2005.

Periasamy K. Novel antimicrobial compounds obtained from some edible mushrooms. *Int. J. Med. Mush.*, **7:** 443-444, 2005.

Peter O. Nutritional aspects and medical use. Manual on Mushroom cultivation. Published by Tool Foundation, Amsterdam, **1:** 23-24, 1991.

Phan CW and Sabaratnam V. Potential uses of spent mushroom substrate and its associated lignocellulosic enzymes. *Appl Microbiol. Biotechnol.*, **96:**863−873, 2012.

Philippoussis A, Zervakis G and Diamantopoulou P. Bioconversion of agricultural lignocellulosic waste through the cultivation of edible mushroom *Agrocybe aegerita, Volriella volvacca and Pleurotus* spp. *World J. Microbiol. Biotechnol.,* **17:**191-200, 2001.

Pill WG, Evans TA and Garrison SA. Forcing white asparagus I various substrates under cool and warm regimes. *Hort. Sci.* **28:**996-998, 1993.

Pokhrel CP, Kalyan N, Budathoki U and Yadav RKP. Cultivation of *Pleurotus sajor-caju* using different agricultural residues. *Int. J. Agric. Pol. Res.* **1(2):** 19-23, 2013.

Poppe J. Use of the agricultural waste materials in the cultivation of mushrooms. *Mush. Sci.* **15:**3-23, 2000.

Raghuramulu N, Madhavan NK and Kalyanasundaram S. *A Manual of Laboratory Techniques, National Institute of Nutrition*. Indian Council of Medical Research, Hyderabad, India, **pp.** 56-58, 2003.

Ramkumar L, Ramanathan T, Thirunavukkarasu P and Arivuselvan N. Antioxidant and radical scavenging activity of nine edible mushrooms extract. *Int. J. Pharmacol.,* **6(6):**950-953, 2010.

Randive S. Cultivation and study of growth of oyster mushroom on different agricultural waste substrate and its nutrient analysis. *Adv. Appl. Sci. Res.,* **3(4):**1938-1949, 2012.

Rao JS, Kumar KR, Vale VK, Yarlagadda PP and Saradhi SV. Bioactive molecules and their Anti-oxidant activity of *Agaricus bisporus. J. Chem. Biol. Phy. Sci.,* **3(2):**1222-1228, 2013.

Ravi B, Renitta RE, Praba ML, Issac R, Naidu S. Evaluation of antidiabetic potential of oyster mushroom (*Pleurotus ostreatus*) in alloxan-induced diabetic mice. *Immunopathol. Toxicol.,* **35(1):**101-109, 2013.

Reguła J and Siwulski M. Dried shitake (*Lentinulla edodes*) and oyster (*Pleurotus ostreatus*) mushrooms as a good source of nutrient. *Acta Sci. Pol. Technol. Aliment.,* **6:**135-142, 2007.

Rout D, Mondal S, Chakraborty I, Pramanik M and Islam SS. Chemical analysis of a new (13), (16) branched glucan from an edible mushroom *Pleurotus florida*. *Carbohydr. Res*. **340:** 2533-2539, 2005.

Roy S, Barman S, Chakraborty U and Chakraborty BN. Cultivation of *Pleurotus djamor* - a new species of oyster mushroom in North Bengal. *J. Mycopathol. Res.* **53(1)**: 59-63, 2015.

Roy S, Barman S, Chakraborty U and Chakraborty BN. Development of an easy and efficient technique for cultivation of different species of *Pleurotus*. *NBU J. Pl. Sci.* **8(1):**13-18, 2014.

Roy S, Chakraborty BN, Barman S and Chakraborty U. Production of *Pleurotus ostreatus* Grown in Different Substrates and Evaluation of Spent Substrate as Organic Manure for Growth Improvement of *Capsicum chinense* Jacq. *J. Mycol. Pl. Pathol.,* **45(3):**267-272, 2015.

Royse DJ, Rhodes TW, Ohga S and Sanchez JE. Yield, mushroom size and time to production of *Pleurotus cornucopiae* (oyster mushroom) grown on switch grass substrate spawned and supplemented at various rates. *Bioresour. Technol.* **91:**85-91, 2004.

Rushita S, Vijayakumar M, Noorlidah A, Abdulla MA and Vikineswary S. Effect of *Pleurotus citrinopileatus* on blood glucose, insulin and catalase of

streptozotocin-induced type 2 diabetes mellitus rats. *J. Ani. Pl. Sci.,* **23(6):** 1566-1571, 2013.

Sachin LB, Shweta NS, Naimesh MP, Prasad AT and Subhash LB. Hypoglycemic Activity of Aqueous Extract of *Pleurotus pulmonarius* in Alloxan-Induced Diabetic Mice. *Pharmaceuti***.** *Biol***., 44(6):** 421-425, 2006.

Sadler M and Saltmarsh M. Functional foods: The consumer, the products and the evidence. Royal Soc. Chem. Cambridge. 1998

Saha AK, Acharya S and Roy A. Antioxidant level of wild edible mushroom: *Pleurotus djamor* (Fr.) Boedijn. *J. Agric. Technol.,* **8(4):** 1343-1351, 2012.

Sanchez A, Ysunza F, Beltran-Gracia M and Esqueda M. Biodegradation of viticulture wastes by *Pleurotus*: a source of microbial and human food and its potential use in animal feeding. *J. Agri. Food Chemistry***, 50:** 2537-2542, 2002.

Sánchez C. Cultivation of *Pleurotus ostreatus* and other edible mushrooms. *Appl. Microbiol. Biotechnol.* **85(5):** 1321-1337, 2010.

Sandven P. Epidemiology of canidemia. *Rev. Iberoam Micol.,* **17:**73-81, 2000.

Sanmee RB, Dell P, Lumyong K, Izumori and Lumyong S. Nutritive value of popular wild edible mushrooms from northern Thailand. *Food Chem.,* **82:** 527-532, 2003.

Sano MK and Yoshino T, Matsuzawa S and Ikekawa T. Inhibitory effects of edible higher basidiomycetes mushroom extracts on mouse type IV allergy. *Int. J. Med. Mush.,* **4:** 37-41, 2002.

Sarangi I, Ghosh D, Bhutia SK, Mallik SK and Maiti TK. Antitumor and anti-immunomodulating effects of *Pleurotus ostreatus* mycelia derived proteoglycans. *Int. Immunopharmacol*., **6:** 1287-1297, 2006.

Sarker NC, Hossain MM, Sultana N, Mian IH, Karim AJMS and Amin SMR. Performance of different substrates on the growth and yield of *Pleurotus ostreatus* (Jacquin ex Fr.) Kummer. *Bangl. J. Mush*.,**1:** 9-20, 2007.

Sato T, Tai Y, Nunoura Y, Yajima Y, Kawashima S and Tanaka K. Dehydrotrametenolic acid induces preadipocyte differentiation and sensitizes

animal models of noninsulin-dependent diabetes mellitus to insulin. *Biol Pharm Bull*, **25:**81–86, 2002.

Shah S, Nasreen S and Kousar S. Efficacy of fungicides against *Trichoderma* spp. causing green mold disease of oyster mushroom (*Pleurotus sajor-caju*). *Res. J. Microbiol.* **8(1):** 13-24, 2013.

Shah ZA, Ashraf M and Ishtiaq M. Comparative study on cultivation and yield performance of oyster mushroom (*Pleurotus ostreatus*) on different substrates (wheat straw, leaves and sawdust). *Pak. J. Nutri*., **3:** 158-160, 2004.

Sharma RK and Bahukhandi D. Studies on shelf life of white button mushroom. *Ann. Pl. Protec. Sci.* **11:** 333-336, 2003.

Sharma S and Madan M. Microbial protein from leguminous and non-leguminous substrates. *Acta. Biotechnologica.,* **13:** 131-139, 1993.

Sharma S, Kailash R, Yadav PC and Pokhrel P. Growth and Yield of Oyster mushroom (*Pleurotus ostreatus*) on different substrates *J. New Biol Rep* **2(1):** 3-8, 2013.

Shashoua VE and Adams DS. New synthetic peptides can enhance gene expression of key antioxidant defence enzymes *in vitro* and *in vivo*. *Brain Res.,* **1024:** 34-43. 2004.

Shu-Ting C and Phillip GM. *Tremella*- increased production by a mixed culture technique mushrooms: cultivation, nutritional value, medicinal effect and environmental impact. (2nd Ed.) Boca Raton, Florida: CRC Press. 2004

Siddhant, Yadav S, Ahmad A and Singh CS. Effect of wheat straw components on the yield of *Pleurotus eous. Int. J. Curr. Microbiol. App. Sci.* **2(8):** 221-225, 2013.

Singh AD, Abdullah N and Vikineswary S. Optimization of extraction of bulk enzymes from spent mushroom compost. *J. Chem. Technol. Biotechnol.* **78:**743−752, 2003.

Singh KL, Kannayan S and Ramasamy K. *Hand Book of Edible Mushrooms-Cultivation of Pleurotus*,. Today and Tomorrow Printers and Publishers, New Delhi, India **pp:** 38–72, 1990.

Singh S, Kumar CG and Singh S. Production, processing and consumption pattern of mushrooms. *Ind. Food Pac.* **14(6):** 38-47, 1995.

Singh VP, Srivastava S, Rastogi J, Singh P, David A and Gupta P. *In vitro* evaluation of carbendazim 50% WP on green mould disease of some *Pleurotus* species. *Mush. Res.* **23 (1):** 119-123, 2014.

Sinha AK and Mehta R. Biochemical properties of different agricultural wastes treated with *Pleurotus ostreatus. Mush. Res.* **23(2):** 175-179, 2014.

Stamets P. Growing Gourmet and Medicinal Mushrooms. Ten Speed Press, Berkeley, USA. **pp.** 150, 2000

Strmiskova G, Strmiska F and Dubravicky J. Mineral composition of oyster mushroom. *Die Nahrung,* **36(2):** 210-212, 1992.

Sultana Z, Chatterjee M, Chakraborty S, Dey P, Paul I, Bankura B, Acharya K and Das M. Augmentation of the Metformin activity using *Pleurotus florida* Polysaccharide to control Hyperglycemia in Mice model: A Preliminary Study. *Int. J. Pharm. Tech. Res.*, **6(1):**147-153, 2014.

Swanston-Flatt SK, Day C, Flatt PR, Gould BJ and Bailey CJ. Glycemic effects of traditional European plant treatments for diabetes: Studies in normal and streptozotocin diabetic mice. *Diabetes Res.* **10:** 69-73, 1989**.**

Syed AA, Kadam JA, Mane VP, Patil SS and Baig MMV. Biological efficiency and nutritional contents of *Pleurotus florida* (Mont.) Singer cultivated on different Agro-wastes, *Nat. Sci.,* **7(1):**44-48, 2009.

Tesfaw A, Tadesse A and Kiros G. Optimization of oyster (*Pleurotus ostreatus*) mushroom cultivation sing locally available substrates and materials in Debre Berhan Ethiopia. *J. Appl. Biol. Biotechnol.* **3(1):** 15-20, 2015.

Thakur MP and Singh HK. Mushrooms, their bioactive compounds and medicinal uses: A review. *Med. Pl.* **5(1):** 1-20, 2013.

Thakur MP, Shukla CS and Yadav V. Biodiversity and conservation of mushroom in Chhattisgarh. In: *Microbial biotechnology and ecology* (Vyas, Deepak, Paliwal, G.S., Khare, P.K. and Gupta, R.K. eds.), Daya Publishing House, New Delhi. **pp.** 320-343, 2011.

Thakur MP. Present status and future prospects of tropical mushroom cultivation in India: A review. *Indian Phytopathol.* **67(2):** 113-125, 2014.

Thekkuttuparambil A, Kainoor A and Janardhanan K. Indian medicinal mushrooms as a source of antioxidant and antitumor agents. *J. Clin. Biochem, Nutr.* **40:**157-162, 2007.

Thomas GV, Prabhu SR, Reeny MZ, Bopaiah BM. Evaluation of lignocellulosic biomass from coconut palm as substrate for cultivation of *Pleurotus sajar-caju*. *World J. Microbiol. Biotechnol*., **14:**879-882, 1998.

Thomson KS and Moland ES. The new blactamases of Gram-negative bacteria at the dawn of the new millennium. *Micro. Inf.*, **2:** 1225-1235, 2000.

Thulasi EP, Danial TP, Ravichandran B and Madhusudhanan K. Mycelial Culture and Spawn Production of two Oyster Mushrooms, *Pleurotus florida* and *Pleurotus eous* on Different Substrates. *Int. J. Biol. Technol.,* **1(3):**39-42, 2010.

Tzianabos A. Polysaccharide immunomodulators as therapeutic agents: Structural aspects and biologic function. *Clin. Microbiol. Rev.*, **13:** 523-533, 2000.

Wang H and Ng TB. Eyringin a novel antifungal peptide from fruiting bodies of the edible mushroom *Pleurotus eryngii. Peptides*. **25:**1-5, 2004

Wang H, Gao J and Ng, TB. A new lactin with highly potent antihepatoma and antisarcoma activities from the oyster mushroom *Pleurotus ostreatus. Biochem. Biophys. Res. Commun.* **275:**810-816, 2000.

Wang HX and Ng TB. Isolation of a novel protein from *Pleurotus ostreatus* mushroom with anti-human immunodeficiency virus, translation-inhibitory and ribonuclease activities. *Biochem. Biophys. Res. Commun.* **276:**587-593, 2000.

Wang HX and Ng TB. Quinqueginsin, a novel protein with anti-human immunodeficiency virus, antifungal, ribonuclease and cell-free translationinhibitory activities from American ginseng roots. *Biochem. Biophys. Res. Commun.*, **269:** 155-159, 2000.

Wang J, Wang HX and Ng TB. A peptide with HIV1 reverse transcriptase inhibitory activity from the medicinal mushroom *Russula paludosa*. *Peptides***. 28:**560-565, 2007

Wang JC, Hu SH, Liang ZC and Yeh CZ. Optimization for the production of water soluble polysaccharides from *Pleurotus citrinopileatus* in submerged culture and its antitumor effect. *Appl. Microbiol. Biotechnol.* **67:**759-766, 2005.

Wang Q, Li H, Chen TT and Han JR. Yield, polysaccharides content and antioxidant properties of *Pleurotus abalones* and *Pleurotus geesteranus* produced on asparagus straw as substrate. *Sci. Horti.* **134:**222-226, 2012.

Wan-Rosli WI, Nurhanan AR and Aishah MS. Effect of Partial Replacement of Wheat Flour with Oyster Mushroom (*Pleurotus sajor-caju*) Powder on Nutritional Composition and Sensory Properties of Butter Biscuit. *Sains Malaysiana* **41(12):** 1565–1570, 2012.

Wan-Rosli WI, Solihah MA, Aishah M, Nik Fakurudin NA and Moshin SSJ. Colour, Textural properties, cooking characteristics and fibre content of chicken patty added with oyster mushroom (*Pleurotus sajor-caju). Int. Food Res. J*., **18:**612-618, 2011

Wassar SP. Medicinal mushrooms as a source of antitumor and immunomodulatory polysaccharides. *Appl. Microbiol. Biotechnol*. **60:** 258-274, 2002.

Wassar SP. Medicinal properties of substances occurring in higher basidiomycete mushrooms: current prospective (review). *Int. J. Med. Mush.* **1:**31-62, 1999.

Weinheim KG. Oyster Mushroom. John Wiley and Sons Inc. New York. 2006.

Wild S, Roglic G, Green A, Sicree R and King H. Global prevalence of diabetes estimates for 2000 and projections for 2030. *Diabetes Care.* **27:** 1047-1053, 2004.

Windholz M. The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals, 10th ed. Merck and Co., New Jersey. 1983

Wolff ERSE, Wisbeck MLL, Silveira RMM, Gern MSL, Pinho and Furlan SA. Antimicrobial and antineoplasic activity of *Pleurotus ostreatus. Appl. Biochem. Biotechnol.,* **151:** 402-412. 2008.

Wood DA and Smith JF. The Cultivation of Mushroom. In essays in agricultural and food microbiology editor by Norris J.R. and Pettipher G.L. John Wiley and Sons Ltd. **pp.** 310-343 1987.

Wood-Kaczmar A, Gandhi S and Wood N. Understanding the molecular causes of Parkinson's disease. *Trends Mol. Med*., **12(11):** 521-528, 2006.

Yadav S, Vats V, Dhunnoo Y and Grover JK. Hypoglycemic and antihyperglycemic activity of *Murraya koenigii* leaves in diabetic rats. *J. Ethnopharmacol.*, **82:**111–116, 2002.

Yadav SS, Ahmad A and Singh CS. Effect of wheat straw components on the yield of *Pleurotus eous. Int.J.Curr.Microbiol.App.Sci.* **2(8):** 221-225, 2013.

Yamac M, Kanbak G, Zeytinoglu M, Senturk H, Bayramoglu G, Dokumacioglu A and Van G. Pancreas protective effect of button mushroom *Agaricus bisporus* (J.E. Lange) Imbach (Agaricomycetidae) extract on rats with streptozotocin induced diabetes. *Int J Med Mushr*, **12(4):** 379–389, 2010.

Yang J, Lin H and Mau J. Non-volatile taste components of several commercial mushrooms. *Food Chem.*, **72:** 465-471, 2001.

Yildiz S, Yildiz UC, Gezer ED and Temiz A. Some lignocellulosic wastes used as raw material in cultivation of the *Pleurotus ostreatus* culture mushroom. *Pro Biochem* **38:** 301-306. 2002.

Yohalem DS, Nordhein EV and Andrew JH. Effect of water extracts of spent mushroom compost on apple scab in the field. *Phytopathol.* **86:**914-922, 1996.

Yoshioka Y, Tabeta R, Saito H, Uehara N and Fukoaka F. Antitumor polysaccharides from *P. ostreatus* (Fr.) Quel. isolation and structure of a β-glucan. *Carbohydrate Res.* **140:** 93-100, 1985.

Yu BP. Cellular defenses against damage from reactive oxygen species. *Physiol. Rev.*, **74:**139–162, 1994.

Yuan Z, He P, Cui J and Takeuchi H. Hypoglycemic effect of water soluble polysaccharide from *Auricularia auricula-judae* Quél. on genetically diabetic KK-Ay mice. *Biosci Biotechnol Biochem*, **62:** 1898–1903, 1998.

Zaidman B, Yassin M, Mahajana J and Wasser SP. Medicinal Mushrooms modulators of molecular targets as cancer therapeutics. *Appl. Microbiol. Biotechnol.* **67:**453-468, 2005.

Zervakis G, Philippoussis A, Ioannidou S and Diamantopoulou P. Mycelial growth, kinetics and optimum temperature conditions for the cultivation of edible mushroom species on lignocellulosic substrates. *Folia Microbiol*. **46(5):** 231-234, 2001.

Zhang HN and Lin ZB. Hypogglycemic effect of *Ganoderma lucidum* polysaccharides. *Acta. Pharmacol. Sin*. **25**: 191-195, 2004.

Zhang M, Cui SW, Cheung PCK and Wang Q. Antitumor polysaccharides from mushrooms: A review on their isolation process, structural characteristics and antitumor activity. *Trends Food Sci. Technol.*, **18:**4-19, 2007.

Zhang M, Zhang L, Cheung PCK and Ooi VEC. Molecular weight and antitumor activity of the water soluble polysaccharides isolated by hot water and ultrasonic treatment from the sclerotia and mycelia of *Pleurotus tuber-regium*. *Carbohydr. Polym.*, **56:** 123-128, 2004.

Zhang R, Li X and Fadel, JG. Oyster mushroom cultivation with rice and wheat straw. *Bioresour. Technol.* **82:** 277-284, 2002.

Zhang Y, Mills GL and Nair MG. Cyclooxygenase inhibitory and antioxidant compounds from the mycelia of edible mushroom *Grifola frondosa*. *J. Agric. Food Chem.* **50(26):** 7581-7585, 2002.

Zimmet PZ, McCarty DJ and deCourten MP. The global epidemiology of non-insulin dependent diabetes mellitus and the metabolic syndrome. *J. Diabetes Compli*. **92:** 1-21, 1997.

APPENDIX A: List of Publications

Chakraborty BN, Chakraborty U, Barman S and Roy S. Effect of different substrates and casing materials on growth and yield of *Calocybe indica* (P&C) in North Bengal, India. *J Appl Nat. Sci.* **8 (2):** 683 - 690 (2016)

Roy S, Chakraborty BN, Barman S and Chakraborty U. Production of *Pleurotus ostreatus* Grown in Different Substrates and Evaluation of Spent Substrate as Organic Manure for Growth Improvement of *Capsicum chinense* Jacq. *J. Mycol Plant Pathol*, **45(3):** 267-272; 2015.

Roy S, Barman S, Chakraborty U and Chakraborty BN. Evaluation of Spent Mushroom Substrate as biofertilizer for growth improvement of *Capsicum annuum* L. *J Appl Biol Biotechnol* **3 (3):**022-027, 2015.

Roy S, Barman S, Chakraborty U and Chakraborty BN. Cultivation of *Pleurotus djamor* - a new species of oyster mushroom in North Bengal. *J. Mycopathol. Res.* **53(1)**: 59-63, 2015.

Barman S, Roy S, U. Chakraborty and Chakraborty BN. Cultivation practices of *Calocybe indica* (P & C) and use of spent mushroom substrate for leafy vegetables in North Bengal. *Global J Biosci Biotechnol*.**4(1):** 74-80, 2015.

Roy S, Barman S, Chakraborty U and Chakraborty BN. Development of an easy and efficient technique for cultivation of different species of *Pleurotus*. *NBU J Pl Sci.* **8(1):**13-18; 2014.

Roy S, Sunar K, De U and Chakraborty BN. Influence of Selective Bioresources on Seedling Vigour and Growth of *Cicer arietinum* L. in Field Conditions. *Adv. Crop Sci*. **3(10):** 663-670, 2013.

APPENDIX B: List of Abbreviations

APS- Ammonium per sulphate
BLAST- Basic local alignment search tool
BSA- Bovine serum albumin
BSS-2- Biclonal seed stock- 2
$Ca_3(PO_4)_2$ - Tri-calcium phosphate
$CaHPO_4$ - Calcium phosphate
CAS- Chrome azurol S
CAT- Catalase
CDA- Chitinase detection agar
cfu- Colony forming unit
CHT- Chitinase
$CuSO_4$- Copper sulphate
DAC-ELISA- Direct antigen coating- Enzyme linked immune-sorbent assay
DAPG- 2,4-Diacetylphloroglucinol
dATP- Deoxy adenosine tri-phosphate
dCTP- Deoxy cytosine tri-phosphate
DEAE cellulose- diethyl aminoethyl cellulose
dGTP- Deoxy guanosine tri-phosphate
DMAB- Di methyl amino benzaldehyde
DNA- Deoxyribonucleic acid
dNTPs- Deoxy nucleotide tri-phosphates
DNSA- Dinitro salicylic acid
dTTP- Deoxy thymidine tri- phosphate
EDTA- Ethylene diamine tetra acetic acid
g- gram
Gfp- Green fluorescent protein
HCl- Hydrochloric acid
HCN- Hydrocyanic acid
HPLC- High performance liquid chromatography
MEGA 4- Molecular Evolutionary Genetics Analysis 4
mg- Mili gram
ml- Mili litre
Na_2CO_3- Sodium carbonate
NaN_3- Sodium azide
$NaNO_2$- Sodium nitrite
NaOH- Sodium Hydroxide
NB- Nutrient Broth
NCBI- National Center for Biotechnology Information
NCM- Nitrocellulose membrane
NH_4Cl- Ammonium chloride
PAL- Phenylalanine ammonia lyase
PBS-Tween- Phosphate buffer saline- Tween
PCA- Phenazine-1-carboxylic acid
PCI- Water saturated phenol: Chloroform: Isoamyl alcohol
PCR- Polymerase chain reaction
SDS- Sodium dodecyl sulphate
SDS-PAGE- Sodium dodecyl sulphate- Poly-acrylamide gel electrophoresis
STZ- Streptozotocin

TE buffer- Tris-EDTA buffer
TEMED- N,N,N',N'-Tetramethylethylenediamine
Tris Hcl- Tris hydrochloric acid
TV- Toklai variety
UP- Upasi variety
UPGMA- Unweighted Pair Group Method with Arithmetic Mean
yr- Year

APPENDIX C: List of Chemicals

Ammonium chloride
Ammonium per sulphate
Bovine serum albumin
Calcium phosphate
Calcium carbonate
Calcium Sulphate
Carboxy methyl cellulose
Chrome azurol S
Colloidal chitin
Copper sulphate
Deoxy nucleotide tri-phosphates
Di methyl amino benzaldehyde
Di sodium hydrogen phosphate
Diethyl aminoethyl cellulose
Dinitro salicylic acid
Ethylene diamine tetra acetic acid
Ferric chloride
Fluorescein isothiocyanate
Helicase (3%)
Hexa-decytrimethyl ammonium bromide
Hydrochloric acid
Hydrocyanic acid
Hydrogen peroxide
Indole acetic acid
O-dianisidine (5 mg/ml methanol)
p- nitrophenyl phosphate
Phosphate buffer saline- Tween
Poly vinyl- pyrollidone
Potassium chloride
Sodium azide
Sodium carbonate
Sodium dodecyl sulphate
Sodium Hydroxide
Sodium molybdate
Sodium nitrite
Streptozotocin
Sulphuric acid
Tri-calcium phosphate
Tris Acetic Acid and EDTA buffer
Tris hydrochloric acid
Tris-EDTA buffer
Water saturated phenol: Chloroform: Isoamyl alcohol
0.3mM borate buffer (pH 8.0)
1 M K-PO4 buffer (pH 7.1)
1M Na-acetate buffer (pH 4)
2 mM β- mercaptoethanol
1 M Na-borate buffer (pH 9.8)
30%-90% absolute alcohol

Research Article

Production of *Pleurotus ostreatus* Grown in Different Substrates and Evaluation of Spent Substrate as Organic Manure for Growth Improvement of *Capsicum chinense* Jacq.

Somnath Roy, Shibu Barman, Usha Chakraborty and Bishwanath Chakraborty

Immuno Phytopathology Laboratory, Department of Botany, University of North Bengal, Siliguri-734013, Darjeeling, West Bengal, India Email: bncnbu@gmail.com

Abstract

Pleurotus ostreatus is one of the most popular oyster mushrooms largely cultivated in North Bengal. Paddy straw and wheat straw is very commonly found in this area and thus it is being used as the main substrates for the cultivation and some time saw dust is also used as an added supplement. Different combinations of substrates are assessed for their effect on the growth, yield and it was found that the paddy straw and wheat straw combined substrates showed faster growth and higher yield in comparison to the other substrates. Spent mushroom substrates of different combinations were further applied on *Capsicum chinense* a variety of chili commonly grown in the hill region of North Bengal. The application of spent mushroom substrate showed significantly better result in terms of growth and yield. It enhances the uptake of soil phosphate to leaf through root. Spent mushroom substrate of paddy straw with saw dust showed better result than the other substrates in increasing the chlorophyll content of leaves. Spent mushroom substrate also helps to increase the total protein content as well as the carotenoid content in fruit.

Key words: *Capsicum chinense, Pleurotus ostreatus*, Spent mushroom substrate (SMS).

Citation: Roy S, Barman S, Chakraborty U and Chakraborty BN. 2015. Production of *Pleurotus ostreatus* Grown in different substrates and evaluation of spent substrate as organic manure for growth improvement of *Capsicum chinense* Jacq. *J Mycol Pl Pathol.* 45 (3): 267-272

Oyster mushroom (*Pleurotus* sp) is commonly called 'Dhingri' in India because of its oyster shell-like structure. The genus *Pleurotus* belongs to the family Tricholomataceae cultivating in different parts of India. Different species of *Pleurotus* can be grown in different temperature and thus it can be cultivated throughout the year. In recent times, *Pleurotus* sp is the second largest cultivated mushroom in the world (Erkel 1992; Chang and Miles 1991). *Pleurotus ostreatus* can be cultivated using a wide range of lignocellulosic substances in different parts of the world depending upon their availability and being cheaper in respective region (Royse 1985; Schmidt 1986). The cultivation of *Pleurotus* mushroom offers one of the most feasible and economic method for bio-conservation of agro-lignocellulosic wastes (Ano et al 1993; Cohen et al 2002).

Capsicum chinense belongs to the family Solanaceae widely known for its pungent taste and strong aroma. Locally known as "Dalle Khorsani", it is very common in the North -Eastern part of India and Nepal. These are red rather smooth without clear tip, looking more like tiny bell pepper that aggressively hot. The pods are valued for their high pungency and good flavour. But they are not used for specific recipes, they are eaten fresh or pickle in brine. Spent mushroom substrate (SMS) is often regarded as an agricultural waste product with little inherent value as reported by Nelson and Crafts (1996). Kadiri and Mustapha (2010) stated that the spent mushroom compost is a very good source of humus formation and humus provided to the plants with micro nutrients, for improving the soil aerations, soil water holding capacity and for maintaining the soil structure. Robbins et al (1986) reported that SMS is a valuable by-product of edible mushroom cultivation. It consists of partially degraded paddy or wheat straw, coconut husk, bagasse or other agricultural waste. After a few cultivation cycles, it is biochemically modified by fungal enzymes into a simpler form and enriched with protein. They also reported that the fresh and aged SMS has been applied to propagation of fruits, vegetables, flower and foliage crops. It is a rich source of carbon, nitrogen and other elements. Nitrogen content varies from 0.4-13.7 per cent with a C: N ratio of 9 to 15: 1 (Chorover et al 2000). Spent mushroom substrate has good physical properties which include the water holding capacity, soil pH, soil porosity salt content and also other properties. Addition of SMS will add great amount of macro nutrients like Nitrogen, Potassium and Phosphorus as reported by Kim et al (2011). After about three flushes of mushroom have been harvested, the growing materials are removed from the growing beds as spent mushroom compost. Storage or transportation of this material lead to environment contamination and cost for mushroom growers. The rich organic matter, moderate nutrient load, near neutral pH and presence of beneficial microbial

population make SMS as the suitable organic waste for its conversion in to quality manure for crops (Pill et al 1993). Ahlawat et al (2010, 2011) stated that *t*he SMS has potential to bioremediate several agricultural grade fungicides and pesticides. After suitable pre-treatment spent mushroom substrate can completely or partially substitute the growing media for cultivation of different economically important horticultural crops (Kaddous and Morgans 1986).According to Hyakumachi (1994), there are other fungi which promote plant growth upon root colonization and functionally designated as plant growth promoting fungi (PGPF). There are also ectomycorrhizal and ectotrophic associations between fungi and plants. They are beneficial to several crop plants not only by promoting growth but also by protecting them from diseases. The present study is to evaluate the effect of spent mushroom substrate of oyster mushroom as soil fertilizer on the growth improvement of *Capsicum chinense*.

Materials and Methods

Preparation of spawn. Spawn was prepared using wheat grains. Wheat grains were boiled for 20 min and the water drained off. Then it was allowed to dry after which 0.5 per cent (w/w) $CaCO_3$ and 2 per cent (w/w) $CaSO_4$ were added and mixed well. The grains (200g) were filled in each polypropylene bag and it was autoclaved at 121 C applying 20ps pressure for 1 hr. The grains were inoculated with actively growing mycelium of the *Pleurotus ostreatus* from PDA slant and incubated at 25-28 C for 14 days until the mycelium fully covered the grains (Sanchez 2010).

Cultivation of mushroom. Different substrates were used for the cultivation of oyster mushroom. Substrates were used either singly or in combination with other substrate. Paddy straw, wheat straw and sawdust were used. The experiment was conducted in different combinations of substrate such as (A) paddy straw, (B) wheat straw, (C) paddy straw + wheat straw and (D) paddy straw + saw dust. Substrates were washed and soaked in water for overnight. Then they were again cleaned and pasteurized at 55-65 C for 20-30 min and allowed to cool at room temperature. Spawning was done using the polypropylene bags. Spawning has been done @100 g kg^{-1} of substrate. The cylinders were then incubated at room temperature (20-30 C) for 10-12 days. After 10-12 days, when white mycelia covered the whole substrate the plastic was removed from the cylinders and 80-90 per cent moisture was maintained by spraying water for 2-3 times in a day for the initiation of pinhead (Sarker et al 2007). The pinheads appeared after 4-5 days of opening the bags. Fruiting body was developed at room temperature and 80-90 per cent relative humidity. The fruiting body was harvested from the base carefully without causing any injury to the mycelia. Humidity was maintained again after the harvest for further flushes.

Application of spent mushroom substrates. The spent mushroom substrates were dried completely under sunlight and mixed with the soil for experiment. In this experiment, treatment was done using the spent mushroom substrate of oyster mushroom with soil (250 g kg^{-1} soil) and a control pot without any treatments. No other organic or inorganic fertilizers were used in the growing media. Seedlings of *Capsicum chinense* were collected from the local market and seedlings were then transferred to the pre-treated pots.

Plant material and planting. Seedlings (15 cm height) were planted directly in untreated and the treated soil in potted condition. The pot diameter was 36 cm and equal amount of soil was used to prepare the bed. Five seedlings of *Capsicum chinense* were planted in each pot. The experiment was carried out in natural condition at the Department of Botany, University of North Bengal.

Evaluation of growth promotion of plant. Data on growth parameters- plant height, number of leaves, and length of leaves, diameter of leaves were recorded in every 7 days intervals after the transfer of plants. Data was also collected during the flowering and fruiting period.

Extraction and estimation of phosphate content. Total phosphate content of soil, root and leaves were done following the methodology. Soil samples (1g, air dried) or plant materials (1g, oven dried) were suspended in 25 ml of the extracting solution (0.025 N H_2SO_4, 0.05N HCl) to which activated charcoal (0.01 g) was also added, shaken well for 30 min on a rotary shaker and filtered through Whatman No 2 filter paper. Quantitative estimation of phosphate was carried out following ammonium molybdate-ascorbic acid method.

Determination of total protein. Protein was extracted from the mushroom using phosphate buffer (pH 7.2) and protein content was determined using BSA as standard.

Extraction and estimation of chlorophyll content. Total chlorophyll of plant leaf was estimated following the methodology as described by Harborne (1973). One gram of leaf sample was grinded using 10 ml of 80 per cent acetone and then it was filtrated using the Whatman No 1 filter paper. The absorbance was taken spectrophotometrically at 663 nm and 645 nm.

Extraction and estimation of carotenoid. One gram of fruit was grinded in dark using 10 ml methanol and then it was filtrated by Whatman No1 paper and used as crude sample for estimation. The absorbance was taken at 480 nm, 645nm and 663nm. Carotenoid content was estimated by the following formula

Total carotenoid = $[A_{480} - (0.114 \text{ X } A_{664}) - (0.638 \text{ X } A_{645})]$ µg g^{-1} tissue

Results and Discussion

Pleurotus ostreatus was cultivated using the different substrates (Fig. 1) and it was observed that the maximum yield was observed in case of saw dust mixed with paddy straw substrate. Besides it was also observed that in case of combined substrate of paddy straw and saw dust, it took less time for initiation of fruiting body (Table 1). It was reported that the use of different substrates for cultivation of *Pleurotus* sp affects the growth of the mycelia, initiation of fruiting body amount of production (Mane et al 2007).

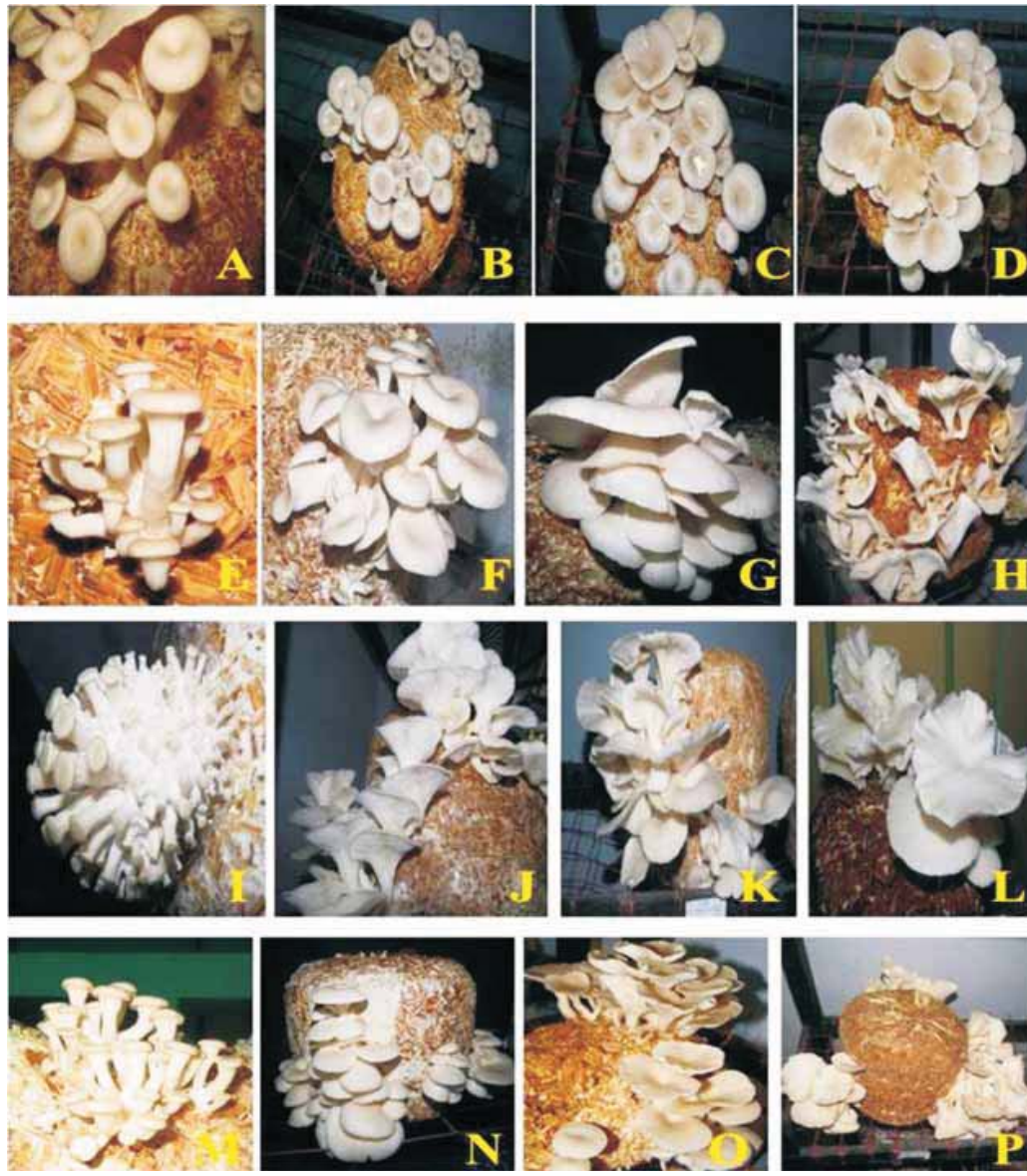


Figure 1. Different stages of growth of *Pleurotus ostreatus* in different substrates; (A-D) Paddy straw, (E-H) Wheat straw, (I-L) Paddy straw + Wheat straw and (M-P) Paddy straw + Saw dust

Table 1. Effect of different substrates on yield of *Pleurotus ostreatus*

Substrate	Fruiting body initiation (days)	Production per bag (g)			Total production (g)
		1st flush	2nd flush	3rd flush	
Paddy Straw	12	250	120	70	440
Wheat Straw	14	200	95	60	355
Paddy straw + Wheat straw	14	275	100	75	450
Paddy straw +Saw dust	10	350	140	90	580

Table 2. Comparison of growth of *Capsicum chinense* grown in spent substrate of *Pleurotus ostreatus* and in untreated soil

Treatment	Height (cm)	No of leaf	Average Leaf size (cm)		Flowering (days)	Yield/plant (g)
			Length	Diameter		
Paddy straw	64.0	35	18.0	8.8	48	30
Paddy straw + Saw dust	64.5	37	20.0	9.0	45	37
Wheat straw	56.0	30	17.5	9.0	54	45
Paddy straw + wheat straw	60.0	35	17.0	7.5	51	42
Control (soil)	21.0	12	7.0	4.5	57	20

Effect of SMS on growth of plants. Spent mushroom substrate of oyster mushroom was tested of their effect on growth promotion of *Capsicum chinense* in potted condition. Spent mushroom were used directly in the soil at 250 g kg^{-1} soil ratio and the growth promotion in terms of height, number of leaves, leaf size and yield were evaluated. The results revealed that all the treated plants showed significant increase in height in comparison to control after 7 days of planting (Fig. 2). After 49 days of interval, the height, number of leaves was showed significant result. It was observed that flowering started after 25 days in case of treated plants while it started 35 days in case of control plants. Size of the leaves also showed very wide variation in comparison to the control plants. In case of treated plants, leaf length ranges from 17 - 20 cm while it is ranges from 7-12.5 cm in case of control plants. Leaf diameter also increased in case of treated plants (7.5-9.5 cm) while it is lower in control plants (2.5-4.5 cm). Final yield also was determined and it was found that both the size and number of fruits was higher in the treated plants (Table 2).

Mobilization of soil phosphate after treatment were evaluated in terms of total phosphate content in soil, roots and leaves of the treated plants in comparison to the control sets. The uptake of phosphate content was significantly increased in spent mushroom substrate treated plants while it shows lower uptake of soil phosphate to the root and leaf in case of control plants (Table 3). Spent mushroom substrate improves the soil quality by having a direct influence on the uptake of phosphate content and thus, aeration and water movements in addition to increasing availability of insoluble sources of phosphorus has also been reported

Figure 2. Effect of spent mushroom substrates on the growth of *Capsicum chinense* (A) Control after 15 days of germination (B) Treated with spent substrate after 15 days of germination (C) Control after 45 days; (D) Treated plant after 45 days (E) flower, (F) Developmental stages of fruit

Table 3. Effect of spent mushroom substrate on total phosphate content of soil, root and leaf of *Capsicum chinense* after 15 days of seedling transfer

Treatment	Total Phosphate Content (µg g^{-1})		
	Soil	Root	Leaf
Control	51.30 ± 2.30	9.12 ± 1.33	7.67 ± 0.83
Paddy straw	44.51 ± 1.31	11.53 ± 0.78	8.47 ± 1.22
Paddy straw + Saw dust	42.50 ± 3.45	15.45 ± 1.30	13.35 ± 1.42
Wheat straw	45.65 ± 1.15	14.25 ± 1.41	10.44 ± 1.22
Paddy straw + wheat straw	40.35 ± 0.83	11.75 ± 1.21	9.33 ± 1.10

by Rillig et al (2002) and Smith and Read (1997). Chlorophyll is the main photosynthetic pigment and it was observed that the use of these spent mushroom substrates affects in the total chlorophyll content of the plant leaves. Chlorophyll content (mg g^{-1} tissue) includes the chlorophyll a and chlorophyll b which significantly increased in the plants treated with spent mushroom substrate of oyster mushroom (Fig. 3).

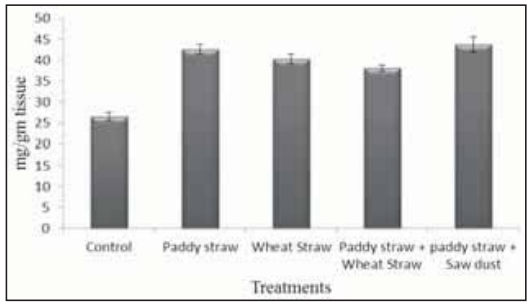


Figure 3. Effect of spent mushroom substrate of oyster mushroom on chlorophyll content of *C. chinense*

Total protein content of leaf and fruit was estimated and it was observed that the total protein content was quite higher in case of leaf of treated plants it is quite higher 37 mg g^{-1} tissue while in case of leaf of control plants, it was lower *i.e.*, 30 mg g^{-1} tissue. Similarly, total protein content of fruit of the treated plants was higher (39 mg g^{-1} tissue) while it lower in case of control plants (32 mg g^{-1} tissue) (Fig. 4).

Carotenoid is one of the most important compounds which show antioxidant activity and this was also estimated to evaluate the effect of spent oyster mushroom substrate on the enhancement of antioxidant compound. The results revealed that the application of spent substrate enhances the carotenoid as the leaf and fruit of the treated plants showed significant higher amount of carotenoid in comparison to the control plants. Howard et al (2000) and Wall et al (2001) reported that mature pepper fruits are also rich in carotenoid which shows antioxidant and anti-

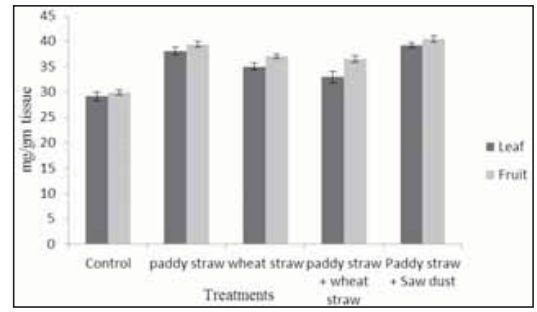


Figure 4. Total protein content of fruit of *C. chinense* treated with spent mushroom substrates

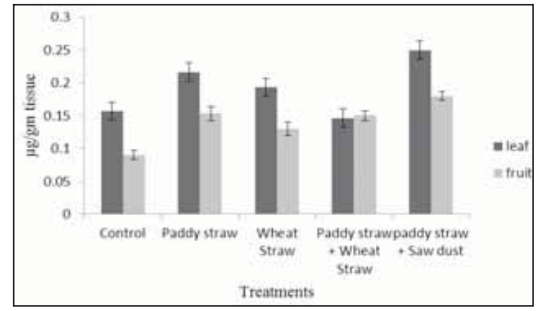


Figure 5. Effect of spent mushroom substrate of oyster mushroom on the carotenoid of *C. chinense*

carcinogenic capacity; furthermore, immature or mature fruits contain a high concentration of antioxidant phenolic compounds. The results also shows that the effect different substrates influence the level of carotenoid compound and it was observed that the combined treatment of spent mushroom substrate of saw dust and paddy straw showed maximum enhancement of carotenoid compound followed by the spent mushroom substrate of paddy straw (Fig. 5).

Therefore from the above study it can be concluded that *Pleurotus ostreatus* can be cultivated in different agricultural substrates. Saw dust can be used as a supplement with paddy and wheat straw to increase the yield of oyster mushroom. The effect of spent mushroom substrate on the cultivation of *C. chinense* also showed

better result in comparison to untreated plants and thus it can be used as an organic fertilizer for the improvement of growth of *C. chinense*.

Acknowledgement

Financial support received from the University Grants Commission (UGC), and Department of Biotechnology, Ministry of Science and Technology, Government of India is acknowledged.

References

Ahlawat OP, Gupta P, Kumar S and Sharma DK. 2010. Bioremediation of fungicides by spent mushroom substrate and its associated micro flora. *Ind J Microbiol* 50: 390-395.

Ahlawat OP, Manikandan K, Sagar MP, Rai D and Vijai B. 2011. Effect of composted button mushroom spent substrate on yield, quality and disease incidence of Pea (*Pisum sativum*). *Mush Res* 20: 87-94.

Ano Z, Sashirekha MN and Rajarathnam S. 1993. Improvement of the bioconversion and biotransformation efficiencies of the oyster mushroom (*Pleurotus sajor-caju*) by supplementation of its rice straw with oilseed cakes. *Enz Microbol Tech* 15:985-989.

Chang ST and Miles PG. 1991. Recent trends in World Production of Cultivated Edible Mushrooms. *Mush J* 503: 15-18.

Chorover RH, Fox H and Romaine CP. 2000. Final Research Project Report–Mushroom Industry Farmer-based Applied Research Program (MIFBAR) (The Pennsylvania State University) 87p.

Cohen R, Persky L and Hadar Y. 2002. Biotechnological applications and potential of wood degrading mushrooms of the genus *Pleurotus*. *Appl Microbiol Biotech* 58: 582-594.

Erkel I. 1992. Effects of different growing medium on yield of *Pleurotus ostreatus* and *Pleurotus florida* cultivation. *Fourth Congress of Edible Mushroom of Turkey*, 1:8.

Harborne JB. 1973. Phytochemical Methods. Champman and Hall Ltd. London. pp.204.

Howard LR, Talcott ST, Brenes CH and Villalon B. 2000. Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* species) as influenced by maturity. *J Agri Food Chem* 48:1713–1720.

Hyakumachi M. 1994. Plant growth promoting fungi from turf grass rhizosphere with potential for disease suppression. *Soil Microorg* 44: 53-68.

Kaddous FGA and Morgan AS. 1986. Spent mushroom compost and deep litter fowl manure as a soil ameliorant for vegetables. Surface soil management. Proceedings New Zealand Society of Soil Science–Australian Society of Soil Science Joint Conference. Rotorua. Forest Research Institute, New Zealand, 138–147.

Kadiri AN and Mustapha D. 2010. The use of spent mushroom substrate of *Agaricus bisporus* as a soil conditioner for vegetables. *J Ind Soc Soil Sci* 40: 518-519.

Kim YL, Cho WM, Hong SK, Oh YK and Kwak WS. 2011. Yield nutrient characteristics Ruminal Solubility and Degradability of spent (*Agaricus bisporus*) substrate of Ruminants. *J Mycol Pl Pathol* 24: 1560-1560.

Mane VP, Patil SS, Syed AA and Baig MMV. 2007. Bioconversion of low quality lignocellulosic agricultural waste into edible protein by *Pleurotus sajor-caju* (Fr.) Singer. *J Zhejiang Univ Sci B* 8: 745-751.

Nelson JS and Crafts C. 1996. Handling and use of spent mushroom substrate *Fusarium oxysporum* (wilt of fenugreek). *J Mycol Pl Pathol* 53: 107-108.

Pill WG, Evans TA and Garrison SA. 1993. Forcing white asparagus in various substrates under cool and warm regimes. *Hort Sci* 28: 996-998.

Rillig MC, Wright SF and Eviner VT. 2002. *Pl Soil Sci* 238: 325-333.

Robbins SH, Righetti TL, Fallahi E, Dixon AR and Chaplin MH. 1986. Influence of trenching, soil amendments, and mulching on the mineral content, growth, yield and quality of "Italian" prunes. *Commun Soil Sci Pl Anal.* 17:457-471.

Royse DJ. 1985. Effect of spawn run time and substrate nutrition on yield and size of shiitake mushroom. *Mycologia* 75: 756-762.

Sanchez C. 2010. Cultivation of *Pleurotus ostreatus* and other edible mushrooms. *Appl Microbio Biotech* 85: 1321-1337.

Sarker NC, Hossain MM, Sultana N, Mian IH, Karim AJMS and Amin SMR. 2007. Performance of different substrates on the growth and yield of *Pleurotus ostreatus* (Jacquin ex Fr.) Kummer.*Bangl J Mush* 1: 9-20.

Schmidt O. 1986. Experiments with Mushroom cultivation on Wood Waste. *Pl Res Dev* 24: 85-92.

Smith SE and Read DJ. 1997. Mycorrhizal symbiosis (London: Academic Press).

Wall MM, Waddell CA and Bosland PW. 2001. Variation in beta-carotene and total carotenoid content in fruits of *Capsicum*. *Hort Sci* 36:746–749.

Received: 13 May 2015 Accepted : 30 Sep 2015

J. Mycopathol. Res. ***53(1)*** *: 59-63, 2015;* (ISSN 0971-3719)

Cultivation of *Pleurotus djamor* - a new species of oyster mushroom in North Bengal

SOMNATH ROY, SHIBU BARMAN, USHA CHAKRABORTY AND BISHWANATH CHAKRABORTY[1]
Immuno-Phytopathology Laboratory, Department of Botany, University of North Bengal, Siliguri 734 013, West Bengal

Received : 16.06.2014 Accepted : 15.12.2014 Published : 27.04.2015

Oyster mushroom is one of the most popular mushrooms cultivated in North Bengal and a large number of mushroom growers are cultivating oyster mushrooms. There are only three species of oyster mushrooms i.e. *Pleurotusostreatus, P. sajor-caju*and *P. florida* have been cultivated widely in this region. However, *Pleurotusdjamor* is a new introduction in North Bengal, which is commonly cultivated in North Western part of India. Cultivation of this mushroom showed significant growth and yield in plastic bag as well as in bottles. Nutritional parameters were assessed and it was observed that it showed very good amount of protein (145-275 mg/g tissue), reducing sugar which ranges from 20-56 mg/g tissue and also showed a good amount of total sugar (100-260 mg/g tissue) in both bag and bottle cultivation.

Key words: *Pleurotus djamor,* oyster mushroom, edible mushroom

INTRODUCTION

Pleurotus species, commonly known as Oyster mushrooms are edible fungi cultivated worldwide especially in south east Asia, India, Europe and Africa. The Oyster mushroom (*Pleurotusostreatus*) is an edible mushroom having excellent flavour and taste. Although mushrooms are often grouped with vegetables and fruits, they are actually fungi. They are macro-fungi which belong either to Basidiomycotina or Ascomycotina and they are very distinct from plants, animals and bacteria (Mushigeni and Chang, 2001). Bioconversion of lignocellulosic residues through cultivation of *Pleurotus* species offers the opportunity to utilize renewable resources in the production of edible, protein-rich food that will sustain food security for people in developing countries (Sanchez *et. al.,* 2002). Mushrooms are valuable health foods which are low in calories and provide essential minerals (Weinheim, 2006). It contains high amount of proteins, fibers, vitamins, minerals and low amount of calorie and cholesterol (Pathak *et. al.,* 1998). It enables us to obtain substrate materials at low prices or even for free and to conserve our surroundings by recycling wastes (Khan *et. al.,* 2012). Mushrooms with their flavour, texture, nutritional value and high productivity per unit area have been identified as an excellent food source to alleviate malnutrition in developing countries

[1]Email : bncnbu@gmail.com

(Eswaran and Ramabadran, 2000).

Pleurotus species are efficient lignin degraders which can grow on wide variety of agricultural wastes with broad adaptability to varied agro-climatic conditions (Jandaik and Goyal, 1995). *Pleurotus* species are rich source of proteins, minerals (Ca, P, Fe, K and Na) and vitamin C, B complex (thiamine, riboflavin, folic acid and niacin) (Çağlarýrmak, 2007). They are consumed for their nutritive as well as medicinal values (Agrahar-Murugkar and Subbulakshmi, 2005). Mushroom protein is intermediate between that of animals and vegetables (Kurtzman, 1976) and is of superior quality because of the presence of all the essential amino acids (Purkayastha and Nayak, 1981).

MATERIALS AND METHODS

Maintenance of mother culture

Mother culture of the fungal mycelia was collected from the Directorate of Mushroom Research (ICAR) Solan and maintained in Potato Dextrose Agar medium and sub-culturing the same following the method suggested by Fritsch (1978).

Preparation of Spawn

Wheat grains are used for the preparation of spawn. Wheat grains were boiled in for 20 min and the water drained off. Then it was allowed to dry for overnight in a clean place after which 0.5% (w/w) $CaCO_3$ and 2% (w/w) $CaSO_4$ were added and mixed well. The grains (200 g) were filled in each polypropylene bag and it was autoclaved at 121^{o}C for 1 h. The grains were inoculated with actively growing mycelium of the *Pleurotus djamor* from PDA slant and incubated at 25-28^{o}C for mycelial growth for 14 days until the mycelium fully covered the grains. Completely covering the grains with mycelium rapidly colonizes the bulk growing substrate (Sánchez, 2010).

Cultivation of mushroom
Substrate preparation

Paddy straw was used for the cultivation of oyster mushroom. Chopped (2-4 cm long) rice straw was washed and soaked in water for overnight. The straw was again cleaned and pasteurized at 55-65^{o}C for 30 min. then it was allowed for cooling at room temperature. Spawning was done using the polypropylene bags.

Spawning

Layer spawning was done using the cooled pasteurized straw. 100 g of spawn was used for 1kg of substrate for spawning. The bottles and bags were then filled using the substrate and spawn and the bags were closed tight and the bottles were closed using the lid. Small holes were made in each bottle and bag for aeration. The bags and bottles were then incubated at room temperature (20-30^{0} C) for 10-12 days. After 10-12 days, the white mycelia covered the whole substrate. Then the plastic was removed and the lid of the bottles was opened. 80-90% moisture was maintained by spraying water on the substrate for 2-3 times in a day for the initiation of pinhead (Sarker*et. al.,* 2007).

Harvesting of fruiting body

The pinhead appeared after 4-5 days of opening the bags and the lids. Fruiting body was developed at room temperature and 80-90% relative humidity.

Extraction and estimation of soluble protein content

Protein was extracted from the mushroom using phosphate buffer (pH 7.2) and protein content was determined.

Extraction and estimation of sugar

One g of fresh mushroom tissue was crushed with 95% ethanol and the alcoholic fraction was evaporated in boiling water bath. Then the fraction was collected and the volume made up to 5 ml using distilled water. Then it was centrifuged at 10,000 rpm for 15 min and the supernatant was collected for estimation.

Total sugar

One ml of extracted sample was taken and 4 ml of Anthrone's reagent was added and incubated on boiling water bath for 10 min. Then it was cooled down in tap water and observed at 620 nm in colorimeter.

Reducing sugar

Ethanol (80%) extract was used for estimating the reducing sugar. Extract (2 ml) was mixed with 2 ml of Alkaline copper tartrate and boiling was done.

Determination of reducing sugar using Arsenomolybdate was carried out at 620 nm.

RESULTS AND DISCUSSION

Cultivation of Pink oyster mushroom was done in winter season and it was observed that the optimum temperature of its growth is about 15-18^{o}C and it requires 70-80% relative humidity for its mycelial growth. It was observed that the mushroom pileus is thinner as compared to other species, leathery in texture and looks like a pink queen on the beds (Fig. 1). The pileus is up to 7.5-10cm in diameter, with little or no stipe and pileus thickness is 7.5-10 at the outer edges. Outer border on pileus top is pink; gills on the lower side are pinkish too. This mushroom is not fleshy as compared to other species of oyster mushrooms as reported by Dhar *et.al.* (2011).

Total yield was estimated on the basis of 1kg of substrate used for cultivation and it was observed that the highest amount harvested in three flushes is about 340 g when cultivated in bag. In bottle cultivation it was found that the highest yield was 400 g (Table 1). Cultivation of oyster mushroom using waste bottle is a new introduction in North Bengal and showed a significant result. Using of waste plastic bottles is a sustainable technique for the mushroom cultivation and it was observed that the rate of contamination is being reduced. Using waste chemical bottles and other waste bottles of water and cold drinks could be another way to recycle the waste bottles in a good way and it reduces the cost of plastic bags. Hence the plastic bottles are very promising in the cultivation of oyster mushroom.

The data in Table 2 show the protein content of different stages of the mushroom and it was observed that the total protein content was higher in pinhead stage (235 mg/g tissue) while it was quite lower in mature stipe (145 mg/g tissue). Breene (1990) reported that the total protein content of oyster mushroom ranged between 190-300 mg / g tissue. On the other hand, total sugar and reducing sugar of the different stages were done and it was observed that the total sugar was higher in young pileus stage (260 mg/g tissue) and lower in case of pin head stage (100 mg/g tissue) and it was also reported by Patil*et. al.,* (2008). In case of reducing sugar, it was observed that the mature pileus contained lower amount of reducing sugar (20 mg/g tissue) while young stipe (56mg/g tissue) and pinhead (50 mg/ g tissue) showing significant amount of reducing sugar.

Table 1 : Yield of Pink oyster mushroom cultivated in bags as well as in bottles

No of container	Flush	Amount of production (g)			
		Bag	Total production	Bottle	Total production
1	1st Flush	100	160	200	400
	2nd Flush	40		110	
	3rd Flush	20		90	
2	1st Flush	150	280	150	310
	2nd Flush	100		100	
	3rd Flush	30		60	
3	1st Flush	100	205	150	165
	2nd Flush	75		90	
	3rd Flush	30		25	
4	1st Flush	180	340	100	195
	2nd Flush	100		75	
	3rd Flush	60		20	
5	1st Flush	150	285	100	205
	2nd Flush	90		75	
	3rd Flush	45		30	

Fig.1 : Cultivation of *Pleurotus djamor* in plastic bag.(A): Pinhead stage,(B-D): Young stage,(E&F): Mature stage,(G-J): Grown in plastic bottles

Table 2 : Total protein, reducing sugar and total sugar content of *P. djamor*

Stages of mushrooms	Content (mg/g tissue)		
	Total protein	Reducing sugar	Total sugar
Pin head	235	50	100
Young Stipe	275	56	110
Young Pileus	200	40	155
Mature Stipe	145	25	145
Mature Pileus	230	20	120

In conclusion, it can be stated that the cultivation of Pink oyster mushroom (*P. djamor*) is a new introduction in North Bengal and it can be cultivated in the prevailing environment easily. In our study, it is also very clear that the species *P. djamor* can be cultivated inplastic bottles as well as in plastic bags. The optimum temperature for its growth ranges from 18-20^{o}C which is common in North Bengal. From the above results it can be concluded that the chemical composition of pink oyster mushrooms determines their nutritional value and sensory properties. They differ according to the atmospheric conditions, age and part of the fructification. In our study we found that the nutritional value differs in different parts of the fructification. And it can be said that Pink oyster mushroom is very good for health and its cultivation is very fruitful for the mushroom growers in North Bengal.

ACKNOWLEDGEMENT

The author acknowledge the financial assistance received from the University Grants Commission

and Department of Biotechnology, Ministry of Science and Technology, Govt. of India.

REFERENCES

Agrahar-Murugkar, D., and Subbulakshmi, G. 2005. Nutritional value of edible wildmushrooms collected from the Khasi hills ofMeghalaya. *Food Chemistry,* **89:** 599-603.

Breene, W. 1990. Nutritional and medicinal value of speciality mushrooms. *Journal of Food Production,***53**: 883-894.

Çağlarýrmak, N. 2007.The nutrients of exoticmushrooms (*Lentinulaedodes*and *Pleurotus*species) and an estimated approach to the volatilecompounds.*Food Chemistry*, **105:** 1188–1194.

Dhar, B.L., Shrivastava, N., Himanshu, Kumar, J., Tyagi, S. and Atrey, P. 2011. Cultivated edible specialty mushrooms- scope in India and EU countries. *Proceedings of the 7th International conference on Mushroom Biology and Mushroom Products*, pp.537-547.

Eswaran, A. and Ramabadran,R. 2000. Studies on somephysiological, cultural and post-harvest aspects of oystermushroom, *Pleurotuseous.Trop. Agri. Res*., **12:** 360-374.

Fritsch, G. 1978.Breeding work in Chang, ST. and Hays, WA. Eds. The biology and cultivation of edible mushrooms.*Academicpress*, New York.pp. 239- 248**.**

Jandaik, C. L., and Goyal, S. P. 1995. Farm and farming of oyster mushroom (*Pleurotus*sp). In: Mushroom Production Technology (Eds. Singh, R.P. and Chaube, H. S.). G. B. Pant Univ. Agril. and Tech., Pantnagar India, pp.72-78.

Khan, N.A., Ajmal, M.,Inam-ul-Haq, M., Javed, N., Ali,M.A., Binyamin, R.and Khan,S.A. 2012. Impact of sawdusts ofdifferent woods for effective cultivation of Oystermushroom I. *Pak. J. Bot.*, **44:** 399-402.

Kurtzman, R. H. 1976. Nitration of *Pleurotus sapidus* effects of lipid. *Mycologia*, **68:** 268-295.

Mushigeni, K.E. and Chang, T.S. 2001. Mushrooms: Theirbiology, nutritional and medicinal properties, cultivationtechnologies and perspectives on mushroom research anddevelopment. *Proceedings of the Mushroom FarmingTraining Workshop* held in Lilongwe, Malawi, pp. 74.

Pathak, V.N., Yadav, N. and Gour, M. 1998. Mushroomproduction and processing technique. *Agrobotanica*, pp. 2-3.

Patil, S.S., Kadam, R.M.,Shinde, S.L. and Deshmukh, S.A. 2008.Effect of different substrate on productivity and proximate composition of *P. florida*. *Int. J. Plant Sci.*; **3:** 151-153

Purkayastha, R. P. and Nayak, D. 1981. Analysis of Protein patterns of an Edible mushroom by Gel-Electrophoresis and its amino acid composition.*Journal Food Science and Technology,* **18:** 89-91.

Sanchez, A., Ysunza, F., Beltran-Gracia, M. and Esqueda,M.2002.Biodegradation of viticulture wastes by *Pleurotus*: asource of microbial and human food and its potential use inanimal feeding. *J. Agri. Food Chemistry*, **50:** 2537-2542.

Sánchez, C. 2010. Cultivation of *Pleurotusostreatus* and other edible mushrooms.*Appl. Microbiol. Biotechnol.***, 85:** 1321-1337.

Sarker, N.C., Hossain, M.M., Sultana, N., Mian, I.H., Karim, A.J.M.S. and Amin, S.M.R. 2007.Performance of different substrates on the growth and yield of *Pleurotusostreatus* (Jacquin ex Fr.) Kummer.*Bangl. J. Mush.*,**1:** 9-20.

Weinheim, K.G. 2006. *Oyster Mushroom*. John Wiley and SonsInc. New York.

Journal of Applied Biology & Biotechnology Vol. 3 (03), pp. 022-027, May-June, 2015
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2015.3305
ISSN: 2347-212X

Evaluation of Spent Mushroom Substrate as biofertilizer for growth improvement of *Capsicum annuum* L.

Somnath Roy, Shibu Barman, Usha Chakraborty and Bishwanath Chakraborty*

Immuno Phytopathology Laboratory, Department of Botany, University of North Bengal, Siliguri- 734013, West Bengal, India.

ARTICLE INFO

Article history:
Received on: 17/01/2015
Revised on: 24/02/2015
Accepted on: 16/04/2015
Available online: 20/05/2015

***Key words**:*
Capsicum annum, Chlorophyll, Plant growth, Spent Mushroom Substrate.

ABSTRACT

Influence of spent mushroom substrate (SMS) of oyster mushroom and button mushroom on the improvement of health status of *Capsicum annuum* L. was investigated. Analysis of growth promotion in terms of height, no of branches, yield and no of leaf drop indicated that the use of the spent mushroom substrate of oyster mushroom and spent compost of button mushroom had a positive effect on the overall growth of the tested plants. SMS had a role in mobilizing the soil phosphate which was evident by a decrease in soil phosphate level and increase in root and leaf phosphate following treatment with SMS. Chlorophyll content of plants increased when treated with oyster mushroom fresh substrate and button mushroom leachate compost. Fruits of plants treated with button mushroom leachate compost and oyster mushroom fresh substrate showed an increase in protein content of about 2.5 times over control. Similarly, carotenoid contents of fruits also increased significantly in the treated plants, but increases in leaves were not significant. It is evident from the present study that the use of different form of spent mushroom substrate of oyster mushroom and spent compost of button mushroom led to the overall increase in growth of *Capsicum annuum* L.

1. INTRODUCTION

After mushroom cultivation, the partially degraded paddy or wheat straw and other agricultural waste, which form as valuable by-products of edible mushroom cultivation, have been termed as Spent Mushroom Substrate (SMS). This SMS, which contains simpler form of protein rich component formed by modification of agricultural materials by the fungus after few cycles of cultivation, can be used as very good soil conditioners for the cultivation of fruits, vegetables flower and foliage crops [1]. Spent mushroom substrate is a good source of carbon, nitrogen and other elements. Nitrogen content varies from 0.4-13.7% with a C: N ratio of 9 to 15: 1 [2] which enhances the growth of plants. The mushroom production is increasing day by day and about 10 million metric tons of spent mushroom compost, a by-product of *Agaricus bisporus* is produced per year [3, 4]. After the mushroom harvest, the substrates have to be removed as storage of this spent substrate may cause environmental contamination. Using the spent mushroom compost as organic manure is one of the solutions to utilize the spent compost in a better way. The rich organic matter, moderate nutrient load, near neutral pH and presence of beneficial microbial population make SMC as a suitable organic waste for its conversion into quality manure for crops. It has been observed that the SMS has potential to bioremediate several agricultural grade fungicides and pesticides [5, 6, 7]. After suitable pre-treatment, spent mushroom substrate can completely or partially substitute the growing media for cultivation of different economically important horticultural crops [8, 9]. There are several methods of using the spent mushroom compost and weathering is one of them. Spent substrates can be spread on the land and allowed to weather for one or more years which allow to reduce the salt and nitrate contents of the spent materials. However, weathering alone is not sufficient and leaching is a better method for reducing salinity of the spent mushroom substrate. Leached spent compost has been reported to have less salinity than the weathered compost and most of the essential elements as well as the microbial properties remain the same as normal spent compost [10, 11, 12]. Thus spent mushroom substrate is considered to be a good source of organic matter and rich in macro and micro elements for plants, which help to increase the soil biological activity [13, 14]. It is also known that roots of most plant supports a wide range of fungal communities which colonize roots intra and intercellularly. Such fungi are known as arbuscular mycorrhizal fungi (AMF) [15]; besides, ectomycorrhizal and ectotrophic associations between fungi and plants [16] are also common. Another group of fungi which are commonly found in soil help in growth promotion upon root colonization are known as plant growth promoting fungi (PGPF) [17].

These are beneficial to several crop plants in respective of growth promotion as well as disease suppression [18]. The present study has been undertaken to evaluate the effect of spent mushroom substrate of different edible mushroom as biofertilizer on the growth and biochemical changes of *Capsicum annuum* L.

* *Corresponding Author*
Email: bncnbu [at] gmail.com

2. MATERIALS AND METHODS

2.1. Experimental design

Cultivation of *Pleurotus ostreatus* was done using the chopped paddy straw spawn and *Agaricus bisporus* on the pasteurized compost consisting of chopped paddy straw, wheat bran, poultry manure and gypsum. After a few cycles of production were completed, the spent mushroom substrate cylinders and the compost of button mushroom were dried completely under sunlight and used for experiment. In this experiment, six treatments were done using the spent mushroom substrate of oyster mushroom and button mushroom, and there was a control without any treatment. The treatments were: Control(only soil), T1=Soil + Oyster mushroom leachate (100ml/kg soil), T2=Soil + oyster mushroom substrate fresh (250gm/kg soil), T3= Soil + Button mushroom leachate (100ml/kg soil), T=4 Soil + Button mushroom spent compost fresh (250gm/ kg soil), T5= Soil + Weathered Spent oyster mushroom substrate (250gm/kg soil), T6= Soil + Weathered Button mushroom compost (250gm/kg soil), T7= Soil + Fresh oyster mushroom substrate + Fresh button mushroom spent compost (250gm/kg soil). The experiment was designed in a complete randomized design which was set up in 3 replicates. Pots were placed in open field condition and no other organic or inorganic fertilizer used in the growing media.Growth promotion was recorded in every 7 days intervals afterthe transfer of plants in terms of plant height, number of branches, leaf drop ratio and yield in comparison to the control sets.

2.2. Plant material

Hybrid seed of *Capsicum annuum* L. (Brand name Bullet) was collected from the local market, germinated in the germination tray at 25-27^0 C and the seedlings were allowed to grow in the tray for 15 days. The 15-day- old seedlings were then transferred to the pre-treated pots.

2.3. Extraction and quantification of phosphate from soil, roots and leaves

Soil samples (1g, air dried) or plant materials (1g, oven dried) were suspended in 25 ml of the extracting solution (0.025N H_2SO_4, 0.05N HCl) and activated charcoal (0.01 g) was also added. The extracted samples were shaken for 30 min in a rotary shaker and filtered through Whatman No. 2 filter paper. Quantitative estimation was carried out following ammonium molybdate-ascorbic acid method as described by Knudsen and Beegle [18].

2.4. Extraction and estimation of Chlorophyll content

For extraction of chlorophyll, 1g of leaf sample was ground using 10 ml of 80% acetone, it was filtered through Whatman No 1 filter paper. The absorbance was taken spectrophotometrically at 663 nm and 645 nm. Calculation of Chlorophyll a, Chlorophyll b and Total Chlorophyll was done following the methodology as described by Arnon [19].

2.5. Extraction and estimation of Carotenoid

1gm of fruit was grinded in dark using 10ml methanol and then it was filtrated by Whatman No1 paper and used as crude sample for estimation. The absorbance was taken at 480 nm, 645nm and 663nm. Carotenoid content was estimated by the following formula

Total carotenoid = $[A_{480} – (0.114 \text{ X } A_{664}) – (0.638 \text{ X } A_{645})]$ µg/gm tissue

2.5. Determination of total protein

Protein was extracted from the plant materials using Phosphate buffer (pH7.2) and protein content was determined following the methods as described by Lowry *et al.,* (1951) using BSA as standard.

3. RESULTS AND DISCUSSION

Spent mushroom substrate of Oyster mushroom and Compost of Button mushroom were tested for their effect on growth promotion of *Capsicum annuum* L. in potted conditions. Spent mushroom substrates were used directly as well as in leached form and weathered compost was also applied either singly or in combination. After this, growth promotion in terms of height, number of branches and root-shoot biomass were evaluated at several intervals. Final yield was also estimated by harvesting the capsicum according to their treatment. The results revealed that all the treated plants showed significant increase of height after 35days out of which, those treated with spent substrate of fresh oyster mushroom, button mushroom leachate and weathered compost of button mushroom showed highest increment in growth (Fig 1 & 2A). On the other hand, it was observed that the number of branches significantly increased in oyster mushroom leachate, button mushroom weathered and button mushroom fresh compost (Fig 2B). In case of yield highest yield was obtained by treatment with SMS of oyster mushroom leachate followed by oyster mushroom weathered SMS (Table 1).

Table 1: Effect of different treatments of spent mushroom substrates on yield of *Capsicum annuum.*

Treatments	Yield in first harvest (g/ plant)
Control	49.67±1.37
Fresh oyster SMS[1]	57.67±0.67
Oyster SMS leachate	67.33± 1.65
Oyster Weathered SMS	59.33±3.57
Button SMC[2] leachate	54.67±4.78
Button weathered Compost	58.00±2.35
Fresh SMC of Button	50.75±1.76
Oyster SMS + Button SMC	53.50±2.20

[1]SMS= Spent Mushroom Substrate (oyster mushroom), [2]SMC=Spent Mushroom Compost (Button mushroom)

It has been reported that the PGPR also stimulate the beneficial plant fungal symbiosis involving both AM fungi and ectomycorrhizae [20]. Results revealed that the spent oyster mushroom leachate, fresh oyster mushroom substrate and button mushroom leachate showed better yield. It was also reported that the ectomycorrhizal treatment influences the growth of plants.

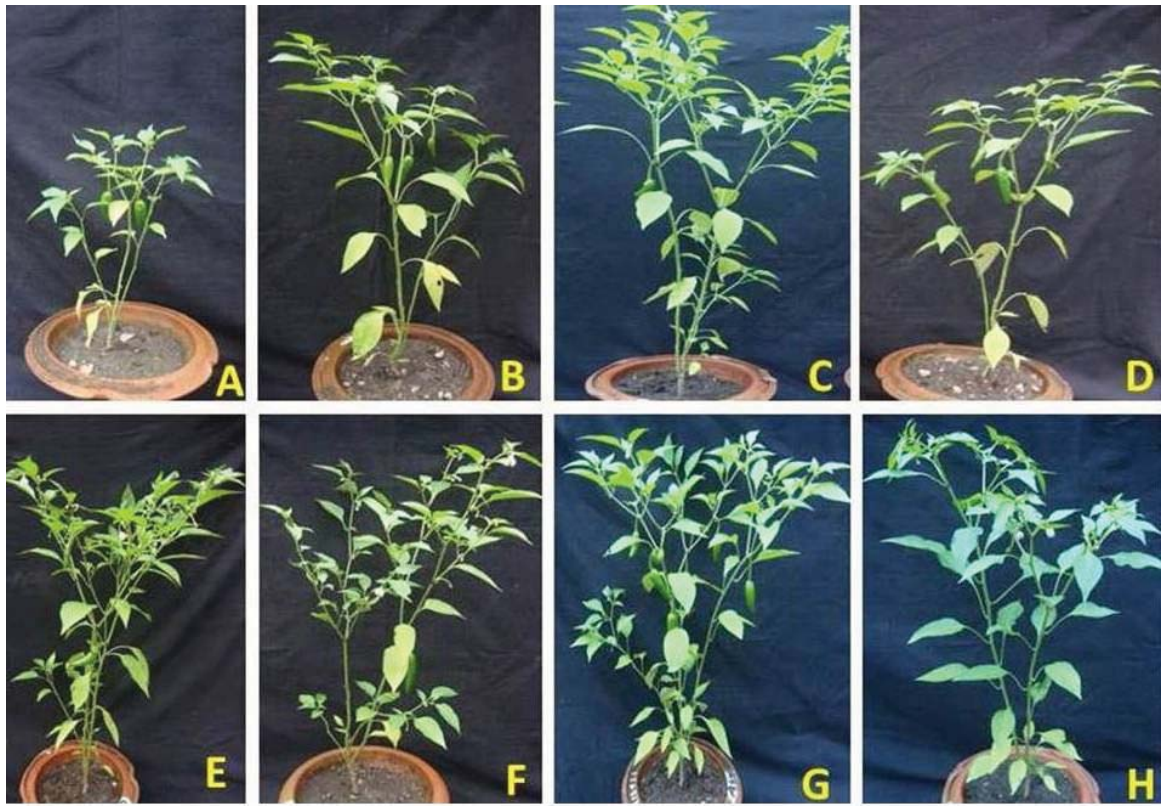


Fig. 1: Effect of Spent mushroom substrates on the growth of *Capsicum annuum* L. (A)= Control; (B)= Fresh spent oyster mushroom substrate; (C)= spent oyster mushroom substrate leachate; (D)= Spent weathered oyster mushroom substrate; (E)=Fresh button mushroom compost; (F)= Spent button mushroom compost leachate; (G)= Spent weathered button mushroom compost; (H)= Combined treatment of Spent oyster mushroom and button mushroom substrate

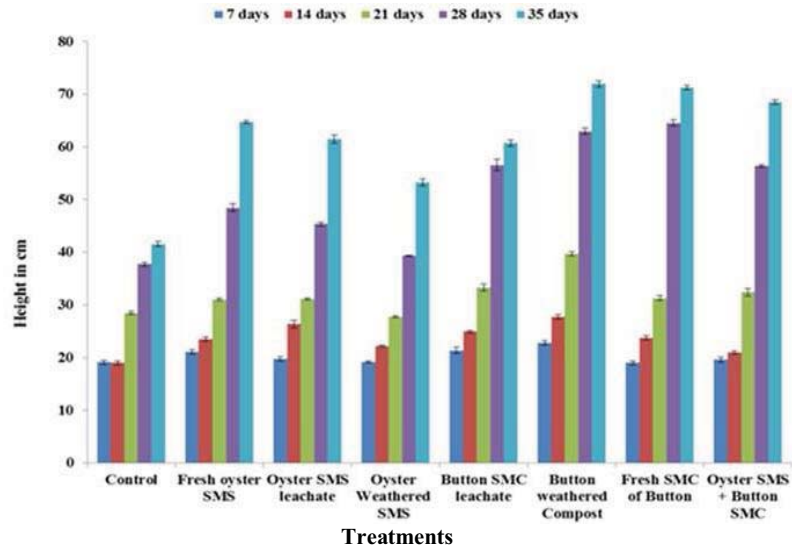


Fig. 2a:

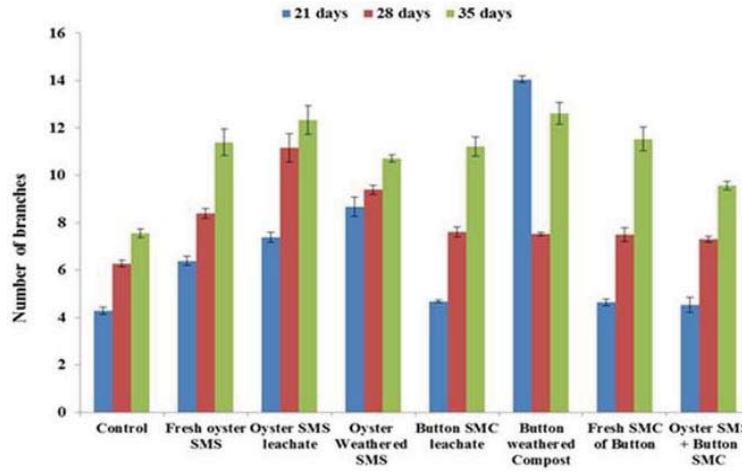


Fig: 2 (B)

Fig. 2: Effect of Spent mushroom substrate on the growth of *Capsicum annuum* L. showing the rate of increase in height (A) and number of branches (B).

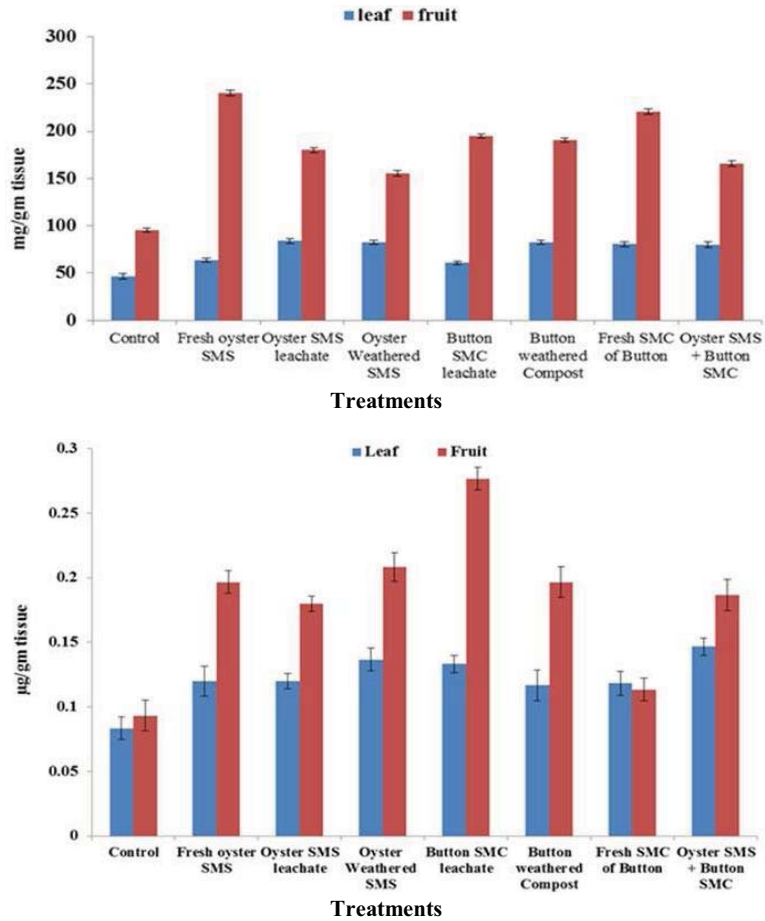


Fig. 3: Total protein content (A) and carotenoid content (B) in leaves and fruits of *Capsicum annuum* L. plants treated with spent mushroom substrates of oyster mushroom and button mushroom.

Apart from this, mobilization of soil phosphate by these treatments were evaluated in terms of total phosphate content in soil, roots and leaves of the treated plants in comparison to the untreated control sets [21]. The uptake of phosphate content was significantly increased in fresh oyster mushroom substrate and in button mushroom weathered compost (Table 2).

Table 2: Effect of spent mushroom substrate on total phosphate content of soil, root and leaf of *Capsicum annuum* L. after 15days of seedling transfer.

Treatment	Total Phosphate Content [µg/gm]		
	Soil	Root	Leaf
Control	53.47±4.73	11.42±1.73	7.67±0.83
Fresh SMS of oyster	43.50±3.45	17.45±1.44	10.33±1.76
Oyster mushroom SMS leachate	47.51±3.33	13.3±1.12	10.65±1.32
Weathered SMS of oyster	39.50±3.21	11.42±1.39	9.05±1.11
SMC leachate of Button	41.1±3.02	12.51±1.12	9.65±0.91
SMC weathered of Button	48.39±4.12	13.70±1.83	10.25±0.93
Fresh SMC of Button	44.56±3.31	12.15±1.19	10.70±1.12
SMS[1] + SMC[2]	41.35±4.17	11.95±1.90	10.25±0.93

[1]SMS= Spent Mushroom Substrate (oyster mushroom), [2]SMC=Spent Mushroom Compost (Button mushroom)

SMS improved soil quality by having a direct influence on soil aggregation and thus, aeration and water movements in addition to increasing availability of insoluble sources of phosphorus [22, 23]. Chlorophyll is the main photosynthetic pigment and it was observed that the use of these spent mushroom substrates enhanced the total chlorophyll content of the leaves of treated plants. Chlorophyll content including chlorophyll a and chlorophyll b was significantly increased in both oyster mushroom and button mushroom leachate, and in fresh oyster mushroom substrate. The dual treatment of both the substrate also showed significant amount of chlorophyll content (Table 3).

Table 3: Effect of spent mushroom substrate on chlorophyll content of *Capsicum annuum* L.

Treatments	Chlotophyll a	Chlorophyll b	Total Chlorophyll
Control	8.38	6.38	14.76
Fresh SMS of oyster	12.92	4.72	17.64
Oyster mushroom SMS leachate	13.51	5.30	18.81
Weathered SMS of oyster	11.40	3.5	14.90
SMC leachate of Button	12.76	4.81	17.57
SMC weathered of Button	11.24	4.35	15.59
Fresh SMC of Button	10.32	3.37	13.69
SMS[1] + SMC[2]	12.40	3.41	15.81

[1]SMS= Spent Mushroom Substrate (oyster mushroom), [2]SMC=Spent Mushroom Compost (Button mushroom)

Total protein content was also evaluated in leaves and fruits. The results revealed that the total protein content was maximum in the fruits of fresh oyster mushroom substrate and fresh button mushroom compost treated plants ranging between 200-250 µg/gm tissue while it was lower in case of treatment with oyster mushroom weathered substrate and the dual treatment of button mushroom and oyster mushroom substrate. Higher leaf protein was also observed in oyster mushroom leachate, button mushroom weathered compost treatment as well as dual application of both the substrates. Among the pigments, carotenoid, being an antioxidant compound is also important and hence carotenoid content was estimated in the study. Carotenoid was estimated in the leaf as well as in fruits of *Capsicum annuum* L. and button mushroom leachate treated plants showed a high range of carotenoid compound (0.25-0.30 µg/gm tissue) followed by the oyster mushroom fresh substrate and button mushroom weathered compost treatment (0.15-0.20 µg/gm tissue). Capsicums were also rich in carotenoids, which is an antioxidant as well as anticarcinogenic compound. It was also observed that immature or mature fruits also contain a high concentration of phenolic compounds [24, 25]. From the above study, it can be concluded that spent mushroom substrate of oyster mushroom and button mushroom compost are good sources of biofertilizer as they influence the growth of *Capsicum annuum* positively. These not only affect the growth but also affect the physiochemical properties. Thus the management of spent mushroom substrate as soil conditioner in the agricultural field can be very effective for crop improvement.

4. ACKNOWLEDGEMENTS

Financial assistance received from the Department of Biotechnology, Ministry of Science and Technology, Govt. of India and University Grants Commission, Govt. of India is highly acknowledged.

5. REFERENCES

1. Robbins, S.H. Reghetti, T.L. Fallahi, E. Dixon, A.R. Chaplin, M.H. Communications in Soil Science and Plant Analysis, 1986, 17, 457-471.
2. Chorover, R.H. Fox Hatcher, C.P. Romaine. (Final Research Project Report – Mushroom Industry Farmer-based Applied Research Program (MIFBAR) (The Pennsylvania State University) 2000; 87.
3. Philippoussis A, Zervakis G I, Diamantpoulou P, Papadopoulou K and Ehaliotis C. Use of spent mushroom compost as a substrate for plant growth and against plant infections caused by *Phytophthora*. Mushroom Science. 2004; 16: 579–584.
4. Lau K L, Tsang Y Y and Chiu S W. Use of spent mushroom compost to bioremediate PAH-contaminated samples. Chemosphere. 2003; 52: 1539–1546.
5. Pill, W.G., T.A. Evans and S.A Garrison. Forcing white asparagus in various substrates under cool and warm regimes. Hort Sci, 1993; 28: 996-998.
6. Ahlawat O.P., Gupta Pardeep, Kumar Satish and Sharma, D.K. Bioremediation of fungicides by spent mushroom substrate and its associated microflora. Indian J. Microbiology, 2010; 50(4) : 390-395.
7. Ahlawat, OP, Manikandan, K, Sagar, MP, Rai Dev and Vijai, B. Effect of composted button mushroom spent substrate on yield, quality and disease incidence of Pea (*Pisum sativum*). Mushroom research, 2011; 20 (2): 87-94.
8. Beyer M. The impact of mushroom industry on the environment. Mushroom News, 1996; 44: 6-13.
9. Kaddous, F.G.A. and A. S. Morgan. Spent mushroom compost and deep litter fowl manure as soil amelioration for vegetables. In: Proc. Of Surface Soil Management, Rotorava, New Zealand, 1986; p. 138-147.
10. Riahi H and Arab A. Spent mushroom compost as an alternative for casing soil. Mushroom Science. 2004; 16: 585–589.
11. Riahi H and Azizi A. Leached SMC as a component and replacement for peat in casing soil and increasing dry matter in mushrooms. In: Proceedings of 2nd International Spent Mushroom Substrate Symposium, ed. K. Paley. 2006; 41–46.
12. Riahi H, Afagh H V and Sheidai M. The first report of spent mushroom compost (SMC) leaching from Iran. Acta Horticulturae. , 1998; 469: 473–480.

13. Debosz K, Petersen S O, Kure L K and Ambus P. Evaluating effects of sewage sludge and household compost on soil physical, chemical and microbiological properties. Applied Soil Ecology, 2002; 19: 237–248,
14. Vandenkoornhuyse, P. Baldauf, S.L. Larval, C. Straczek, J. Young, J.P.W. Science, 2002, 295, 2051.
15. Crecchio C, Curci M, Mininni R, Ricciuti P and Ruggiero P. Short term effects of municipal solid waste compost amendments on soil carbon and nitrogen content, some enzyme activities and genetic diversity. Biology and Fertility of Soils, 2001; 34: 311–318.
16. Eom AH, Hartnett DC and Wilson GWT. Oceologia, 2000; 122, 435-444.
17. Hyakumachi M. Plant-growth-promoting fungi from turfgrass rhizosphere with potential for disease suppression. Soil Microorganisms. 1994;44:53–68.
18. Shivanna MB, Meera MS and Hyakumachi M. Crop Protection, 1996; 15: 497-504.
19. Knudsen D, Beegle D. Recommended Phosphorous tests. In: Recommended chemical soil tests procedures for the north central region. Dahnke W.C. (ed), Bull North Dakota Agric Exp Stn. North Dakota USA, 1988; 499.122-125.
20. Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris.* Plant Physiology, 1994; 4(1):1-16.
21. Bending GD, Poole EJ, Whipps JA and Read DJ. *FEMS Microbiology Ecology,* 2002; 39: 219-227.
22. Roy S, Sunar K., De U. and Chakraborty BN. Influence of Selective Bioresources on Seedling Vigour and Growth of *Cicer arietinum* L. in Field Conditions. Advanced Crop Science, 2013; 3(10): 662–670
23. Rillig M C, Wright S F and Eviner V T. Plant and Soil Sciences, 2002; 238: 325-333.
24. Smith SE and Read DJ. Mycorrhizal symbiosis (London: Academic Press), 1997.
25. Howard LR, Talcott ST, Brenes CH and Villalon B.Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* species) as influenced by maturity. J. Agric. Food Chem., 2000; 48:1713–1720.
26. Wall MM, Waddell CA and Bosland PW. Variation in beta-carotene and total carotenoid content in fruits of *Capsicum*. Hort Science*,* 2001; 36:746–749.

How to cite this article:

Somnath Roy, Shibu Barman, Usha Chakraborty and Bishwanath Chakraborty. Evaluation of Spent Mushroom Substrate as biofertilizer for growth improvement of *Capsicum annuum* L. J App Biol Biotech. 2015; 3 (03): 022-027.

NBU Journal of
Plant Sciences

Vol. 8, No. 1 March 2014 p. 13-18
ISSN No. 09746927

Development of an easy and efficient technique for cultivation of different species of *Pleurotus*

Somnath Roy, Shibu Barman, Usha Chakraborty and Bishwanath Chakraborty*
Immuno-Phytopathology Laboratory, Department of Botany, University of North Bengal, Siliguri-734013, West Bengal, India.

Abstract
Oyster mushroom is one of the most popular mushrooms in North Bengal and a large number of growers are now cultivating oyster mushroom throughout the year. There are mainly three species of oyster mushroom (*Pleurotus ostreatus, P. sajor-caju, P. florida*) which are being cultivated in this region. Out of these three mushrooms, *P. ostreatus* and *P. sajor-caju* are generally cultivated in summer as it grows in a temperature between 25-33^{0}C while *P. florida* is generally cultivated during winter as it requires 15-20^{0}C for its growth. Bottle cultivation is a technique of mushroom cultivation where it reduces the cost of plastic bags and the plastic bottles can be recycled. Besides plastic bottles, other used laboratory chemical plastic containers were also tested and gave good results.

Key words: *Pleurotus ostreatus, P. sajor-caju, P. florida*, Bottle Cultivation.

Introduction:

Mushrooms are prized for their delicacy and distinctive flavor. Because of their unique nutritional status, they are known as "the ultimate health food" (King, 1993). They are non-conventional sources of human food and are delicious, nutritionally rich and have their own importance as medicines. Mushroom has been defined as a "Macro-fungus with a distinctive fruiting body which can be either epigeous (above ground) or hypogeous (underground) and large enough to be seen with the naked eye and to be picked by hand'' (Chang and Miles, 1993). Mushrooms are rich in protein, some essential amino acids, fiber, potassium, and vitamins and have low cholesterol and fat levels (Rafique, 1996). Mushroom cultivation represents the only current economically viable biotechnology process for the conversion of waste plant residues from forests and agriculture (Wood and Smith, 1987). It is a highly efficient method of disposing of agricultural residues as well as producing nutritious food (Chang *et. al.,* 1981).

Oyster Mushroom belongs to the genus *Pleurotus* from the class Basidiomycetes with a shell like fleshy stipe and white, brownish, pink or dark grey in colour. It is now ranks second among the cultivated mushrooms in world (Chang and Miles, 1991). Almost all the available lignocellulosic substances can be used as substrate for *Pleurotus* sp. It is a popular mushroom due to its nutritional, medicinal and potential commercial value (Saidu *et. al.*, 2011). They have ability to grow at wide range of temperatures and utilize various lignocellulose substrates (Khan and Garcha, 1984). Species of *Pleurotus* are usually found to be most efficient in the degradation of lignocellulose substrates among all types of white rot fungi (Das and Mukherjee, 2007). It is composed of 90% water and 10% dry matter (Morais *et.al*., 2000; Sánchez, 2004). Fruiting bodies as well as active mycelia of *Pleurotus* sp. possess a number of therapeutic properties like anti-inflamatory, immuno-stimultory and immuno-modulatory (Asfors*et. al.,* 1993), anticancer activity (Wasser, 2002), and many more medicinal activities.Wide spread malnutrition with ever increasing protein gap in our country has necessitated the search for alternative source of protein because the production of pulses has not kept pace with our requirement due to high population growth. Animal protein is beyond the reach of most people in this country. Edible mushrooms are recommended by the FAO as food, contributing to the protein nutrition of developing countries dependent largely on cereals. Human have been eating different food groups such as meat

Corresponding author:
E-mail: bncnbu@gmail.com

and plant-based including fungi or edible mushrooms for thousands of years. Edible mushrooms have been consumed as food and medicine in many cultures (Bobek *et. al*., 1997; Yang *et al*., 2001; Chocksaisawasdee *et. al*., 2010; Wan Rosli *et al*., 2011). Mushrooms are fungi which commonly grown in the shady area and prolifically propagated through its spores. They are versatile and may be eaten fresh or cooked entirely. Mushrooms are eaten by people for their unique flavour, texture as well as for the health benefits they accord. Mushrooms have been consumed and appreciated for their flavour, economical and ecological values and medical properties for many years (Sanchez, 2010). Mushrooms are healthy foods, low in calories and fat, but high in vegetable proteins, chitin, vitamins and minerals (Manzi *et al*., 1999). Some species of wild mushrooms are sold in the local wet markets as vegetable and many researchers focused on their therapeutic effects and cultivation methods (Tan and Wahab 1997; Pathmashini *et. al*., 2008; Rashad *et al*., 2009; Beluhan and Ranogajec 2011).

Materials Methods

Preparation of Spawn

Wheat grains are used for the preparation of spawn. Wheat grains were boiled in for 20 min and the water drained off. Then it was allowed to dry for overnight in a clean place after which 0.5% (w/w) $CaCO_3$ and 2% (w/w) $CaSO_4$ were added and mixed well. The grains (200gm) were filled in each polypropylene bag and it was autoclaved at 20lb pressure for 1hour. The grains were inoculated with actively growing mycelium of the *Pleurotus ostreatus, P.sajor-caju* or *P.florida* from PDA slant and incubated at 25-28^{0}C for mycelial growth for 14 days until the mycelium fully covered the grains. Completely covering the grains with mycelium rapidly colonizes the bulk growing substrate (Sánchez, 2010).

Cultivation of mushroom

Maintenance of mother culture

Mother culture of the fungal mycelia was maintained by preparing proper selective media and sub-culturing the same following the method suggested by Fritsch (1978) and Jong (1978).

Substrate preparation

Rice straw was used for the cultivation of oyster mushroom. Chopped (2-4 cm long) rice straw was washed and soaked in water for overnight. The straw was again cleaned and pasteurized at 55-65^{0}C for 20-30 min. then it was allowed for cooling at room temperature. Spawning was done using the polypropylene bags, waste bottles of chemicals, waste water bottles, and broken laboratory glass goods.

Spawning

Polypropylene bags are generally used for spawning while using the bottles is a new introduction in the cultivation practices in North Bengal. Besides, used plastic bottles and empty laboratory chemical bottles were also tested as container. Layer spawning was done using the cooled pasteurized straw. 100 gm of spawn was used for 1kg of substrate for spawning. The bottles and bags were then filled using the substrate and spawn and the bags were closed tight and the bottles were closed using the lid. Small holes were made in each bottle and bag for aeration. The bags and bottles were then incubated at room temperature (20-30^0 C) for 10-12 days. After 10-12 days, the white mycelia covered the whole substrate. Then the plastic was removed and the lid of the bottles was opened. 80-90% moisture was maintained by spraying water on the substrate for 2-3 times in a day for the initiation of pinhead (Sarker*et. al*. 2007).

Initiation of Fruiting body

The pinhead appeared after 4-5 days of opening the bags and the lids. Fruiting body was developed at room temperature and 80-90% relative humidity. The fruiting body was harvested from the base carefully so that there should be no injury of the mycelia. Humidity was maintained again after the harvest for further flushes.

Determination of total protein

Protein was extracted from the mushroom using Phosphate buffer (pH7.2) and protein content was determined following the methods as described by

Lowry *et al.,* (1951) using BSA as standard.

Determination of total sugar

1gm of fresh mushroom tissue was crushed with 95% ethanol and the alcoholic fraction was evaporated in boiling water bath. Then the fraction was collected and the volume made upto 5 ml using distilled water. Then it was centrifuged at 10,000 rpm for 15 min and the supernatant was collected for estimation.

1 ml of extracted sample was taken and 4ml of Anthrone's reagent was added and incubated on boiling water bath for 10 min. Then it was cooled down in tap water and observed at 620 nm in colorimeter.

Determination of reducing sugar

Ethanol (80%) extract was used for estimating the reducing sugar. Extract (2 ml) was mixed with 2 ml of Alkaline copper tartrate and boiling was done. Determination of reducing sugar using Arseno-molybdate was carried out at 620 nm following Nelson-Somogyis' Method (1952).

Results and discussion

Three species of *Pleurotus* cultivated in different season. It was observed that the optimum temperature for the cultivation of *Pleurotus sajor-caju* is 20-28^0 C, *P. ostreatus* 20-25^0 C and *P. florida* was 18-20^0 C as reported by Dhar *et. al.* (2011). They also stated that the Fruit body of *P. sajor-caju* fan shaped with thick texture grey in colour, 3-4 inches long when mature while in case of *P. ostreatus* the mature pileus is quite dark and small as compared to *P. sajor-caju* and in case of *P. florida,* the mature pileus is small and white in colour with thin margin and smooth fleshy. *P. florida* look like a white disc growing on a thick stipe with decurrent gills extended up to the base of the stipeunlike *P. sajor-caju* and *P. ostreatus* (Figure 1). It was also observed that the amount of production of *P. ostreatus* is higher followed by the production of *P. sajor-caju* and *P. florida* (Figure 2).

Cultivation of oyster mushroom using waste bottle is a new introduction in North Bengal and showed a significant result. Using of waste plastic bottles as reported by Hyunjong Kwon is a sustainable technique for the mushroom cultivation and it was observed that the rate of contamination is being reduced when plastic bottles are used. Using waste chemical bottles and other waste bottles of water and cold drinks could be another way to recycle

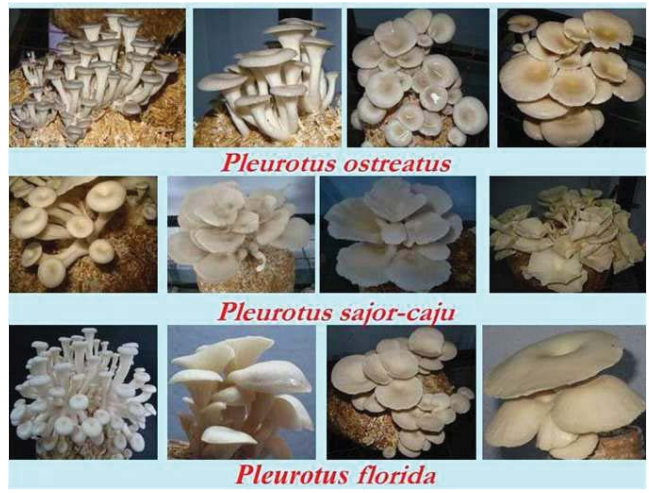


Fig 1: Different stages of three species of oyster mushroom

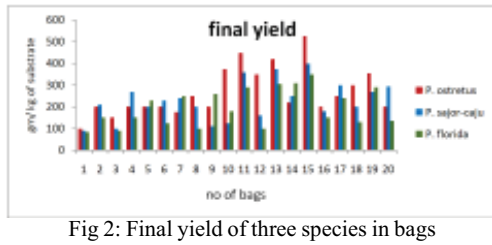


Fig 2: Final yield of three species in bags

the waste bottles in a good way and it reduces the cost of plastic bags. Different species of oyster mushroom was cultivated and it was observed that the production of *P. ostreatus* and *P. sajor-caju* was much higher than *P. florida* (Figure 3).

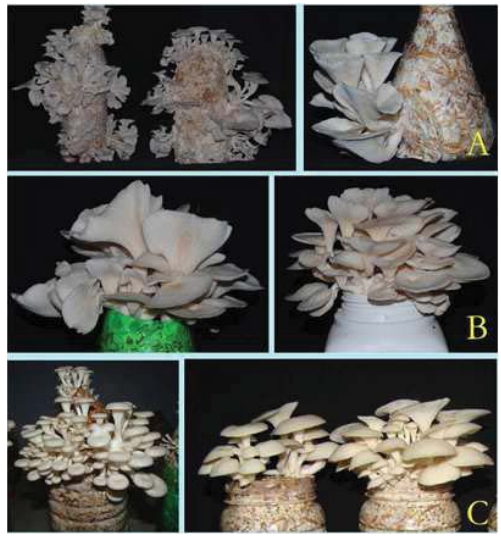


Fig 3: Bottle cultivation of three different species, A: *P. ostreatus;* B: *P. sajor-caju;* C: *P. florida* using waste plastic bottle, laboratory chemical bottles.

Total protein content was estimated and it was observed that total protein is quite higher in *P. ostreatus* and *P. sajor-caju*in comparison to *P. florida* (Table 1). It was also observed that in case of *Pleurotus ostreatus* the protein content was high in mature pileus (390 mg/gm tissue) and young pileus (230 mg/gm tissue) while in case of mature stipe and young stipe the total protein content was lower (190-280 mg/gm tissue). Pinhead of *P. ostreatus* also shows significant amount of protein content.

In case of *Pleurotus sajor-caju* the total protein content is high in mature pileus (370 mg/gm tissue) while it was observed that the protein content is lower in mature stipe (170 mg/gm tissue) and other stages. On the other hand, it was observed that the protein content was quite higher in pinhead stage (265 mg/gm tissue) of *P. florida.* While in *P. florida* the total protein content was lower in mature pileus (220 mg/gm tissue). It was observed that the total protein content was higher in *P. sajor-caju* as described by Khan *et. al.* (2008).

Table 1: Activity of total soluble protein content of different stages of the three species

Stages	Protein content (mg/gm tissue)		
	P. ostreatus	*P sajor-caju*	*P. florida*
Pin head	270	240	265
Young Stipe	190	185	135
Young Pileus	230	220	230
Mature Stipe	280	170	190
Mature Pileus	390	370	220

It was also observed that the total sugar content and reducing sugar was higher in *P. sajor-caju* followed by *P. ostreatus* and *P. florida* as reported by Alam *et. al.* (2008). In case of *P. ostreatus*, total sugar content ranges from 100-300 mg/gm tissue and maximum total sugar content observed in young pileus (300 mg/gm tissue) while in case of reducing sugar, it ranges from 20-50 mg/gm tissue and maximum activity found in young pileus (Table 2).

In case of *P. sajor-caju* total sugar content ranges from 120-310 mg/gm tissue and maximum activity observed in young pileus (310 mg/gm tissue). While in case of reducing sugar it was observed that the activity ranges from 29-55 mg/gm tissue and maximum activity showed by the young pileus and pinhead stage (55 mg/gm tissue). On the contrary, in case of *P. florida* total sugar content ranges from 140-290 mg/gm tissue and reducing sugar content ranges from 26-60 mg/gm tissue. It was also observed that the maximum total sugar content showed by young pileus (290 mg/gm tissue) and maximum reducing sugar activity showed by young pileus (60 mg/gm tissue).

Table 2: Estimation of Total sugar content and reducing sugar activity in different stages of three mushrooms

Stages	Reducing sugar (mg/gm tissue)			Total sugar (mg/gm tissue)		
	P. ostreatus	*P sajor-caju*	*P. florida*	*P. ostreatus*	*P sajor-caju*	*P. florida*
Pin head	52	55	49	100	120	140
Young Stipe	49	48	45	210	225	210
Young Pileus	50	55	60	300	310	290
Mature Stipe	32	39	33	170	180	185
Mature Pileus	20	29	26	300	280	270

In conclusion, the chemical composition of edible mushrooms determines their nutritional value and sensory properties as also mentioned by Manzi *et. al.* (2001). They differ according to the species and also on the atmospheric conditions, age and part of the fructification. In our study we found that the nutritional values differ in different parts of the fructification. These data suggests that the three species cultivated in North Bengal *P. ostreatus, P. sajor-caju* and *P. florida* are a good source of nutrients specially in protein and sugar. These data also indicated that the mushrooms have a good nutritive value for human.

Acknowledgement:

Financial assistance received from University Grants Commission and Department of Biotechnology, Ministry of Science and Technology, Government of India is acknowledged.

Reference

Alam, N., Ami, R., Khan, A., Ara, I., Shim, M.J., Lee, M.W. and Lee, T.S. (2008). Nutritional analysis of cultivated mushrooms in Bangladesh- *Pleurotus ostreatus, Pleurotus sajor-caju, Pleurotus florida* and *Calocybe indica. Mycobiology;* 36(4): 228-232.

Beluhan S. and Ranogajec A. (2011).Chemical composition and non-volatile components of Croatian wild edible mushrooms. *Food Chemistry;* 124: 1076–1082.

Bobek, P., Ozdín, L. and Kuniak, L. (1997). Effect of oyster mushroom and isolated â-glucan on lipid peroxidation and on the activities of antioxidative enzymes in rats fed the cholesterol diet. *The Journal of Nutritional Biochemistry;* 8(8): 469-471.

Chang, S.T. and Miles, P.G. (1991). Cultivation of edible mushrooms. *Chung Chi, J. Hongkong*; 4: 76-86.

Chang, S.T. and Miles, P.G. (1993). Recent trends in world production of cultivated edible mushrooms. *Mushroom J.*, 504: 15-17.

Chockchaisawasdee S., Namjaidee S., Pochana S. and Stathopoulos C.E. (2010). Development of fermented oyster-mushroom sausage. *Asian Journal of Food and Agro-Industry* 3(1): 35-43.

Das, N. and Mukherjee, M. (2007).Cultivation of *Pleurotus ostreatus* on weed plants. *Bioresour. Technol.*, 98(14): 2723-2726.

Dhar, B.L., Shrivastava, N., Himanshu, Kumar, J.,Tyagi, S. and Atrey, P. (2011). Cultivated edible specialty mushrooms- scope in India and EU countries. Proceedings of the 7th International conference on Mushroom Biology and Mushroom products (ICMBMP), pp. 537-547.

Fritsch, G. (1978).Breeding work in Chang, ST. and Hays, WA. Eds. The biology and cultivation of edible mushrooms. Academic press, New York. pp. 239- 248.

Jong, S.C. (1978). Conservation of the cultures in Chang, S.T. and Hays, WA. Eds. The biology and cultivation of edible mushrooms. Academic press, New York. p.119-133.

Khan, P. and Garcha, H.S. (1984). *Pleurotus* mushroom, a source of food protein. *Mushroom News letter Trop.*, 4: 9-14.

Khan, Md. A., Amin, R.S.M., Uddin, Md. N., Tania, M. and Alam, N. (2008). Comparative study of the nutritional composition of oyster mushroom cultivated in Bangladesh. *Bangladesh J. Mushroom;* 2(1): 9-14.

King, T.A. (1993). Mushrooms - The ultimate health food but little research in US to prove it. *Mush. News*, 4(12): 26-29.

Manzi, P., Gambelli, L., Marconi, S., Vivanti, V. and Pizzoferrato, L. (1999). Nutrients in edible mushrooms: an inter-species comparative study**.** *Food Chemistry,* 65(4): 477-482.

Manzi, P., Aguzzi, A. and Pizzoferrato, L.(2001). Nutritional value of mushrooms widely consumed in Italy. *Food Chem.,* 13: 21-26.

Morais, M.H., Ramos, A.C., Matos, N. and Oliveira, E.J.S. (2000). Production of shiitake mushroom (*Lentinus edodes*) on lignocellulosic residues / *Nota. Cultivodelhongo shiitake (Lentinus edodes) en residues lignocelulósicos. Food Sci. Technol. Intl.*, 6(2): 123-128.

Pathmashini, L., Arulnandhy, V. and Wijeratnam, R.S.W. (2008). Cultivation of oyster mushroom (*Pleurotus ostreatus*) on sawdust. *Ceylon Journal of Science (Biological Sciences),* 37(2): 177-182.

Rafique, A. N. (1996). Studies on the Cultivation of Mushroom *Pleurotus* species in Gujarat. Ph. D. Thesis, Department of Microbiology. M.G. Science Institute, Navrangpura, Ahmedabad, India.

Rashad M.M., Abdou H.M., Mahmoud A.E. and Nooman M.U. (2009). Nutritional analysis and enzyme activities of *Pleurotus ostreatus* cultivated on *Citrus limonium* and *Carica papaya* wastes. *Australian Journal of Basic and Applied Sciences,* 3(4): 3352-3360.

Saidu, Md., Salim, Md. R. and Yuzir Md. A. M. (2011).Cultivation of oyster mushroom (*Pleurotus* spp.) on palm oil mesocarp fiber. *African Journal of Biotechnology*, 10 (71): 15973-15976.

Sánchez, C. (2004). Modern aspects of mushroom culture technology. *Appl. Microbiol. Biotechnol.*, 64(6): 756-762.

Sánchez, C. (2010). Cultivation of *Pleurotus ostreatus* and other edible mushrooms. *Appl. Microbiol. Biotechnol.*, 85(5): 1321-1337.

Sarker, N.C., Hossain, M.M., Sultana, N., Mian, I.H., Karim, A.J.M.S. and Amin, S.M.R. (2007). Performance of different substrates on the growth and yield of *Pleurotus ostreatus* (Jacquin ex Fr.) Kummer. *Bangl. J. Mush*., 1(2): 9-20.

Tan, Y.H. and Wahab, M.N. (1997). Extracellular enzyme production during anamorphic growth in the edible mushroom, *Pleurotus sajor-caju*. *World Journal of Microbiology and Biotechnology* 13(6): 613-617.

Wan Rosli, W.I., Solihah, M.A., Aishah, M., Nik Fakurudin, N.A. and Mohsin, S.S.J. (2011). Colour, textural properties, cooking characteristics and fibre content of chicken patty added with oyster mushroom (*Pleurotus sajor-caju*). *International Food Research Journal,* 18: 612-618.

Wasser, S.P., (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides (mini review). *Appl. Microbiol. Biotechnol.*, 60: 258-274.

Wood, D.A. and J.F. Smith. (1987). The Cultivation of Mushroom. In essays in agricultural and food microbiology editor by Norris J.R. and Pettipher G.L. John Wiley and Sons Ltd. pp 310-343

Yang, J.H., Lin, H.C. and Mau, J.L. (2001). Non-volatile taste components of several commercial mushrooms. *Food Chemistry* 72(4): 465-471