Characterization and exploitation of jelly mushrooms (*Auricularia* spp. / *Tremella* spp.)

by

PRIYA R. U.

(2014-21-133)

THESIS

Submitted in partial fulfilment of the

requirements for the degree of

DOCTOR OF PHILOSOPHY IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF AGRICULTURE

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KERALA, INDIA

2017

DECLARATION

I, hereby declare that this thesis entitled **"Characterization and exploitation of jelly mushrooms (*Auricularia* spp. / *Tremella* spp.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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ACKNOWLEDGEMENT

Gratitude takes three forms, "An emotion from the heart, an expression in words and a giving in return ..." At very last the time has come to look deep into the layers of my heart which is clumped with the feeling of togetherness, exquisiteness, support and satisfaction. Some are everlasting and some are transitory but both occupy a number of persons to whom I acknowledge my warm regards. First and foremost I would like to bow my head to the feet of God Almighty for giving strength to my soul, helping me to encourage during the entire period of research and for giving good health and spirit to complete this work successfully.

I express my deep sense and heartfelt reverence to Dr. D. Geetha, Professor (Pl. Path.), Instructional Farm, College of Agriculture, Vellayani and the most esteemed chairperson of my advisory committee. I am overwhelmingly thankful to her for constant encouragement, wholehearted interest, support, positive condemnation, care and tremendous guidance from conceptualization to completion of the thesis, everlasting patience throughout the endeavour and devoting her time amidst her busy schedule. I proudly acknowledge that this work has gained most importance under the kind supervision and truthful inspiration of my guide and I also genuinely and humbly confess that it has been a great privilege for me to have been one of her students.

The ethical zeal and constant pledge at each count bestowed by members of my advisory committee, Dr. V. K. Girija., Professor and Head, Department of Plant Pathology, I extend my sincere and obedient thanks for her valuable suggestions, constructive denigration and support throughout the research studies.

I am very much grateful to Dr. Lulu Das, Professor, Department of Plant Pathology for her timely suggestions, valuable counsel and keen interest into my research work.

I owe my sincere gratitudes towards Dr. C. Nirmala, Associate Professor and Head, Department of Community Science, College of Agriculture, Vellayani for her expert suggestions, everlasting patience and technical support for the current investigation.

My special and heartfelt thanks to Dr. Biju Joseph, Assistant professor, Instructional Farm, College of Agriculture, Vellayani for his kind and truthful support and also helping me timely.

I have gigantic pleasure in expressing my deep sense of indebtedness to Dr. Babu Mathew, Professor and Head, Instructional Farm for facilitating from the starting of this investigation, which helped me to complete the work.

I specially thankful to Dr. M. Joy, Associate Professor, Department of Plant Pathology for ever willing support, highly appreciable suggestions and technical corrections during the research programme.

I am so much grateful to Dr. C. Gokulapalan, retired Professor and Head, Department of Plant Pathology, College of Agriculture, Vellayani for his constant and valuable suggestions and support during the present study.

I express my wholehearted acknowledgement and sincere respect to Dr. K. K. Sulochana, retired Professor and Head, Dr. A. Naseema, retired Professor Department of Plant Pathology, for their valuable blessings and suggestions during the course of my work.

I extend my sincere gratitude to Dr. K. Umamaheshwaran, Professor and Research Co-ordinator, College of Agriculture, Vellayani, Dr. Susha S. Thara Assistant Professor and Dr. G. Heera, Assistant Professor for their valuable suggestions and timely help.

I take this opportunity to specially acknowledge Dr. Kamala Nayar, retired Professor (Plant Pathology), for all the technical suggestions, immense support, critical evaluation of manuscript and ever willing help throughout my study. I also express my sincere gratitude to all the teaching and non teaching staffs of Instructional Farm, College of Agriculture, Vellayani for their constant support and suggestions.

I deeply express my wholehearted thanks to Radhika chechi, Assistant Professor and my classmate for supporting academically, morally and personally throughout the study time and I extend my extreme and wholehearted thanks to my seniors Deepa chechi, Asha chechi, Manal chechi, Nisha, Saritha chechi, Rejitha chechi, teaching and non teaching staff of Department of Plant Pathology for their kind help, hopeful suggestions and academic support.

I express my sincere gratitudes to Smt. Brigit Joseph, Associate Professor, Department of Agricultural Statistics, who gave me the proper guidance for data analysis, Dr A. S. Anil Kumar, Professor, Department of Agronomy, Dr. K. N. Anith, Professor,

Department of Microbiology, Dr. B. Aparna, Assistant Professor, Dept. of Soil science and Agricultural Chemistry, Dr. Reji Rani, Assistant Professor, Biocontrol Lab, Dr. R. V. Manju, Professor and Head, Department of Plant Physiology and Dr. T. Sajitha Rani, Professor, Department of Agronomy for their constant and technical support throughout the research work. I sincerely express my gratitudes to Dr. V. P. Sharma, Directorate of Mushroom Research, Solan for technical guidance in the present study. Austin, Athimic Biotech Solutions, Kalliyoor, Biogenix, Valiyavilla and Rajiv Gandhi Center for Biotechnology (RGCB), Thycaud for analyzing and helping me to carry out the anti-cancerous activity studies.

I specially convey my wholehearted thanks to Nithya Jim for making efforts to correct my thesis and I express my heartfelt thanks to Selvi chechi, Sheeja chechi , Mushroom Lab, Instructional Farm, Vellayani . Manoj chetan and Saju chetans for their immense support, co-ordination, willingness and constant encouragement during the course of study.

The encouragement and care I experienced at each instant by my friends. I will be forever grateful to my beloved friends Darshan, Ashwini, Bhavya, Gowrish, Jeevan, Geetha, Sowmya, Bhavya Reddy, Suresha, Kirana, Subbu, Brunda, Sainath, Sreekanth, Prittin, Rathna, Guna Bhagya, Mamatha, Ashwini, M., Jyothi, Pritam, Vineeth Verma, Pintu, Sree lakshmi , Sreya and Thejashree.

I sincerely thankful to my ever supporting seniors Shivu anna, Mallinath anna, Kavitha Madam, Nagaraj Sir, Gangadhar anna and Vidyakka and Narmada chichi for their constant support, help, love and encouragement.

I extend my heartfelt wishes to Sumi, Krishnapriya, Agnes, Shivu, Brinda, Shilpa Chakoo, Lishma , Niranjan, Madhu , Aparna, Ananthu, Chinnu, Karthika, Saffir, Gireesh, Aswini Devi, Theresa, Nimisha and Lishma special hearty thanks to Jaslam for helping me out in all the analysis problems, invariable help and support during the research work. I also wish to extend my kindness and love to my beloved juniors, Shruthi, Sathya, Shalu, Lakshmi, Asha, Keerthishree, Usha, Harshitha, Archana, Gana and Manasa for their mental support and constant togetherness.

I owe extreme gratitude to The Dean, Academic Head, College of Agriculture, Vellayani and Kerala Agricultural University and its staff for the financial, technical and academic support and specially granting the award of a KAU Doctoral Fellowship and contingency fund that helped to provide the necessary financial support required by the present work.

The praise of my rise in scholastic livelihood goes entirely to my parents who have invited me to this beautiful world of education and were there to lend a hand to me when I necessitate them the most. I feel scanty of words to enormity the boundless love, care, tireless sacrifice and unimaginable affection showed by my father Shri. Ravi Kumar B.J, my mother Smt. Uma Devi C, brothers Pradeep Kumar, Prathap Kumar, grandmother Late Smt. Onkaramma, grandfather Late Sri. Jayanna, and my best relatives, Keerthi Prasad, Veeresh, Raghu, Ramya atthige, Ranju, Manjula, Meenakshi, Shantha, Bhagya, Swamy mama, Kumar uncle, Prema, Sudha, Pushpa, Pooja, Monika, Chandu and Harshi. It would have been impossible to complete this task without their overwhelming support, constant encouragement and everlasting love, affection and admiration. I would like to specially convey my sincere heartfelt thanks to Shambhu uncle, Kamala aunty and my ever loving Chinni for their blessed support, constant love and encouragement.

Above all, I express my unconditional credit to those who directly and indirectly helped me to achieve this level

... Any exemption in this note of acknowledgement does not specify lack of gratitude.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
lbs	Pound per square inch
PDA	Potato dextrose agar
OMA	Oat meal agar
MEA	Malt extract agar
CEA	Carrot extract agar
mm	Milli meter
^{0}C	Degree Celcius
CD	Critical difference
CRD	Completely randomized design
S. Em±	Standard error of mean
cm	Centimetre
OD	Optical density
et al.	And other co workers
g	Gram
h	Hours
i. e.	That is
mL	Milli Litre
µL	Micro Litre
K cal	Kilo calorie
DMEM	Dulbecos modified Eagles medium
MTT	3-(4,5- dimethylthiazol-2,5- diphenyl tetrazolium bromide
PBS	Phosphate buffer saline
kg	Kilo gram
min.	Minutes
mg	Milli gram
Sl. No.	Serial number

sp. or spp.	Species (Singular and plural)
viz.	Namely
pH	Negative logarithm of hydrogen ions
µm	Micron meter
ppm	parts per million
wt.	weight
B. E	Biological efficiency
temp.	temperature
mt	Million ton
N	Normal
nm	Nano meter
mM	Milli molar
µg	Micro gram

Introduction

1. INTRODUCTION

A mushroom is a macro fungus that has a fleshy, spore-bearing fruiting body, with varied shape and is typically produced above ground, either on soil or on its food source. Mushrooms have been recognised as ingredients of special cuisine across the globe, especially due to their distinctive flavour. More than 2,000 species of mushrooms exist in nature of which, only 25 are widely accepted as food and still fewer are commercially cultivated (Chang and Miles, 2008). They are also accepted as nutraceutical food and are of significant interest due to their organoleptic worth, medicinal properties and economic significance (Ergonul *et al.*, 2013).

Agriculture sector and its related industries produce large amounts of solid wastes, residues and by-products throughout the world, and they accumulate every day and cause tremendous environmental pollution. One of the efficient solutions for recycling such challenging wastes is the production of mushrooms using such agricultural wastes as substrates. Cultivation of edible mushrooms on agricultural residues/wastes is a value-added practice for translation of waste resources into food. Subsequently, value-added products are obtained for human consumption.

There are five major genera representing 85 per cent of the world's mushroom supply of which principally *Agaricus* (*A. bisporus* and a few *A. brasilensis*) is the major genus, contributing about 30 per cent of the world's cultivated mushrooms. *Pleurotus* comes second, with five to six cultivated species, constituting about 27 per cent of the world's production whereas, *Lentinula edodes* (shiitake), contributes about 17 per cent. The other two genera *viz.*, *Auricularia* and *Flammulina* are accountable for six per cent and five per cent of the production, respectively (Royse, 2014). India produces about 2.5 lakh tons of edible mushrooms annually (Dhar, 2014).

Although mushroom is considered as a delicacy with high nutritional and functional value there is no clear discrimination between edible and medicinal

mushrooms. Many of the common edible species have therapeutic properties and many of the medicinal ones are edible too, *viz.*, the jelly mushrooms.

Jelly mushrooms are species of fungi that have gelatine like consistency because of their exceptional structural hyphae. Unlike the thin and rigid texture observed in many fungi, the walls of jelly mushrooms spread out to an indefinite extent. These are the fungi that emerge earlier during spring as they have remained dry and inconspicuous throughout the winter. The group of jelly mushrooms are mainly accommodated in orders: Dacrymycetales, Tremellales, Sebacinales and Auriculariales (Deacon, 2005).

The systematic positions of *Auricularia* spp. and *Tremella* spp. are as follows:

Kingdom- Fungi	Kingdom- Fungi
Division- Mycota	Division- Mycota
Sub division- Basidiomycotina	Sub division- Basidiomycotina
Class- Heterobasidiomycetes	Class- Heterobasidiomycetes
Order-Auriculariales	Order- Tremellales
Family-Auriculariaceae	Family- Tremellaceae
Genus- *Auricularia*	Genus- *Tremella*

The name *Auricularia* is derived from the Greek word "*Auricula*" meaning "ear" and therefore is commonly known as the Jew's ear, wood ear or jelly ear. The fruiting body is distinguished by its earlike shape and brown colour. It grows upon wood especially of trees like the elder tree (*Sambucus nigricans* L.). The occurrence of *Tremella* spp. was first reported from China during 1832 and is best known by the Chinese name "Yin Er" (silver ear) due to the white chrysanthemum like appearance and transparent lobes (Wang *et al.*, 2015). Both

mushrooms grow upon dead and living wood, and can be found throughout the year in temperate regions worldwide.

Auricularia sp. was recorded as first cultivated in China during 600 A.D. and has been a popular one ever since. Among the ten species identified by Lowy (1952) from worldwide collection, *Auricularia auricula* (Hook.) Underw. and *Auricularia polytricha* (Mont.) Sacc. are the two most popular edible species that can be cultivated on sawdust amended with different supplements. Production of wood ear accounts for only six per cent of the world's total output of mushrooms. However, annual production of *Auricularia* spp. in China reached nearly 3.6 mt and was ranked the second most widely cultivated mushroom there (Li., 2012). Today, this mushroom is a popular ingredient in many Chinese dishes such as hot and sour soup as well as in Chinese medicine (Chang and Miles, 2004).

In case of the other jelly mushroom *Tremella*, among the different identified species, it is *Tremella fuciformis* that is being commonly cultivated and was reported to be cultivated on natural logs in China during 1894. However, the cultivation of *Tremella* spp. is not as easy as the cultivation of other mushrooms because it is basically mycoparasitic in nature and requires another ascomycetes fungus as its host for parasitization. Therefore, a mixed culture cultivation is essential and the most preferred host is *Annulohypoxylon archeri* whose pure cultures are fairly available (Chang and Miles, 2004; Wang *et al.*, 2015).

The two jelly mushrooms *Auricularia* spp. and *Tremella* spp. have always been popular subjects of research due to their possible medicinal applications. Fruiting bodies of these mushrooms are rich in carbohydrates, proteins and minerals (Ca, P and Fe). The polysaccharides obtained from *Auricularia* extract include carbohydrate *viz.*, rhamnose, xylose and glucose, smaller amounts of mannose, galactose, arabinose and its active constituents *viz.*, beta (13) and (16) D glucans. These components have been reported to have antitumor, anticoagulant, hypocholesterolemic and antiplatelet aggregation activities.

In spite of the excellent qualities of jelly mushrooms, their large-scale production and utilization have not been fully explored in Kerala, a place blessed with good climatic conditions most suited for mushroom cultivation. Although preliminary trials on jelly mushrooms conducted at the College of Agriculture, Vellayani, by Vidyaresmi (2008) and Geetha (2011) indicated the scope for popularising these mushrooms, systematic studies on the different aspects of its cultivation, palatability or medicinal properties were not undertaken.

In this context, the present project was proposed with the objective of standardizing the techniques for cultivation of jelly mushrooms (*Auricularia* spp. /*Tremella* spp.) in agricultural wastes and to study their morphological, physiological, cultural characteristics as well as nutritional and organoleptic qualities.

Review of Literature

2. REVIEW OF LITERATURE

"Jelly mushrooms" comprise a large variety of fungi that share the characteristic feature of gelatinous sporocarps, meaning, the hyphae and reproductive structures are embedded in a gelatine-like matrix. Hypothetically, the gelatinous material results from the breakdown of outer hyphal layers. However, hardly enough evidence exists to prove this theory. The different types of jelly mushrooms are Dacrymycetales, Tremellales, Sebacinales and Auriculariales (Deacon, 2005). The usual edible ones come under Auriculariales and Tremellales.

Auricularia spp. are known as the Jew's ear, wood ear or jelly ear. The fruiting body is differentiated by its noticeably earlike shape and brown colouration and it grows upon wood, particularly of the elder tree (*Sambucus nigricans* L.). Its specific epithet was resulting from the belief that Judas Iscariot hanged himself from an elder tree, and these mushrooms were first discovered growing on elder trees. Therefore, they were named Judas' ear, which later became Jew's ear. The fungus is found throughout the year in temperate regions worldwide, where it grows upon both dead and living wood. Even though it was not extensively consumed in the West, it had long been admired in China, to the level that Australia exported great volumes to China in the early twentieth century (Chang and Miles, 2004).

Tremella spp. known to produce white, frond like, gelatinous basidiocarps, and are extensive, especially in the tropics, where it could be found on the dead branches of broad-leaved trees. It is one of the most accepted fungi in the cuisine and medicine of China and this species universally known as snow fungus, snow ear, silver ear fungus and white jelly mushroom (Stamets, 2000).

Taxonomy

The term *Auricularia* was related with Judas Iscariot because of the belief that he hanged himself on an elder tree (*Sambucus nigricans*) after betraying Jesus Christ. These mushrooms were first discovered growing on the wood of elder trees. Hence, they were named after Judas, which later became the Jew's ear. Gerarde (1597) first reported it as "*Fungus sambicinus*" and later as *Agaricum auriculae-forma* by Micheli (1728). Linnaeus (1753) named it as *Tremella auricula-judae*, later Fries (1823) named it as *Exidia auricula judae* and Schroeter (1889) finally confirmed the scientific name as *Auricularia auricula judae*. *Tremella fuciformis* was first described by the English mycologist (Berkeley, 1856) later showed that the fruit bodies were those of *Tremella fuciformis* parasitized by an ascomycete, *Ceratocystis epigloeum*, that formed the dark spines and *Nakaiomyces nipponicus* was therefore a synonym of *T. fuciformis* (Guerrero, 1971).

The genus *Auricularia* accomodates 10 species, the most popular ones being *A. auricularia* and *A. polytricha* (Stamets, 1993; Chang and Miles, 2004). Alexopoulos *et al.* (1996) classified the genus *Auricularia* in the order *Auriculariales* in the basidiomycete group of fungi and *Auriculariales* was the largest order of jelly fungi and the fruiting bodies or basidiocarps of many of the species were produced on wood. *Auricularia polytricha* (Mont.) Sacc. belonged to Basidiomycota, Auriculariaceae and known as wood ear, Jew's ear, or red ear (Yu *et al.*, 2008).

Looney *et al.* (2013) reported that the taxonomy and nomenclature of *Peziza nigricans* which was the earliest apprporiate name for *A. polytricha sensu auct. amer.*, then *Auricularia nigricans* was proposed and the genus *Auricularia* comprised 10-15 recognized species worldwide, and most are recognised to have intercontinental to cosmopolitan distributions. Five species, *A. fuscosuccinea*, *A. auricula-judae*, *A. mesenterica*, *A. polytricha* and *A. delicata* have been recorded and new taxonomic trait, the schizomedulla, was discussed and revealed to

distinguish two novel species, *A. subglabra* and *A. scissa* from the morphologically similar species of *A. delicata.*

Choudhury and Sarma (2014) stated that the widespread woodland of Nameri national park provided congenial condition for the growth of different species of *Auricularia* and collected four different species of *Auricularia* of which three species were identified and one species remain unidentified. They also studied the taxonomy and diversity of different species of *Auricularia* and their ethnomycological uses.

At present there are 29 species of *Auricularia* in the world, among which *A. americana*, *A. auricula*, *A. fuscosuccinea*, *A. mesenterica*, *A. polytricha*, *A. scissa* and *A. subglobra* had been commonly occurring and *A. auricula* and *A. polytricha* under cultivation (Kirk, 2015). Bandara *et al.* (2015) reported a new species of *Auricularia*, *A. thailandica*, from fresh collections of Philippines and described that *Auricularia thailandica* differs from other species by having short and loosely arranged abhymenial hairs on the basidiomata and in the different size of the zones in a cross section of the basidiomata. Phylogenetic relationships were inferred based on the nuclear ribosomal internal transcribed spacer (ITS) region.

2.1. SURVEY

Cheng and Tu (1978) and Ingold (1982) stated that species of *Auricularia* are found worldwide and *A. polytricha* occurs in both tropical and subtropical regions. They are brown, rubbery, earlike structures which reach 4 to 6 inches in diameter and produced on dead stumps, logs, and branches of hardwood trees. Well (1984) reported that there are many *Auricularia* species of which *A. polytricha, A. fuscosuccinea* and *A. auriculu judea* are the mainly grown and *A. polytricha* is the most suitable species to be cultivated in tropical regions where temperature is high.

Tremella fuciformis is recognised to be a parasite of *Hypoxylon* species and this species not only occurred in tropical and subtropical areas but also in

temperate areas *viz.*, Asia, North, South and Central America, Caribbean and Africa (Roberts, 2001). Musngi *et al.* (2005) collected four species of *Auricularia* (*A. auricula*, *A. fuscossucinea*, *A. polytricha* and *A. tenuis*) in the campus of the Central Luzon State University, Philippines and the study revealed that identification was based on their hyphal zonation. From each species, various strains were identified and seven host trees were recorded *viz.*, rain tree (*Samanea saman*), coconut (*Cocos nucifera*), ipil - ipil (*Leucaena leucocephala*), mahogany (*Sweitenia mahogany*), mango (*Mangifera indica*) and rubber tree (*Hevea brasiliensis*). The study also reported that among these host species, rain tree supported the highest number of *Auricularia* species. They also reported that *Auricularia* spp. had a worldwide distribution from the temperate to the tropics growing on living and dead broad-leaved trees, decayed stumps or logs and could be found across Europe, North America, Asia, Australia, South America and Africa.

A survey of macrofungi and their ethnomycology was conducted by Osemwegie *et al.* (2006) in Edo and Delta states (latitudes 4.87^0 to 7.12^0 E and longitudes 5^0 to 7.6^0 N) of Nigeria and reported 53 species of macrofungi, comprising 23 basidiomycetes including *A. auricula*, *Pleurotus* spp., *Schizophyllum commune* and *Volvariella volvaceae.* Similarly, Sibounnavong *et al.* (2008) collected mushrooms from Philippines during dry season and identified seven species among which one species belonged to the Order Tremellales (Jelly Fungi) and another in the Order Auriculariales (*Auricularia fuscosuccinea*). In Australia, *Auricularia* was found in *Eucalyptus* woodland and rainforests where it grew in very large colonies on fallen logs (Harding and Patrick, 2008).

In Kerala, *Auricularia* spp. is widely distributed. Vidyaresmi (2008) in a survey conducted at different parts of Thiruvananthapuram district found the occurrence of *Auricularia* at Vellayani, Balaramapuram, Kalliyoor, Palappur and Vanchiyoor. She reported that *Auricularia* was found on coconut tree basin, fallen wooden logs, dead stumps of mango and anjili trees from all the locations. Regular survey and collection of macrofungi were carried out in reserve forest of

erstwhile Goalpara district of Western Assam and the study revealed that the major collection was of macrofungi *Ganoderma lucidum* followed by *Cantharellus tubaeformis*, *Agaricus bisporus*, *S. commune*, *A. delicata*, *Boletus luteus*, *Cantherallus cibarius*, *Lycoperdon cladopus* and *Termitomyces clypeatus* (Sarma *et al.*, 2010). Geetha (2011) reported the occurrence of *Auricularia* spp. from dried logs of mango, moringa and casuarina and *Tremella* species from dead logs of coconut and cashew from Western ghats and forest areas of Kerala. Mohanan (2011) reported that *A. polytricha* was widely distributed in moist deciduous to wet evergreen forests of the Western Ghats of Kerala where it occurred in bunches on rotting branches, twigs, decayed stumps and logs.

Du *et al.* (2011) reported that *Auricularia* spp. are saprotrophs and have a worldwide distribution from the temperate to the tropics, growing on living and dead deciduous trees, decayed stumps or logs. They collected and cultivated *A. polytricha* extensively in China. The genetic diversity of 20 wild *A. polytricha* strains from Yunnan Province and four cultivated strains from Henan and Sichuan Province were profiled by using 10 pairs of sequence-related amplified polymorphism (SRAP) primers.

Haryati and Azrianingsih (2012) studied on the three edible mushrooms which were consumed mostly by the villagers and tribes of East Java Indonesia, *i.e.*, *Pleurotus* sp., *Clitocybe nebularis* and *A. auricula*. *A. auricula* was characterized by distinct soft flesh of the fruit body, dark colour and absence of ring on the stipe. They stated that the mushrooms occurred on dead trunks of pasang tree (*Lithocarpus sundaicus*), danglu (*Engelhardia spicata*), kemlandingan (*Albizia montana*), casuarina (*Casuarina junghuhniana*) and acacia (*Acacia decurens*). Gogoi and Sarma (2012) collected a total of 12 edible macrofungi from macrofungal biodiversity in Dhemaji district of Assam, which comprised *A. auricula*, *Lycoperdon perlatum* and *Tricholoma luscinatum* which were extensively distributed around the district.

Leon *et al.* (2012) reported fourteen fungal species from Northern Philippines which included *Auricularia auricula* and *A. polytricha.* Nwordu *et al.* (2013) conducted a national survey on mushroom and many species were identified and documented in Nigeria, the most common species found were *Termitomyces*, *Auricularia*, *Chlorophyllum*, *Corprinus*, *Russula*, *Pleurotus*, *Fomes lignosus*, *Panus fulvus*, *Lepiota procera*, *Lepista flaccid*, *Lactarius deliciosus*, *Auricularia polytricha* and *Coprinus* spp.

Devi *et al.* (2013) conducted survey in the hills of Nilgiris, Shervoys and lower Pulneys during rainy season and collected wood ear mushroom (*Auricularia* sp.) from coffee plantations of Horticultural Research Station, Yercaud. Survey was conducted by Toma *et al.* (2013) to identify the wild mushroom that grew naturally in different area and different season in Erbil Governorate, Kurdistan region of Iraq and identified several mushrooms namely *Agaricus* spp., *Coprinus* spp., *Cortinarius* spp., *Exidia* sp., *Fomes* spp. and *A. auricula-judae.* A survey was conducted by Gateri *et al.* (2014) in coastal Kenya to document the edible species of mushrooms based on the habitat, morphological and phenotypic features and the edible mushrooms collected were *Cantharellus* spp., *Agaricus* spp., *Pleurotus* spp., *Russula* spp. and *Auricularia* spp. from dead and decayed wood. Choudhury and Sarma (2014) reported the occurrence of *A. auricula* on decaying *Bambusa* spp., *A. auricula- judae* from decaying branches of unknown tree, *A. polytricha* from *Delonix regia* and *Auricularia* sp. from *Albizzia procera* and they also studied the detailed descriptions of these *Auricularia* spp. from Sonitpur District of Assam.

A survey was carried out by Sylvester *et al.* (2014) to identify the edible mushrooms in Enugu State, Nigeria. The survey covered the seventeen local government areas in Enugu State and results showed that eight edible mushrooms namely *Pleurotus tuber-regium*, *A. auricula-judae*, *Lactarium triviralis*, *Russulla vesca*, *Termitomyces mammiformis*, *S. commune* and *Lentinus squarrosulus* were commonly found in all the local government areas surveyed. Lazo *et al.* (2015) collected and identified *A. auricula, A. fuscosuccinea* and some other mushrooms

like *Schizophyllum commune*, *Volvariella volvacea*, *Lentinus* sp., *Pleurotus* sp. and *Polyporus* sp.

A survey on mushroom was carried out by Adebiyi and Yakubu (2016) in Ado and Ikere local government areas of Ekiti State, Nigeria. The collected mushrooms were taxonomically identified. Among 109 samples, 19 species were identified out of which 11 were edible while eight were inedible. The edible ones included *Auricularia* spp., *Lentinus squarrosulus*, *Termitomyces robustus and Agaricus campestris*. These mushrooms were distributed among different substrates which included soils, bark of trees, dead wood/trees and dead leaves.

Toshinungla *et al.* (2016) studied the diverse flora of wild edible mushroom of Nagaland and a total of 33 species belonging to Auriculariaceae, Cantharellaceae, Russulaceae, Polyporaceae, Hericiaceae, Dacrymycetaceae, Schizophyllaceae, Boletaceae, Amanitaceae, Tricholomataceae, Pleurotaceae, Clavariaceae, Tremellaceae, Hygrophoraceae, Suillaceae, Hydnangiaceae, Pyronemataceae, Agaricaceae and Lyophyllaceae were collected and identified.

2.2. MORPHOLOGICAL CHARACTERS

2.2.1. Macroscopic Studies

Wong *et al.* (1985) stated that basidiocarp of *Tremella mesenterica* resembled brain with irregular clustered folds consisting of one to several distorted lobes measuring 0.5 cm × 0.3 cm in individual basidiocarps. They also reported that the stipe of the mushroom was absent and the colour was pale yellow, more rarely whitish or almost colourless. Contradictorily, Roberts and Meijer (1997) reported that the fruiting body of *Tremella fuciformis* was gelatinous, watery white in colour, diameter up to 7.5 cm and consisted of thin sea weed like branching fronds with crisped edges.

Wong and Wells (1987) studied the comparative morphology of *A. cornea*, *A. polytricha* and *A. tenius* and they reported that the shape was ranging from bell shaped to ear-shaped and flattened and variations were also observed in the

texture of the basidiocarps. Pegler (2001) reported the morphological characters of jelly mushrooms and reported that, the fruiting body of *Auricularia* spp. was purplish grey-brown, human ear shaped, gelatinous, 2-15 cm width with soft velvety coat on the upper surface whereas, *Tremella* spp. resembled a white chrysanthemum flower and measured 10-15 cm width with many semi-transparent and leaf-like lobes. Musngi *et al.* (2005) examined the morpho-anatomical characters of *A. auricula*, *A. fuscosuccinea*, *A. polytricha* and *A. tenuis*. *A. auricula* had tough, gelatinous texture and yellow brown coloured basidiocarps, *A. fuscosuccinea* possessed thin translucent basidiocarp, *A. polytricha* with convex shaped larger sized (5-6 cm) sporocarps and *A. tenuis* showed tough, gelatinous basidiocarp with solitary nature.

Sobal *et al.* (2007) reported that the species of *A. auricula* had brown coloured basidiocarps despite the fact that lighter basidiocarps were also observed. With respect to basidiocarp shape, marked variations were observed ranging from auriform, discoid to campanulate. Sibounnavong *et al.* (2008) described the fruiting body of *A. fuscosuccinea* as irregularly cup-shaped or ear-shaped, reddish brown, gelatinous-textured, measured 3–6 cm diagonally and laterally attached without a stipe. The outer surface was covered with very short and greyish hairs whereas surface layer was pale brown, wrinkled or veined and flesh was thin and semi translucent. Sterry *et al.* (2009) reported that the species of *Auricularia* had a tough, gelatinous and elastic texture when fresh, but hard and brittle while dried. The outer surface showed a bright reddish tan with a purplish tint, covered with grey coloured thin and downy hairs with undulating folds and wrinkles.

The fruiting body of *A. auricula* was three to eight centimetres (1.2 to 3.1 in) and extended up to 12 centimetres (4.7 in) with droppy ear shape and laterally attached to the substrate with a very short stalk (Sterry *et al.*, 2009). Mohanan (2011) studied the fruiting body characters and reported that *Auricularia* spp. were resupinate or pileate, loosely attached and elastic textured, yellowish brown to dark brown coloured with greyish brown silky hair. Choudhury and Sarma

(2014) reported that the fruiting body of *A. polytricha* was about 6 to 8 cm, smooth, fan shaped and occured in group. The upper surface was dark brown and the stipe was absent. *A. auricula* exhibited wavy irregular margin without hairs at lower surface and occurred solitary on the substrate.

2.2.2. Microscopic Studies

Lowy (1952) studied the classification of jelly mushrooms based on the internal stratification of hyphae, presence of abhymenial hair and of a strongly or weakly differentiated medulla. He defined medulla as a zone composed of hyphae of 6-10 micrometer broad, positioned centrally and arranged parallelly through the middle of the basidiocarp. The other different zones observed were zona pilosa, zona compacta, zona subcompacta, zona laxa, schizomedulla and cortical layer.

Roberts and Meijer (1997) explained that haustorial cells of *Tremella* spp. arose on the hyphae, produced filaments that attached and penetrated the hyphae of the host and the basidia were tremelloid and measured 10-13 × 6.5-10 µm. The basidiospores were ellipsoidal, smooth, 5-8 × 4-6 µm in size and germinated through hyphal tube. Spores of *Auricularia* spp. were smooth, hyaline, reniform to allantoid, measuring 14-18 × 68 µm and guttulate (Philips and Roger, 1981). Young *et al.* (2005) reported that the spores of *Auricularia* were long and sausage shaped, ranging in size from 16 to 18 µm long with a thickness of 6 to 8 µm and the spore colour was white, creamy, yellowish or hyaline.

Musngi *et al.* (2005) made extensive studies by taking cross sections of mushroom *Auricularia* spp. and reported marked hyphal organization with varied desirable zonations *viz.*, zona pilosa, zona compacta, zona sub compacta superiosis, medullary region and zona laxa inferiosis. The hymenium was single layered with dense structure and the basidia were cylindrical and transversely triseptate with elongated sterigmata.

Weber and Webster (2006) studied the spore characters of *A. auricula-judae* and reported that the mushroom produced basidiospores from cylindrical basidia which were divided into four cells by three transverse septae and each cell produced an elongated epibasidium with a single basidiospore at the hymenial surface of the fruit body. Mohanan (2011) reported that hymenium of *Auricularia* was smooth and the hairs were thick walled, 0.6 mm length and cylindrical. The basidium was hyaline and septate measuring 46-60 × 4-5.5 µm with 1-3 lateral sterigmata. Individual spores were hyaline, reniform to allantoid, 13-16 × 4-5.5 µm and the spores occurred as a whitish mass underneath the fruiting body. Similar observations were reported by Choudhury and Sarma (2014) who mentioned that *A. polytricha* had smooth hymenium, hyaline, 8–10 µm in length basidiospores and cylindrical, hyaline basidia with 2-3 septate, whereas *A. auricula* spores had elongated shape, measured 12-16 µm and the hyphae of both the species were smooth, hyaline and septate.

Bandara *et al.* (2015) described the microscopic features of a new species *viz.*, *A. thailandica* with different measurements of hyphal zonations. Zona pilosa measured ≤ 80 µm, zona compacta 23-46 µm, zona subcompacta superioris 10-29 µm, zona laxa superioris 35-181 µm, medulla 31-98 µm, zona laxa inferioris 98-307 µm, zona subcompacta inferioris 21-78 µm, hymenium 44-78 µm and basidia 34.67-48.41 × 2.73-6.31 µm. The shape of basidia was reported to be cylindrical with tapered or blunt ends. Onyango *et al.* (2016) observed the different zonations of two groups of *Auricularia* sp. and stated that except for the size of medulla, all the other hyphal zonations were similar to each other.

2.3. ISOLATION AND PURE CULTURING

The pure cultures have traditionally been maintained through periodic sub-culturing and/or cold storage under refrigeration at a temperature range of 2-5 ^{0}C (Smith and Onions, 1983).

Upadhyay (1999) isolated internal tissue from basidiocarp of *A. mesenterica* and raised pure culture on two per cent malt extract agar medium. Pure cultures

could also be obtained from either single or multispore culturing but mainly tissue isolation from a high yielding basidiocarp is beneficial (Marcel, 2004). Liu (2005) isolated pure culture of *A. auricula-judae* on potato dextrose agar (PDA) and germinated into hyphae under favourable conditions. Kushwaha *et al.* (2006) maintained the pure culture of *A. polytricha* and multiplied on 2% malt extract agar medium. Wu *et al.* (2006) maintained the strain of *A. auricula* - AA2 on potato dextrose agar (PDA) slants and incubated at 25 ^{0}C for seven days.

The suitability of 2 % malt extract medium for isolation and culturing of Kenyan native strains of wood ear mushroom *A. auricula* was also reported by Onyango *et al.* (2011a) and that of potato dextrose agar medium for *Flammulina velutipes* by Hassan *et al.* (2012). Ma *et al.* (2014) prepared pure cultures of *A. auricula-judae* strain Techan 2 which was isolated from a wild *A. auricula-judae* and maintained in potato dextrose agar (PDA). Devi *et al.* (2015a) utilised the sporophores of *Auricularia* spp. for tissue culturing and mycelial culture obtained was maintained on PDA medium.

2.3.1. Strainal Variations

Wong and Wells (1987) reported three strains of *Auricularia* spp. namely, yellow brown soft strains, gelatinous brown strains and dark brown tough and leathery strains. Musngi *et al.* (2005) classified the different strains of *Auricularia* species based on the apparent characteristics of the mature basidiocarp. The exterior characteristics included for strain selection were colour and texture of the basidiocarp, shape, type of margin and hymenial layer. Thirteen strains were identified in *A. polytricha*, five strains in *A. fuscosuccinea,* four strains in *A. tenuis* and three strains in *A. auricula.*

Palapala *et al.* (2006) reported the occurrence of three native strains of *A. auricula* in Kakamega Forest of Western Kenya. Sobal *et al.* (2007) reported that most of the yellow brown strains of *Auricularia* were ear shaped, brown strains were discoid while that of dark brown strains were flattened. Du *et al.* (2011) obtained 24 strains of *A. polytricha* and classified into six groups. Group I

consisted of 10 strains, group II four cultivated strains, group III and IV with three and five strains respectively but group V and VI had only one strain each and wild strains had more morphological and genetic diversity than cultivated strains. Onyango *et al.* (2011b) stated that most of yellow brown strains of *Auricularia* were ear shaped, brown strains were discoid and campanulate while the dark brown strains were flattened.

2.4. PHYSIOLOGICAL STUDIES

2.4.1. Growth in Different Media

Every living being requires food for its growth and reproduction and they derive food from the substrate upon which they grow. In order to culture fungi artificially, the essential nutrients needed for the growth and development are to be supplemented in the medium.

Tabata and Ogura (2003) reported that *A. polytricha* grew well on PSA (potato sucrose agar) supplemented with calcium sulphate and calcium phosphate @ 1, 5 and 10 % concentration and magnesium sulphate and magnesium chloride @ 0. 5, 1 and 5 % concentration. Several media were reported to be favourable for the mycelial growth of different mushrooms. Hur *et al.* (2008) studied the effects of nine different growth culture medias on the mycelial growth of *Phellinus* spp. and concluded that PDA yielded maximum growth. Ukoima *et al.* (2009) reported that the highest mycelial growth of *P. sajor-caju* was obtained from rice bran/soil culture medium (7.8 cm), *P. tuber-regium* on cassava peels soil culture medium (5.8 cm) whereas, *V. volvaceae* had maximum growth from palm fibre culture medium (7.1 cm).

Kibar and Peksen (2011) reported that culture media were important as they supplied the required nutrients for mycelial growth and malt extract agar supported the maximum mycelial growth rate of *Tricholoma terreum.* Kapoor *et al.* (2011) reported that among seven different media, optimum growth of *A. polytricha* was recorded on three media namely Czapeck's, malt extract and potato dextrose with adjusted pH of 6.5 and temperature 25 and 30 ^{0}C. Jing *et al.* (2014)

studied the influence of carbon and nitrogen sources on the cultural characteristics of *A. delicata*, *A. auricula* and *A. polytricha.* The most preferred carbon source were sugars for *A. delicata*, *A. auricula* and *A. polytricha* while that for *Auricularia* sp. was lactose. The best nitrogen sources for *A. delicata* and *A. polytricha* were beef extract and for *A. auricula* and *Auricularia* sp. yeast extract and peptone supported well. Devi *et al.* (2015b) recorded potato dextrose medium as the best to support the maximum biomass production of three *Auricularia* species and for both *A. delicata* and *A. polytricha*, the next best medium was yeast potato dextrose and malt extract for *A. auricula.*

2.4.2. Growth in Different Temperature

Of all the physical factors influencing fungal growth, temperature is certainly one of the most important and frequently studied factor. The temperature extremes (maximum and minimum) are of great importance in determining the survival and distribution of a mushroom species in nature. Kibar and Peksen (2011) stated that increase in temperature led to denaturation of important enzymes which catalysed fungal metabolic processes.

Upadhyay (1999) reported the optimal temperature of 30 ^{0}C to complete 90 mm radial growth of *A. mesenterica* within ten days and growth was inhibited completely at 10 ^{0}C and 40 ^{0}C. Zervakis *et al.* (2001) studied the influence of environmental parameters on linear mycelial growth of *A. auricular-judae* with a varied range of temperature and opined that a temperature of 20 ^{0}C or 25 ^{0}C favoured the growth of *A. auricular-judae*. Reports by Shim *et al.* (2005), Imtiaj and Rahman (2008), Jayasinghe *et al.* (2008) and Lai *et al.* (2011) concluded that the favourable mycelial growth of *Schizophyllum commune*, *Lignosus rhinoceros*, *Macrolepiota procera* and *Ganoderma lucidum* was at 30 ^{0}C.

Jing *et al.* (2014) studied the influence of environmental conditions on the cultural characteristics of four main wild species of *Auricularia* and concluded that the optimum temperature for *A. auricula* was found at 30 ^{0}C and for *A. polytricha*, *Auricularia* sp. and *A. delicata*, 25 ^{0}C as the optimum condition.

Mbaluto (2015) reported that the optimum temperature was 25 ^{0}C for the mycelial growth and density of tested *Macrolepiota* accessions and also opined that with increased temperature growth was suppressed with lowest mycelial growth. Devi *et al.* (2015b) reported the growth response of *Auricularia* species at seven different incubation temperatures and indicated that at a temperature of 28 ^{0}C, both *A. delicata* and *A. polytricha* attained their maximum biomass production.

2.4.3. Growth in Different pH

pH affects almost every function of the fungi. For each fungus, there is a particular pH below which it will not grow. Likewise, there is a particular pH above which growth ceases. Hydrogen ion concentration of the medium has a profound effect upon the rate and amount of growth and many other life processes of the fungus (Lilly and Barnett, 1951). Kinjo and Kondo (1979) found the optimum pH for the growth of *A. polytricha* as 7-8 and even in lower pH of 4.0 the mycelia grew over 50 %. Ma and Luo (1992) reported that *Auricularia* attained vigorous mycelial growth at a pH range of 5.5 to 6.5 whereas at pH below 5 and above 7 the mycelial growth was significantly decreased.

Shim *et al.* (2005) showed the rapid mycelial growth of *Macrolepiota procera* at pH 7. Jayasinghe *et al.* (2008) also reported that *Ganoderma lucidum* strain grown with a pH range of 5 to 9 but optimum growth was obtained at neutral pH. Imtiaj and Rahman (2008) and Lai *et al.* (2011) experimented on *Lignosus rhinoceros* and *Schizophyllum commune* and reported that pH 6 and 7 were ideal. Acidic conditions were found favourable for mycorrhizal fungi but ectomycorrhizal members of *Agaricales* were favoured in neutral to near neutral pH (Kibar and Peksen, 2011). Mbaluto (2015) stated that the pH of the medium is an important factor for mycelial growth of macrofungi which preferred different pH conditions and also reported that favorable mycelial growth of *Macrolepiota* spp. was obtained at a pH range of 7-8.

2.4.4. Growth in Light and Dark Conditions

The phototropic response of fungus for the production of reproductive structures is noteworthy. The formation of primordia of fruiting bodies of many basidiomycetes was triggered by light (Chang and Miles, 2004).

Vidyaresmi (2008) tested different ranges of light conditions like sun light, room light, intermittent light and darkness for the efficacy in the production of biomass of *Auricularia* spp. and recorded that room light produced maximum mycelial growth and biomass production of 0.95 g. Kalaw *et al.* (2016) reported that in the presence of light *A. polytricha* attained comparatively higher radial growth (2.42 cm) than in dark conditions (2.39 cm). Contradictorily, the suitability of dark condition for mycelial growth of mushroom has been reported by many researchers. Dulay *et al.* (2012) reported that *Lentinus tigrinus* (Bull.) Fr. had maximum mycelial growth under dark conditions (86.67 mm) when compared to light conditions (83.33 mm). Sumi (2016) reported that mycelial growth of *Hypsizygus ulmarius* a took minimum time of 8.4 days under dark conditions whereas, a maximum time of 8.9 days was taken in light conditions. Deepa (2016) also recorded maximum growth of *Lentinula edodes* (9.0 cm) in dark conditions as well as in ambient light conditions (400 lux at day time + darkness at night time).

2.5. SPAWN PRODUCTION

The word spawn is derived from the Latin word *espandre*, meaning, "to spread", and spawn is a substrate into which mushroom mycelium has been impregnated and developed to serve as the main inoculum, or seed, for the substrate in mushroom cultivation (Chang, 2001; Chang and Miles, 2004). The success or failure of mushroom production is mainly influenced by the genetic makeup of the specific inoculant and quality of the substrates used for the preparation of spawn.

Different media such as grain of sorghum, millet, rye or wheat can be used for spawn production (Sinden, 1934; Marcel, 2004). Upadhyay (1999) prepared the spawn of *A. mesenterica* on wheat grains in glass bottles. Stamets (2000) suggested dense and thick mycelia to be used for inoculation of grains for spawn preparation. Oei (2003) attributed that quality of the inoculants and aeration influenced the rate of mycelial growth. Kushwaha *et al.* (2006) prepared the spawn of *A. polytricha* in glass bottles using boiled and autoclaved wheat grain, inoculated a small bit of 10 days old mycelial culture under aseptic condition and incubated at 25 ^{0}C for 20 days.

Vidyaresmi (2008) prepared the grain spawns of *Auricularia* spp. with paddy grains and maize and sawdust spawns with rubber, anjili, jack, mango and coconut sawdusts. Among these substrates, maize grain and rubber sawdust took minimum days for spawn run and she also reported that coir pith was the most unsuitable substrate without any spawn run. Ekpo *et al.* (2009) opined vigorous substrate colonization by the mycelium during spawn run as a desirable factor for reducing mushroom cropping time and for overcoming the competitors in the substrate. Onyango *et al.* (2011a) produced spawns of three Kenyan native strains of *A. auricula* following bottle culture technology which resulted in high quality spawns on supplemented with millet and sorghum grains. They also reported that suitable combination of nutritional ingredients like carbon, nitrogen, lipids and minerals helped in the highest rate of mycelial colonization. Razak (2013) agreed that the smaller size of crushed corn provided a larger area and fetched more nutrients for the mycelial growth compared to whole corn. Crushed corn without the addition of nitrogen source recorded the highest mycelial growth rate of 6±0 mm/day for *A. polytricha.*

2.6. SUBMERGED CULTURING

Lopez and Vazquez (1997) compared the biomass production of *A. fuscosuccinea* in different liquid media like water from soaking maize (WSM), water from coffee pulp (WCP), sorghum infusion (SI), cheese whey (CW), water

from washing coffee beans (WCB) and synthetic glucose extract medium (SGE), and the maximum yield was obtained from SGE (3.68 g/L). Lomberh *et al.* (2002) worked on the laboratory scale submerged culture production of medicinal mushrooms including *Auricularia auricula*, *Flammulina velutipes*, *Ganoderma lucidum*, *Grifola frondosa*, *Hypsizygus marmoreus*, *Lentinula edodes*, *Piptoporus betulinus* and *Pleurotus ostreatus* and reported that the mycelial dry weight ranged from 6-25 g/L.

Yang *et al.* (2002) studied the dry mycelial weight and hypolipidemic effect of an exo-biopolymer (EBP) produced from *A. polytricha* under submerged culture production for which pH 4 and a temperature of 20 ^{0}C were found optimum. They obtained maximum dry weight of 11.1 g/L and 1.2 g/L of EBP. Xu and Yun (2003) optimized the submerged culture conditions for mycelial growth and exobiopolymer production of *A. polytricha*, and recorded the maximum dry weight of mycelium as 6.14g/L and 2.12. g/L of exobiopolymer at a temperature of 25 ^{0}C and pH of 5.0. Jeong *et al.* (2004) reported that dry mycelial weight of 11.3 g/L and exo-polymer (EP) of 0.5 g/L were obtained in submerged culture of *A. auricula-judae* at optimal pH of 5-6 and temperature of 25 ^{0}C.

Cho *et al.* (2006) reported on the optimization of submerged culture conditions for mycelial growth and exopolysaccharide (EPS) production of *Tremella fuciformis* in shake flasks and bioreactors and observed that a temperature of 28 ^{0}C and pH 8 were optimum. The influence of pH conditions on exopolysaccharide (EPS) and dry mycelial production of *A. auricula* was investigated in submerged culture by Wu *et al.* (2006) and indicated that the highest EPS concentration (7.5 ± 0.14 g/L) and dry mycelial growth rate (3.9 ± 0.04 g/L) were obtained at pH 5.0 and 5.5.

Confortin *et al.* (2008) recorded 8.18 g/L of biomass when glucose was used as a carbon source whereas, 5.94 g/L of biomass for sucrose with 1.58 g /L of exopolysaccharide were obtained from submerged culture production of *Pleurotus sajor-caju*. Xu *et al.* (2008) studied the most efficient and highly economical

production of ganoderic acid (GA) and mycelial biomass of *Ganoderma lucidum* SB97 in the submerged culture conditions.

Jonathan *et al.* (2009) reported that the mushroom *A. polytricha* produced the highest mycelial biomass of 340 mg cm^{-3} at 25 ^{0}C and pH of 6.5 after ten to fifteen days of incubation whereas glucose supported the maximum biomass production of 375 mg cm^{-3} at 1.6 % concentration and 0.8 % peptone produced a biomass yield of 320 mg cm^{-3} under submerged culture conditions.

According to Hassan *et al.* (2012), the maximum mycelial biomass of *Flammulina velutipes* was obtained from optimal submerged culture conditions with a temperature of 25 ^{0}C and initial pH of 6.5. Zhang *et al.* (2015) studied the production and characterization of melanin from *A. auricula* under submerged culture and obtained the maximum yield of 1008.08 mg/L in optimum culture conditions which contained appropriate amount of glucose, tyrosine, peptone and calcium carbonate.

2.7. CULTIVATION

Mushrooms require lignocellulosic materials for their growth and development of fruiting bodies. The cultivation of *Auricularia* was first reported in China during 600 A. D. *Auricularia auricula-judae* and *A. fuscosuccinea* were first cultivated in China. The Chinese *Materia Medica* was a classical Chinese book, which showed the older method of wood ear cultivation in Tang dynasty (Lou, 1978; Quimio, 1979).

Wong and Wells (1987) cultivated *Auricularia auricula* and *A. polytricha* using sugarcane bagasse and rolled oats medium in 6:1 ratio in one litre beaker and they reported that fruiting body formation was noticed after 3-4 weeks. However, there was a report of successful usage of wheat straw as fruiting substrate for this species (Jianjung, 1991). Similarly, Wong (1993) reported the successful fruiting of *A. fuscosuccinea* and *A. delicata* in the sugarcane bagasse-rolled oats media. Upadhyay (1999) conducted experiment on cultivation of *A.*

polytricha in wheat straw substrate and reported that the mycelial growth and fructification were maximum at 25-30 ^{0}C with a relative humidity of 70-80 %. Biological efficiency of 137.7 % and 174 % was recorded at 4 and 8 weeks of cropping duration.

Zervakis *et al.* (2001) recorded the mycelial extension rates of *A. auricula-judae* on seven mushroom cultivation substrates like wheat straw, poplar sawdust, oak sawdust, cotton waste, peanut shells, corn cobs and olive press cake, and reported that the mycelium extension rate was the highest in wheat straw. Carrera *et al.* (2002) reported that the capacity of wood ear mushrooms to grow on agricultural wastes such as maize cobs is due to the production of lignolytic enzymes that are necessary for degradation of such substrates. Petcharat and Tongwised (2003) reported the suitability of oil palm kernel meal on production of *A. polytricha* and *Lentinus squarrosulus.*

Chang and Miles (2004) stated that the dual culture method was commercially used for the cultivation of *Tremella fuciformis* using sawdust mix with preferred host like *Annulohypoxylon archeri.* Sharma *et al.* (2005) cultivated *Auricularia polytricha* by sterilizing the substrates with formaldehyde and carbendazim under *in vitro* and *in vivo* condition. According to Shashireka *et al.* (2005) substrates with higher nutrient bases exhibited higher quality mushrooms as compared to substrates deficient in important carbon and nitrogen sources. Kwon (2004) reported the use of various hardwood sawdust supplemented with nitrogen-rich additives like rice bran for mushroom cultivation.

Kushwaha *et al.* (2006) evaluated different substrate combinations for the cultivation of *A. polytricha* for two years *i.e.*, 2002–03 and 2003–04 and reported that the yield performance was superior in rapeseed straw + 4 % wheat bran *i.e.*, 483.00 and 473.15 g in both the years. Palapala *et al.* (2006) reported that Kenyan native wood ear mushrooms grew well on locally available substrates like wheat straw, sawdust, maize cobs, maize stalks and sugarcane bagasse.

Vidyaresmi (2008) studied on the suitability of six different locally available cheap substrates for the cultivation of *Auricularia* spp. and reported that rubber sawdust gave maximum yield of 180.67 g in 3 harvests and the lowest yield was obtained from paddy straw (131.73 g). Similarly, Kapoor *et al.* (2011) cultivated *A. polytricha* on corn cobs, maize stalks, sawdust and wheat straw and recorded maximum biological efficiency of 73.2% from non-supplemented wheat straw and wheat straw supplemented with four per cent wheat bran (73.6 %). Onyango *et al.* (2011b) studied the performance of different agricultural wastes as substrates for the cultivation of *A. auricula* and obtained the maximum yield from maize cob (67.0 %) for brown strains and 63.7 % for black strains. Irawati *et al.* (2012) reported the biological conversion of *Auricularia polytricha* on wood meals of *Falcataria moluccana*, *Shorea* sp. and *Tectona grandis*. Among them, *F. moluccana* had the highest biological conversion (15.6 %) with minimum period for harvesting.

Devi *et al.* (2013) reported the minimum number of days for completing spawn run of *A. polytricha* (21.3 days), for pinhead formation (31.3 days), for first harvest (35.6 days) and biological efficiency (59.04 %) from paddy straw + wheat bran (3:1). Report from Razak (2013) indicated that the maximum yield of *A. polytricha* was produced from sawdust + empty fruit bunch (50:50) with a moisture content of 85 % and yielded 25.2±1.45 % biological efficiency while sawdust alone with 65 % moisture content recorded low biological eficiency of 18.7±6.64 %. The optimal conditions for spawn run of *A. polytricha* was studied by Devi *et al.* (2015a) who reported that temperature ranging from 20±2 ^{0}C to 24±2 ^{0}C is required for primordial formation and 22±2 ^{0}C with a relative humidity of 80-85 % is favourable for fruiting body induction.

2.8. AMENDMENTS

Amendments are the nutrient supplements that regulates the production of mushroom. Royse *et al.* (1991) opined that supplements *viz.*, soybean meal, rice and wheat bran as nutrient bases along with substrates were essential to achieve

maximum mushroom yields. Mycelial growth of mushrooms could be increased two times when supplemented with bran and it acted as a rich source of protein to the basal substrate (Royse, 1997). Similarly, Hadwan *et al.* (1997) recommended the use of different supplements namely rice bran, spent grain and wheat grain with the substrates before spawning in order to increase the yield as well as optimum growth for the cultivation of mushroom. Royse and Vazquez (2001) suggested different starch-based supplements namely wheat bran, rice bran, rye, millet and maize powder to sawdust basal substrate as important sources of major nutrients.

Quimio (1982) reported a mixture of sterilised sawdust with 2-20 % rice bran as a medium for the production of *Auricularia* spp., which provided essential nutrient compounds for the growth and development of mushrooms. As per the reports of Chang and Quimio (1982), *A. polytricha* could be produced on a synthetic medium consisting of nutrient supplements like cotton seed hulls, bran, and other cereal grains. Bhandal and Mehta (1986) obtained 60-80 % biological efficiency of *A. polytricha* on both fresh and composted wheat straw supplemented with rice bran. Tabata and Ogura (2003) supplemented 1-5 % of calcium phosphate, calcium carbonate and 0.5 % of magnesium carbonate, magnesium hydroxide, magnesium sulphate and magnesium chloride to the sawdust medium and reported that calcium content of *A. polytricha* increased 1.1-1.5 times and magnesium content by 1.7-2.2 times.

Shashireka *et al.* (2005) attributed the presence of several amino acids, protease and transaminase enzyme activities on wheat bran which influenced the increased rate of mycelial growth of mushrooms. Ayodele and Akpaja (2007) stated that supplementation of substrates is one of the important factors for increased mushroom production. Uhart *et al.* (2008) reported increased biological efficiency of 1.2 fold times when supplemented with rolled oats and soybean flour to the wheat straw for the cultivation of *Agrocybe cylindraceae*.

Onyango *et al.* (2013) recorded the maximum yield of *Auricularia* with supplementation of 10 % wheat bran to the composted maize cobs (282 g) while fresh maize cobs with same supplementation produced 230 g of fresh mushrooms and the least yield was obtained from fresh grass straw without supplementation (78.4 g). Lau *et al.* (2014) reported the highest biological efficiency of *A. polytricha* (34.3-37.4 %) from rubber sawdust supplemented with rice bran in a ratio of 80:20 and rubber sawdust with empty oil palm fruit bunch (75:25) but empty oil palm fruit bunch and sago waste alone significantly reduced the yield. Ma *et al.* (2014) cultivated *A. auricula-judae* by supplementing sawdust 80 % with wheat bran 10 %, rice bran 6 %, corn meal 2 %, gypsum 1 % and lime 1 % and adjusting the moisture content to 65 %. Devi *et al.* (2015a) amended paddy straw with wheat bran, rice bran and sawdust for the production of *A. polytricha* and recorded the highest yield of 155 g with 62 % biological efficiency and maximum fruiting body weight (6.10 g) for 25 fruiting bodies in paddy straw + wheat bran (3:1).

2.9. PROXIMATE CONSTITUENTS

Mushrooms are consumed as food and/or nutritional attributes and determination of nutritional value requires plenty of scientific work which involves the analysis of the proximate constituents present in the particular mushroom. The composition of a given mushroom is highly influenced by the diversity of its genetic makeup and environmental conditions. Crisan and Sands (1978) pointed out that cultivation practices and inaccuracies that were inherited in different methods of analyses also introduce variation in the analytical data.

Nutritional value of edible black fungus, *Auricularia* spp., was endorsed to their chemical constituents. The chemical composition of mushroom depended mainly on the species, substrate where they had grown and age of fruiting bodies (Manzi *et al.*, 2001). Several genera of mushroom were edible and rich sources of essential nutrients *viz.*, carbohydrates, proteins, vitamins, minerals, fat, fibre and various amino acids (Okwulehie and Odunze, 2004) and they received global

consideration for their antioxidant compounds like ascorbic acid, β-carotene and α-tocopherol (Jagadish *et al.*, 2009). *A. auricula* contained melanin which increased the level of antioxidants (Tu *et al.*, 2009).

A. polytricha was reported to contain potassium (K), phosphorus (P), sodium (Na), calcium (Ca) and magnesium (Mg) (Kaneda and Tokuda, 1966). Lu and Tang (1986) stated that *Auricularia polytricha* was rich in protein content (7.59 %) and fibre (3.69 %), with appropriate amounts of calcium and zinc, while fat content observed was low (1.12 %). Yagi and Tadera (1988) isolated a lectin compound with a molecular weight of approximately 23 kDa from *A. polytricha.* Chang and Miles (2004) reported that fruiting bodies of *Auricularia* spp. contained high levels of protein (about 30 %) on dry weight basis and other essential elements like vitamins, minerals and polysaccharides. Lo *et al.* (2012) identified small amounts of lovastatin (16 mg/kg) and 1.4 mg/kg of ergothioneine in dried fruiting bodies of *A. polytricha.* Kadnikova *et al.* (2015) reported that *A. auricula* was proved to be a good source of almost all essential amino acids.

2.9.1. Moisture Content (%)

Chang *et al.* (1981) and Ragunathan and Swaminathan (2003) reported that fresh mushrooms contained about 90 % moisture and 10 % of dry matter. Gbolagade *et al.* (2006) reported that the moisture content of *A. polytricha* was 97.1 % and dry matter content of 2.9 %. *Calocybe indica* contained 86.0 % of moisture (Alam *et al.*, 2008). Johnsy *et al.* (2011) observed that the moisture content of collected mushrooms, such as *Pleurotus roseus*, *Pleurotus ostreatus*, *Pleurotus sajor-caju*, *Termitomyces microcarpus*, *Termitomyces heimii*, *Auricularia auricula*, *Volvariella volvacea*, *Lentinus squarrosulus*, *Lentinus tuber-regium* and *Grifola frondosa* ranged from 87.13 % to 95.17 %.

Kakon *et al.* (2012) reported that *A. polytricha* contained 87.1 % moisture and Celestine *et al.* (2013) recorded 82.17 % of moisture content in *A. polytricha.* Usha and Suguna (2014) reported that the moisture content of *A. polytricha* and *Pleurotus ostreatus* ranged from 90.6 % to 93.3 % and dry matter of 6.7 % to 9.4

% respectively. Kadnikova *et al.* (2015) reported that the moisture content of dried *A. auricula-judae* was 12.3 %.

2.9.2. Carbohydrate Content

Edible mushrooms are reported to be good suppliers of carbohydrates and their contents ranged from 40.6 % to 53.3 % on dry weight basis (Khanna *et al.*, 1992; Ragunathan *et al.*,1996). Carbohydrate compounds were present in a variety of polysaccharides with different particle sizes *viz.*, glycogen and indigestible forms as dietary fibre, cellulose, chitin, mannans and glucans (Manzi and Pizzoferrato 2000; Pizzoferrato *et al.*, 2000; Manzi *et al.*, 2001). Carbohydrate content of *P. ostreatus* was 46.3 % (Wang *et al.*, 2001) whereas, for *Calocybe indica* it was 48.50 % (Alam *et al.*, 2008).

A. polytricha contained less amount of sugar (5.45 %) as reported by Sanchez (2010). Johnsy *et al.* (2011) indicated that the carbohydrate content of *A. auricula* was 33.23 % and in *Pleurotus ostreatus* it was 43.4 %. Hung and Nhi (2012) reported that the total carbohydrate content of *Pleurotus ostreatus* was 61.3 %, 52.5 % in *Volvariella volvacea* and 65.1 % in *Lentinula edodes.* However, significantly higher amount of carbohydrate (87.6 %) in *A. polytricha* was reported by Kakon *et al.* (2012). Similar report was made by Celestine *et al.* (2013) who recorded 80.85 % of carbohydrates. According to Usha and Suguna (2014) the carbohydrate content of *Auricularia polytricha* was 28.5 % and that of *Pleurotus ostreatus* (44.7 %). Kadnikova *et al.* (2015) revealed that *A. auricula-judae* contained 66.1 g/100 g of carbohydrates which was the major compound and were in the form of polysaccharides represented by indigestible forms of uronic acids (38.8 %), water-soluble polysaccharides like mannans and glucans (10.2 %), pectin (7.4 %), chitin (5.4 %) and 4.3 % of cellulose.

2.9.3. Protein Content

According to Chan (1981) and Jonathan (2002), the protein content of mushroom was generally higher than those found in green vegetables. The

genetic structure, physical and chemical differences in growing medium showed variations in the protein content of mushroom (Ragunathan and Swaminathan, 2003; Sanmee *et al.*, 2003 and Murugkar and Subbulakshmi, 2005). Wang *et al.* (2001) reported that the crude protein content of *P. ostreatus* was 46.3 %. The protein content of *Calocybe indica* was reported to be 21.4 % (Alam *et al.*, 2008) and that of *P. florida* it was 23.5 % (Ahmed *et al.*, 2009).

The protein content of *A. polytricha* was reported to be low (8.90 %) compared to other mushrooms (Sanchez, 2010). Pushpa and Purushothama (2010) recorded 21.60 % of protein content in *Calocybe indica*, 41.06 % in *Agaricus bisporus*, 27.83 % in *Pleurotus florida* and 26.25 % in *Russula delica*.

The protein content of different mushrooms *viz.*, *Volvariella volvacea* (36.5 %), *Pleurotus ostreatus* (28.6 %), *Lentinus edodes* (26.3 %), *Genoderma lucidum* (13.3 %), *Auricularia polytricha* (7.2 %) and *A. auricula-judae* (12.5 %) was reported by Hung and Nhi (2012). Kakon *et al.* (2012) reported that *A. polytricha* contained 7.7 % of protein on fresh weight basis and Celestine *et al.* (2013) analysed it to be 3.75 %. However, Usha and Suguna (2014) reported a higher protein content of 36 % in *A. polytricha* and 33.3 % in *Pleurotus ostreatus*.

2.9.4. Fat Content

There is an ample variation observed in the fat content of different mushrooms as stated by Wang *et al.* (2001), Khan *et al.* (2008) and Ahmed *et al.* (2009) who reported a lower content of 4.4 % in *P. ostreatus*, 26.2 % in *Pleurotus sajor-caju*, 27 % in *Pleurotus ostreatus*, 26.8 % in *Pleurotus florida*, 25.5 % in *Pleurotus cystidiosus* and 26.3 % in *Pleurotus gestaranus*. The lipid content of *Calocybe indica* was reported to be 4.95 % by Alam *et al.* (2008).

The fat content of *A. polytricha* was reported to be 3.45 % by Sanchez (2010) whereas, Celestine *et al.* (2013) reported 2.01 % fat content. Usha and Suguna (2014) reported the crude lipid content in *A. polytricha* and *Pleurotus*

ostreatus as 4.37 % and 3.8 % respectively in their studies. Comparatively low fat content (1.75 %) was reported in *A. auricula-judae* by Kadnikova *et al.* (2015).

2.9.5. Fibre Content

Normally, fresh mushrooms contained relatively higher amounts of fibre which may be responsible for their high amount of ash content (Cheung, 1998). Fibre content of *Calocybe indica* was 12.9 % (Alam *et al.*, 2008). Celestine *et al.* (2013) recorded 14.01 % fibre in *A. polytricha* while Usha and Suguna (2014) reported 17.85 %.

2.9.6. Ash Content

The wide variation in ash content of mushrooms ranging from 6.60 % in *P. florida*, 7.3 % in *P. ostreatus* to 13.1 % in *Calocybe indica* was reported by Wang *et al.* (2001) and Alam *et al.* (2008). However, lower ash content of 5.2 % and 4.95 % in *A. polytricha* was reported by Gbolagade *et al.* (2006) and Sanchez (2010). Johnsy *et al.* (2011) recorded 8.7 % ash content in *Lentinus* and *Auricularia* sp. Kakon *et al.* (2012) stated that *A. polytricha* contained 14.0 % of ash content on fresh weight basis and 2.39 % of ash content was reported in *A. polytricha* by Celestine *et al.* (2013). Usha and Suguna (2014) observed the ash content of *A. polytricha* and *P. ostreatus* as ranging from 5.2 % to 7.93 %. Prasad *et al.* (2015) recorded 9.7 % ash content in *Agaricus bisporus*, 6.7 % in *Lentinus edodes*, 6.3 % in *P. sajor caju*, 8.26 % in *Hypsizygus marmoreus* and 7.2 % in *Flammulina velutipes* whereas, Kadnikova *et al.* (2015) reported the ash content of *A. auricula-judae* as 3.6 %.

2.9.7. Total Antioxidants

Emre *et al.* (2016) reported the antioxidant activity of distilled water extract of *A. polytricha* as 0.91 µmol/L and in contrast, ethanol extract had 0.73 µmol/L. They also reported that when the antioxidant capacities of the extracts were compared, the distilled water extract of *A. polytricha* displayed higher capacity

than ethanol extract and phenolic extract showed the lowest anti-oxidant capacity (0.7 %). The total antioxidant capacity of *A. polytricha* was 17.93 mg/g (Packialakshmi *et al.*, 2016).

2.9.8. β-carotene Content

Haxo (1950) reported that the edible mushroom *Cantharellus cinnabarinus* contained a complex mixture of carotenoid pigments and major crystalline compounds, which were isolated and named as canthaxanthin, while the minor pigment identified was beta-carotene. Barros (2007) concluded that very low amount of ascorbic acid, β-carotene and lycopene were present in the mushroom extracts of three Portuguese wild edible mushroom species such as, *Leucopaxillus giganteus, Sarcodon imbricatus,* and *Agaricus arvensis.* Ullah *et al.* (2011) stated beta-carotene as the principal compound synthesized by few mushrooms which play a very important role in the bio-synthesis of several bio-molecules.

2.9.10. Polyphenol Content

Phenolic compounds are a large group of secondary plant metabolites which play a major role in the protection of oxidation processes and these compounds have antioxidant properties and can act as free radical scavengers, hydrogen donators and singlet oxygen quenchers. In addition, phenolics exhibit a wide range of biological effects including antibacterial, anti-inflammatory and antihyperglycemic activities (Li *et al.*, 2012).

Afiukwa *et al.* (2013) reported 0.058 % phenol content in *A. polytricha.* Mushrooms contained naturally occurring polyphenols with antioxidant capacity which could scavenge reactive chemical compounds as well as reduce oxidative damage. The total phenolic content of water extract of *A. fuscosuccinea* was 35.9 mg GAE/g and ethanolic extract, 195.5 mg GAE/g, as reported by Liao *et al.* (2014). Packialakshmi *et al.* (2016) reported the total phenolic content of *A. polytricha* as 7.20 mg CE/g of the sample.

2.9.11. Energy Value

Energy value of mushrooms varied from 1442 to 1881 KJ/100 g of dry matter (Colak *et al.*, 2009). The energy value of *P. ostreatus* was reported to be 330.3 Kcal/100 g by Wang *et al.* (2001). Mattila *et al.* (2002) opined that mushrooms were low-calorie food and their energy value varied from 113 to 125 KJ/100 g of fresh matter. Regula and Siwulski (2007) obtained an energy value of 1442 KJ/100 g *in P. ostreatus.*

Kakon *et al.* (2012) reported that *A. polytricha* contained 347 kcal of energy on fresh weight basis and Kadnikova *et al.* (2015) reported that *A. auricula-judae* had a calorific value of 1370 KJ/100 g of dry matter. Prasad *et al.* (2015) reported 325 Kcal/Kg of calorific value in *Agaricus bisporus*, 772 Kcal/Kg in *Lentinus edodes* and 467 Kcal/Kg in *Flammulina velutipes.*

2.10. SENSORY EVALUATION

Mushrooms blended well with most of the vegetables and spices and produced delicious recipes which included salads, soups, snacks, pickles and sweets. They could be dried under sun and stored or marketed as mushroom powder (Das, 1992). The Greeks believed that mushrooms gave strength to their warriors and the Romans considered them as 'food of the Gods'. Kakon *et al.* (2012) regarded mushrooms as nutrient dense versatile food which could eradicate malnutrition since these contained more proteins, less fat and sufficient vitamins and minerals.

According to Acton *et al.* (2001) the young specimens *of Auricularia* were ideal for cooking purposes. They could also be consumed raw after thorough washing. Das (2011) reported that Jew's ear mushrooms were excellent for wine preparations, and milky and oyster mushrooms for snacks such as burgers, cutlets, fritters, etc. She also stated that mushroom served excellently as main as well as side dishes and combined well with fish and meat products.

Cha *et al.* (2014) formulated pork patties with *Tremella fuciformis* and reported higher cooking quality in *T. fuciformis* preparations than control sample and the acceptance was also higher in jelly mushroom formulation (4.9-5.8) than the control sample (3.8). They also concluded that the oil holding capacity of mushroom preparations showed an affirmative effect on the cooking yield of patty.

2.10.1. Keeping Quality

Mushrooms usually possess low keeping quality compared to vegetables and the short shelf life is mainly because of their increased respiration, loss of water and fast metabolic activity (Ares *et al.*, 2007). Mehta and Jandaik (1989) reported storage of freshly harvested fruit bodies of *Pleurotus* spp. in non-perforated polythene bags up to 72 h at room temperature and up to 15 days at low temperature of 0-5 ^{0}C. Kim *et al.* (2006) worked on the modified atmosphere packaging of whole and sliced fresh mushrooms of *Agaricus bisporus* in PVC wrap and two polyolefins films with subsequent coating of $CaCl_2$ and chitosan, and recorded a weight loss of 3 to 7 g/100g after six days of storage in both packages. Mota *et al.* (2006) packed mushrooms with perforated PVC films and refrigerated for storage purposes.

Rai and Arumuganathan (2008) reported that shelf life of mushrooms is less due to its increased respiration rate of about 28.2-43.6 mg CO_2 per kg fresh weight per hour at 0 ^{0}C and 280 mg CO_2 per kg fresh weight per hour at 19 ^{0}C. Fernandes *et al.* (2012) recommended the use of gamma, electron beam and UV irradiation as potential tools for extending the postharvest shelf life of fresh mushrooms such as *A. bisporus*, *L. edodes* and *P. ostreatus.* As per Yanjie *et al.* (2013), high oxygen packaging with 100 per cent oxygen increased quality of fresh mushrooms.

2.11. OUTDOOR CULTIVATION

Krishnamoorthy *et al.* (2005) experimented a new approach for outdoor cultivation of paddy straw mushroom (*Volvariella volvacea*) and obtained an average yield of 1.734 kg/bed with a biological efficiency of 8.75 % in 15 days. Prakasam *et al.* (2013) reported that under open field conditions, *P. florida* recorded maximum yield of 504 g followed by *P. djamor-roseus* (495 g), *P. eous* (462 g) and *P. platypus* (439 g) at Sathyamangalam area of Tamil Nadu. Ma *et al.* (2014) reported that the bags of *A. auricula-judae* placed in open fields and watered three times per day produced uniform fruiting bodies of 2.0 cm in diameter.

2.12. YIELD PERFORMANCE OF OYSTER AND MILKY MUSHROOMS

Gupta *et al.* (2011) studied the fruiting body production of *P. ostreatus* on varied substrates and reported that wheat straw exhibited the highest mean yield of 996 gm per kg of dry weight substrate with a biological efficiency of 99.6 %, while cotton spinning substrate showed 98.5 % and the lowest yield of 50 g was obtained from sawdust substrate. They also reported that spawn run period ranged between 12-21 days for different substrates, minimum of 12 days on coir pith and maximum of 21 days on sawdust substrates.

Kumar *et al.* (2011) evaluated different strains of *Calocybe indica* for mushroom production and recorded that CI-6 took a minimum of 16 days for spawn run and pinhead formation, 24 days for first harvest, maximum number of pinheads (64/bag), average weight of sporophore (32 g) and the highest yield of 620 g/kg of dry substrate with biological efficiency of 62 %. Sharma *et al.* (2012) studied different sterilization methods of casing medium, including autoclaving, steam pasteurization and chemical treatment on the fully spawn run bags of different strains of *Calocybe indica* from which, autoclaved casing soil resulted in the highest yield for all the strains tested and the lowest yield was recorded in chemically treated soil and fruiting body weight of 51g to 88g was recorded in all the strains.

Kumar *et al.* (2012) examined the effects of eleven organic supplements on wheat straw at 4 % and casing mixture at 3 % for the cultivation of two strains of milky mushroom (CI-6 and CI-4) and reported that maximum yield was recorded from supplementation with soybean flour (648.3 and 599.7 g/kg) for two strains whereas 616.0 and 588.3g/kg of yield was obtained from neem cake supplemented casing mixture and among the tested casing thickness, maximum yield (559.0 and 544.7g/kg) was obtained in casing thickness of 2.5 cm.

Chandra *et al.* (2013) studied the yield performance of *Pleurotus sajor-caju* on three different substrates namely maize stalk, pea residue (tendrils) and banana leaves with and without the supplementation of rice bran and chicken manure and results indicated that the highest yield of 348.13 g with 87.03 per cent biological efficiency was obtained from maize stalk with rice bran. Sharma *et al.* (2013) cultivated *Pleurotus ostreatus* on different substrates *viz.*, rice straw without amendment, wheat straw with amendment, paper waste, sugarcane bagasse and sawdust and reported that the highest yield (381.85 gm) with a biological efficiency of 95.46 % was recorded from rice straw. Vijaykumar *et al.* (2014) studied the effect of six different substrates namely paddy straw, wheat straw, soybean straw, coconut coir pith, cotton waste and sugarcane baggase for the cultivation of milky mushroom and reported that minimum days taken for spawn run (15.67 days), pinhead formation (28.67 days), first harvest (33.67 days) and highest biological efficiency of 146.3 per cent were recorded from paddy straw.

Fakkiravva (2014) studied the comparative performance of *Pleurotus eous* and *Calocybe indica* on paddy straw amended with $MgSO_4$ and $CaSO_4$, and recorded the highest biological efficiency of 113.50 % and 527.50 % respectively. Alananbeh *et al.* (2014) studied the efficacy of date palm wastes with other agricultural wastes on the cultivation of *Pleurotus ostreatus* and reported that the highest biological efficiency (184.79 %) was obtained from date palm-wheat straw combination (25:75).

Koutrotsios *et al.* (2014) cultivated *P. ostreatus* on nine agro-industrial and forestry by-products from which grape waste plus cotton gin trash was the best combination (1:1) to obtain the highest biological efficiency of 137.2 % in a cultivation period of 67 days. Navathe *et al.* (2014) cultivated *Calocybe indica* with locally available substrates *viz.*, paddy straw, horse gram waste, wild grass, bamboo leaves and different casing materials such as vermicompost, dried biogas spent slurry, combination of sand + soil (1:1) and sand + soil + dried biogas spent slurry (1:1:1). The study reported that among four different substrates evaluated for cultivation of milky mushroom, the highest biological efficiency was obtained from paddy straw (81.05 %) and casing material combination of sand + soil + dried biogas spent slurry (180.32 %).

Dhakad *et al.* (2015) compared the growth behavior and yield potential of five strains of *Calocybe indica* and reported that the strain CI-14 recorded the highest average yield for first, second and third flushes (441.67g, 285g and 85 g) compared to the other four strains. Owaid *et al.* (2015a) recorded the highest yield (204.28 g) and biological efficiency (51.78 %) of *P.ostreatus* from substrate combination of 50 % wheat straw, 30 % sawdust and 20 % date palm whereas poorer yield (12.5 g) was obtained from sawdust alone.

Owaid *et al.* (2015b) studied four different *Pleurotus* spp. *viz.*, *P. salmoneostramineus* (pink), *P. ostreatus* (grey and white) and *P. cornucopiae* var. *citrinopileatus* (bright yellow) on sawdust and rice bran combination. The study reported that among the four different species of *Pleurotus*, *P. salmoneostramineus* (pink) took minimum period for pinhead appearance (2 days), maximum number of fruiting bodies (11.0) and the highest average weight of 9.23 g. Subramanian and Shanmugasundaram (2015) cultivated *Calocybe indica* on paddy straw with different casing thickness and recorded the maximum biological efficiency of 140.3 % in beds prepared with 2-2.5 cm casing thickness, minimum period for pinhead formation (31.17 days) and production of first flush (35.67 days).

Shamsi (2016) studied the comparative performance of *Calocybe indica* and *Pleurotus sajor-caju* on agricultural wastes from date palm bunches, date palm leaf wastes and mowed turf grass wastes and reported significant interaction between mushrooms and substrates. The highest yield was obtained from *Calocybe indica* on date palm bunches (466.6 g) while *Pleurotus sajor-caju* showed the lowest yield (252.03 g). In the case of substrates, date palm leaf wastes recorded the highest yield for both *Calocybe indica* (340.18 g) and *Pleurotus sajor- caju* (294.82).

Jadhav *et al.* (2017) cultivated wild strains of *Pleurotus* spp. along with *P. sajor-caju* and reported that the strain PN-14-41 took minimum days to complete spawn run (16.08 days), pinhead formation (3.67 days), first harvest (24 days) and recorded the highest yield of 977.42 g/kg on wheat straw substrate whereas only 896.25 g/kg was obtained from the native strain of *P. sajor-caju*. Sharma *et al.* (2017) studied the utilization of nitrogen rich supplements in wheat straw *viz.*, leafy biomass of *Syzygium cumini*, *Cassia fistula* and *Bauhinia variegate* for the cultivation of *C. indica* and reported that the highest biological efficiency was recorded from the beds amended with *B. variegate* (82.93 %) whereas control had 72.6 % of biological efficiency.

2.13. DEVELOPMENTAL MORPHOLOGY

The mushroom development consists of both vegetative as well as reproductive stages and the shift from vegetative to reproductive stage is mainly regulated by physical environmental conditions namely, temperature, light, moisture content, CO_2 concentration and relative humidity.

Onyango *et al.* (2011a) studied the morphological changes of *Auricularia* sp. and reported that *Auricularia* had four different stages *viz.*, cup-shaped/ discoid mushrooms of <10 mm diameter with soft texture, the second stage with lobed mushrooms of 11-20 mm diameter with rubbery texture, the third stage with flattened/appressed stage of 21-40 mm diameter and leathery texture and the last stage with ear-shaped mushrooms of diameter >40 mm with gelatinous texture.

2.14. LOG METHOD OF CULTIVATION

The cultivation of *Auricularia* was first reported in China during 600 A.D. under log method of cultivation. Kwon (2004) described that the log method of cultivation was done primarily in the cooler spring and autumn months and is an open, non-sterile procedure and therefore, contamination with other wood rotting fungi is common. Schenck and Dudley (1999) reported that pepeiao (*Auricularia polytricha*) started to produce fruiting bodies on the koa (*Acacia koa*) logs after three months of inoculation, then on kukui (*Aleurites moluccana*) log and the most prolific yield was produced from kukui (624.1 g) which was approximately eight times higher than koa (73.7 g). However, the least yield (14.9 g) was obtained from hau (*Hibiscus tiliaceus*) logs and they also noted that production of fruiting bodies correlated with rainfall.

Mahmud *et al.* (2013) experimented the cultivation of *Auricularia* spp. on twigs and stems of 3 species of trees, namely teak (*Tectona grandis*), jabon (*Anthocephalus cadamba*) and sengon (*Falcataria moluccana*) stems with 10-15 cm in diameter, cut into 20 cm long pieces with 20 holes of 1.3 cm diameter. After pasteurization, logs were inoculated with mycelia of ear mushroom and the results indicated the potentiality of these three host species for the cultivation of ear mushroom.

Mbaluto (2015) opined that log method of cultivation is applied to mushroom species which were saprophytic in nature and though the method is simple, the main disadvantage is the slowness in comparison with sterilised sawdust mixtures and that this method is technically not sound, is seasonal and could not secure high productivity to meet demands.

2.15. PESTS AND DISEASES

Pests and diseases pose a major threat to mushroom production all through its various stages *viz.*, composting, spawning, spawn running and mushroom development. Chang and Miles (2004) reported that there are plenty of ways the

organisms *viz.*, bacteria, viruses, fungi, nematodes, mites and insects, can enter and grow in the substrate or on the mycelium and fruiting bodies, and cause drastic decrease in the yield and even total loss of the crop.

Back *et al.* (2012) confirmed the cross pathogenecity test for cobweb disease causing pathogens *viz.*, *Cladobotryum dendroides*, *C. mycophilum*, *C. varium*, *C. multiseptatum*, *C. verticillatum*. *C. mycophilum* and *C. varium* on *Agaricus bisporus*, *Pleurotus eryngii*, *Flammulina velutipes* and *Hypsizygus marmoreus*.

Sun and Bian (2012) reported a new disease, slippery scar from the cultivated bags of *A. polytricha* which was isolated from the infected mycelia and identified as the *Scytalidium lignicola* based on morphological observation, rDNA internal transcribed spacer and 18S sequence analysis. This disease caused 30 % yield loss annually. Gea *et al.* (2014) reported the dry bubble disease (*Lecanicillium fungicola*) in button mushroom and recommended the use of compost teas made from spent mushroom substrate as it significantly inhibited the growth of *L. fungicola* (100 %) under *in vitro* and 73 % under *in vivo* conditions. Peng *et al.* (2014) studied the new causative pathogen of slippery scar, *Scytalidium auriculariicola* in *A. polytricha* and made the taxonomic classification of the pathogen by morphological observations, *in vivo* pathogenicity tests, molecular evidences of ITS and RPB2 sequences. Singh *et al.* (2014) stated that *Trichoderma* spp. caused green mould disease and resulted in extensive yield loss of varied species of both cultivated and medicinal mushrooms. *Trichoderma harzianum* caused green mould disease in oyster mushroom and inhibited the growth of mycelium and fruiting bodies and considerably lowered the yield.

Kumar *et al.* (2017) reported the holistic approaches for management of pest and diseases in mushrooms and recommended that empty rooms are to be treated with 2 % formalin, sprayed with 150 ppm bleaching powder for controlling bacterial diseases, set up with light traps for monitoring and controlling the insect

vectors and the bags to be drenched in 2 % formalin before disposing off. Kounsar *et al.* (2017) studied the efficacy of antagonists against the wet bubble disease (*Mycogene perniciosa*) of white button mushroom and concluded that among the antagonists tested, *Pseudomonas fluorescens*-103 exhibited stimulatory effect of white button mushroom and inhibited the mycelial growth of the pathogen (100 %).

2.16. ANTI-CANCEROUS ACTIVITIES

Medicinal mushrooms gained interest for their enormous pharmacological effects as well as their nutritional values. Basidiomycetes contained highly efficient polysaccharides and protein complexes that showed antitumor, immunomodulation, anticardiovascular, antiviral, antibacterial and antiparasitic activities. Antitumor activities of mushrooms had been broadly investigated because of modern chemotherapeutic relevance of antitumor drugs obtained from natural sources and continued to occupy vital role in current cancer treatments (Wasser and Weis, 1999).

Reza *et al.* (2011) reported the MTT assay for the cytotoxic activities of 70% ethanol extract of *A. auricula* and indicated that the effect was dosage dependent which inhibited proliferation of tumour cells in all the tested solvent fractions and doxorubicin in both P388D1 macrophages and Sarcoma 180 cells. Song and Du (2012) indicated that 1,3 β glucan, 1,4 α glucan, 1,3 α glucan and glucopyranosyl present in *A. auricula* significantly inhibited the growth of transplantable Sarcoma180 (S) in mice compared with the model controls with the inhibitory rate being 43.61 %. Wang *et al.* (2013) proved the inhibitory properties of crude polysaccharides and purified polysaccharide from *A. polytricha* (APS-2) with cyclophosphamide as a positive control on the growth of transplantable S180 tumors in mice and it showed inhibition of 20.35 %, 47.64 % and 53.30 % respectively.

Yu *et al.* (2014) investigated the anticancer activity and its underlying mechanisms of *Auricularia polytricha* polysaccharides (APPs) on A549 human

lung cancer cells and reported that APPs inhibited the proliferation and DNA synthesis of A549 cells in a concentration dependent manner. The compound also induced apoptosis in A549 cells by arresting cell cycle progression at the G0/G1 phase. Meng *et al.* (2016) reported that the antitumor mechanisms of mushroom polysaccharides were mediated by stimulated T cells or other immune cells and the polysaccharides were able to trigger various cellular responses such as the expression of cytokines and nitric oxide. Most polysaccharides could bind other conjugate molecules *viz.*, polypeptides and proteins whose conjugation possessed strong antitumor activities.

Materials and Methods

3. MATERIALS AND METHODS

The materials used and methods followed in accomplishing the objectives of the study are explained as follows.

3.1. SURVEY

Survey was conducted during pre and post monsoon seasons from November 2014 to July 2016 in ten different locations of Thiruvananthapuram and Kollam districts of Kerala. Locations *viz.*, Vellayani, Venganoor, Vanchiyoor, Neyyattinkara, Kattakada, Ponmudi, Nedumangad and Palode of Thiruvananthapuram district and Arippa and Kulathupuzha of Kollam district were selected to collect and identify different strains of Jelly mushrooms. The study was undertaken in places having dense vegetation of trees, forest ranges, meadows or fallen wooden logs in the selected locations. Specimens were collected at different stages of development and general observations on the natural occurrence and habitat of the spotted mushrooms were recorded. The specimens were taken to the laboratory for further detailed studies. The collections were serially numbered.

Mushroom sporocarps obtained from the surveyed locations were brought to the Mushroom laboratory, College of Agriculture, Vellayani, in order to examine the specimens and to isolate the fungal culture by the standard technique (Suharban, 1987). Subsequently the morphological studies of collected mushrooms were also undertaken by detailed examination of macroscopic and microscopic characters of the sporocarps. The detailed characters were recorded following the techniques and proforma developed by Nair (1990), as given in Appendix I.

3.2. MORPHOLOGICAL CHARACTERS

Morphological studies on the macroscopic and microscopic characters were undertaken to identify the collected jelly mushrooms.

3.2.1. Macroscopic Studies

Macroscopic studies were conducted on the colour and texture of pileus and stipe. The characteristics of lamella and gills were also observed and the spore prints were taken. Sporocarps closely resembling jelly mushrooms in their external features, obtained during the survey were examined. Spore print was made by cutting and keeping portions of the pileus on black and dark pink paper sheets. A medium mature sporocarp was selected for obtaining the spore print. The pileus was detached and placed on a piece of paper facing opposite to the surface of the paper. A bell jar was placed over this to keep moist and to protect from air flow. This was done in an air conditioned room and after ten hours, the bell jar was removed and the pileus was taken away from the paper to obtain the spore print (Deepa, 2016).

3.2.2. Microscopic Studies

Microscopic studies were done by taking thin sections of freshly formed sporocarps of jelly mushrooms by standard technique (Deepa, 2016). The sections were transferred to the glass slide were stained using lactophenol cotton blue (Appendix II) and examined under microscope (10X and 40 X) for observing the hyphal and mycelial characters. The spores from the jelly mushrooms were measured directly soon after mounting in lacto phenol on a clean slide. Spores were mixed with lacto phenol thoroughly to obtain a uniform spread and then a cover slip was placed over it. Spores were measured under microscope (10X and 40X). The average size of spores was then determined and the shape of the spores were recorded. Microphotographs were taken to depict the typical spore morphology of the fungus. Hyphal characters were also recorded by taking and observing growing mycelium from culture. The internal hyphal stratified zonations of hyphae was also studied by taking thin cross section of pileus and they were microphotographed.

3.3. ISOLATION AND PURE CULTURING

The pure culture of jelly mushrooms obtained from ten different locations of Thiruvananthapuram and Kollam districts were isolated using standard tissue culture method (Suharban, 1987). The collected mushrooms were cleaned up to remove adhered soil particles. The pest and disease free, medium aged, healthy mushrooms were thoroughly cleaned and surface sterilized with ethyl alcohol (90-95 %). After splitting longitudinally, a small portion of the tissue from the junction of pileus and stipe was taken out using a sterile inoculation needle. The detached tissue was placed aseptically in petri plates containing sterile solidified Potato Dextrose Agar (PDA) medium and incubation time was given for seven to ten days at 28±1 ^{0}C to obtain pure culture of mycelium. These cultures were again purified using hyphal tip method by transferring the hyphal tips of fungal growth aseptically (Rangaswamy and Mahadevan, 2008).

The fungal cultures of jelly mushroom isolates were sub cultured on potato dextrose agar slants by cutting circular discs from the tip portion of mycelia of jelly mushrooms with the help of a sterile cork borer of 5 mm size and kept at 28±1 ^{0}C for 15 days and subsequently renewed once after every 25 days. Such isolates were stored in a refrigerator at 5 ^{0}C and were revived at monthly interval for preservation of quality and further studies. The isolates obtained were grown on Petri plates containing PDA and the fast growing cultures were selected for further studies. The same cultures were sent to the DMR, Solan, for identification and getting accession number.

3.3.1. Strainal Variations

The five mushroom cultures obtained from survey were screened for selecting fast growing strains after taking observations *viz.*, the number of days taken to complete mycelial coverage on Petri plates, nature of mycelial growth and morphology of sporocarps. The details of the cultures are given below (Table 1).

Table. 1. Isolates of jelly mushrooms obtained from survey

Sl no.	Collections	Host (Fallen logs)	Location	Designation no.
1.	*Auricularia* sp.	Rubber (*Hevea brasiliensis* (Mull.) Arg.)	Vellayani	A1
2.	*Auricularia* sp.	Coconut (*Cocos nucifera* L.)	Neyyatinkara	A2
3.	*Auricularia* sp.	Coconut (*Cocos nucifera* L.)	Vellayani	A3
4.	*Auricularia* sp.	Rubber (*Hevea brasiliensis* (Mull.) Arg.)	Kattakada	A4
5.	*Tremella* sp.	Jack fruit tree (*Artocarpus heterophyllus* Lamn.)	Vellayani	T1

3.4. PHYSIOLOGICAL STUDIES

3.4.1. Growth of *Auricularia polytricha* (Mont.) Sacc. - A1 and A2 in Different Media

The growth characters of *A. polytricha* A1 and A2 were studied on four different solid media *viz.*, Potato dextrose agar (PDA), Oat meal agar (OMA), Carrot extract agar (CEA) and Malt extract agar (MEA).

The media were prepared and autoclaved at 121 ^{0}C and 15 lbs pressure for 20 minutes. Twenty millilitres of each of the sterilized medium was poured into the sterilized petri plates and allowed to solidify. Inoculation was made by transferring a five millimeter disc of mycelial mat, taken from the periphery of seven days old culture. The plates were incubated at 28±1^{0}C. Observations on colony radial growth was taken when the maximum growth was attained in any one of the media tested. Other cultural characters *viz.*, rate of growth, colour and diameter of mycelial growth were also recorded when it attained fifty per cent and

hundred per cent growth. Analysis was carried out by using Completely Randomized Design (CRD) with four treatments, each treatment replicated four times. The growth was observed in every two days interval. The best medium supported for the growth of *A. polytricha* A1 and A2 was selected for further physiological studies.

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

3.4.2. Growth of *A. polytricha* – A1 and A2 in Different Temperature

The effect of temperature on the growth of *A. polytricha* - A1 and A2 was studied at three different temperature levels *viz.* 20, 25 and 30 ^{0}C using MEA as the basal medium. Twenty millilitre of sterilized basal medium was poured into sterile petri plates. A five milimeter mycelial disc of each of the selected strains was cut from actively growing cultures, inoculated and incubated at different temperature levels and the diameter of mycelial growth, nature of mycelial growth and time taken for complete colonization were recorded. The results were analyzed statistically and interpreted by using CRD tool with three treatments and five replications.

3.4.3. Growth of *A. polytricha* – A1 and A2 in Different pH

Using malt extract agar as basal medium the experimentation on growth characteristics of jelly mushroom with respect to hydrogen ion concentration was studied with different pH levels. The pH of the medium was determined by pH meter and adjustment of pH was done by adding 0.1N alkali (NaOH) or acid (HCl). The pH of basal medium was adjusted to 5.0, 6.0, 7.0 and 8.0. From seven day old cultures of two selected jelly mushroom strains, the mycelial discs were cut and inoculated in 20 ml basal medium in 9 cm diameter petri plates and incubated at best temperature of 28±1^{0}C and the time taken for complete growth

as well as nature of growth were recorded. The experiment was laid out in CRD with four replications for each treatment.

3.4.4. Growth of *A. polytricha* – A1 and A2 in Light and Dark Conditions

Sterile molten malt extract medium was poured into sterile petri plates of 9 cm diameter under aseptic conditions and allowed to solidify. Culture discs of 5 mm size were cut out from actively growing culture of jelly mushrooms and inoculated at the centre of plate containing solidified medium. The plates were incubated in light and dark conditions. Light condition was facilitated by switching on the tube light (1400 lux) and keeping the inoculated plates under it until the growth completes and dark condition was facilitated by wrapping in carbon sheets and storing in closed cupboard in order to provide darkness. The time taken for complete mycelial growth and nature of growth were recorded. The experiment was analysed using student t-test with 10 observations for each treatment.

3.5. SPAWN PRODUCTION

To acquaint with the efficacy of different substrates for spawn production, different grains *viz*., paddy, wheat, sorghum, ragi, sawdust of hard wood trees and rubber sawdust were used for spawn production as per the technique of Sinden (1934). The grains were cooked in boiling water until the seed coat had just begun to split open. Then the excess water was drained off and the grains were spread on a clean cloth for drying. Each substrate after sufficient drying was mixed with calcium carbonate at the rate of 30 g per kg of grains. These were separately packed in polypropylene bags (12×6 inches) at the rate of 300 g per bag and sterilized by autoclaving at 15 lbs pressure and 121 ^{0}C for 2 h. After cooling, the bags were inoculated aseptically with mycelial bits of equal sizes from 10 days old culture of *A. polytricha* and incubated at 28 $\pm$ 2 ^{0}C for 15 days. The time taken for spawn run, nature of mycelial growth and presence of contaminants were observed and recorded. The spawn thus obtained as mother spawn was maintained for further spawn production.

The sawdust spawn was prepared as procedure of Deepa (2016).

Composition

i) Sawdust + rice bran - 1 kg

ii) Calcium carbonate - 30 g

Procedure

Fresh sawdust was taken after removing clods and other impurities, soaked for 12 h in water after which the excess water was drained off and sawdust allowed for drying until moisture content became 60 %. It was mixed thoroughly with calcium carbonate and this mixture was then filled in poly propylene bags and sterilized at 121 ^{0}C for 2 hours in an autoclave. Mycelial bits from 10 days old pure culture of jelly mushroom was inoculated under aseptic conditions and incubated at 28$\pm$2 ^{0}C until complete spawn run occurred. The best substrate for spawn production was selected based on the minimum time required for complete spawn run, nature of mycelial growth and presence of contaminants. Experiment was conducted with four replications for each treatment and was statistically analysed using CRD.

3.5.1. Time Taken for Spawn Run

Evaluation was done by recording the time taken from the day of inoculation to the complete colonization of the spawn substrates by the fungal mycelium of *A. polytricha* A1 and A2.

3.5.2. Growth of Mycelia

The growth pattern of mycelia, colour change and nature of spawn run were recorded.

3.5.3. Presence of Contaminants

Periodic observations were recorded on the occurrence of fungal or bacterial contaminants and per cent contamination was recorded.

Good quality mother spawns were selected based on the above characteristics, grower's spawns were prepared from mother spawns and used for preparation of beds.

3.6. SUBMERGED CULTURING

For submerged culture production, the liquid media *viz.*, potato dextrose broth, malt extract broth and carrot extract broth were used and the method of preparation was the same as that of the solid media, with the exemption of agar-agar. All the liquid media were sterilized at 15 lbs pressure. The flasks were inoculated with 5 mm mycelial disc obtained from the actively growing colony of *A. polytricha* - A1 and A2. After one day of inoculation, the conical flasks were placed in a shaker and kept at 120 rpm for fifteen days in order to obtain the mycelial pellets. Each treatment was replicated five times and observations were recorded on the dry mycelial weight and the data were analyzed statistically in CRD.

3.7. CULTIVATION

In order to assess the efficacy of different substrates on mushroom production, mushroom beds were laid out using different locally available substrates such as paddy straw, sawdust of softwood trees, sawdust of hardwood trees, banana pseudostem, sugarcane bagasse and neopeat as per the procedure described by Baskaran *et al.* (1978).

Paddy straw was soaked for 18 h in water containing carbendazim (bavistin) 75 ppm and formalin 500 ppm for sterilization. Then the excess water was drained off and spread over a silpaulin sheet under sun to reduce the moisture content to 60%. The beds were prepared by polybag method by using polythene

bags of 60×30 cm size. Paddy straw were made into twists and placed in bag along with spawn laid in sides, above which again paddy straw twists were laid and spawning was done. Likewise, four layers were prepared and the upper layer was fully covered with spawn. The bags were made compact, tied at the top and given 15 pin holes for air circulation.

Sawdust of hardwood trees (*Hevea brasiliensis* (Mull.) Arg.) and softwood trees (*Alianthus excelsa* Roxb.) were soaked in water for a day, excess water was drained out and the material was sun dried and mixed with 30 g calcium carbonate and filled in polypropylene cover. The mixture was sterilized at 121 ^{0}C for 2 hours in an autoclave. After sterilizing, the beds were prepared by placing four layers of sterilized sawdust mixture in polythene covers and inoculated with spawn. The bags were tied tightly and holes were made. The bags were then transferred to an incubation chamber having appropriate conditions like temperature, humidity, aeration and darkness. After complete spawn run, 8-10 long slits were put in polybags for the emergence of pinheads.

Banana pseudostem and sugarcane bagasse were prepared by chopping the materials into small pieces and drying properly. The substrates were sterilized by chemical treatment and beds were prepared following standard procedures. Neopeat blocks were soaked in water first at the rate of four litre water per block (650 g), excess water was drained out and sterilized to prepare the beds. Two kg sterilized substrate and 150 g spawn were used for preparing beds and the best substrate was selected based on the time taken for complete spawn run, pinhead formation, first flush, total yield, total crop period, average weight of sporocarp, number of sporocarps and biological efficiency. The experiment was laid out in CRD using four replications for each treatment.

3.7.1. Days Taken for Complete Spawn Run

This was evaluated by taking into an account of period in days from time of spawn inoculation up to the complete colonization of the bed substrates by the mycelium of the fungus.

3.7.2. Days Taken for Pinhead Formation

Pinheads started to appear after complete mycelial run and observations on the days taken from spawn run to pinhead formation were noted and number of pinheads was also counted.

3.7.3. Days Taken for First Harvest

First flush of mushrooms occur after pinhead formation and observations were made from the time taken for pinhead formation to complete growth and expansion of mushroom which was ready to harvest.

3.7.4. Total Yield Per Bed from Three Harvests

Total yield per bed was calculated by taking the weight of all the mushrooms from each bed to the dry weight of substrate used for cultivation. The same calculation was made for three and eight harvests of mushrooms.

3.7.5. Total Crop Period

The period in days from the time of spawn inoculation stage of the bed till the last harvest of sporocarps from bed of each strain laid out in the experiment was counted as the total crop period.

3.7.6. Average Weight of Sporocarp

Average weight of sporocarps was calculated as given below

$$\text{Average weight of sporocarp} = \frac{\text{Total weight of mushrooms}}{\text{Total number of mushrooms harvested}}$$

3.7.7. Number of Sporocarpsi

Number of sporocarps was calculated by counting total number of mushrooms picked at each harvests.

3.7.8. Biological Efficiency

Biological efficiency was determined by following formula

$$\text{Biological efficiency} = \frac{\text{Fresh weight of mushrooms harvested}}{\text{Dry weight of substrates used for cultivation}} \times 100$$

3.7.8. Pest and Diseases

Periodic observations were done for the occurrence of fungal or bacterial contaminants and other pests.

The best substrate was selected for further studies.

3.8. AMENDMENTS

The best substrate for cultivation of mushroom *viz,*. rubber sawdust obtained from (3.7.) was taken as the base medium for the amendment trials. The comparative efficacy of wheat bran, rice bran, cotton seed hull, neem cake and groundnut as amendments was evaluated. All the above amendments were mixed with rubber sawdust at two and four per cent concentration. Rubber sawdust was soaked in water for 24 h, excess water was drained off, dried in sun light and mixed with amendments @ 20 g/kg and 40 g/kg of substrate and beds were laid. Separate mixtures were prepared for each amendment and concentration. The mixture was filled in polypropylene cover and sterilized at 121 ^{0}C for 2 hours in an autoclave. After autoclaving and cooling, the beds were laid out in polythene bags of 60×45 cm. Spawning was done layer by layer and polythene bags were made compact, tied at the top and provided with a few holes for air circulation. The spawned bags were then transferred to an incubation chamber for spawn run. After completion of spawn run the bags were kept for fruiting in cropping room with high relative humidity of 80-85 % by opening the bag from top to get a good yield. The best amendment was selected for further studies based on the criteria as explained in experiment 3.7 (time taken for complete spawn run, pinhead formation, first flush, total yield, total crop period, average weight of sporocarp, number of sporocarps and biological efficiency) for cultivation studies. The

experiment was laid out in CRD with ten treatments along with control and three replications and the results were analysed.

3.9. ANALYSIS OF PROXIMATE CONSTITUENTS

The proximate constituents namely moisture content, carbohydrate, protein, fat, fibre, ash, total antioxidants, β-carotene, polyphenols and energy were estimated for two strains of jelly mushroom.

3.9.1. Moisture Content (%)

Hundred grams (w_1) fresh sample of jelly mushroom was dried in an oven until a constant weight was reached (w_2). Weight of dried sample was noted and the difference between fresh weight and dry weight of sample gave the result which was converted into per cent (Geetha, 1993).

$$\% \text{ of moisture content} = \frac{w_1 - w_2}{100} \times 100$$

3.9.2. Carbohydrate Content

Estimation of total carbohydrate content was done using anthrone method (Aminoff *et al.*, 1970).

One hundred mg of mushroom powder was weighed in to boiling tube. It was hydrolysed by keeping in a boiling water bath for three hours with 5 mL of 2.5N hydrochloric acid. It was cooled to room temperature and neutralised with solid sodium carbonate until the effervescence ceased. The volume was made to hundred ml and centrifuged at 5000 rpm for 10 min. The supernatant was collected and the aliquot was used for analysis. 0.5 mL aliquot was taken from the supernatant and made up to one ml by addition of distilled water. Four mL anthrone reagent was added to the solution and heated for eight minutes in a boiling water bath. The solution was cooled rapidly and the absorbance was read in spectrophotometer at 630 nm.

3.9.3. Protein Content

Microkjeldhal method was followed as per procedure given by Jackson (1973). Ten mL of digested mushroom sample was taken in the microkjeldhal flask and loaded on the left side of the distillation unit and 10 mL of 40 % NaOH was added automatically. On the right side, 250 mL conical flask was placed into which, 10 mL of 4 % boric acid and 2-3 drops of mixed indicator was added. Processing time was set for 6 min and RUN key was pressed (After complete processing, colour of the solution in the conical flask, changed from pink to green). The ammonia collected after processing in the conical flask was taken out and titrated against 0.02 N H_2SO_4. End point was denoted by the appearance of light pink colour. The obtained N value was converted to protein value by multiplying with 6.25 multiplication factor.

Calculation

$$\% \text{ of total N} = \frac{\text{Titre value} \times N \times 0.014 \times 100 \times 100}{W \times 10}$$

1 ml of 1 N H_2SO_4 = 0.014 g N_2
N -Normality of acid (0.02 N)
W -Weight of dried and ground mushroom sample taken (0.5 g)
Protein content = N × 6.25

3.9.4. Fat Content

Fat estimation was carried out using Soxhlet extraction apparatus (Lees, 1975).

Five grams of mushroom powder was taken in a thimble and placed inside the extractor. A piece of cotton wool was placed at the top of thimble in order to properly distribute the solvent on the sample during the process of extraction. Extraction was done with the use of petroleum ether for 16 h. Then the extract was transferred into a pre-weighed beaker (w_1), cooled in a desiccator and weighed (w_2). The per cent of fat was analysed using the following equation.

$$\% \text{ of fat content} = \frac{W_2 - W_1}{w \text{ (sample)}} \times 100$$

3.9.5. Fibre Content

Estimation of crude fibre content in two strains of jelly mushroom was done by following the procedure of De (1965).

Fat content was removed by extracting two grams of powdered sample with petroleum ether. Dried sample was boiled with 200 ml concentrated sulphuric acid (1.25 %) for 30 min with bumping chips. Then the digested sample was filtered through a muslin cloth and washed with boiling water until washings are no longer acidic. The sample was again boiled with 200 ml of sodium hydroxide solution for 30 min and filtered through muslin cloth and washed with 1.25 % sulphuric acid, three 50 ml portions of water and 25 ml alcohol. The residue was transferred to pre-weighed ashing dish (W_1) and the residue was dried for two hours at 130±2 ^{0}C. It was then cooled in a desiccator and the weight was recorded (W_2). The residue was further ignited for 30 min. at 600±15 ^{0}C, cooled in a desiccator and reweighed.

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

3.9.6. Ash Content

Five gram mushroom powder was transferred to a pre-weighed silica crucible (W_1) and heated at low flame over a Bunsen burner and while the substrate gets charred, transferred to a muffle furnace. Then it was again heated to 500 ^{0}C for two hours until a white ash was obtained and cooled in a desiccator then the weight (W_2) was recorded. Per cent content of ash was determined using the following formula (Raghuramulu *et al.*, 1983).

$$\% \text{ of ash content} = \frac{W_2 - W_1}{5} \times 100$$

3.9.7. Total Antioxidants

The total antioxidants of the extracts were evaluated by the phospho molybdenum method according to the procedure described by Prieto *et al.* (1999).

0.3 mL of extract was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95 ^{0}C for 90 min. Then, the absorbance of the solution was measured at 695 nm using a UV-VIS spectrophotometer against blank after cooling to room temperature. Methanol (0.3 mL) in the place of extract was used as the blank. The total antioxidant activity was expressed as the number of gram equivalent of ascorbic acid.

3.9.8. β-carotene Content

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

$$\beta\text{-carotene } (\mu g/100g) = \frac{O.D. \times 13.9 \times 104 \times 100}{Wt. \text{ of sample} \times 560 \times 1000}$$

3.9.9. Polyphenol Content

The Folin-Ciocalteu method was used to measure total amount of polyphenol content as per reference given by Ondo *et al.* (2013).

Aliquots of 0.25 ml of extracts (1 mg/mL) were mixed with 1.25 mL Folin–Ciocalteu reagent (0.2 N diluted in methanol). A reagent blank using methanol instead of sample was prepared. After 5 min incubation at room temperature, 1 Ml sodium carbonate solution (75 g/L) was added. Samples were incubated at room temperature for 2 h and the absorbance was measured at 765 nm.

3.9.10. Energy Value

The energy value of *A. polytricha* A1 and A2 was estimated based on the content of protein, fat and carbohydrate in the mushroom respectively using the factors 2.62, 8.37 and 4.2 Kcal /g of each component (Crisan and Sands, 1978).

Energy value (Kcal per 100 g dry weight) = 2.62 × (% protein) + 8.37 × (% fat) + 4.2 × (% carbohydrate)

3.10. SENSORY EVALUATION

Fresh mushrooms were cooked with minimum salt and oil in order to record the time taken for cooking, development of off odour, colour change and to assess the real taste of mushroom.

Fresh mushroom was cooked by sauting method and organoleptic characters like colour, appearance, texture, flavor, taste and overall acceptability were recorded. Hedonic rating scale was used for the preference study of the cooked sample as per the procedure obtained by Jellinick (1985). The nine point Hedonic rating scale is given in Appendix IV and the method of preparation of recipe is given in Appendix V.

3.10.1. Keeping Quality

In order to evaluate the keeping quality, jelly mushrooms of medium maturity were harvested, cleaned and packed. Packing was done in perforated polypropylene covers with ten holes of 5 mm diameter. Similarly one set was stored at room temperature of 26±2 ^{0}C and another set in refrigerated condition (4

^{0}C). Visual observations on the morphological characters and smell were recorded at 24 h interval. Per cent reduction in weight was estimated by periodically checking the weight of different sets (100 g in each cover) of stored mushrooms at 1, 2, 3, 5, 10, 15, 20 and 30 days.

3.11. OUTDOOR CULTIVATION

In order to determine the efficiency of outdoor cultivation, the mushroom beds of A1 and A2 were prepared on the best substrate and amendment, *i.e.,* rubber sawdust amended with rice bran @ 2 % per kg following the standard polybag method. One set of beds were kept in open field condition having enough shade, humidity and aeration and control beds were kept in mushroom house in order to compare the yield performance. The observations were carried out on the time taken for complete spawn run, pinhead formation, first flush, total yield, total crop period, average weight of sporocarp, number of sporocarps and biological efficiency. Statistical analysis was done using student t test with ten observations for each treatment.

3.12. YIELD PERFORMANCE OF OYSTER AND MILKY MUSHROOM

Comparative performance of *A. polyticha* - A1 and A2, *Pleurotus florida* (Mont.) Singer and *Calocybe gambosa* (Fr.) Donk. on the best substrate for spawn and mushroom production was done after selecting the best spawn as well as cultivation substrate for jelly mushrooms. Spawn production was done by inoculating the pure culture bits of *P. florida* and *C. gambosa* on the best substrate as per the standard procedure. Likewise, mushroom beds for *P. florida* and *C. gambosa* were prepared by adopting polybag method as done for jelly mushrooms. Observations on the time taken for spawn run and nature of mycelial growth were recorded for spawn studies. The time taken for complete spawn run, pinhead formation, first flush, total yield, total crop period, average weight of sporocarp, number of sporocarps and biological efficiency were recorded for mushroom production. The results obtained for jelly mushrooms, *P. florida* and

C. gambosa were compared and interpreted. All the mushrooms under study were replicated four times and CRD was used for analysis.

3.13. DEVELOPMENTAL MORPHOLOGY

Developmental morphology was studied by recording and observing the developmental changes of sporocarps from formation of pinhead stage to harvesting stage. Observations like colour, size and shape of the sporocarps were recorded and the number of days for development from pinhead stage to maturity stage was noticed.

3.14. LOG METHOD OF CULTIVATION

30 × 15 cm sized logs of bottle brush tree (*Callistemon citrinus*) were used for trying the log method of cultivation and the procedure was followed as per modified method of Cheng and Tu (1978). The logs were allowed to wither for 15 to 20 days and 15 to 20 holes of one cm diameter were made on them. The logs were sterilized by immersing in formalin-carbendazim solution for twelve hours. Excess water was drained out, spawning was done on the holes and were covered with sterilized cotton. The cultured logs were piled up and hung in mushroom house. The observations on the time taken for primordial formation, total yield and biological efficiency were recorded. The result was analysed with four replications in CRD.

3.15. ANTI-CANCEROUS ACTIVITIES

3.15.1. *In vitro* Antiproliferative Effect Determination by MTT Assay

HeLa (cervical cancer) cell line was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained on Dulbecos modified Eagles medium (Gibco, Invitrogen).

The cell line was cultured in 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic

solution containing: Penicillin (100 U/mL), Streptomycin (100 µg/mL), and Amphoteracin B (2.5µg/mL). Cultured cell lines were kept at 37 ^{0}C in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany).

The viability of cells was evaluated by direct observation of cells by Inverted phase contrast microscope followed by MTT assay.

3.15.1.1. Cells Seeding in 96 Well Plate

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10 % growth medium, 100 µl cell suspension (5×10^4 cells/well) was seeded in 96 well tissue culture plate and incubated at 37 ^{0}C in a humidified 5% CO_2 incubator.

3.15.1.2. Preparation of Extracts and Compound Stock

1 mg of ethanol mushroom extract was added to 1ml of DMEM and dissolved completely by cyclomixer. After that the extract solution was filtered through 0.22 µm millipore syringe filter to ensure the sterility.

3.15.1.3. Antiproliferative Evaluation

After 24 hours the growth medium was removed, freshly prepared samples in 5 % DMEM were five times serially diluted by two fold dilution (100 µg, 50 µg, 25 µg, 12.5 µg, 6.25 µg in 100 µL of 5 % MEM) and each concentration of 100 µl were added in triplicates to the respective wells and incubated at 37 ^{0}C in a humidified 5 % CO_2 incubator.

3.15.1.4. Antiproliferative Assay by Direct Microscopic Observation

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

shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

3.15.1.5. Antiproliferative Assay by MTT Method

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization.

After 24 hours of incubation period, the sample content in wells were removed and 30 µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37 ^{0}C in a humidified 5 % CO_2 incubator for 4 hours. After the incubation period, the supernatant was removed and 100 µl of MTT Solubilization Solution (DMSO was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 570 nm (Dinesh *et al.,* 2016).

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD Samples} \times 100}{\text{Mean OD of control group}}$$

Similar procedure was followed for cervical as well as liver cancer cells.

Results

4. RESULTS

Jelly mushrooms are gelatinous fungi which belong to the orders, Auriculariales, Dacrymycetales and Tremellales, that come under the class Heterobasidiomycete. Although these mushrooms possess appreciable nutritional as well as medicinal qualities, their low biological efficiency has been bottleneck in their production and popularization. Under these circumstances there is a need for an extensive study on the biology and cultivation of these mushrooms. In this regard, an investigation was undertaken during 2014-2017 on the characterization and exploitation of jelly mushrooms (*Auricularia* spp. / *Tremella* spp.) in College of Agriculture, Vellayani and the results of the study are presented below.

4.1. SURVEY

Random surveys were conducted purposively in locations having comparatively dense vegetations and observations during these surveys were recorded based on the data sheet given by Nair (1990) (Appendix I). Mushrooms were collected from ten different locations of Thiruvananthapuram and Kollam districts of Kerala *viz.*, Vellayani, Venganoor, Vanchiyoor, Neyyattinkara, Kattakada, Ponmudi, Nedumangad, Palode, Arippa and Kulathupuzha (Plate 1) to identify indigenous jelly mushrooms (*Auricularia* spp. / *Tremella* spp.) growing under natural conditions during pre and post- monsoon showers of 2014-2016.

Mushrooms collected from all the locations were gregarious in habit and lignicolous in nature. In Vellayani, wood logs of mango (*Mangifera indica* L.), fallen coconut wood logs (*Cocos nucifera* L.), bottle brush tree stumps (*Callistemon citrinus* (Curtis) Skeels.), copper pod tree stumps (*Peltophorum pterocarpum* (DC.) Backer.), yellow bells (*Tecoma stans* (L.) Juss.), pine tree (*Casuarina equisetifolia* L.) wattles/acacia (*Acasia senegal* (L.) Wild.), cashew (*Anacardium occidentale* L.), golden temple tree (*Macaranga indica* Wight.), rubber *(Hevea brasiliensis* (Mull.) Arg.), teak wood (*Tectona grandis* L.F.), arecanut (*Areca catechu* L.) and drumstick *(Moringa oleifera* Lam.) were found to

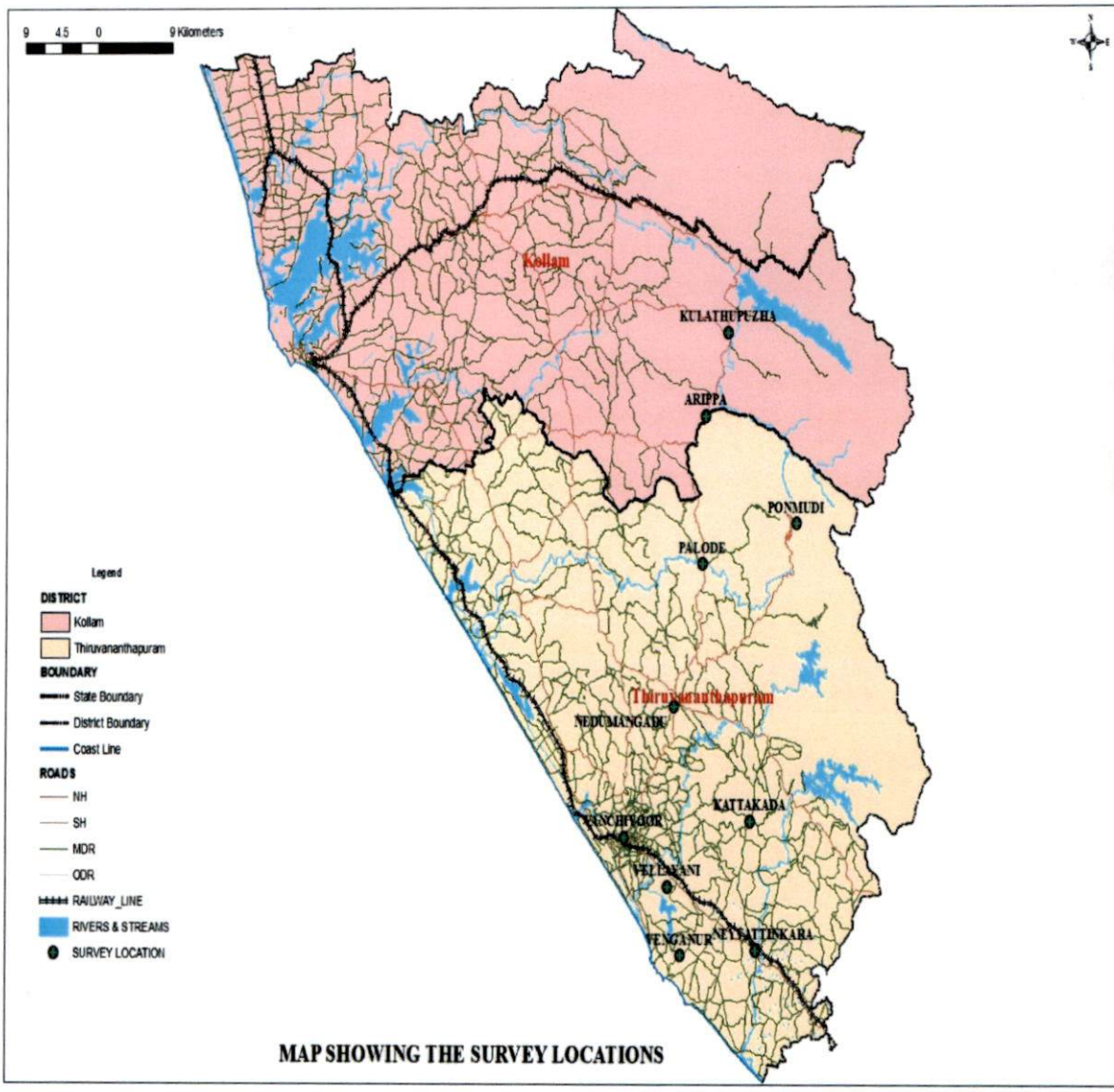


Plate 1.1. Surveyed locations for the collection and identification of different strains of jelly mushrooms (*Auricularia* spp. / *Tremella* spp.).

be the hosts for *Auricularia* spp. The jelly mushroom, *Tremella* sp., was collected only from Vellayani and was found growing on dead logs of jack tree (*Artocarpus heterophyllus* Lamn.) (Plate 1.2a). Coconut and drumstick logs were found to be the hosts for collections of *Auricularia* spp. in Venganoor and Ponmudi (Plate 1.2b). Rubber and coconut saw mills were observed as the hot spots for collections of *Auricularia* spp. in urban areas of Thiruvanathapuram district (Vanchiyoor), Nedumangad and Palode and in Arippa and Kulathupuzha of Kollam district (Plate 1.2c). Drumstick was found to be the host for *Auricularia* spp. in Kulathupuzha also. Jelly mushrooms were obtained from coconut, tamarind and rubber trees in surveyed locations of Neyyatinkara whereas in Kattakada, these mushrooms were obtained from teak and rubber woods. Tree stumps of mango, coconut, drumstick, teak and rubber wood were also found to be the usual spots for occurrence of these jelly mushrooms. The details of the survey are given in Table 2.

4.2. MORPHOLOGICAL CHARACTERS

Macroscopic morphological characters like colour and texture of pileus and stipe and microscopic characters of hyphae and spores of collected jelly mushrooms were studied in detail and their descriptions are given below (Table 3).

4.2.1. Macroscopic Studies

4.2.1.1. Macroscopic Studies of Vellayani Collections (Auricularia sp. *and Tremella* sp.)

Pileus : Brown to dark brown colour, incurved margin, ear shaped, leathery textured with a diameter of 2.6 cm for *Auricularia* spp. and *Tremella* spp.- white in colour, chrysanthemum like appearance (Plate 2.1), with a diameter of 6.2 cm and texture was soft and leathery.

Stipe : Rudimentary, 0.6 × 0.4 cm^2 for *Auricularia* spp.

Table 2. Details of jelly mushrooms from ten different locations in Thiruvananthapuram and Kollam districts

Sl no.	Locations	Habit	Habitat	Host plant	Month of collection
1	Vellayani (A)	Gregarious	Lignicolous	Mango (*Mangifera indica* L.), Coconut (*Cocos nucifera* L.), Bottle brush (*Callistemon citrinus* (Curtis.) Skeels), Copper pod tree (*Peltophorum pterocarpum* (DC.) Backer.), Yellow bells (*Tecoma stans* (L.) Juss.), Pine tree (*Casuarina equisetifolia* L.) Wattles/Acacias (*Acacia senegal* (L.) Willd.), Cashew (*Anacardium occidentale* L.), Golden temple tree (*Macaranga indica* Wight.), Rubber (*Hevea brasiliensis* (Mull.) Arg.), Teak wood (*Tectona grandis* L.F.), Arecanut (*Areca catechu* L.) and Drumstick (*Moringa oleifera* Lam.)	November 2014 to July 2016
2	Vellayani (T)	Gregarious	Lignicolous	Logs of Jack fruit (*Artocarpus heterophyllus* Lamn.)	November 2014
3	Venganoor (A)	Gregarious	Lignicolous	Coconut (*Cocos nucifera*) and Drumstick (*Moringa* sp.)	September, 2015

4	Vanchiyoor (A)	Gregarious	Lignicolous	Rubber (*Hevea brasiliensis*) and Coconut (*Cocos nucifera*)	September, 2015
5	Neyyattinkara (A)	Gregarious	Lignicolous	Coconut (*Cocos nucifera*), Rubber (*Hevea brasiliensis*) and Tamarind (*Tamarindus indica*)	September, 2015
6	Kattakada (A)	Gregarious	Lignicolous	Teak wood (*Tectona grandis*) and Rubber (*Hevea brasiliensis*)	September, 2015
7	Ponmudi (A)	Gregarious	Lignicolous	Coconut (*Cocos nucifera*) and Drumstick (*Moringa* sp.)	September, 2015
8	Nedumangad (A)	Gregarious	Lignicolous	Rubber (*Hevea brasiliensis*), *stans*) *Casuarina equisetifolia* and Coconut (*Cocos nucifera*)	December, 2015
9	Palode (A)	Gregarious	Lignicolous	Rubber (*Hevea brasiliensis*) and Coconut (*Cocos nucifera*)	December, 2015
10	Arippa (A)	Gregarious	Lignicolous	Rubber (*Hevea brasiliensis*) and Coconut (*Cocos nucifera*)	December, 2015
11	Kulathupuzha (A)	Gregarious	Lignicolous	Rubber (*Hevea brasiliensis*), Drumstick (*Moringa* sp.) and Coconut (*Cocos nucifera*)	December, 2015

A- *Auricularia* sp. T- *Tremella* sp.

Table 3.Morphological characters of jelly mushrooms from ten different locations in Thiruvananthapuram and Kollam districts

Collections	Colour of pileus and stipe	Shape of pileus	Diameter (cm) (Pileus)	Texture of pileus and stipe	Size of Stipe (l × b) (cm^2)	Volva and Annulus
Vellayani (A)	Brown to dark brown	Margin incurved and ear shaped	2.6	Leathery	0.6 × 0.4	Absent
Vellayani (T)	White	Chrysanthemum flower like appearance	6.2	Soft and leathery	0.2 × 0.2	Absent
Venganoor (A)	Light brown	Spathulate	3.6	Leathery and spreaded	1 × 0.9	Absent
Vanchiyoor (A)	Brown	Ear shaped	3.8	Tomentose	0.7 × 0.7	Absent
Neyyattinkara (A)	Brown	Ear shaped to flabelliform	2.5	Smooth and velvetty	0.6 × 0.4	Absent
Kattakada (A)	Dark brown	Ear shaped	3.8	Leathery and Caespitose	0.8 × 0.4	Absent
Ponmudi (A)	Brown	Flabelliform	3.8	Soft textured	0.8 × 0.6	Absent
Nedumangad (A)	Brown	Ear shaped	3.2	Velvety	0.4 × 0.4	Absent
Palode (A)	Dark brown	Margin incurved	2.6	Rougher at border and	0.5× 0.5	Absent

				smoother inside		
Arippa (A)	Brown	Ear shaped	3.2	Soft and velvety	0.9× 0.8	Absent
Kulathupuzha (A)	Brown	Ear shaped	3.2	Leathery	0.9× 0.7	Absent

Rubber

Drum stick

Yellow bells

Mango

Bottle brush

Pine tree

Teak wood

Copper pod

Teak wood

Coconut

Acasia/Wattle

Cashew

Arecanut

Jack tree

Plate 1.2a. Jelly mushrooms in different hosts at Vellayani

Coconut Drumstick Coconut Rubber

Venganoor collections Vanchiyoor collections

Rubber Tamarind Coconut

Neyyattinkara collections

Teak wood Rubber Coconut Drumstick

Kattakada collections Ponmudi collections

Plate 1.2b. Jelly mushrooms obtained in different hosts in the surveyed area

Coconut Rubber Rubber Coconut

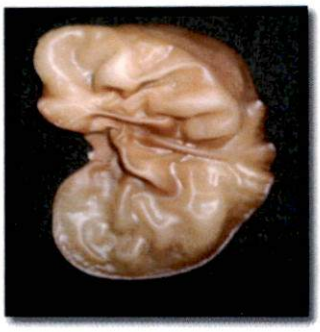
Nedumangad collections Palode collections

Rubber Coconut Drum stick Rubber

Arippa collections Kulathupuzha collections

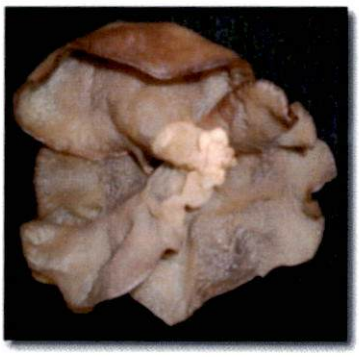
Plate 1.2c. Jelly mushrooms obtained in different hosts in the surveyed area

Chrysanthemum like



Ear shaped

Spathulate



Flabelliform

Tomentose

Caespitose

Incurved margin

Rubbery

Soft and velvetty

Plate 2.1.Morphological characters of the jelly mushrooms

and 0.2 × 0.2 cm^2 for *Tremella* spp.

Volva and Annulus : Absent in both *Auricularia* spp. and *Tremella* spp.

Spore print : White coloured.

4.2.1.2. Morphological Studies of Venganoor Collections (Auricularia spp.)

Pileus : Light brown in colour, spathulate (Like a spoon), 3.6 cm in diameter, leathery textured and spreaded .

Stipe : Rudimentary, 1.0 × 0.9 cm^2

Volva and Annulus : Absent

Spore print : White

4.2.1.3. Morphological Studies of Vanchiyoor Collections (Auricularia spp.)

Pileus : Brown coloured, ear shaped, 3.8 cm diameter with tomentose (Covering of soft and matted hairs) textured.

Stipe : Rudimentary, 0.7 × 0.7 cm^2

Volva and Annulus : Absent

Spore print : White

4.2.1.4. Morphological Studies of Neyyatinkara Collections (Auricularia spp.)

Pileus : Brown coloured, ear shaped to flabelliform (Fan like), 2.5 cm in diameter with smooth and velvety texture.

Stipe : Rudimentary, 0.6 × 0.4 cm^2

Volva and Annulus : Absent

Spore print : White

4.2.1.5. Morphological Studies of Kattakada Collections (Auricularia spp.)

Pileus : Dark brown coloured, ear shaped, 3.8 cm diameter,

leathery textured and caespitose (in groups).

Stipe : Rudimentary, 0.8 × 0.4 cm^2

Volva and Annulus : Absent

Spore print : White

***4.2.1.6. Morphological Studies of Ponmudi and Nedumangad Collections (Auricularia* spp.)**

Pileus : Brown coloured, flabelliform and ear shaped, 3.8 and 3.2 cm diameter respectively with soft and velvety textured.

Stipe : Rudimentary, 0.8 × 0.6 and 0.4 × 0.4 cm^2

Volva and Annulus : Absent

Spore print : White

***4.2.1.7. Morphological Studies of Palode Collections (Auricularia* spp.)**

Pileus :Dark brown coloured, margin incurved and 2.6 cm in diameter with a rough texture at border and smoother inside.

Stipe : Rudimentary, 0.5 × 0.5 cm^2

Volva and Annulus : Absent

Spore print : White

***4.2.1.7. Morphological studies of Arippa and Kulathupuzha Collections (Auricularia* spp.)**

Pileus : Brown coloured, ear shaped, 3.2 cm in diameter and texture varied from soft and velvety to leathery.

Stipe : Rudimentary, 0.9 × 0.8 and 0.9 × 0.7 cm^2

Volva and Annulus : Absent

Spore print : White

4.2.2. Microscopic Studies

***4.2.2.1. Hyphal Characters and Hyphal Stratification*:** Hyphae were septate, branched, hyaline, aerial as well as submerged for both *Auricularia* and *Tremella* spp. and measured 1.8-3.5 μm in width.

The cross section of *Auricularia* spp. showed eight different zonations like zona pilosa, zona compacta, zona subcompacta superiosis, zona laxa superiosis, medulla, zona laxa inferiosis, zona compacta inferiosis and hymenium (Plate 2.2).

Zona pilosa: The sterile, abhymenial zone composed of abhymenial hairs.

Zona compacta: The abhymenial surface from which the hairs arised including the very dense, narrow zone of compacted hyphae that surrounded hair bases which was typically the most darkly pigmented zone.

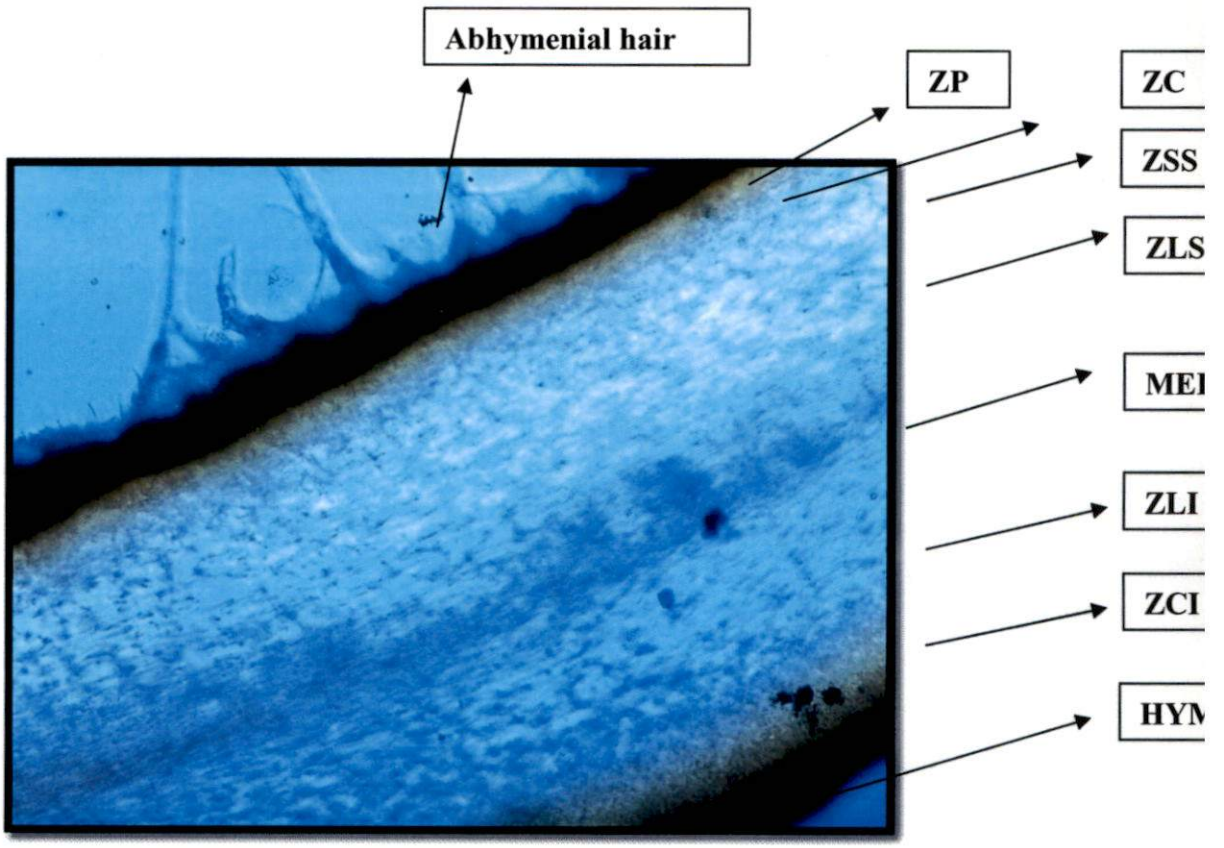
Zona subcompacta superiosis: Two zones proceeded from the zona compacta (superioris) or the hymenium (inferioris) where hyphae became less dense towards the centre of a section.

Zona laxa superiosis: A zone of loosely arranged hyphae in a gelatinous matrix that surrounded the medulla when present.

Medulla: A dense, central zone of hyphae characterized by its arrangement centrally and parallel with the surface and by the frequent presence of pigmentation. This particular zone had significant taxonomic importance. The presence of this zone indicated that the collected mushrooms belonged to *Auricularia polyticha* group where it had tropical importance and this zone was followed by zona laxa inferiosis, zona compacta inferiosis and hymenium.

Hymenium: A fertile layer bearing basidia as well as basidiospores.

Abhymenial hair: Hairs on the fruit body of *Auricularia* spp. They were hyaline, lacked a central strand and had rounded tips and they did not grow in dense tufts.



ZP-Zona pilosa, ZC- Zona compacta, ZSS – Zona subcompacta superiosis, ZLS – Zona laxa superiosis, MED – Medulla, ZLI – Zona laxa inferiosis, ZCI – Zona compacta inferiosis and HYM - Hymenium

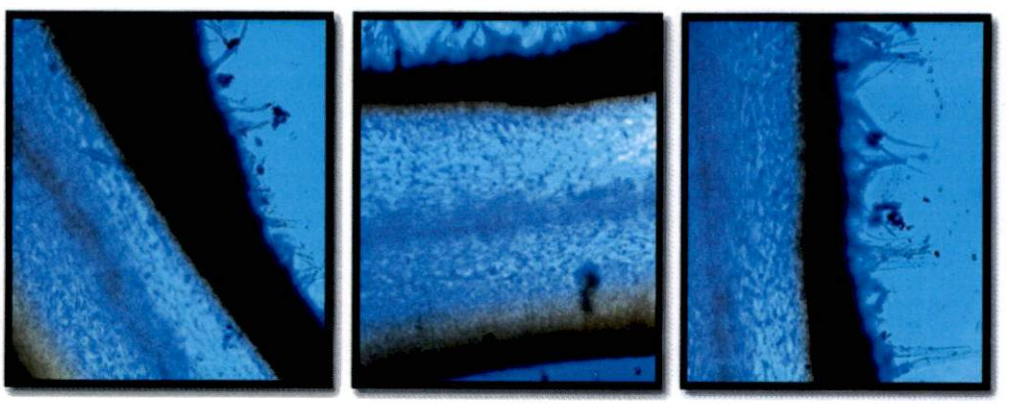


Plate 2.2. Microscopic view of cross section of mushroom with different zonations (40 X)

4.2.2.2. Basidiospores

Spore Print: Pure white in colour (Plate 2.3a).

Basidiospores: Hyaline (Plate 2.3b) oval, sub cylindrinical to cylindrical shaped (Plate 2.3c) and ranging from 10.8 × 3.2 to 12.8 × 3.4 µm in size

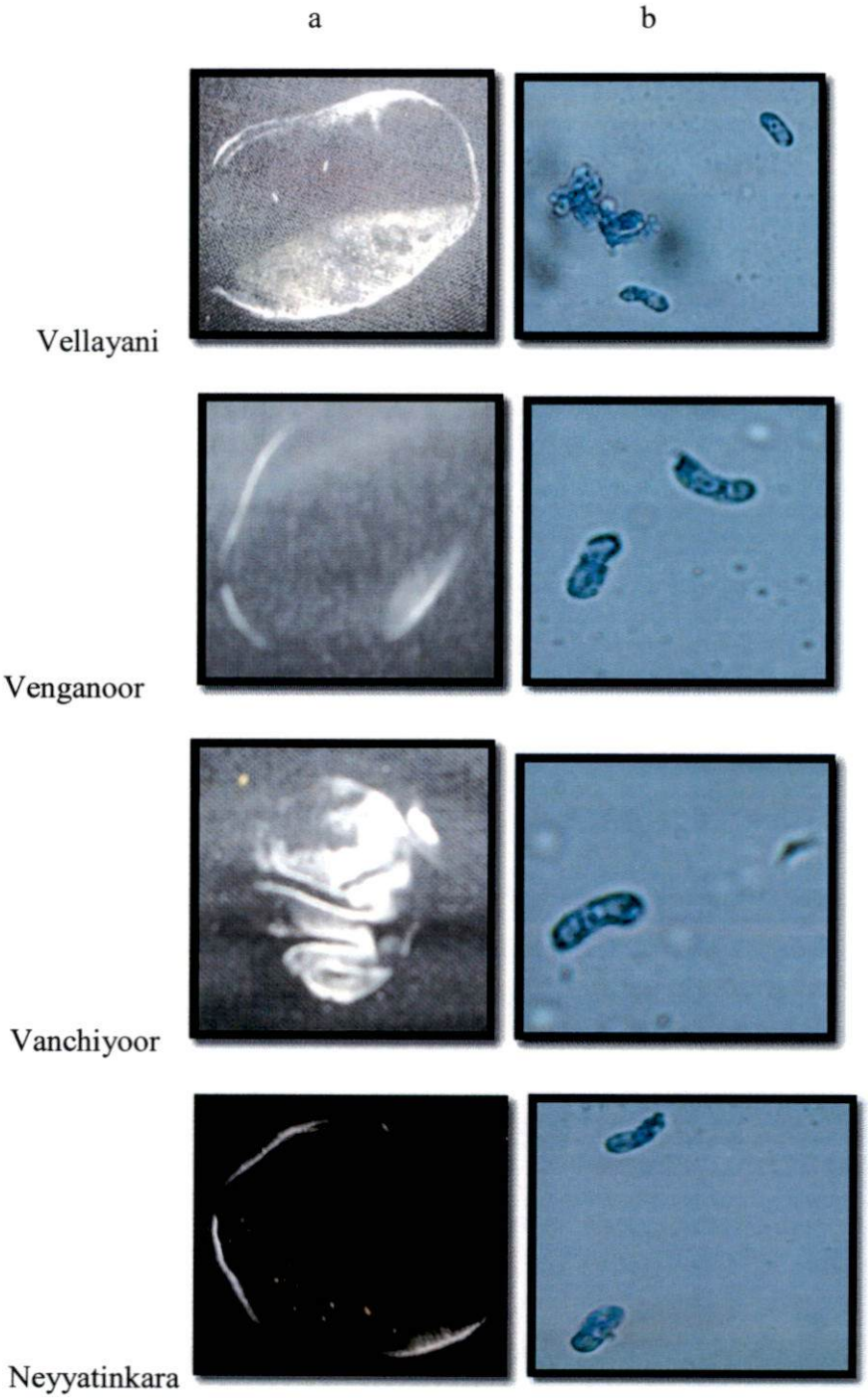
4.3. ISOLATION AND PURE CULTURING

The collections of jelly mushrooms obtained during the survey were isolated to obtain pure culture of the fungus, at the Department of Plant Pathology, College of Agriculture, Vellayani. Partially matured, pests and disease free (*Auricularia* and *Tremella* spp.) sporocarps obtained during the survey, were selected for isolation. The tissue culturing as per the standard method (Suharban, 1987) was adopted for isolation.

Two days after inoculation, white hair like strands of mycelial growth started growing from the tissues inoculated on PDA medium. As the growth continued the mycelium turned white, thick, cottony and branched and was completed within 14 to 15 days. The pure cultures of five collections *viz.*, four isolates of *Auricularia* spp. and one isolate of *Tremella* sp. obtained were maintained, sub cultured and designated as A1, A2, A3, A4 and T1 respectively.

4.3.1. Strainal Variations

Observations on the growth of the fungal cultures of jelly mushroom and the corresponding time taken for the same were recorded and statistically analysed to screen fast growing isolates from the collections obtained during the survey. Among the five isolates of *Auricularia* spp. and *Tremella* sp., the *Auricularia* isolate, A1, recorded maximum growth of 8.90 cm diameter which was significantly superior to the remaining isolates (Table 4). With regard to the time taken for completing the diameter of growth, it took 10.25 days, and was found to be significantly superior to all the other isolates. The next best results were



. Plate 2.3a Spore print and spores of jelly mushrooms from ten different locations

a- Spore print b- Spores (40 X)

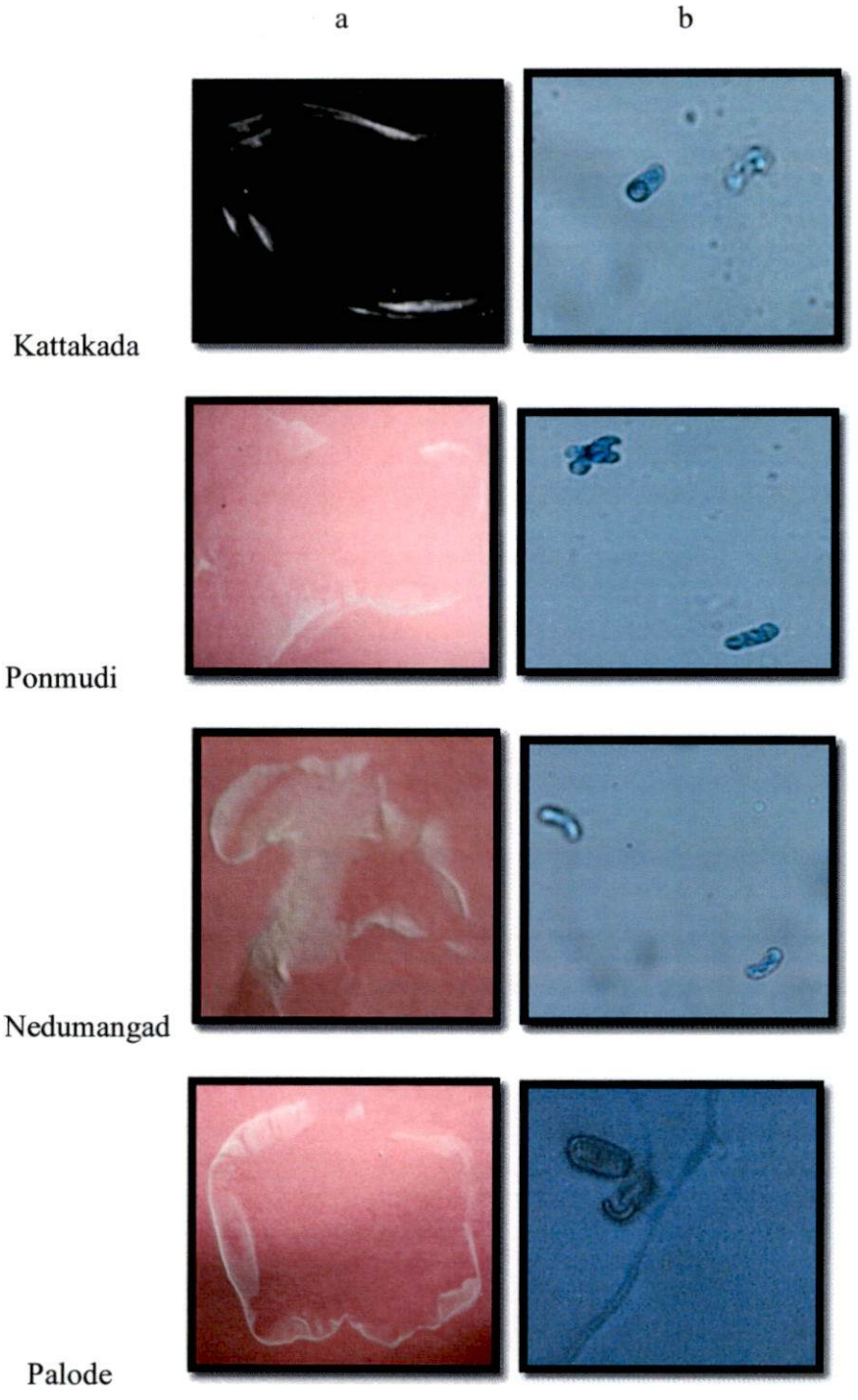


Plate 2.3b. Spore print and spores of jelly mushrooms from ten different locations

a- Spore print b- Spores (40 X)

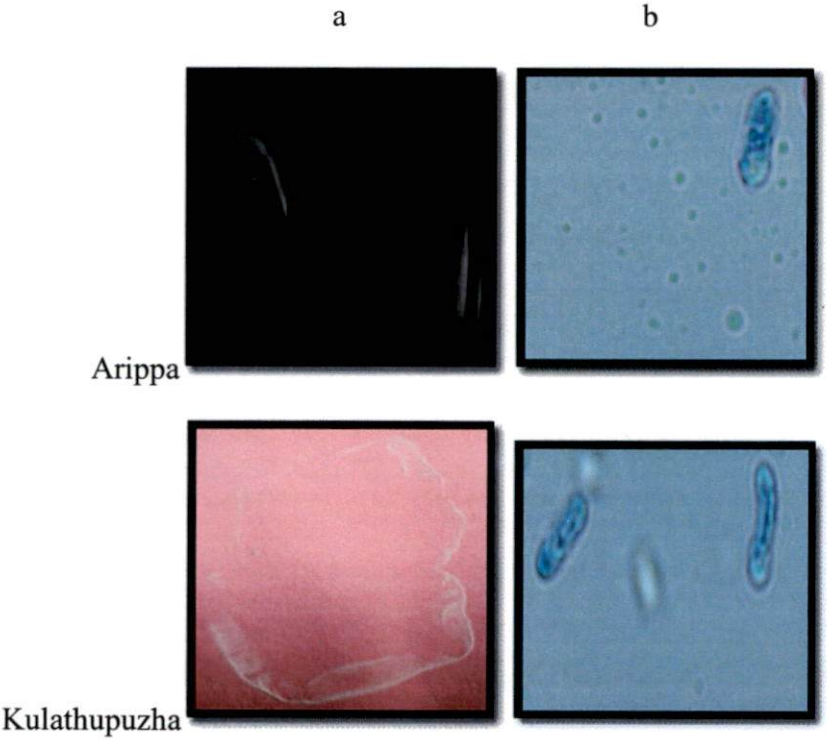


Plate 2.3c. Spore print and spores of jelly mushrooms from ten different locations

a- Spore print b- Spores (40 X)

recorded by A2 (11.25 days), A3 (12.0 days), A4 (12.75 days) and the least performance was given by T1 which took a maximum of 14.25 days for complete mycelial growth and correspondingly the diameter of growth was 8.45, 8.05, 7.50 and 6.92 cm.

The nature of mycelial growth of A1 was thick, cottony and fluffy with irregular margin, whereas in A2 it was cottony with smooth margin. The nature of mycelial growth of A3 was thick and fluffy. Thin growth with smooth margin was observed in Kattakada isolate (A4) and thin growth with irregular margin was observed in *Tremella* isolate (T). The mushrooms of A1 were dark brown coloured and leathery in texture whereas A2 were brown coloured, smooth and velvety. Correspondingly, the aerial growth of culture also showed marked differences particularly in A1 which had irregular margins with more number of zonations (four), compared to A1, A2 which showed smooth margin and two to three zonations (Plate 3).

Selection of isolates of jelly mushrooms identified as *Auricularia polytricha* (Mont.) Sacc. with accession number DMRO-825 for A1 and DMRO-826 for A2 at the Directorate of Mushroom Research (DMR), ICAR, Solan, Himachal Pradesh.

4.4. PHYSIOLOGICAL STUDIES

The two isolates A1 and A2 which were screened for fast growth and identified as *Auricularia polytricha*, were used in the subsequent studies of this investigation.

4.4.1. Growth of *Auricularia polytricha* (Mont.) Sacc. - A1 and A2 in Different Media

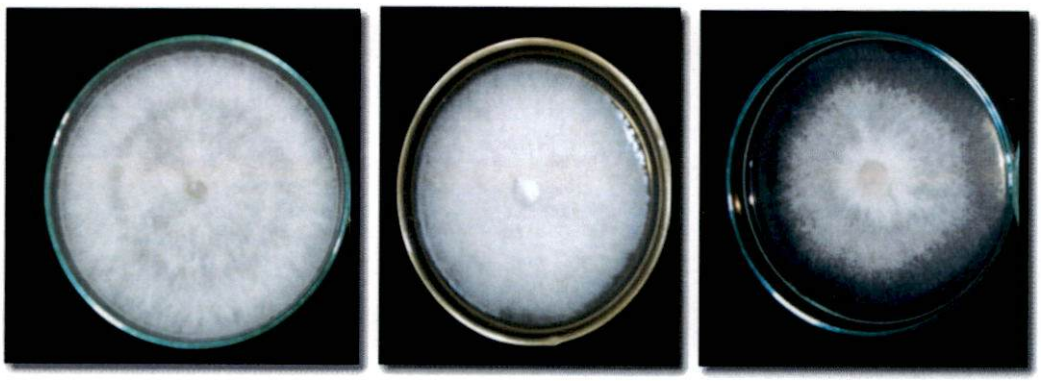
Cultural characteristics of isolates A1 and A2 of *Auricularia polytricha* were studied on four different media, the results of which are presented in Table 5 and 6.

Table 4. Cultural characteristics of different isolates of jelly mushrooms

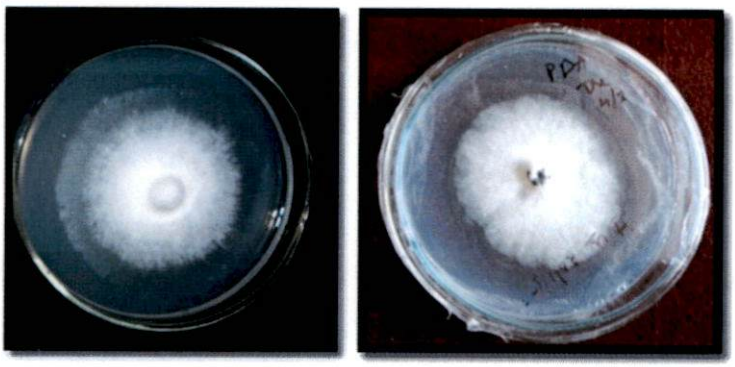
Sl. No.	Cultures	Time taken for completing 90mm radial growth (days)*	Radial growth of mycelia after 10 days of inoculation (cm)*	Nature of mycelial growth	Colour of mycelia
1.	Vellayani (A1)	10.25^{e}	8.90^{a}	Thick cottony and fluffy growth with irregular margin	White
2.	Neyyatinkara (A2)	11.25^{d}	8.45^{b}	Cottony growth with smooth margin	White
3.	Vellayani - (A3)	12.00^{c}	8.05^{c}	Thick and fluffy growth	White
4.	Kattakada (A4)	12.75^{b}	7.50^{d}	Thin growth with smooth margin	White
5.	Vellayani (T1)	14.25^{a}	6.92^{e}	Thin growth with irregular margin	White
	CD (0.05)	0.68	0.15		
	S. Em±	0.22	0.05		

* Mean of four replications

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



Vellayani - 1 (A) Neyyatinkara (A) Vellayani - 2 (A)



Kattakada (A) Vellayani (T)

Plate 3.Cultural characteristics of different isolates of jelly mushrooms

The results revealed that among the different media tested for the growth of A1 and A2, malt extract agar was found to be the best medium on which it took a minimum of 10.50 days to complete mycelial growth for both A1 and A2 on petri plates. The next best treatment was potato dextrose agar (11.25 days and 11.50 days respectively for A1 and A2) followed by carrot extract agar (12.50 and 12.25 days respectively). Both the isolates took maximum time to complete mycelial growth (13.75 days and 13.0 days respectively for A1 and A2) on oat meal agar. The time taken by the two isolates to complete growth on carrot extract and oat meal agar were found to be on par for A2. No primordial growth observed in any of the media tested.

Maximum growth for each of the two isolates A1 and A2 was measured on malt extract agar medium (9.0 cm diameter), ten days after inoculation and was significantly superior to all the other media. The next best results were obtained on potato dextrose agar on which the diameter of growth measured for the two isolates were 7.52 cm and 8.20 cm respectively and was on par with oat meal agar (7.02 cm and 7.90 cm diameter, respectively). Growth of each of the two isolates was least on carrot extract agar as indicated by the diameter of 6.90 cm and 7.60 cm respectively.

Thick, cottony and fluffy growth was observed in malt extract agar with three to four zonations both for A1 (Plate 4.1a) and A2 (Plate 4.1b), whereas in potato dextrose agar medium fluffy growth with irregular margin and two zonations were observed. Thin growth with irregular margin and two zonations were observed in carrot extract agar, and oat meal agar showed thick cottony with irregular margin for A1, whereas A2 showed smooth margin in almost all the media tested. The mycelial growth was white in colour.

The best medium *viz.*, MEM which was screened in this experiment based on the maximum growth produced by the two isolates within minimum time, was used in the subsequent studies.

Table 5. Mycelial characters of *Auricularia polytricha* – A1 as influenced by growing media

Sl. No.	Media	Time taken for complete mycelial growth (days)*	Diameter of growth on 10th day (cm)*	Nature of mycelial growth	Colour of mycelia
1.	Potato Dextrose Agar	11.25[c]	7.52[b]	Fluffy growth and irregular margin	White
2.	Oat Meal Agar	13.75[a]	7.02[b]	Thick cottony with irregular margin	White
3.	Carrot Extract Agar	12.50[b]	6.90[b]	Thin growth with irregular margin	White
4.	Malt Extract Agar	10.50[c]	9.00[a]	Thick cottony and fluffy growth	White
	CD (0.05)	1.05	0.93		
	S. Em±	0.33	0.30		

* Mean of four replications

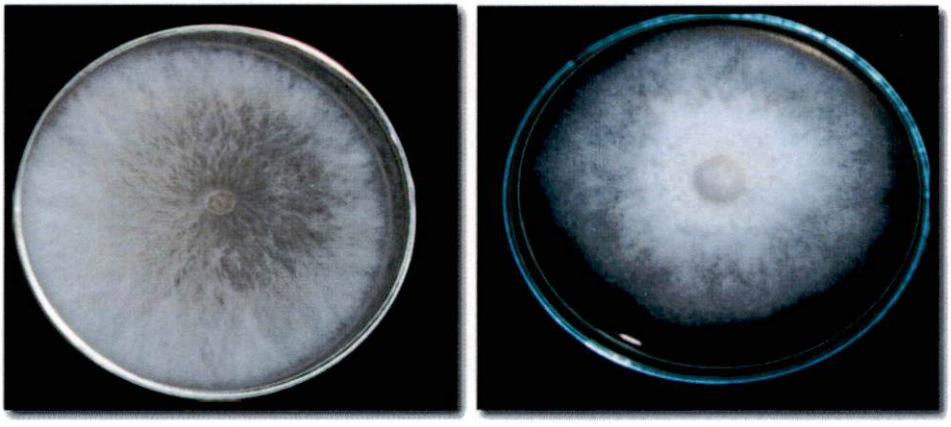
*Means followed by similar superscripts are not significantly different at 5% level

Table 6. Mycelial characters of *A. polytricha* – A2 as influenced by growing media

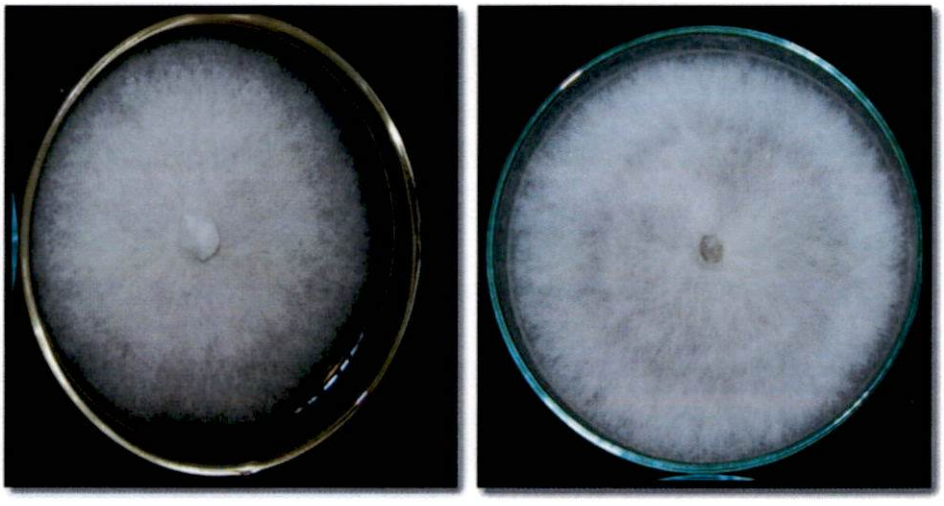
Sl. No.	Media	Time taken for complete mycelial growth (days)*	Diameter of growth on 10th day (cm)*	Nature of mycelial growth	Colour of mycelia
1.	Potato Dextrose Agar	11.50^{b}	8.20^{b}	Cottony growth and smooth margin	White
2.	Oat Meal Agar	13.00^{a}	7.90^{bc}	Thick cottony with smooth margin	White
3.	Carrot Extract Agar	12.25^{ab}	7.60^{c}	Thin growth with smooth margin	White
4.	Malt Extract Agar	10.50^{c}	9.00^{a}	Thick cottony and fluffy growth	White
	CD (0.05)	0.980	0.547		
	S. Em±	0.315	0.176		

* Mean of four replications

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

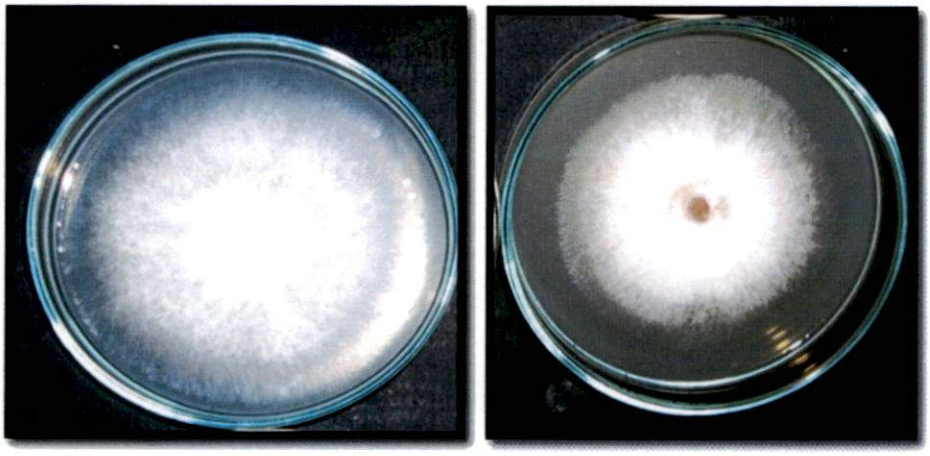


Potato dextrose agar | Oat meal agar



Carrot extract agar | Malt extract agar

Plate 4.1a.Mycelial characters of *Auriculariapolytricha*– A1 as influenced by growing media



Potato dextrose agar Oat meal agar

Carrot extract agar Malt extract agar

Plate 4.1b.Mycelial characters of *A. polytricha*– A2 as influenced by growing media

4.4.2. Growth of *A. polytricha* - A1 and A2 in Different Temperature

The physiological characteristics of A1 and A2 isolates of *A. polytricha* were studied at three different temperature levels as described in material and methods and the results of the study are presented below.

Among the different temperature levels tested, a temperature of 25 ^{0}C was found to be the best and took minimum of 9.60 and 10.20 days to complete mycelial growth of A1 (Table 7) and A2 (Table 8) respectively. The next best treatment was 30 ^{0}C (11.20 and 11.60 days) and temperature at 20 ^{0}C took maximum time to complete the mycelial growth (11.80 and 12.40 days). In terms of diameter of growth produced after 10 days of inoculation, maximum measurements of 9.0 cm and 8.84 cm, respectively were recorded at temperature of 25 ^{0}C which was found to be on par with 30 ^{0}C (8.8 and 8.68 cm). The least growth was observed at temperature of 20 ^{0}C (8.52 cm and 7.74 cm diameter, respectively). Thick cottony white coloured mycelial growth of both isolates A1 (Plate 4.2a) and A2 (Plate 4.2b) was observed in all the treatments and no primordial formation was noticed in any of the treatments.

4.4.3. Growth of *A. polytricha* - A1 and A2 in Different pH

The physiological characteristics of isolates A1 and A2 were studied in four different pH levels and the results of the study are presented in Table 9 and 10.

The results revealed that minimum time for completion of mycelial growth for both A1 and A2 isolates (9.50 days) occurred at pH 7 and was significantly different from all other pH levels tested. This was followed by growth at pH 6 (11.50 days and 10.50 days respectively), pH 8 (11.75 days and 12.0 days, respectively). The maximum time taken to complete mycelial growth for A1 and A2 isolates was 12.75 days and 12.50 days respectively at pH 5.

Maximum growth was measured for A1 and A2 isolates at pH 7 (9.0 cm and 8.95 cm diameter, respectively) 10 days after inoculation and was significantly superior to all other pH levels tested. This was followed by growth at pH 6 (8.67 cm and 8.77 cm) which was on par with the results of growth at pH 8 (8.65 and

Table 7. Mycelial characters of *A. polytricha* – A1 as influenced by different temperature

Sl. No.	Temperature (^{0} C)	Time taken for complete mycelial growth (days) *	Diameter of growth on 9th day (cm)*	Nature of mycelial growth	Colour of mycelia
1.	20	11.80^a	8.52^b	Thick cottony	White
2.	25	9.60^b	9.00^a	Thick cottony	White
3.	30	11.20^a	8.88^a	Thick cottony	White
	CD (0.05)	0.67	0.35		
	S. Em±	0.21	0.11		

Table 8. Mycelial characters of *A. polytricha* – A2 as influenced by different temperature

Sl. No.	Temperature (^{0} C)	Time taken for complete mycelial growth (days) *	Diameter of growth on 10th day (cm)*	Nature of mycelial growth	Colour of mycelia
1.	20	12.40^a	7.74^b	Thick and cottony	White
2.	25	10.20^c	8.84^a	Thick and cottony	White
3.	30	11.60^b	8.68^a	Thick and cottony	White
	CD (0.05)	0.719	0.53		
	S. Em±	0.231	0.17		

* Mean of five replications

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

Table 9. Mycelial characters of *A. polytricha* – A1 as influenced by different pH

Sl. No.	pH	Time taken for complete mycelial growth (days) *	Diameter of growth on 9th day (cm)*	Nature of mycelial growth	Colour of mycelia
1.	5	12.75[a]	8.45[b]	Thin and cottony	White
2.	6	11.50[b]	8.67[b]	Thick and cottony	White
3.	7	9.50[c]	9.00[a]	Thick and cottony	White
4.	8	11.75[b]	8.65[b]	Cottony growth	White
	CD (0.05)	0.84	0.32		
	S. Em±	0.27	0.10		

* Mean of four replications

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

Table 10. Mycelial characters of *A. polytricha* – A2 as influenced by different pH

Sl. No.	pH	Time taken for complete mycelial growth (days) *	Diameter of growth on 9th day (cm)*	Nature of mycelial growth	Colour of mycelia
1.	5	12.50[a]	8.42[c]	Thin and cottony	White
2.	6	10.50[b]	8.77[b]	Thick and cottony	White
3.	7	9.50[c]	8.95[a]	Thick and cottony	White
4.	8	12.00[a]	8.70[b]	Cottony growth	White
	CD (0.05)	1.006	0.17		
	S. Em±	0.323	0.05		

* Mean of four replications

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

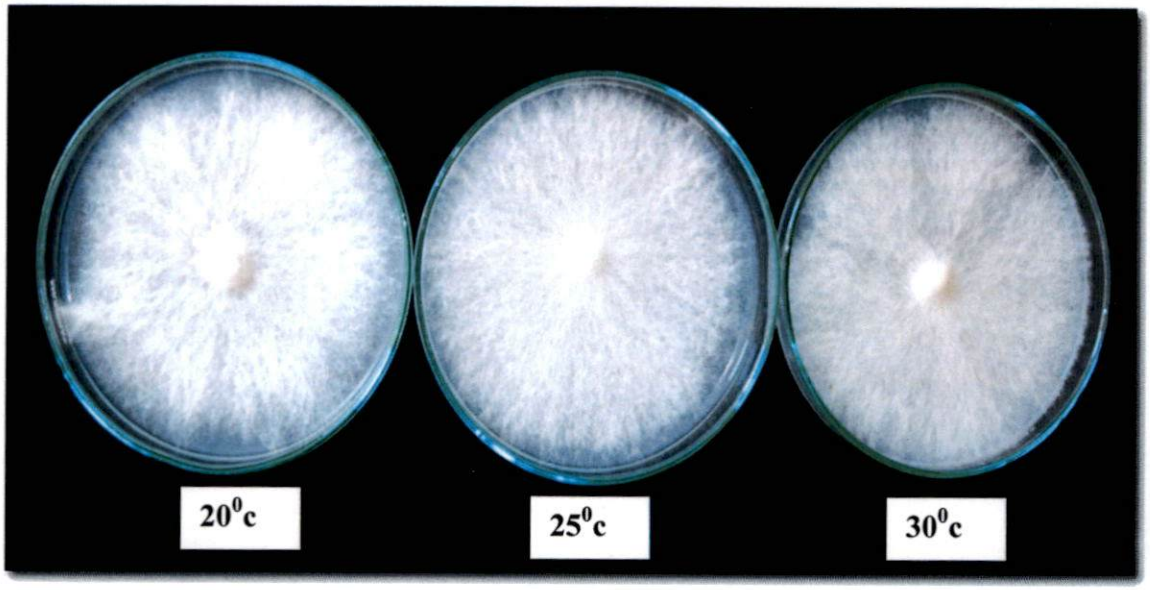


Plate 4.2a.Mycelial characters of *A. polytricha*– A1 as influenced by different temperature

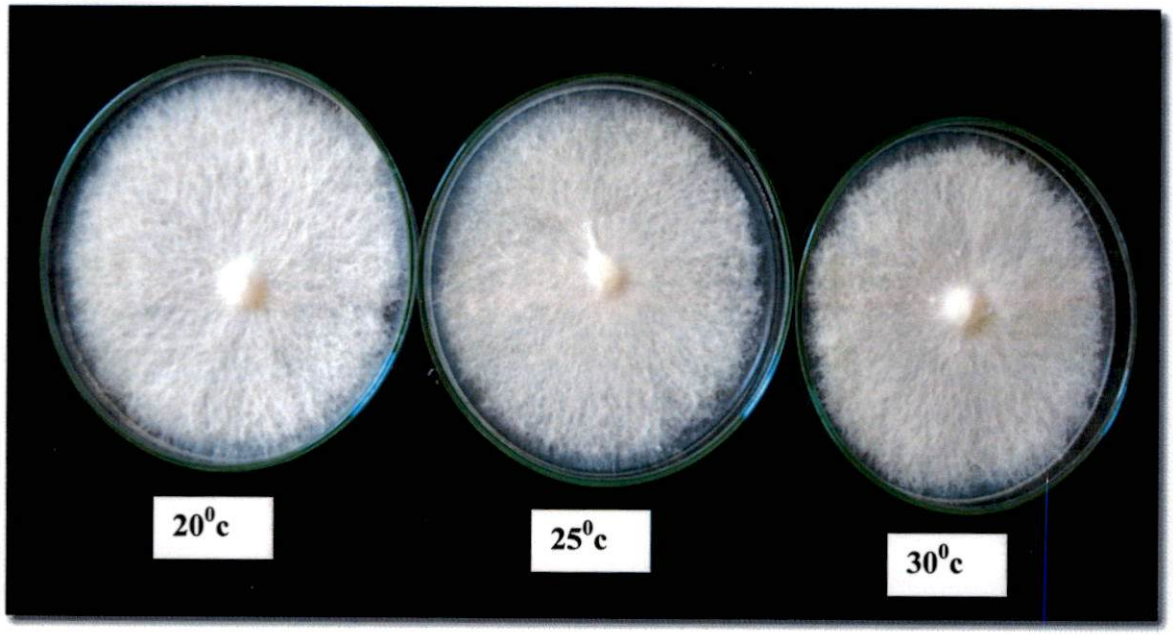


Plate 4.2bMycelial characters of *A. polytricha*– A2 as influenced by different temperature

8.70 cm). The least performance in growth of the two isolates occurred at pH 5 (8.45 cm and 8.42 cm diameter, respectively). It was observed that the growth was thin to thick, cottony and white in colour without any primordial formation in all the pH levels tested (Plate 4.3a. and 4.3b.).

4.4.4. Growth of *A. polytricha* – A1 and A2 in Light and Dark Conditions

The physiological characteristics of A1 and A2 were studied in two different light conditions.

The time taken to complete mycelial growth on exposure to light were 9.70 days and 9.40 days respectively for A1 and A2 isolates. Under dark conditions the isolates took more time (11.70 days and 12.30 days) to complete the mycelial growth and were found to differ significantly (Table 11 and 12). Exposure to light resulted in a maximum growth of 8.82 and 8.93 cm diameter respectively for the two isolates after 10 days of inoculation, which significantly differed from their growth under dark conditions which was 7.78 cm diameter for both the isolates. Nature of growth observed for both the isolates was thick and cottony on exposure to light. Thin cottony growth was observed in the two isolates A1 and A2 under dark conditions. Both light and dark conditions produced white mycelial growth (Plate 4.4a and 4.4b) without any primordia in the two isolates A1 and A2.

4.5. SPAWN PRODUCTION

Six substrates (Table 13 and 14) were evaluated for production of spawn on the basis of time taken for spawn run, nature of mycelial growth and presence of contaminants. The substrates tested were paddy grains, wheat, sorghum, ragi, sawdust of hardwood trees (teak) and rubber sawdust (Plate 5.1).

A1 and A2 isolates took minimum period (16.75 and 16.50 days) for completing spawn run in paddy grains (Plate 5.2a.) wherein the mycelial growth was thicker and fluffy. This was followed by sorghum grains where a similar growth pattern was observed. The number of days taken for spawn run on this substrate were 17.0 days and 19.50 days respectively for the two isolates A1 and A2 (Plate 5.2c.) which also had thicker and fluffy growth. This was followed by

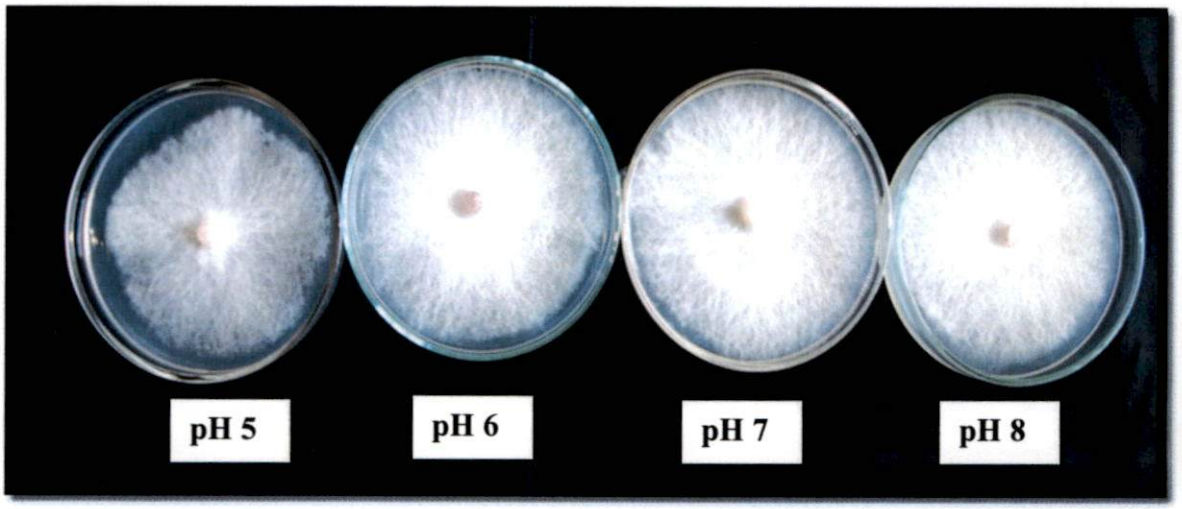


Plate 4.3a.Mycelial characters of *A. polytricha*– A1 as influenced by different pH

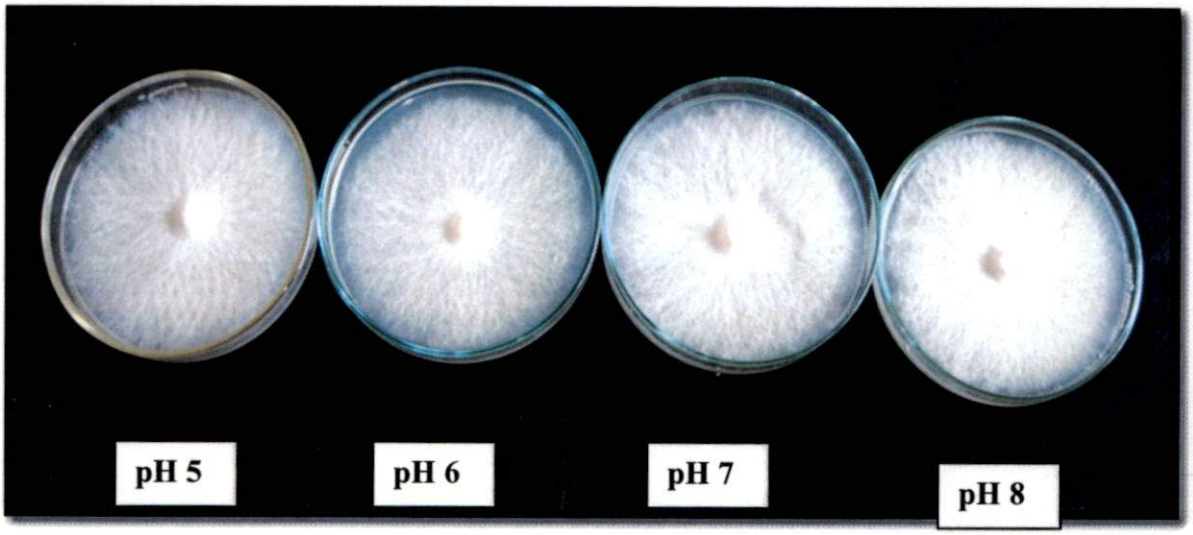


Plate 4.3b.Mycelial characters of *A. polytricha*– A2 as influenced by different pH

Table 11. Mycelial characters of *A. polytricha* – A1 as affected by light and dark conditions

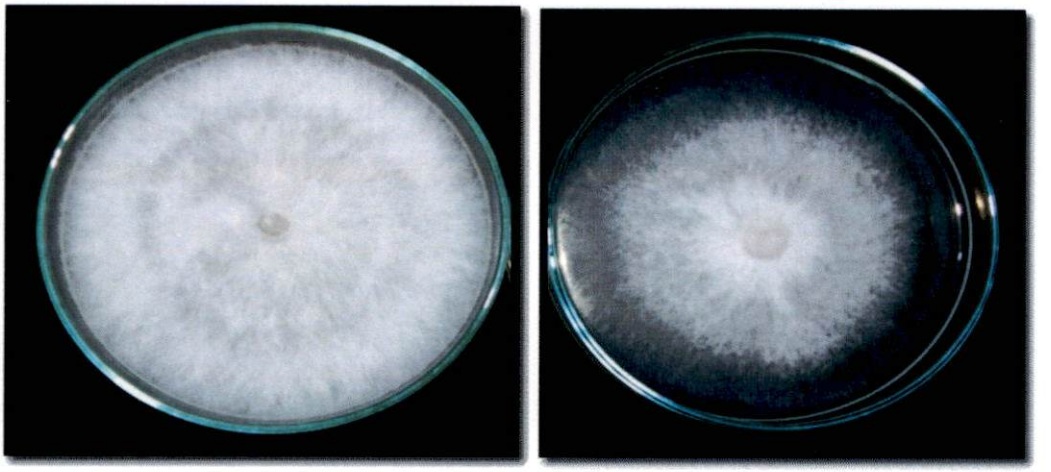
Sl. No.	Light and dark conditions	Time taken for complete mycelial growth (days) *	Diameter of growth on 9th day (cm)*	Nature of mycelial growth	Colour of mycelia
1.	Light	9.70	8.82	Thick cottony	White
2.	Dark	11.70	7.78	Thin cottony	White
	t (Value)	3.579**	8.96**		

Table 12. Mycelial characters of *A. polytricha* – A2 as affected by light and dark conditions

Sl. No.	Light and dark conditions	Time taken for complete mycelial growth (days) *	Diameter of growth on 9th day (cm)*	Nature of mycelial growth	Colour of mycelia
1.	Light	9.40	8.93	Thick cottony	White
2.	Dark	12.30	7.78	Thin cottony	White
	t (Value)	4.320**	24.58**		

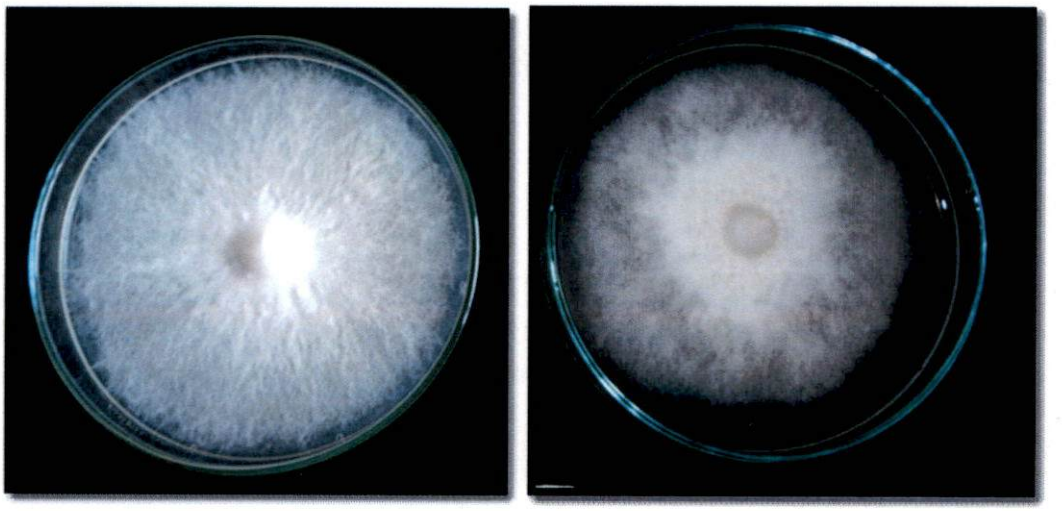
* Mean of ten observations

** Treatments are significantly different at 5% level



Light Dark

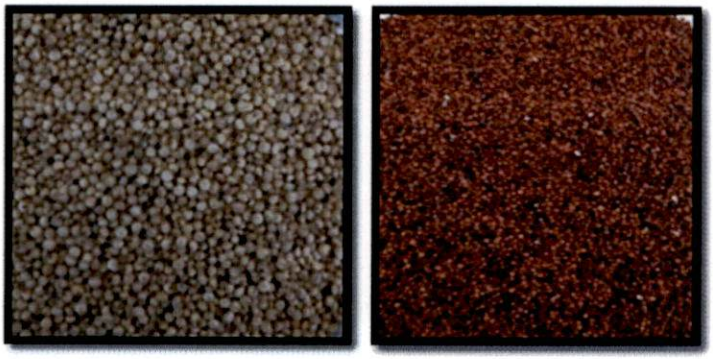
Plate 4.4a.Mycelial characters of *A. polytricha*– A1 as affected by light and dark conditions



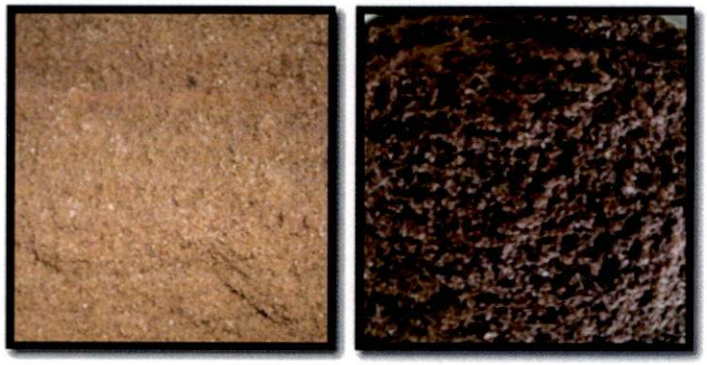
Light Dark

Plate 4.4b.Mycelial characters of *A. polytricha*– A2 as affected by light and dark conditions

Paddy grains Wheat grains



Sorghum grains Ragi grains



Sawdust of hardwood trees Rubber sawdust

Plate 5.1. Substrates used for spawn preparation of *A. polytricha*- A1 and A2

wheat substrate on which the spawn run was completed in 20.0 days for both the isolates. On ragi, spawn run was completed by the isolates in 21.75 and 22.25 days respectively. Both wheat and ragi produced thick mycelial growth. The spawn run was poor on the hard wood sawdust for the two isolates A1 and A2 which took 22.50 and 23.0 days to complete spawn run respectively which were on par with ragi. On rubber sawdust the isolates A1 and A2 took maximum period of 27.0 days and 27.50 days for completing spawn run which had thick mycelial growth.

Presence of contaminants like *Aspergillus* sp., *Rhizopus* sp. and *Trichoderma* spp. were observed in almost all the substrates (Plate 5.2b.) and the predominant contaminants recorded were *Aspergillus* spp. and *Trichoderma* spp. in paddy grains and *Aspergillus* spp. in sorghum, wheat, sawdust of teak and rubber sawdust. *Rhizopus* sp. was predominant in ragi spawn. The maximum contamination percentage was found in wheat (48 % and 52 %) followed by paddy grains (40 % and 44 %), ragi (36 % and 40 %), sorghum (32 % and 36 %) and rubber sawdust (28 % and 32 %). The least per cent contamination was observed in sawdust of hard wood trees (24 % and 28 %).

The keeping quality was the highest in rubber sawdust spawn (82.50 and 80.25 days) and was significantly superior to all other substrates. The next best substrate found was paddy grains (76.25 and 74.0 days) which were found to be on par with sawdust of hard wood trees (75.0 and 72.50 days). The keeping quality of ragi spawn was 62.75 and 61 days, followed by sorghum (45.0 and 43.50 days) and wheat (27.0 and 26.50 days). Among the different substrates tried for spawn production, rubber sawdust was the only substrate which could produce primordia as well as fruiting bodies in the spawn itself (Plate 5.2b.).

4.6. SUBMERGED CULTURING

The submerged culture production of A1 and A2 isolates of *A. polytricha* was studied in three different liquid media and dry weight of mycelial pellets obtained for each isolate was determined (Plate 6.1 and 6.2).

Table 13. Influence of different substrates on spawn production of *A. polytricha* -A1

Sl. No	Substrates	Time taken for spawn run (days)*	Nature of mycelial growth	Contaminants observed	Per cent spawns contaminated	Keeping quality of spawns (days) *	Primordia/ fruiting body formation
1.	Paddy grains	16.75[d]	++++ (100%)	*Trichoderma* spp. and *Aspergillus* sp.	40	76.25 [b]	Nil
2.	Wheat	20.00[c]	+++ (75%)	*Aspergillus* sp.	48	27.00 [e]	Nil
3.	Sorghum	17.00[d]	++++ (100%)	*Aspergillus* sp.	32	45.00 [d]	Nil
4.	Ragi	21.75[b]	+++ (75%)	*Rhizopus* sp.	36	62.75 [c]	Nil
5.	Sawdust of hard wood trees	22.50[b]	++ (50%)	*Aspergillus* sp.	24	75.00 [b]	Nil
6.	Rubber sawdust	27.00[a]	+++ (75%)	*Aspergillus* sp.	28	82.50 [a]	Present
	CD (0.05)	1.51				5.42	
	S. Em±	0.49				1.78	

++++ - Thicker and fluffy growth +++ - Thick growth ++ - Poor growth * Mean of four replications

Table 14. Influence of different substrates on spawn production of *A. polytricha* - A2

Sl. No	Substrates	Time taken for spawn run (days)*	Nature of mycelial growth	Contaminants observed	Per cent spawns contaminated	Keeping quality of spawns (days) *	Primordia/ fruiting body formation
1.	Paddy grains	16.50^{e}	++++	*Trichoderma* spp., *Aspergillus* sp.	44	74.00^{b}	Nil
2.	Wheat	20.00^{cd}	+++	*Aspergillus* sp.	52	26.50^{e}	Nil
3.	Sorghum	19.50^{d}	++++	*Aspergillus* sp.	36	43.50^{d}	Nil
4.	Ragi	22.25^{bc}	+++	*Rhizopus* sp.	40	61.00^{c}	Nil
5.	Sawdust of hard wood trees	23.00^{b}	++	*Aspergillus* sp.	28	72.50^{b}	Nil
6.	Rubber sawdust	27.50^{a}	+++	*Aspergillus* sp.	32	80.25^{a}	Present
	CD (0.05)	2.36				3.75	
	S. Em±	0.77				1.23	

++++ - Thicker and fluffy growth +++ - Thick growth ++ - Poor growth * Mean of four replications

1 Paddy

2 Wheat

3 Sorghum

4 Ragi

5 Saw dust of hard wood trees

6 Rubber saw dust

Plate 5.2a. Influence of different substrates on spawn production of *A. polytricha*A1

Primordia / fruiting bodies *Trichoderma* spp. *Aspergillus*spp.

Plate 5.2b.Primordia/ fruiting body formation and contaminants observed in spawn of *A. polytricha*– A1

1 Paddy

2 Wheat

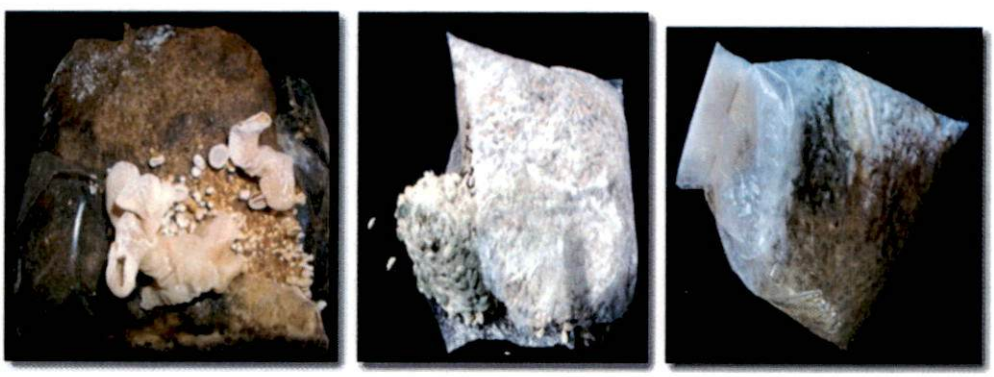
3 Sorghum

4 Ragi

5 Saw dust of hard wood trees

6 Rubber saw dust

Plate 5.2c. Influence of different substrates on spawn production of A2



Primordia / fruiting bodies *Trichoderma* spp. *Aspergillus*spp.

Plate 5.2d.Contaminants observed in grain spawns of *A. polytricha*– A2

Among three different liquid media (potato dextrose broth, malt extract broth and carrot extract broth) used for submerged culture production, malt extract medium was found to be significantly superior to the remaining two treatments resulting in the highest dry weight of mycelial pellets for the isolate A1 (1.05 g) and A2 (1.01 g). Dry weight of mycelial pellets in potato dextrose broth was 0.61g for isolate A1 and 0.57 g for isolate A2, which was followed by carrot extract broth (0.37 g and 0.32 g) for isolates A1 and A2 respectively (Table 15 and 16) and the pellets were light cream to white in colour.

4.7. CULTIVATION

Different substrates *viz.*, paddy straw, sawdust of softwood trees (*Alianthus excelsa* Roxb.) and hardwood trees (rubber), banana pseudostem, sugarcane bagasse and neopeat (cocopeat) were evaluated for screening the most suitable substrate for fruiting body production of the two isolates of *A. polytricha* - A1 and A2. The time taken for complete spawn run, time taken for pinhead formation, time taken for first flush, total crop period, average weight of sporocarp, number of sporocarps, total yield from three harvests and biological efficiency (BE) were recorded and presence of pests and competitor moulds were also recorded.

Spawn run was found to be significantly superior in paddy straw compared to all the other substrates taking 27.25 days and 27.75 days for A1 and A2 isolates respectively (Table 17 and 19). A white coloured mycelium was observed (Plate 7.1a and 7.2a). This was followed by rubber sawdust (33.0 days and 33.75 days for A1 and A2, respectively), sawdust of soft wood trees (36.0 days and 36.75 days) for A1 and A2, respectively and sugarcane bagasse (37.0 days and 38.0 days) for A1 and A2, respectively. The average time taken for spawn run in banana pseudostem and neopeat was maximum (37.75 days and 38.25 days for A1 and A2, 38.25 days and 38.75 days) respectively and they did not differ significantly.

In case of time taken for pinhead formation (Plate 7.1b and 7.2b.) paddy straw was found to be significantly superior over other substrates which took 38.75 and 34.50 days respectively for both A1 and A2. The next best substrate

Table 15. Evaluation of different liquid media on submerged culture production of *A. polytricha* - A1

Sl No	Liquid media	Dry mycelial pellets weight of A1(g)
1	Liquid Potato dextrose medium (LPDM)	0.61[b]
2	Liquid Malt extract medium (LMEM)	1.05[a]
3	Liquid Carrot extract medium (LCEM)	0.37[c]
CD (0.05)		0.09
S. Em±		0.03

Table 16. Evaluation of different liquid media on submerged culture production of *A. polytricha* –A2

Sl No	Liquid media	Dry mycelial pellets weight of A2(g)
1	Liquid Potato Dextrose Medium (LPDM)	0.57[b]
2	Liquid Malt Extract Medium (LMEM)	1.01[a]
3	Liquid Carrot Extract Medium (LCEM)	0.32[c]
CD (0.05)		0.10
S. Em±		0.03

* Mean of five replications

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

LPDM LMEM LCEM

Plate 6.1. Evaluation of different liquid media for submerged culture production of *A. polytricha*- A1

LPDM LMEM LCEM

Plate 6.2. Evaluation of different liquid media for submerged culture production of *A. polytricha*– A2

observed was rubber sawdust (41.75 days and 42.75 days for both A1 and A2 respectively) which showed on par results with sawdust of softwood trees (42.50 and 43.50 days for A1 and A2 respectively) followed by sugarcane bagasse (43.50 days and 44.25 days for A1 and A2 respectively) and neopeat (44.0 days and 45.0 days for A1 and A2 respectively) which did not differ significantly. Primordial formation was absent in beds prepared using banana pseudostem.

When rubber sawdust was used as substrate for jelly mushroom cultivation, A1 and A2 took 48.75 and 49.75 days respectively for first harvest in which the average weight of sporocarp was 3.65 g and 3.30 g respectively. The average number of sporocarps for the three harvests of A1 and A2 isolates was 38.00 and 36.50 respectively, total yield 0.148 kg and 0.122 kg respectively and biological efficiency was 14.8 per cent and 12.2 per cent respectively for the two isolates A1 and A2 (Table 18 and 20). Flushes of the mushrooms appeared even after the period of three harvests and the total crop period extended up to 88 days and 84.25 days for A1 and A2 respectively, reaching a total of eight harvests (Plate 7.1c and 7.2c.). The total yield from eight harvests was 0.506 kg and 0.486 kg for which the biological efficiency was 50.6 per cent for A1 and 48.6 per cent for A2.

Table 21 indicated that the major insect pests in different substrates were phorid flies (*Megaselia* spp.), springtails (*Seira* spp.) and staphylinid beetles in paddy straw, springtails and mites in sawdust of softwood trees and phorid flies, springtails, staphylinid beetles and mites in sawdust of hardwood tress. Phorid files and springtails were observed in banana pseudostem, phorid flies in sugarcane bagasse and staphylinid beetles in neopeat substrate (Plate 7.3). *Coprinus* spp., *Aspergillus* spp., *Penicillium* spp. and *Trichoderma* spp. were the major microbial contaminants found in the substrates of paddy straw, *Aspergillus* sp. and *Trichoderma* sp. in sawdust of softwood trees, *Aspergillus* sp. and *Trichoderma* sp. in sawdust of hardwood trees, *Trichoderma* sp. in banana pseudostem and sugarcane bagasse and *Aspergillus* sp. in neopeat (Plate 7.3b).

Table 17. Influence of different substrates on growth stages of *A. polytricha* –A1

Sl. No.	Substrates	Time taken for complete spawn run (days)*	Time taken for pinhead formation (days)*	Time taken for first flush (days)*	Total crop period (days)*
1.	Paddy straw	27.25^{d}	38.75^{c}	0.00	0.00
2.	Sawdust of softwood trees	36.00^{b}	42.50^{ab}	0.00	0.00
3.	Sawdust of hardwood trees	33.00^{c}	41.75^{b}	48.75	88.00
4.	Banana pseudostem	37.75^{a}	0.000^{d}	0.00	0.00
5.	Sugarcane bagasse	37.00^{ab}	43.50^{a}	0.00	0.00
6.	Neopeat	38.25^{a}	44.00^{a}	0.00	0.00
	CD (0.05)	1.332	1.71		
	S. Em±	0.445	0.57		

* Mean of four replications *Means followed by similar superscripts are not significantly different at 5% level

Table 18. Influence of different substrates on the yield parameters of *A. polytricha* – A1

Substrates	Average weight sporocarp (g)*	Number of sporocarps*	Total yield from three harvests (kg/kg dry weight of substrate)*	Biological efficiency (BE) (%)(Fresh wt. of mushrooms/ dry wt. of substrate used ×100)	Total yield from eight harvests (kg/kg dry weight of substrate)*	BE for eight harvests
Paddy straw	0.00	0.00	0.00	0	0	0
Sawdust of softwood trees	0.00	0.00	0.00	0	0	0
Sawdust of hardwood trees	3.65	38.00	0.148	14.8	0.506	50.6
Banana pseudostem	0.00	0.00	0.00	0	0	0
Sugarcane bagasse	0.00	0.00	0.00	0	0	0
Neopeat	0.00	0.00	0.00	0	0	0

Table 19. Influence of different substrates on growth stages of *A. polytricha* -A2

Substrates	Time taken for complete spawn run (days)*	Time taken for pinhead formation (days)*	Time taken for first flush (days)*	Total crop period (days)*
Paddy straw	27.75[d]	34.50[c]	0.00	0.00
Sawdust of softwood trees	36.75[b]	43.50[ab]	0.00	0.00
Sawdust of hard wood trees	33.75[c]	42.75[b]	49.75	84.25
Banana pseudostem	38.25[a]	0.000[d]	0.00	0.00
Sugarcane bagasse	38.00[a]	44.25[ab]	0.00	0.00
Neopeat	38.75[a]	45.00[a]	0.00	0.00
CD (0.05)	0.982	1.55		
S. Em±	0.328	0.52		

*Mean of four replications *Means followed by similar superscripts are not significantly different at 5% level

Table 20. Influence of different substrates on the yield parameters of *A. polytricha* – A2

Substrates	Average weight sporocarp (g)*	Number of sporocarps*	Total yield from three harvests (kg/kg dry weight of substrate)*	Biological efficiency (BE) (%) (Fresh wt. of mushrooms/ dry wt. of substrate used ×100)	Total yield from eight harvests (kg/kg dry weight of substrate)*	BE for 8 harvests
Paddy straw	0.00	0.00	0.00	0	0	0
Sawdust of softwood trees	0.00	0.00	0.00	0	0	0
Sawdust of hard wood trees	3.30	36.50	0.122	12.2	0.486	48.6
Banana pseudostem	0.00	0.00	0.00	0	0	0
Sugarcane bagasse	0.00	0.00	0.00	0	0	0
Neopeat	0.00	0.00	0.00	0	0	0

Table 21. Pests and competitor moulds observed in mushroom beds of *A. polytricha* – A1 and A2

Sl. No.	Substrates	Major pests observed	Major competitor moulds
1.	Paddy straw	Phorid flies *(Megaselia* sp.), Springtails (*Seira* sp.), and *Staphylinus* sp.	*Trichoderma* sp., *Aspergillus, Penicillium* sp. sp., *Coprinus* sp.,
2	Sawdust of softwood trees	*Staphylinus* sp. and Mites	*Aspergillus* sp., *Trichoderma* sp.
3.	Sawdust of hardwood trees	*Megaselia* sp, *Seira* pp ., *Staphylinus* sp. and Mites	*Aspergillus* sp., *Trichoderma* sp.
4.	Banana pseudostem	*Megaselia* sp, *Seira* sp	*Trichoderma* sp.
5.	Sugarcane bagasse	*Megaselia* sp.	*Trichoderma* sp
6.	Neopeat	*Staphylinus* sp.	*Aspergillus* sp

Paddy straw

Sawdust of hardwood trees

Sawdust of softwood trees

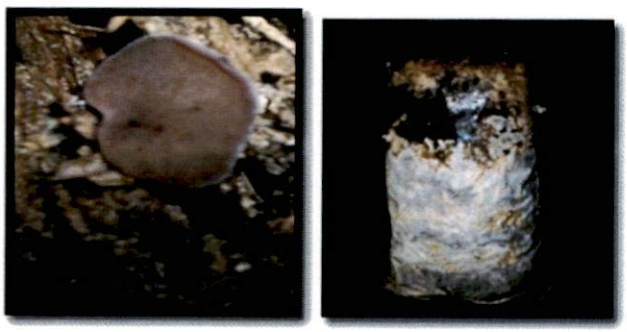
Sugarcane bagasse

Banana pseudostem

Neopeat

Plate 7.1a. Influence of different substrates on mushroom production of *A. polytricha*– A1

Paddy straw Sawdust of hard wood trees Sawdust of Soft wood trees



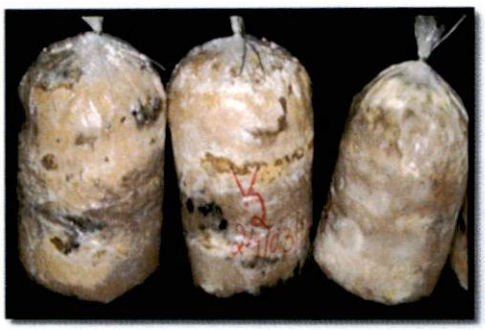
Sugarcane bagasse Neopeat

Plate 7.1b. Appearance of primordia of *A. polytricha*- A1 on different substrates

42 days after bed preparation 45 days after bed preparation 48 days after bed preparation

Plate 7.1c.Different stages of fruiting body formation of *A. polytricha*-A1 on rubber sawdust substrate

Paddy straw



Sawdust of hard wood trees

Sawdust of softwood trees

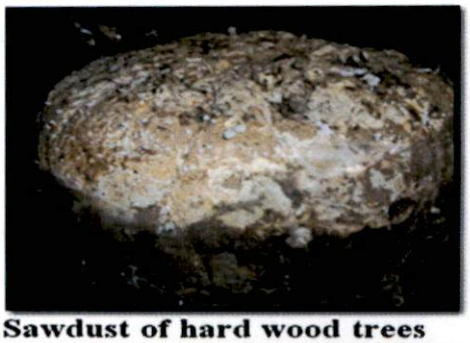
Sugarcane bagasse

Neopeat

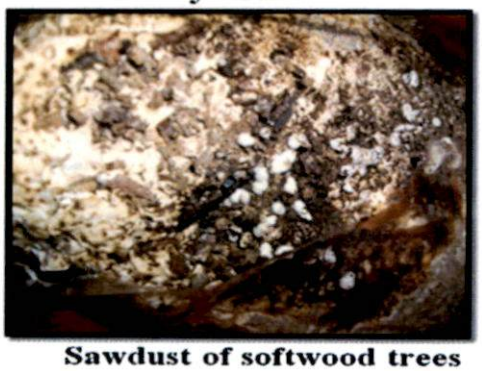
Banana pseudostem

Plate 7.2a . Influence of different substrates on mushroom production of *A. polytricha* – A2

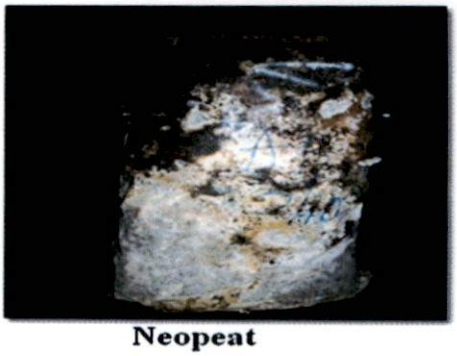
Paddy straw



Sawdust of hard wood trees

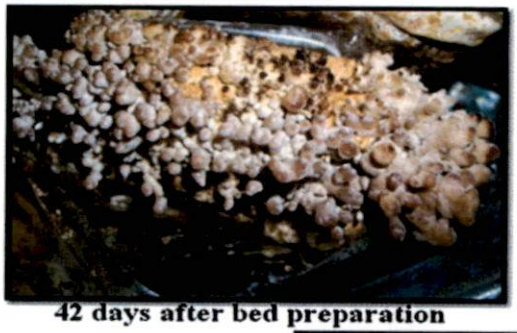


Sawdust of softwood trees



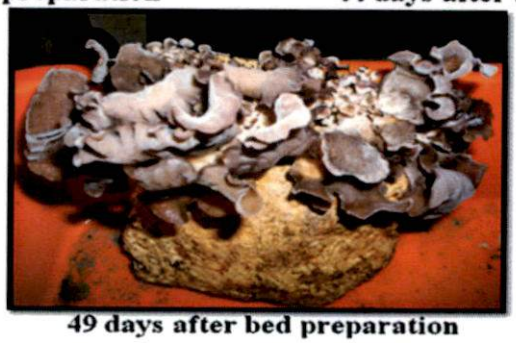
Neopeat

Plate 7.2b. Appearance of primordia of *A. polytricha* (A2) on different substrates



42 days after bed preparation

44 days after bed preparation



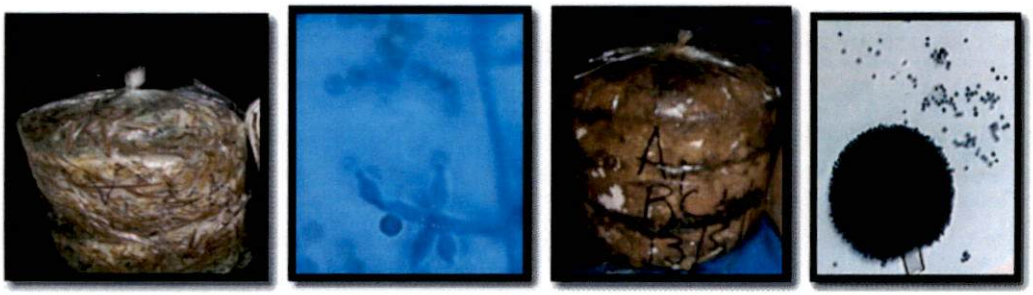
49 days after bed preparation

Plate 7.2c.Different stages of fruiting body formation of *A. polytricha*-A2 on rubber sawdust substrate

Staphylinus sp. *Seira* sp. *Megaselia* sp.

Plate 7.3a. Presence of pests observed in beds of *A. polytricha*– A1 and A2

Penicillium sp. *Rhizopus* spp. *Coprinus* sp.



Trichoderma sp. *Aspergillus*sp.

Plate 7.3b. Contaminant fungi in beds of *A. polytricha*– A1 and A2

4.8. AMENDMENTS

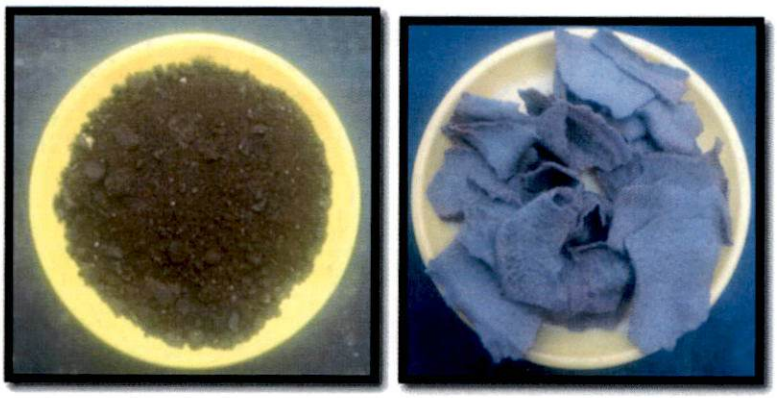
Five different amendments (Plate 8) were evaluated for determining their impact on the substrates, in enhancing fruiting body production of the two isolates A1 and A2 of *A. polytricha*. The criteria for evaluation were similar to those assessed in the evaluation of different substrates in mushroom cultivation technology *i.e.*, the time taken for complete spawn run, time taken for pinhead formation, time taken for first flush, total crop period, average weight of sporocarp, number of sporocarps, total yield from three harvests and biological efficiency.

4.8.1. Evaluation of Amendments for Mushroom Production of *A. polytricha* - A1

The experiment was conducted by raising mushroom beds using the basal substrate sawdust that was evaluated as the best based on the criteria mentioned in (4.7.). The amendements tested with this substrate were wheat bran, rice bran, cotton seed hull, neem cake and groundnut cake @ two and four per cent concentrations (20 g and 40 g amendments in 1000 g of rubber sawdust).

Time taken for the isolate A1 of *A. polytricha* to complete spawn run on amendment of the basal substrate with two per cent wheat bran was shortest taking 32.33 days which was on par with four per cent wheat bran (32.66 days) and control (32.66 days). Time for completion of spawn run on amendment with two per cent groundnut cake was 33.66 days followed by amendment with four per cent groundnut cake (34.66 days) and two per cent rice bran (35.66 days) which did not differ significantly (Table 22). Four per cent rice bran took 37.0 days for spawn run and was statistically on par with the other amendments *viz.*, two per cent cotton seed hull (37.0 days), four per cent cotton seed hull (37.66 days), two per cent neem cake (37.66 days) and four per cent neem cake (38.0 days).

Wheat bran Rice bran Cotton seed hull



Neem cake Groundnut cake

Plate 8. Amendments used for the production of *A. polytricha*- A1 and A2.

Time taken for pinhead formation by amendment with two per cent wheat bran was 37.66 days which was on par with time taken by amendment with four per cent wheat bran (38.33 days) followed by control (38.66 days), two per cent groundnut cake (40.33 days), four per cent groundnut cake (41.66 days), two per cent rice bran (42.33 days), four per cent rice bran (43.33 days), two per cent cotton seed hull (44.0 days) and four per cent cotton seed hull (44.66 days). Results of all the above mentioned treatments were on par with each other and significantly superior to the amendments with two and four per cent neem cake in which the maximum time of 45.33 days and 45.66 days was taken for completion of spawn run which did not differ significantly.

Fourty four days after bed preparation, two per cent wheat bran amended beds started to produce first flush of A1 and was on par with the time taken by amendment with four per cent wheat bran and control (45.33 days) followed by two per cent groundnut cake (47.33 days), four per cent groundnut cake (47.66 days) and these two treatments did no differ significantly. Effects of all the above treatments were significantly superior to the remaining treatments in which two per cent rice bran took 50.33 days to produce first flush and was on par with four per cent rice bran (51.33 days) followed by two per cent cotton seed hull (52.33 days), four per cent cotton seed hull (53.00 days), two and four per cent neem cake (53.33 days) and they also showed on par results.

Total crop period was the highest in amendment with two per cent rice bran (95.66 days) which was on par with amendment with two per cent wheat bran (94.66 days) followed by amendment with four per cent rice bran (94.33 days) and four per cent wheat bran (92.66 days) and the above treatments were significantly superior to the remaining treatments. Two per cent groundnut cake had a crop period of 89.66 days followed by control (88.33 days), two per cent cotton seed hull (88.0 days), four per cent cotton seed hull (87.0 days), four per cent groundnut cake (87.0 days) and two per cent neem cake (86.66 days) and these treatments did not differ significantly. The shortest cropping period was

recorded in beds amended with two per cent (86.66 days) and four per cent neem cake (86.33 days).

The average weight of sporocarp was the highest in amendment with two per cent rice bran (4.26 g) which was on par with average weight obtained by amendment with four per cent rice bran (4.13 g) and also they were found to be significantly different from all the other amendments tested. This was followed by amendment with four per cent wheat bran (3.76 g), two per cent wheat bran (3.70 g), control (3.63 g) and two per cent groundnut cake (3.53 g). Four per cent groundnut cake amended beds produced mushrooms with average weight of 3.43 g followed by two per cent cotton seed hull (2.40 g), four per cent neem cake (2.36 g), two per cent neem cake (2.33 g) and the least weight (2.30 g) was observed in four per cent cotton seed hull amended beds and these treatments did not differ significantly from each other.

The number of sporocarps was the highest in beds amended with two and four per cent rice bran (42.0 and 41.66) and they were on par with each other and also showed significant results with other treatments tested. This was followed by amendment with two per cent wheat bran (39.66), control without any amendment (38.66) and two per cent groundnut cake (38.33) and these two treatments did not differ significantly. Four per cent wheat bran amended beds recorded 38.0 number of sporocarps followed by four per cent groundnut cake (37.66), two per cent cotton seed hull (32.66), four per cent cotton seed hull (30), two per cent neem cake (22.66) and four per cent neem cake (17.33) amended beds.

The total yield from three harvests as well as biological efficiency were the highest in amendment with two per cent rice bran (0.186 kg and 18.6 % respectively) which was significantly superior to all the other amendments (Table 23). This was followed by amendment with four per cent rice bran (0.175 kg and 17.5 % respectively) followed by four per cent wheat bran (0.145 kg and 14.5 %), two per cent wheat bran and control (0.143 kg and 14.3 % respectively) and these three treatments did not show significant difference. These results were followed

by two per cent groundnut cake (0.136 kg and 13.6 % respectively) which was on par with four per cent groundnut cake (0.133 kg and 13.3 % respectively). The results were again followed by two per cent cotton seed hull (0.080 kg and 8.0 % respectively), four per cent cotton seed hull (0.063 kg and 6.3 % respectively), two per cent neem cake (0.056 kg and 5.6 % respectively) and four per cent neem cake (0.042 kg and 4.2 % respectively).

The total yield from eight harvests and biological efficiency indicated that two per cent rice bran was found to be significantly superior to all the other treatments tested and the maximum yield obtained was 0.563 kg and 56.3 % biological efficiency (Plate 8.1a). The next best results were recorded by four per cent rice bran (0.533 kg and 53.3 % respectively), control (0.517 kg and 51.7 % respectively), two per cent wheat bran (0.487 kg and 48.7 % respectively), two per cent groundnut cake (0.473 kg and 47.3 % respectively), four per cent wheat bran (0.363 kg and 36.3 %), two per cent cotton seed hull, four per cent groundnut cake (0.313 kg and 31.3 % respectively), four per cent cotton seed hull (0.287 kg and 28.7 %), two per cent neem cake (0.277 kg and 27.7 % respectively) and the least yield was obtained from four per cent neem cake (0.263 kg and 26.3 % respectively). Except for amendment with two per cent cotton seed hull and four per cent groundnut cake (0.313 kg and 31.3 %), all the other amendments showed significant differences.

Major pests observed were mites and phorid flies. Major contaminants observed were *Trichoderma* spp., *Aspergillus* spp., *Penicillium* spp. and *Coprinus* spp.

4.8.2. Evaluation of Amendments for Mushroom Production of *A. polytricha* –A2

Spawn run in non-amended beds took a minimum of 33.33 days and was on par with two per cent wheat bran (34.00 days). This was followed by amendment with four per cent wheat bran (35.00 days) followed by two per cent groundnut cake amendment (35.66 days), four per cent groundnut cake amendment (36.33

Table 22. Influence of different amendments on growth stages of *A. polytricha* A1

Amendments	Time taken for complete spawn run (days)*	Time taken for pinhead formation (days)*	Time taken for first flush (days)*	Total crop period (days)*
Wheat bran 2%	32.33^{e}	37.66^{h}	44.33^{e}	94.66^{a}
Wheat bran 4%	32.66^{de}	38.33^{gh}	45.33^{e}	92.66^{ab}
Rice bran 2%	35.66^{b}	42.33^{e}	50.33^{c}	95.66^{a}
Rice bran 4%	37.00^{a}	43.33^{d}	51.33^{bc}	94.33^{a}
Cotton seed hull 2%	37.00^{a}	44.00^{cd}	52.33^{ab}	88.00^{c}
Cotton seed hull 4%	37.66^{a}	44.66^{bc}	53.00^{a}	87.00^{c}
Neem cake 2 %	37.66^{a}	45.33^{ab}	53.33^{a}	86.66^{c}
Neem cake 4 %	38.00^{a}	45.66^{a}	53.33^{a}	86.33^{c}
Groundnut cake 2 %	33.66^{cd}	40.33^{f}	47.33^{d}	89.66^{bc}
Groundnut cake	34.66^{bc}	41.66^{e}	47.66^{d}	87.00^{c}

4%				
Control	32.66^{de}	38.66^{g}	45.33^{e}	88.33^{c}
CD (0.05%)	1.110	0.938	1.187	3.658
S. Em±	0.376	0.318	0.402	1.239

*Mean of three replications

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

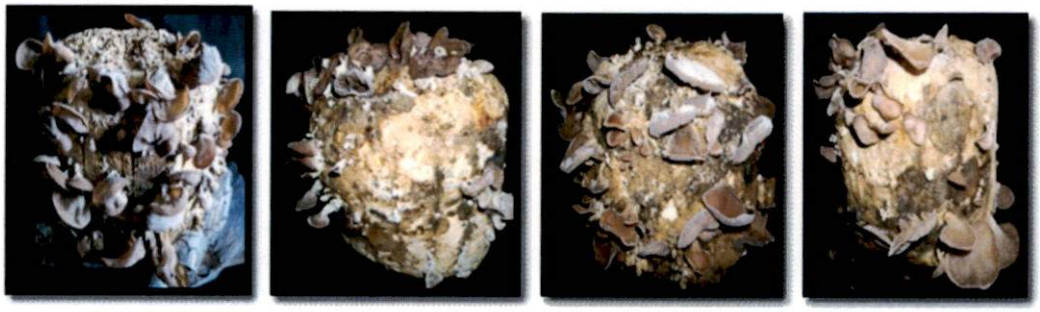
Table 23. Influence of different amendments on the yield parameters of *A. polytricha*- A1

Amendments	Average weight of sporocarp (g)*	Number of sporocarps*	Total yield from three harvests (kg)*	Biological efficiency (%) for three harvests	Total yield from eight harvests (kg)*	Biological efficiency (%) for 8 harvests
Wheat bran 2%	3.70^{b}	39.66^{b}	0.14^{c}	14.3	0.487^{d}	48.7
Wheat bran 4%	3.76^{b}	38.00^{bc}	0.14^{c}	14.5	0.363^{f}	36.3
Rice bran 2%	4.26^{a}	42.00^{a}	0.18^{a}	18.6	0.563^{a}	56.3
Rice bran 4%	4.13^{a}	41.66^{a}	0.17^{b}	17.5	0.533^{b}	53.3
Cotton seed hull 2%	2.40^{d}	32.66^{d}	0.08^{e}	8.0	0.313^{g}	31.3
Cotton seed hull 4%	2.30^{d}	30.00^{e}	0.06^{f}	6.3	0.287^{h}	28.7
Neem cake 2 %	2.33^{d}	22.66^{f}	0.05^{g}	5.6	0.277^{i}	27.7

Neem cake 4 %	2.36^{d}	17.33^{g}	0.04^{h}	4.2	0.263^{j}	26.3
Groundnut cake 2%	3.53^{bc}	38.33^{bc}	0.13^{d}	13.6	0.473^{e}	47.3
Groundnut cake 4%	3.43^{c}	37.66^{c}	0.13^{d}	13.3	0.313^{g}	31.3
Control	3.63^{bc}	38.66^{bc}	0.14^{c}	14.2	0.517^{c}	51.7
CD (0.05%)	0.255	1.968	0.008		0.010	
S. Em±	0.086	0.667	0.003		0.003	

*Mean of three replications

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



Rice bran 2% Rice bran 4% Wheat bran 2% Wheat bran 4%

Cotton seed hull 2% Cotton seed hull 4% Neem cake 2 % Neem cake 4 %

Groundnut cake 2% Groundnut cake 4% Control

Plate 8.1a. Fruiting bodies of *A. polytricha* (A1) on rubber sawdust substrate with different amendments

days), two per cent rice bran amendment (36.66 days), four per cent rice bran amendment (37.33 days), two per cent cotton seed hull amendment (37.66 days), four per cent cotton seed hull amendment (38.33 days), two per cent neem cake amendment (38.66 days) and four per cent neem cake amendment (39.00 days) and these treatments did not differ significantly from each other (Table 24).

In case of time taken for pinhead formation also non-amended beds were found to be significantly superior over all the other treatments by taking a minimum of 39.33 days followed by two per cent wheat bran (40.66 days), four per cent wheat bran, two per cent groundnut cake (41.66 days), four per cent groundnut cake (42.0 days), two per cent rice bran (42.33 days), four per cent rice bran (43.33 days), two per cent cotton seed hull (43.66 days) , four per cent cotton seed hull (44.0 days), two per cent neem cake (44.66 days) and four per cent neem cake (45.66 days) which took maximum days to produce pinheads.

Non-amended beds and beds amended with two per cent wheat bran took 48.33 days to produce first flush of mushroom and they were on par with four per cent wheat bran amendment (48.66 days). Groundnut cake amendment at two and four per cent took 51.0 and 51.66 days respectively followed by four per cent rice bran amendment (53.33 days), two per cent cotton seed hull amendment (54.0 days), four per cent cotton seed hull amendment (54.33 days), two per cent neem cake amendment (54.66 days) and four per cent neem cake which took maximum of 55.33 days for producing first flush.

The total crop period was the highest in two per cent rice bran amendment (94.66 days) which was on par with four per cent rice bran and two per cent wheat bran amendment (92.66 days) followed by four per cent wheat bran amendment (90.66 days) and two per cent groundnut cake amendment (88.33 days). Non-amended beds recorded a cropping period of 88.0 days and two per cent cotton seed hull, four percent groundnut cake, four per cent neem cake and two per cent neem cake amended beds recorded a cropping period of 86.66 days, 86.0 days, 85.33 days and 85.0 days respectively. Four per cent cotton seed hull amended

beds recorded the shortest cropping period of 84.66 days and all these treatments did not differ significantly.

The average weight of sporocarp was the highest in beds amended with two and four per cent rice bran (3.93 g and 3.86 g respectively). This was followed by average weight of sporocarp obtained from beds amended with two per cent wheat bran, four per cent wheat bran and non-amended beds (3.36 g) which were equal and on par with those obtained from beds amended with two per cent groundnut cake (3.30 g) and four per cent groundnut cake (3.13 g). These results were followed by four per cent neem cake amendment (2.40 g) and two per cent neem cake (2.26 g) which did not differ significantly. Average weight was observed minimum in two and four per cent cotton seed hull amendment (1.93 g and 1.90 g).

Rice bran amendment at two per cent concentration produced the highest number of sporocarps (40.33) which was on par with the production with four per cent rice bran amendment (Table 25). This was followed by amendment with two per cent wheat bran (38.0), non-amended control, two per cent groundnut cake amendment, four per cent wheat bran amendment, four per cent groundnut cake amendment, two per cent cotton seed hull amendment, four per cent cotton seed hull amendment and two per cent neem cake amendment that produced 37.0, 36.66, 36.33, 36.0, 30.0, 27.33 and 20.0 sporocarps, respectively. The least number of sporocarps was obtained in beds amended with four per cent neem cake (15.0).

In *A. polytricha* -A2 also, the total yield from three harvests and biological efficiency was the highest in substrate amended with two per cent rice bran (0.159 kg and 15.9 %) which was on par with four per cent rice bran (0.155 kg and 15.5 %). The next best treatment was two per cent wheat bran (0.136 kg and 13.6 %) followed by control, four per cent wheat bran and two per cent groundnut cake, four per cent groundnut cake, two per cent cotton seed hull, four per cent cotton seed hull, two per cent neem cake respectively of 0.126 kg and 12.6 %, 0.123 kg

and 12.3 %, 0.113 kg and 11.3 %, 0.056 kg and 5.6 %, 0.054 kg and 5.4 %, 0.046 kg and 4.6 % and the least yield was obtained from beds amended with four per cent neem cake (0.036 kg and 3.6 %).

Up to eight harvests were procured from the amended mushroom beds and the highest yield and biological efficiency were recorded from beds amended with two and four per cent rice bran (0.52 kg and 52 %) which was significantly superior to all other treatments (Plate 8.1b). This was followed by non-amended control (0.47 kg and 47 %), two per cent groundnut cake and two percent wheat bran (0.41 kg and 41 %) and four per cent wheat bran amended beds (0.30 kg and 30 %). Similar results were obtained from beds amended with two per cent cotton seed hull, two per cent neem cake and four per cent cotton seed hull which recorded total yield and biological efficiency respectively of 0.28 kg and 28 %, 0.25 kg and 25 %, 0.24 kg and 24 % and the least performance was given by beds amended with four per cent neem cake (0.22 kg and 22 %).

Major pests observed were mites and phorid flies. Major contaminants observed were *Trichoderma* spp., *Aspergillus* spp., *Penicillium* sp. and *Coprinus* sp. (Table 26; Plate 8.2).

4.9. PROXIMATE CONSTITUENTS

Proximate constituents of the two isolates of jelly mushrooms *viz.*, A1 and A2 were evaluated by analysing the percentage of moisture content, carbohydrate, protein, fat, fibre, ash, total antioxidants, β-carotene, polyphenols followed by estimation of their energy value, the results of which are depicted in Table 27.

4.9.1. Per Cent Moisture Content

The moisture content of A1 was found to be (90.12 %) on fresh weight basis and was higher than that of A2 (89.91 %).

Table 24. Influence of different amendments on growth stages of *A. polytricha* -A2

Amendments	Time taken for complete spawn run (days)*	Time taken for pinhead formation (days)*	Time taken for first flush (days)*	Total crop period (days)*
Wheat bran 2%	34.00^{hi}	40.66^{f}	48.33^{e}	92.66^{ab}
Wheat bran 4%	35.00^{gh}	41.66^{ef}	48.66^{e}	90.66^{bc}
Rice bran 2%	36.66^{def}	42.33^{de}	51.66^{d}	94.66^{a}
Rice bran 4%	37.33^{cde}	43.33^{cd}	53.33^{c}	92.66^{ab}
Cotton seed hull 2%	37.66^{bcd}	43.66^{bc}	54.00^{bc}	86.66^{de}
Cotton seed hull 4%	38.33^{abc}	44.00^{bc}	54.33^{abc}	84.66^{e}
Neem cake 2 %	38.66^{ab}	44.66^{ab}	54.66^{ab}	85.00^{de}
Neem cake 4 %	39.00^{a}	45.66^{a}	55.33^{a}	85.33^{de}
Groundnut cake 2 %	35.66^{fg}	41.66^{ef}	51.00^{d}	88.33^{cd}

Groundnut cake 4%	36.33^{ef}	42.00^{e}	51.66^{d}	86.00^{de}
Control	33.33^{f}	39.33^{g}	48.33^{e}	88.00^{cde}
CD (0.05%)	1.223	1.256	1.028	3.434
S. Em±	0.414	0.426	0.348	1.163

*Mean of three replications

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

Table 25. Influence of different amendments on the yield parameters of *A. polytricha* –A2

Amendments	Average weight of sporocarp (g)*	Number of sporocarps*	Total yield from three harvests (kg)*	Biological efficiency (%) for three harvests	Total yield from eight harvests (kg)*	Biological efficiency for 8 harvests (%)
Wheat bran 2%	3.36^{b}	38.00^{b}	0.13^{b}	13.6	0.41^{c}	41
Wheat bran 4%	3.36^{b}	36.33^{bc}	0.12^{c}	12.3	0.30^{d}	30
Rice bran 2%	3.93^{a}	40.33^{a}	0.15^{a}	15.9	0.52^{a}	52
Rice bran 4%	3.86^{a}	40.00^{a}	0.15^{a}	15.5	0.52^{a}	52
Cotton seed hull 2%	1.93^{d}	30.00^{d}	0.06^{e}	5.6	0.28^{e}	28
Cotton seed hull 4%	1.90^{d}	27.00^{e}	0.05^{e}	5.4	0.24^{f}	24
Neem cake 2 %	2.26^{c}	20.00^{f}	0.04^{g}	4.6	0.25^{f}	25

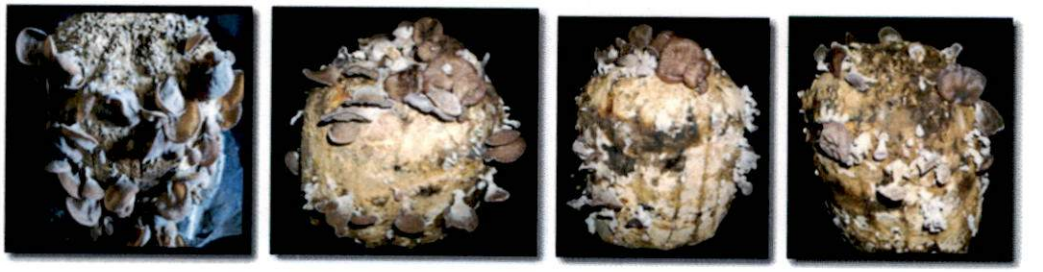
Neem cake 4 %	2.40^{c}	15.00^{g}	0.03^{h}	3.6	0.22^{g}	22
Groundnut cake 2 %	3.30^{b}	36.66^{bc}	0.12^{c}	12.3	0.41^{c}	41
Groundnut cake 4%	3.13^{b}	36.00^{c}	0.11^{d}	11.3	0.28^{e}	28
Control	3.36^{b}	37.00^{bc}	0.12^{c}	12.6	0.47^{b}	47
CD (0.05%)	0.31	1.92	0.010		0.011	
S. Em±	0.10	0.65	0.003		0.004	

*Mean of three replications

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

Table 26. Pests and competitor moulds observed in mushroom beds of *A. polytricha* – A1 and A2

Amendments	Pests	Competitor moulds
Wheat bran 2%	Mites and Phorid flies	*Trichoderma* sp., *Aspergillus* sp. and *Coprinus* sp.
Wheat bran 4%	Mites and Phorid flies	*Trichoderma* sp., *Aspergillus* sp. and *Coprinus* sp.
Rice bran 2%	Mites and Phorid flies	*Trichoderma* sp., *Aspergillus* sp. and *Coprinus* sp.
Rice bran 4%	Mites and Phorid flies	*Trichoderma* sp., *Aspergillus* sp. and *Coprinus* sp.
Cotton seed hull 2%	Phorid flies	*Trichoderma* sp., *Aspergillus* sp., *Coprinus* sp. and *Penicillium* sp.
Cotton seed hull 4%	Phorid flies	*Trichoderma* sp.
Neem cake 2 %	Phorid flies	*Trichoderma* sp.
Neem cake 4 %	Phorid flies	*Trichoderma* sp.
Groundnut cake 2 %	Mites and , Phorid flies	*Trichoderma* sp.
Groundnut cake 4%	Mites and , Phorid flies	*Trichoderma* sp.
Control	Phorid flies	*Trichoderma* sp.

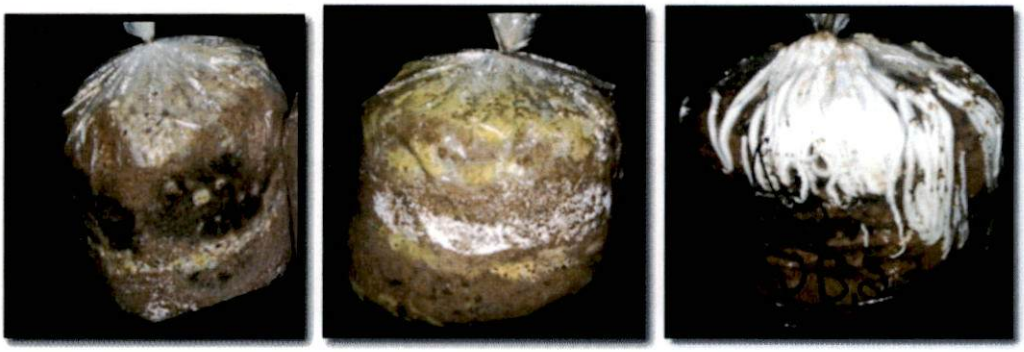


Rice bran 2% Rice bran 4% Wheat bran 2% Wheat bran 4%

Cotton seed hull 2% Cotton seed hull 4% Neem cake 2 % Neem cake 4 %

Groundnut cake 2 % Groundnut cake 4% Control

Plate 8.1b.Fruiting bodies of *A. polytricha* (A2) on rubber sawdust substrate with different amendments



Aspergillus sp. *Penicillium* sp. *Coprinus* sp.

Trichoderma sp. *Staphylinus* sp.

Plate 8.2.Pests and competitor moulds observed in beds of *A. polytricha*– A1 and A2

4.9.2. Carbohydrate Content

Estimation of carbohydrates was done using anthrone method. Lower amount of carbohydrates was estimated in A1 (47.1 %) compared to A2 which recorded 48.8 % carbohydrate on dry weight basis.

4.9.3. Protein Content

Microkjeldhal method was used to find out the protein content of dried mushroom. A1 recorded protein content of 18.06 % and A2 recorded 20.75 % on dry weight basis.

4.9.4. Fat Content

Fat content in mushroom powder was estimated for which Soxhlet extraction apparatus was used. The fat content estimated for A1 was (0.76 %) on dry weight basis and that of A2 was (0.56 %).

4.9.5. Fibre Content

Studies conducted on analysis of crude fibre content revealed that A1 had more fibre (17.69 %) than A2 (15.49 %) on dry weight basis.

4.9.6. Ash Content

A1 recorded an ash content of 5.5 % on dry weight basis and A2, 5.3%.

4.9.7. Total Antioxidants

Total antioxidants were the highest in A1 (116 µg) whereas for A2 it was 74 µg.

4.9.8. β-carotene Content

β-carotene content was found to be the highest in A1 (0.178 µg) and 0.150 µg in A2.

4.9.10. Polyphenol Content

Analysis of polyphenol content was done by Folin-ciocalteu method and the results showed that A1 had a higher amount of polyphenols (9.53 µg) than A2 (6.89 µg).

4.9.11. Energy Value

The energy value was calculated based on the conversion factors of carbohydrate, protein and fat present in the sample. Energy value was 251.49 Kcal in A1 and 264 Kcal in A2.

4.10. SENSORY EVALUATION

Seven different recipes were prepared separately using A1 and A2 isolates in order to assess their cooking quality and sensory evaluation for which nine point Hedonic rating scale was used, selecting ten panel members for evaluation. The recipes prepared for the evaluation were pepper masala, pizza, pakoda, mushroom tomato sauce, soup, mushroom vegetable mix curry and mushroom bajji (Plate 9.1).

Among the different recipes tried, mushroom tomato sauce had maximum score for appearance (9.0), colour (8.9), flavor (8.9), texture (8.8), taste (9.0) aroma (8.8) and overall acceptability (8.9 and 9.0 respectively) for both A1 (Table 28) and A2 (Table 29). This was followed by overall acceptability for pizza (8.0), pakoda (7.9), soup and mushroom veg curry (7.8) whereas pepper masala had scores of 7.6, 7.2, 7.4, 7.7, 7.6, 7.3 and 7.5 respectively for each of the above mentioned sensory criteria. Pepper masala and bajji had an overall acceptability value of 7.5.

4.10.1. Keeping Quality

Both the samples could be kept fresh for 24 h under ordinary conditions and the mushrooms started to wither thereafter. Mushrooms kept under

Table 27. Proximate constituents in *A. polytricha* (A1and A2)

Sl.No.	Proximate constituents	Percentage (%)	
		A1	A2
1.	Moisture #	90.12	89.91
2.	Carbohydrate *	47.1	48.8
3.	Protein *	18.06	20.75
4.	Fat *	0.76	0.56
5.	Fibre*	17.69	15.49
6.	Ash *	5.5	5.3
7.	Total antioxidants*	116 µg	74 µg
8.	β-carotine*	0.178 µg	0.150 µg
9.	Polyphenols*	9.53 µg	6.89 µg
10.	Energy *	251.49 Kcal	264 Kcal

#- Fresh weight basis

*-Dry weight basis

Table 28. Sensory evaluation for recipes of *A. polytricha* -A1

Products	Appearance*	Colour*	Flavor*	Texture*	Taste*	Aroma*	Overall acceptability*
Pepper masala	7.6	7.2	7.4	7.7	7.6	7.3	7.5
Pizza	8.1	8.2	8.7	8.2	8.0	8.6	8.0
Pakoda	8.5	8.3	8.2	8.1	8.1	8.1	7.9
Mushroom tomato sauce	9.0	8.9	8.9	8.8	9.0	8.8	8.9
Soup	7.6	7.7	7.6	7.7	7.9	7.5	7.8
Mushroom veg curry	7.7	7.6	7.6	7.6	7.8	7.5	7.8
Bajji	7.1	7.2	7.1	7.2	7.3	7.2	7.5

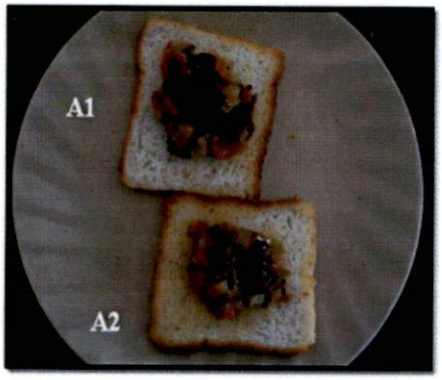
*Average of 10 Panel members

Table 29. Sensory evaluation for recipes of *A. polytricha* –A2

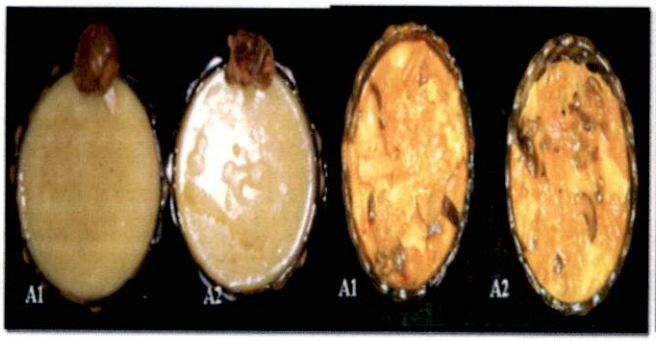
Products	Appearance*	Colour*	Flavor*	Texture*	Taste*	Aroma*	Overall acceptability*
Pepper masala	7.6	7.2	7.4	7.7	7.6	7.3	7.5
Pizza	8.1	8.2	8.7	8.2	8.0	8.6	8.0
Pakoda	8.5	8.3	8.2	8.1	8.1	8.1	7.9
Mushroom tomato sauce	9.0	8.9	8.9	8.8	9.0	8.8	9.0
Soup	7.6	7.7	7.6	7.7	7.9	7.5	7.8
Mushroom veg curry	7.7	7.6	7.6	7.6	7.8	7.5	7.8
Bajji	7.1	7.2	7.1	7.2	7.3	7.2	7.5

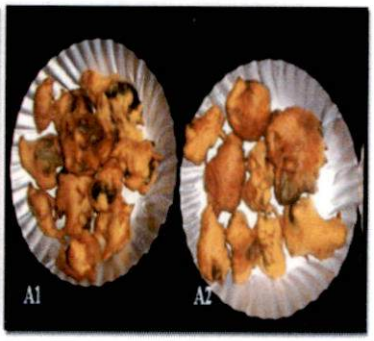
*Average of 10 Panel members

Pepper masala Pizza



Pakoda Mushroom tomato sauce





Soup Mushroom veg curry Bajji

Plate 9.1. Mushroom recipes prepared with *A.polytricha* (A1 and A2)

refrigerated conditions (4 ^{0}C) remained fresh, turned brown, possessed no stench smell and had a watery appearance upto 72 h, and they were also found ideal for cooking (Table 30). After five days, mushrooms became soft, and after ten days the freshness reduced, a colour and an unpleasant odour developed and had a sticky nature with no watery appearance. After 15 days of preservation, they turned creamy brown, possessed a stench, and had more watery and sticky appearance. After 20 days, they were found unfit and had a prominent stench. After 30 days of refrigeration, mushrooms possessed watery texture, putrefied smell, underwent complete tissue disintegration and liquefication (Plate 9.2 and 9.3).

Per cent reduction in fresh weight for A1 and A2 was 1.6 and 2.4, after keeping for 24 h under refrigerated conditions, and after three days it showed 4.8 and 5.6 per cent reduction. Hence the results indicated that mushrooms could be preserved under refrigerated conditions upto three days without any quality changes.

4.11. OUTDOOR CULTIVATION

Mushrooms grow well under in indoor conditions. Under proper conditions, they can be grown outdoor too. In this regard both A1 and A2 were cultivated both under indoor and outdoor conditions to assess their comparative performance under the two different conditions (Plate 10). The weather parameters during the cultivation period are fiven in Appendix VI.

Outdoor cultivation took 34.1 and 34.80 days whereas indoor cultivation took 33.1 and 34.40 days for complete spawn run and there was no significant difference observed between these two conditions (Table 31 and 32). Similarly, for pinhead formation, minimum time was taken by indoor conditions (42.6 and 43.10 days) followed by outdoor cultivation method (43.2 and 44.20 days). A significant difference was observed between indoor and outdoor cultivation for

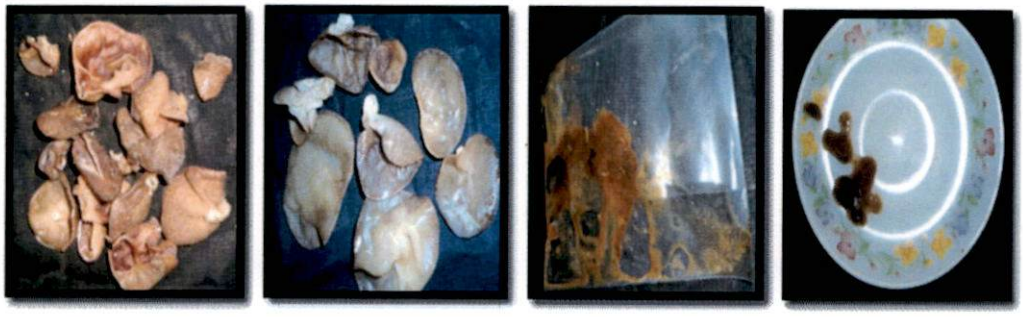
the time taken for first flush in A1. Indoor conditions took (50.6 and 51.5 days) to produce first flush whereas outdoor took (52.5 and 52.0 days). Indoor cultivation

Table 30. Keeping quality of *A. polytricha* – (A1 and A2) in refrigerated condition (4^0 C)

Sl. No.	Time after harvest	Observation	Per cent reduction in fresh weight of A1	Per cent reduction in fresh weight of A2
1	One day	Fresh, brown, no smell, no watery appearance and good for cooking	1.6	2.4
2	Two days	Fresh, brown, no smell, no watery appearance and good for cooking	3.2	3.6
3	Three days	Fresh, brown, no smell, no watery appearance and good for cooking	4.8	5.6
4	Five days	Fresh, brown, no smell, no watery appearance and became soft	7.6	8.0
5	Ten days	No freshness, brown colour, off smell, slightly sticky and no watery appearance	12.0	12.8
6	Fifteen days	Creamy brown colour, off smell, no watery texture, more sticky like appearence	15.2	16.0
7	Twenty days	Creamy brown colour, no watery texture, prominent bad smell,	18.4	19.6
8	Thirty days	Very light brown colour, watery texture, putrefied smell and completely liquified	24	24.8

Fresh mushrooms Discolouration Tissue disintegration Liquification

Plate 9.2. Observations on keeping quality of *A. polytricha* (A1) under refrigerated condition



Fresh mushrooms Discolouration Tissue disintegration Liquification

Plate 9.3. Observations on keeping quality of *A. polytricha* (A2) under refrigerated condition

conditions recorded a maximum total cropping period of (88.9 and 88.3 days) followed by outdoor conditions (80.7 and 81.3 days) and they were found to be significantly different. Average weight of sporocarp was also the highest in indoor cultivation (4.2 and 3.8 g) and outdoor cultivation recorded 4.14 and 3.5 g. A significant difference in the number of sporocarps was observed with both the cultivation conditions for A1. Indoor cultivation condition produced 34.0 and 34.4 numbers of sporocarps, while in outdoor cultivation conditions only 31.2 and 34.1 sporocarps were produced (Table 32 and 34). Total yield from three harvests and biological efficiency were also the highest in indoor cultivation conditions (0.147 kg and 14.7 % for A1 and for A2 and 0.136 kg and 13.6 %). In outdoor cultivation conditions A1 yielded 0.138 kg mushroom with biological efficiency of 13.8 % and 0.123 kg mushroom with 12.3 % biological efficiency for A2 and significant difference was observed between the two isolates.

Major pests observed were phorid flies, spring tails and mites. *Trichoderma* sp., *Aspergillus* sp., *Penicillium* sp. and *Coprinus* sp. were the major contaminants (Table 35).

4.12. YIELD PERFORMANCE OF OYSTER AND MILKY MUSHROOM

Pleurotus florida and *Calocybe gambosa* are the widely cultivated mushroom species in Kerala state. Hence, they were selected for comparative studies with A1 and A2 of *A. polytricha* on paddy grains for spawn production as it was proved to be the best substrate in spawn studies while cultivation trials for mushroom production were conducted on rubber sawdust amended with two per cent rice bran.

4.12.1. Spawn Production

Spawn production studies indicated that *P. florida* completed the spawn run in 14.75 days with thick, white cottony growth of mycelia and was found to be significantly superior over other mushrooms (Table 36). This was followed by *A. polytricha* -A1 (17.0 days) with thick growth of mycelia and was found to be on

Table 31. Influence of outdoor and indoor conditions for the growth stages of *A. polytricha*- A1

Cultivation conditions	Time taken for complete spawn run (days)*	Time taken for pinhead formation (days)*	Time taken for first flush (days)*	Total crop period (days)*
Outdoor	34.1	43.2	52.5	80.7
Indoor	33.1	42.6	50.6	88.9
t (Value)	1.68	1.16	8.14**	11.34**

Table 32. Influence of outdoor and indoor conditions on the yield parameters of *A. polytricha*-A1

Cultivation conditions	Average weight sporocarp (g)*	Number of sporocarps*	Total yield from three harvests (kg/kg dry weight of substrate)*	Biological efficiency (%)(Fresh wt. of mushrooms/ dry wt. of substrate used ×100)
Outdoor	4.1	31.2	0.138	13.8
Indoor	4.2	34.0	0.147	14.7
t (Value)	0.86	6.0**	5.226**	

*Mean of ten observations

** Treatments are significantly different at 5% level

Table 33. Influence of outdoor and indoor conditions for the growth stages of *A. polytricha* – A2

Cultivation conditions	Time taken for complete spawn run (days)*	Time taken for pinhead formation (days)*	Time taken for first flush (days)*	Total crop period (days)*
Outdoor	34.80	44.20	52.0	81.3
Indoor	34.40	43.10	51.5	88.3
t (Value)	1.34	2.95**	1.24	7.81**

Table 34. Influence of outdoor and indoor cultivation conditions on the yield parameters of *A. polytricha* – A2

Cultivation conditions	Average weight sporocarp (g)*	Number of sporocarps*	Total yield from three harvests (kg/kg dry weight of substrate)*	Biological efficiency (%)(Fresh wt. of mushrooms/ dry wt. of substrate used ×100)
Outdoor	3.5	34.1	0.123	12.3
Indoor	3.8	34.4	0.136	13.6
t (Value)	5.77**	0.64	5.417**	

*Mean of ten observations

** Treatments are significantly different at 5% level

Table 35. Pests and competitor moulds observed under outdoor and indoor cultivation *A. polytricha* – A1 and A2

Sl. No.	Cultivation conditions	Major pests observed	Major competitor moulds
1.	Outdoor	Phorid flies *(Megaselia* sp.), Springtails (*Seira* sp.) and *Staphylinus* sp.	*Trichoderma* sp., *Aspergillus, Penicillium* sp. sp. and *Coprinus* sp.
2	Indoor	*Staphylinus* sp and Mites	*Aspergillus* sp., *Trichoderma* sp. and *Coprinus* sp.

Table 36. Comparative performance of Jelly mushrooms (*A. polytricha* -A1 and A2) with Milky and Oyster mushrooms for spawn production in paddy grains

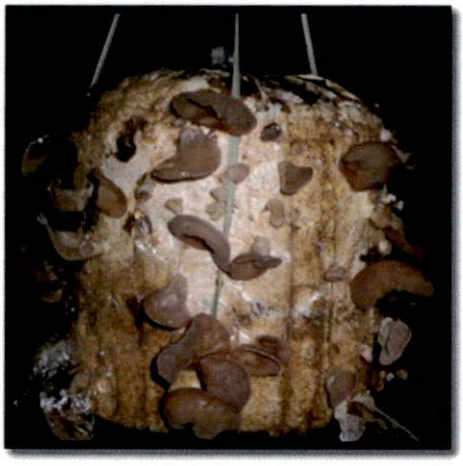
Treatments	Time taken for spawn run (days)*	Nature of mycelial growth
Jelly mushroom-A1 (*Auricularia polytricha*)	17.00[b]	Thick growth
Jelly mushroom-A2 (*Auricularia polytricha*)	17.25[b]	Thick growth
Milky mushroom (*Calocybe jambosa* (Fr.) Donk.)	21.0[a]	Thick mat like white growth
Oyster mushroom (*Pleurotus florida* (Mont.)Singer.)	14.75[c]	Uniformly thick and cottony white growth
CD (0.05)	1.23	
S. Em±	0.39	

* Mean of four replications

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

Indoor method- A1

Outdoor method- A1



Indoor method- A2

Outdoor method- A2

Plate 10. Fruiting bodies of *A. polytricha* (A1 and A2) under outdoor and indoor cultivation

par with *A. polytricha* - A2 (17.25 days) which also attained thick growth of mycelia. *C. gambosa* took maximum of (21.0 days) for completing mycelial growth with thick mat like white mycelia.

4.12.2. Mushroom Production

Comparative performance of different mushrooms indicated that *P. florida* was found to be significantly superior to other mushrooms and took minimum of 16.25 days for completing spawn run (Table 37). This was followed by *C. gambosa* (21.50 days) and *A. polytricha* -A1 (34.75 days) which was found to be on par with A2 (35.25 days) (Plate 11.1). For pinhead formation also *P. florida* was found to be significantly superior to other mushrooms and it took a minimum of (22.75 days) for first flush followed by *C. gambosa* (26.50 days) and *A. polytricha* -A1 and A2 which were found to be on par with each other (47.0 days) (Plate 11.2).

Time taken for first flush was found to be minimum in *P. florida* (25.25 days) followed by *C. gambosa* (32.25 days) and *A. polytricha* – A2 (50.50 days) which was found to be on par with *A. polytricha* – A1 (51.50 days). *A. polytricha* -A2 had attained maximum cropping period (88.50 days) and was found to be significantly superior over all the other mushrooms. The results were followed by *A. polytricha* - A1 (85.0 days), *C. gambosa* (66.50 days) and *P. florida* (57.0 days). *C. gambosa* had the highest average weight of sporocarp (146.85 g) and was significantly superior to other treatments like *P. florida* (16.60 g) and *A. polytricha* - A1 (4.17 g) and it was found to be statistically on par with *A. polytricha* - A2 (3.97 g). The number of sporocarps was maximum in *P. florida* (37.50) and it was statistically on par with *A. polytricha* - A2 and A1 (37.25 and 36.50 respectively). The total yield from three harvests and biological efficiency was also the highest in *C. gambosa* (0.661 kg and 66.1 %) and was statistically on par with *P. florida* (0.623 kg and 62.3 %) followed by *A. polytricha* - A1 (0.152 kg and 15.2 %) and A2 (0.148 kg and 14.8 %) and they were found to be on par with each other, whereas the total yield from maximum harvests and biological

efficiency was the highest in *C. gambosa* (0.67 kg and 67 %) and was found to be on par with *P. florida* (0.62 kg and 62 %) followed by *A. polytricha* – A1 and A2 with a total yield of (0.528 kg and 0.508 kg) and biological efficiency of (52.8 % and 50.8 %) respectively and they were found to be on par with each other (Table 38; Plate 11.3).

Common pests observed were phorid flies and staphylinid beetles. The competitor moulds *viz.*, *Trichoderma* sp., *Aspergillus* sp. and *Coprinus* sp. as well as bacterial contamination were observed on the beds (Table 39 and Plate 11.4).

4.13. DEVELOPMENTAL MORPHOLOGY

The sporocarps took an average of six days from the day of pinhead formation to complete maturity. The pileus of the pinhead was creamy white colour with 2 mm diameter, rubbery and soft textured. The stipe was rudimentary. The colour became creamy to light brown after two days and the pileus started to attain cup/discoid shape with a diameter of 8 mm and a very small stipe was observed (0.3 mm diameter). The pileus became lobed after three days with a diameter of 15 mm and colour changed to brown. The mushrooms grew flat and became appressed after 4-5 days with a diameter of 30 mm and the colour was predominantly brown. On the sixth day the mushroom attained full maturity with exact ear shape and measured 40 mm diameter. As the pileus opened fully, the length of the stipe was reduced and no prominent stipe was observed. Sporocarps were normally produced singly. They were rarely produced in clusters. Even within a bunch, the sporocarps differed in their size and morphology. After complete maturity the sporocarps were loaded with abhymenial hair on the pileus and it started to produce basidiospores from the under surface of the pileus. The maturity of sporocarps was greatly influenced by temperature, humidity and aeration. Increased temperature and low humidity inside the cultivation room caused drying of pinheads on the very next day of formation (Plate 12.1 and 12.2).

Table 37. Comparison of growth parameters of Jelly mushrooms (*A. polytricha* -A1 and A2) with milky and oyster mushroom

Sl. No.	Mushrooms	Time taken for complete spawn run (days)*	Time taken for pinhead formation (days)*	Time taken for first flush (days)*	Total crop period (days)*
1.	Jelly mushroom-A1 (*Auricularia polytricha*)	34.75[a]	47.0[a]	51.50[a]	85.00[b]
2.	Jelly mushroom-A2 (*Auricularia polytricha*)	35.25[a]	47.0[a]	50.50[a]	88.50[a]
3.	Milky mushroom (*Calocybe jambosa*)	21.50[b]	26.50[b]	32.25[b]	66.50[c]
4.	Oyster mushroom (*Pleurotus florida*)	16.25[c]	22.75[c]	25.25[c]	57.00[d]
	CD (0.05%)	2.817	1.998	1.881	3.180
	S. Em±	0.904	0.641	0.604	1.021

* Mean of four replications *Means followed by similar superscripts are not significantly different at 5% level

Table 38. Comparison of yield parameters of Jelly mushroom (*A. polytricha* -A1) with milky and oyster mushroom

Mushrooms	Average weight of sporocarp (g)*	Number of sporocarps*	Total yield from three harvests (kg/kg dry weight of substrate)*	Biological efficiency (%)(Fresh wt. of mushrooms/ dry wt. of substrate used ×100)	Total yield	Biological efficiency (%)
Jelly mushroom (A1)	4.175^{c}	36.50^{a}	0.152^{b}	15.2	0.528^{b}	52.8
Jelly mushroom (A2)	3.975^{c}	37.25^{a}	0.148^{b}	14.8	0.508^{b}	50.8
Milky mushroom	146.85^{a}	4.5^{b}	0.661^{a}	66.1	0.67^{a}	67.0
Oyster mushroom	16.60^{b}	37.50^{a}	0.623^{a}	62.3	0.629^{a}	62.9
CD (0.05%)	1.305	4.212	0.077		0.088	
S. Em±	0.419	1.352	0.025		0.028	

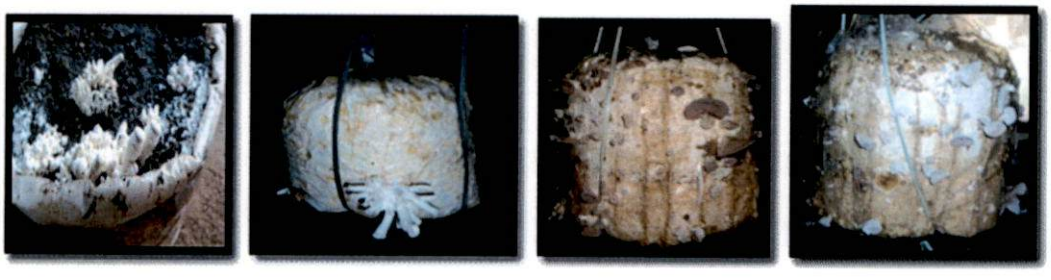
* Mean of four replications *Means followed by similar superscripts are not significantly different at 5% level

Table 39. Pests and competitor moulds observed in Jelly mushroom (*A. polytricha* -A1 and A2) with milky and oyster mushroom

Sl. No.	Mushrooms	Major pests observed	Major competitor moulds
1.	Jelly mushroom (A1)	Phorid flies *(Megaselia* sp.) and *Staphylinus* sp.	*Trichoderma* sp., *Aspergillus* sp. and *Coprinus* sp.
2	Jelly mushroom (A2)	Phorid flies *(Megaselia* sp.) and *Staphylinus* sp.	*Trichoderma* sp., *Aspergillus* sp. and *Coprinus* sp.
3	Milky mushroom	Phorid flies *(Megaselia* sp.) and *Staphylinus* sp.	Bacterial contamination
4	Oyster mushroom	Phorid flies *(Megaselia* sp.) and *Staphylinus* sp.	Mat of *Trichoderma* sp.

Milky mushroom Oyster mushroom Jelly mushroom (A1) Jelly mushroom (A2)

Plate 11.1. Comparison of growth parameters of jelly mushroom *A. polytricha* - (A1 and A2) with milky and oyster mushroom- Spawn run

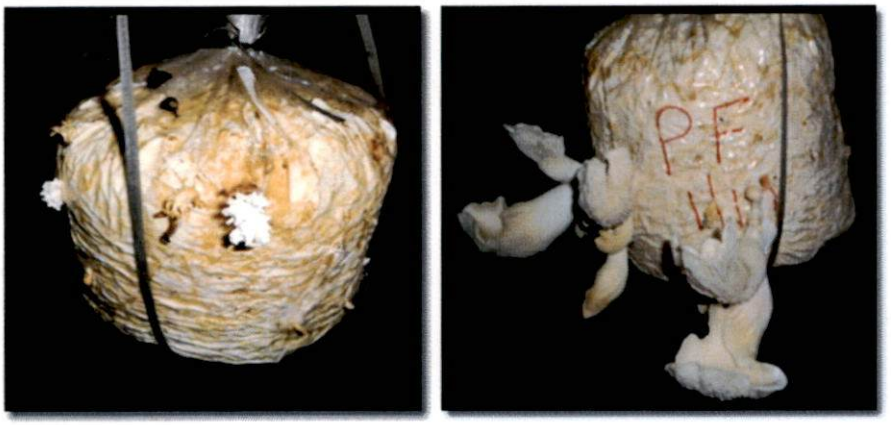


Milky mushroom Oyster mushroom Jelly mushroom (A1) Jelly mushroom (A2)

Plate 11. 2. Comparison of growth parameters of Jelly mushroom (*A. polytricha* - A1 and A2 with milky and oyster mushroom- Pinhead formation

Milky mushroom Oyster mushroom Jelly mushroom (A1) Jelly mushroom (A2)

Plate 11. 3. Appearance of fruiting bodies of Jelly mushroom (*A. polytricha* – A1 and A2), milky and oyster mushroom



Bacterial contamination

Coprinus sp. Mycelial mat of *Trichoderma*sp.

Plate 11. 4. Contaminants observed in the beds of three different mushroom

1 day after PHF 2 days after PHF 3 days after PHF

4 days after PHF 5 days after PHF 6 days after PHF

Plate 12.1.Developmental morphology of fruiting bodies on beds

Plate 12.2. Developmental stages of *Auricularia*fruiting bodies on beds

PHF- Pin head formation

4.14. LOG METHOD OF CULTIVATION

Comparison between log method of cultivation and polybag method of cultivation indicated that polybag method of cultivation for A1 and A2 was significantly superior to log method of cultivation (Table 40). For primordial formation, A1 took minimum of 42.50 days followed by A2 (44.25 days), whereas log method of cultivation took maximum of 58.75 days for A1 and 60.50 days for A2. The total yield from three harvests was maximum in polybag method of cultivation which attained a yield of 0.136 kg and was found to be on par with A2 (0.130 kg). Log method of cultivation for A1 recorded 0.037 kg yield and was on par with A2 (0.035 kg). Biological efficiency followed the similar trend and it was found to be the highest in polybag method of cultivation for A1 (13.6 %) and A2 (13.0 %), followed by log method of cultivation for A1 (3.7 %) and for A2 (3.5 %). The study indicated that maximum biological efficiency was obtained from polybag method of cultivation compared to log method of cultivation (Plate 13).

4.15. ANTI-CANCEROUS ACTIVITIES

The results of study from anti-cancerous activities of *A. polytricha* (A1) revealed that the activity was dosage dependent and as the concentration of the sample increased, the percentage viability of cancer cells decreased. The concentrations ranging from 6.25, 12.5, 25, 50 and 100 µg/ml showed decreased per cent viability of cervical cancer cells (79.34, 73.69, 66.95, 61.10 and 53.75 % respectively) (Plate 14a), colon cancer cells (87.24, 73.37, 59.0, 46.86 and 30.74%) (Plate 14b) and liver cancer cells (98.27, 92.77, 81.79, 74.50 and 53.59 percentage) (Plate 14c). Control sample showed 100 percentage cell viability in all the cancer cells tested (Table 41). The detectable changes observed in the morphology of the cells such as rounding and shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

Table 40. Log method of cultivation of *A. polytricha* (A1 and A2)

Sl. No.	Method of cultivation	Time taken for primordia formation	Total yield from three harvests	Biological efficiency
1.	Poly bag method (A1)	42.50[d]	0.136[a]	13.6
2.	Poly bag method (A2)	44.25[c]	0.130[a]	13.0
3.	Log method (A1)	58.75[b]	0.037[b]	3.7
4.	Log method (A2)	60.50[a]	0.035[b]	3.5
	CD (0.05)	1.525	0.008	
	S. Em±	0.489	0.003	

* Mean of four replications

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

Table 41. *In vitro* antiproliferative effect of *A. polytricha* (A1) by MTT* assay (cervical, colon and liver cancer)

Sample Concentration (µg/ml)	Average OD at 540nm			Percentage Viability		
	Cervical cancer	Colon cancer	Liver cancer	Cervical cancer	Colon cancer	Liver cancer
6.25	0.72	1.90	1.78	79.34	87.24	98.27
12.5	0.67	1.60	1.68	73.69	73.37	92.77
25	0.61	1.29	1.48	66.95	59.0	81.79
50	0.55	1.02	1.35	61.10	46.86	74.50
100	0.48	0.67	0.97	53.75	30.74	53.59
Control	0.91	2.18	1.82	100	100	100

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

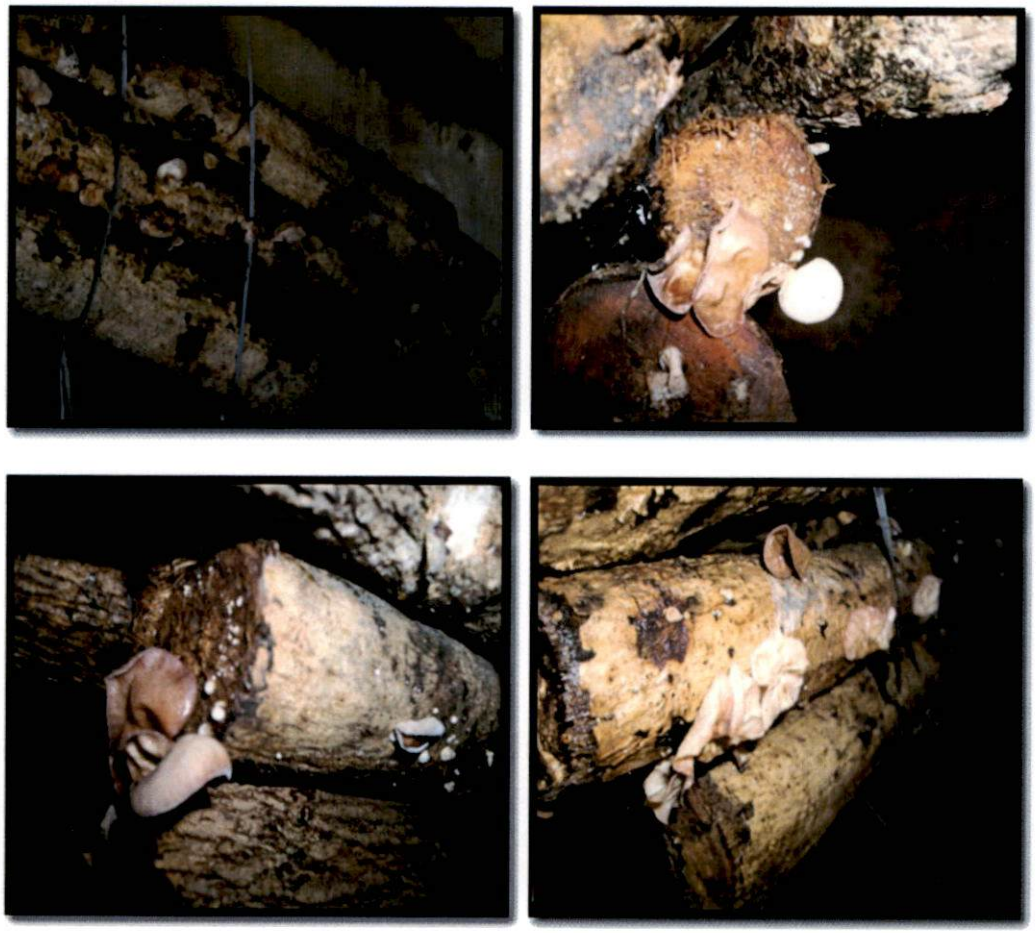
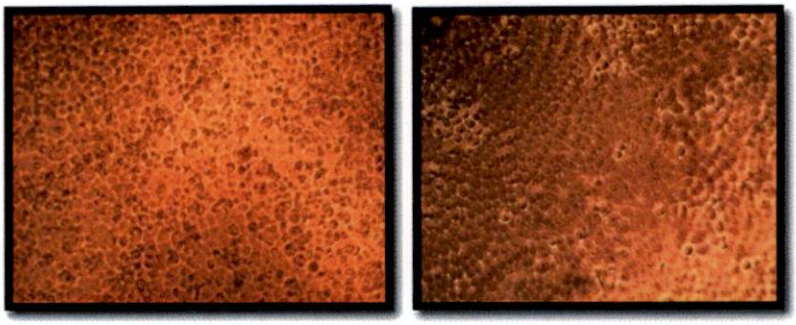
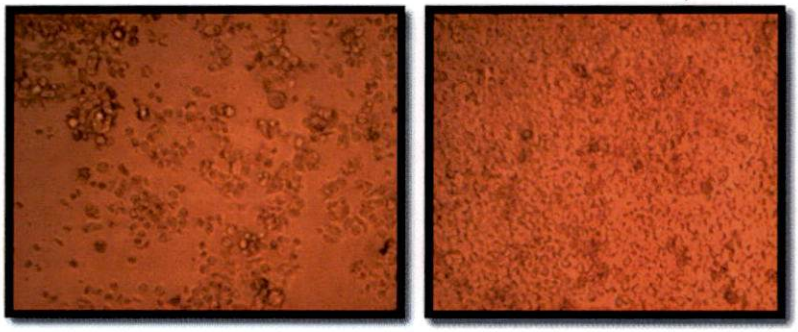


Plate 13. Fruiting bodies *of A. polytricha* (A1 and A2) on wood logs



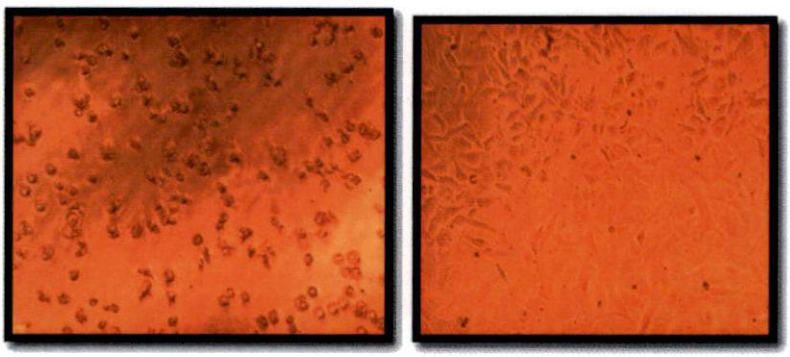
100 (µg/ml) Control

Plate 14a. Microscopic observation of cervical cancer cells



100 (µg/ml) Control

Plate 14b. Microscopic observation of colon cancer cells



100 (µg/ml) Control

Plate 14c.Microscopic observation of colon cancer cells (Inverted phase contrast tissue culture microscope- 40 X)

Discussion

5. DISCUSSION

Jelly fungi are commonly found growing on logs, twigs as well as tree stumps. They are typically white, orange, pink, rose, brown, or black coloured. Among mushrooms, jelly fungi are very unique and they are first to be seen on the substrates after every monsoon showers. *Auricularia auricula* was reported to be the first artificially cultivated mushroom in the world about 600 AD Nowadays. *Auricularia* mushrooms are among the top four most important cultivated mushrooms in the world as well as highly prized mushroom in Southeast Asia and this mushroom can be cultivated in both temperate and tropical regions depending on its varieties (Razak, 2013). *Auricularia* has a long standing reputation in Chinese traditional medicine for its ability to increase health benefits with special reference to anti-cancerous activities.

There are many *Auricularia* species of which *A. polytricha* and *A. auricula* are the most commonly grown. However, *A. polytricha* is the most suitable species to cultivate in tropical regions where temperatures are high (Devi *et al.*, 2015a). In this prospects Kerala is much suited for the cultivation of this highly nutritionally as well as medicinally important mushroom. Eventhough, listed as one of the top culinary mushrooms, the production technology for large scale cultivation is still insufficient and inefficient. Hence, focusing light on this point, the present study has been undertaken with the objective of evolving suitable strains by survey and standardizing their cultural, physiological, spawn production and cultivation aspects that could be beneficial for mushroom growers. The nutritional, post-harvest and anti-cancerous activities of these mushrooms were also undertaken.

5.1. SURVEY

Every mushroom has its own unique ecological niche and occurs only in convinced habitats. They are found growing effectively all over the world (Smith, 1964).

In the present study, surveys were conducted in ten different locations of Thiruvananthapuram and Kollam districts of Kerala *viz.*, Vellayani, Venganoor, Vanchiyoor, Neyyattinkara, Kattakada, Ponmudi, Nedumangad, Palode, Arippa and Kulathupuzha to identify different strains of *Auricularia* spp. and *Tremella* sp. which were growing under natural conditions, during pre and post monsoon seasons of 2014-2016.

All the collections of jelly mushroom obtained from surveyed locations were invariably gregarious and lignicolous. The collected specimens were mainly obtained from logs and dead stumps/branches of trees. In the surveyed locations of Vellayani the jelly mushrooms were found on logs of several types of host plants including both soft wood and hard wood trees like mango, fallen coconut wood logs, bottle brush tree stumps, copper pod tree stumps, yellow bells, pine, wattles/acacias, cashew, golden temple tree logs, rubber, teak logs, arecanut and drumstick (soft wood) which also indicates the diversified range of trees present in and around College of Agriculture, Vellayani. In all the other locations surveyed, logs of coconut and rubber were the common hosts for these mushrooms. Dried stumps of the soft wood tree, drum stick were the hosts for jelly mushrooms in elevated areas like Ponmudi and Kulathupuzha whereas they were found growing on teak wood in Kattakada. *Tremella* sp. was found only on logs of jack fruit tree. The growth of jelly mushroom on a wide range of host justifies the gregarious nature and adaptability of these mushrooms to survive on the wood of different tree species. In the present study *Auricularia* spp. could be collected from 13 host plants and also more collections were obtained during the rainy seasons (May-June to November-December). Dried sporocarps were observed in dead twigs and branches during summer season (February to April). The majority of collections were of *Auricularia polytricha.*

Sohi and Upadhyay (1990) collected 15 species of *Auricularia* from dead wooden logs in Himachal Pradesh and identified eight species from which *A. mesenterica* was selected for artificial cultivation. Sharma *et al.* (1992) reported the occurrence of *A. delicata, A. auricula and A. mesenterica* from North India.

Hemmes and Desjardin (2004) collected *A. polytricha* from dead stumps and fallen wooden logs.

Results of the survey conducted by Vidyaresmi (2008) was in accordance with the present study, wherein the native strains of *A. polytricha* were collected from dead wood and logs in coconut basins from locations such as Vellayani, Balaramapuram, Kalliyoor, Palappur and Vanchiyoor panchayats of Thiruvananthapuram district during June to November, 2007. Similarly, Geetha (2011) identified and collected different strains of *Auricularia* spp. from several locations in Western Ghats of Kerala and Tamil Nadu where they were observed on dead woods of mango tree, moringa, coconut and broad-leaved woods. Mohanan (2011) reported that *A. polytricha* was widely distributed in moist deciduous to wet evergreen forests of the Western Ghats of Kerala and this species occurred in clusters on rotting branches, twigs, decaying stumps and logs. Kumari *et al.* (2013) reported a new species of *Auricularia*, namely *A. olivaceus* from North India after studying in detail their macroscopic and microscopic characters. Choudhury and Sarma (2014) collected *A. auricula* from decayed *Bambusa* sp. and *A. polytricha* from *Delonix regia* from Sonitpur district of Assam.

Musngi *et al.* (2005) reported that basidiocarps of *Auricularia* spp. grow saprobically, usually solitary to gregarious, as observed in the present survey. They identified these mushrooms abundantly on dead trunk and branches of coconut, mango, rain tree, rubber tree and mahogany. *Auricularia* was found in woodland and rainforests where it was growing in large colonies on fallen logs of *Eucalyptus* (Harding and Patrick, 2008). Du *et al.* (2011) reported that *Auricularia* spp. are saprotrophs and have a worldwide distribution from temperate to the tropics, growing on living and dead deciduous trees, decayed stumps or logs.

Cheng and Tu (1978) stated that species of *Auricularia* are found worldwide and *A. polytricha* occurs in both tropical and subtropical regions. Gogoi and

Sarma (2012) recorded the wide distribution of *A. auricula* from Dhemaji district of Assam. Gateri *et al.* (2014) conducted a survey in coastal Kenya and identified *Auricularia* spp. as lignicolous in nature, growing on dead decaying wood and were found in clusters. Geetha (2011) reported the occurrence of *Tremella fuciformis* on the dried barks of cashew tree and coconut palm.

From the results of the survey it is concluded that *Auricularia* spp. have a wide host range and are gregarious and lignicolous in nature whereas the occurrence of *Tremella* spp. is limited and was obtained only from dead stumps of jack fruit tree.

5.2. MORPHOLOGICAL CHARACTERS

The morphological studies revealed that the pileus of *Auricularia* spp. collected from different places had brown to dark brown colour, incurved margin, ear shape, soft velvety texture and their diameter ranged from 2.6 cm to 3.8 cm. The collected *Auricularia* spp. and *Tremella* spp. mushrooms from ten different locations were devoid of stipe, volva and annulus. In some cases, rudimentary stipes were observed. Similar observations were recorded by Sobal *et al.* (2007) and Devi *et al.* (2015a) who reported that the fruiting body of *A. polytricha* was brown in colour, ear shaped, 2-15 cm broad and rubbery textured with a rudimentary stalk of 7 mm long and without veil.

The present study is in agreement with the observations made by earlier researchers who have reported that the basidiocarp of *Auricularia* spp. was rubbery and gelatinous when young and brittle on drying. Its structure varied from discoid, capulate, auriform, fan shaped, smooth, curved, elastic, irregularly cup shaped, resupinate with reflex margins, laterally attached and gathered together and attached at a central position in groups with sessile stipe and measured 4-7.9 cm in breadth and 0.5-1.6 mm in thickness (Musngi *et al.*, 2005; Sarma *et al.*, 2010; Onyango *et al.*, 2011a; Gogoi and Sarma, 2012; Choudhary and Sarma, 2014). On the contrary, reddish brown colour of fruiting body of *Auricularia fuscosuccinea* was reported by Sibounnavong *et al.* (2008) and Sterry

et al. (2009) and a dark brown colour with a purplish tint was observed in another species of *Auricularia* by Gateri *et al.* (2014). Sterry *et al.* (2009) also mentioned that the outer surface of fruiting bodies of *Auricularia* spp. was bright reddish to tan brown and the inner surface was lighter grey brown in colour. Choudhary and Sarma (2014) reported dark brown colouration of upper surface compared to lower surface.

Kumari *et al.* (2013) reported a new species *viz.*, *A. olivaceus* which showed marked and unique olivaceous colouration on the pileus, presence of medulla, multi-lobed margin and variation on the surface and prominent central strand in hymenial hairs. But in the present study, the hyaline abhymenial hair lacked a central strand, had rounded tips and did not grow in dense tufts. Musngi *et al.* (2005) also reported the same characteristics of abhymenial hairs, but a prominent central strand was seen as well.

The collected *Tremella* spp. were white in colour, chrysanthemum like with a diameter of 6.2 cm and had a soft and leathery texture. Similar observations were made by Pegler (2001) who reported that *Tremella* spp. resembled a white chrysanthemum flower in appearance, about 10-15 cm in diameter and had numerous, semi-transparent and leaf-like lobes. Wong *et al.* (1985) reported that the basidiocarp of *Tremella mesenterica* was brain like with irregular clustered folds and consisted of one to several distorted lobes and measured 0.5 cm × 0.3 cm. Geetha (2011) also reported the chrysanthemum like white transparent nature of *Tremella* spp. collected from coconut trunks and thin transparent spreaded nature of *Tremella* spp. from cashew tree.

The present studies on the hyphal characters revealed that they are septate, branched, hyaline, aerial as well as submerged and internal stratification of hyphae showed different zonations. A dense, central zone of hyphae was found in medulla and this particular zone had significant taxonomical importance as the presence of this zone indicated that the collected mushrooms belonged to *Auricularia polyticha* group where it had tropical importance and these

observations were in line with the study conducted for the first time by Lowy (1952) who classified *Auricularia*, based on the internal stratification of hyphae with special emphasis on a strongly or weakly differentiated medullary zone and abhymenial hair.

Musngi *et al.* (2005) also reported the presence of different zonations of *A. polytricha* namely, zona compacta zona subcompacta superioris, zona laxa superioris, zona laxa inferioris, zona subcompacta inferioris and medulla. The hyphae were reported to be smooth and hyaline with septations (Choudhury and Sarma, 2014). Bandara *et al.* (2015) reported the presence of medulla in *A. thailandica.* Hymenium is the fertile layer bearing basidia as well as basidiospores and it is a gelatinous layer found on the underside of the pileus through which the cylindrical basidiospores emerged (Cheng and Tu, 1978; Musngi *et al.*, 2005).

A wide variation in the shape and size of basidiospores has been reported by many workers and the shape of spores ranged from oval, cylindrical, sausage and reniform to allantoid. In the present study the spore print produced was pure white in colour. The basidiospores were hyaline, oval, sub cylindrical to cylindrical and measured 12.8 × 3.4 µm in size. Similar observations were given by Young *et al.* (2005) who reported that the spores of *Auricularia* were long and sausage shaped (6 to 8 µm thick) and the spores were white, cream yellowish or hyaline. Weber and Webster (2006) made detailed studies on spores of *A. auricula-judae* and explained that the fungus produced basidiospores from cylindrical basidia which became divided into four cells by three transverse septa. Sarma *et al.* (2010) and Choudhury and Sarma (2014) stated that the basidia of *Auricularia* were cylindrical, tri septate and measured 50-60 × 4-5µm whereas basidiospores were curved, cylindrical and 12.3-15 × 4.5-6 µm in measurement. Mohanan (2011) and Choudhury and Sarma (2014) observed the spores of *Auricularia polytricha* and reported that they were hyaline, reniform to allantoid, 13-16 × 4-5.5 µm, guttulate and the spores can sometimes be seen in a whitish mass on the underside of the fruit body. Similar observation of mass of

basidiospores on the undersurface of fruiting bodies was also observed in the present study.

5.3. ISOLATION AND PURE CULTURING

Isolation of jelly mushrooms was done by adopting tissue culture method after sterilizing with 99.9 per cent ethyl alcohol. The internal tissue was selected from the junction of pileus and stipe and hair like mycelial growth started after 48 h of inoculation in potato dextrose agar medium. A pure fungal culture was isolated from medium-aged pest and disease-free mushrooms. Fluffy hair-like white mycelial growth started after two days from the inoculated bits on PDA medium. The growth was white, cottony and branched, which completed growth in 14 to 15 days. The pure culture obtained was periodically subcultured for further use and stored under refrigerated conditions (4 ^{0}C).

The present study is in accordance with Ling *et al.* (2005) who isolated *A. polytricha* on PDA medium from the sporophore. Marcel (2004) also isolated pure culture from healthy and mature basidiocarp of *Agaricus bisporus* after surface sterilizing with alcohol. Similar observations were made by Vidyaresmi (2008) who maintained the cultures of *Auricularia* by periodical subculturing in PDA medium and Ma *et al.* (2014) who isolated pure culture of *Auricularia auricula-judae* strain Techan 2 from a wild *A. auricula-judae*, and maintained in potato dextrose agar (PDA). But Onyango *et al.* (2011a) reported the suitability of malt extract agar medium for pure culturing of *Auricularia* spp. Pure cultures have been traditionally maintained by periodic sub-culturing in PDA and/or cold storing between 2-5 ^{0}C which is costly and time consuming (Smith and Onions, 1983). The present study also indicated the suitability of PDA for the isolation and culturing of collected *Auricularia* spp.

Evaluation of morphological and mycelial characters of mushrooms will provide vital information for characterization and selection of strains. Sobal *et al.* (2007) stated that the mycelial and morphological traits such as color, density,

texture and presence of aerial hyphae were crucial in distinguishing various *Auricularia* species.

In the current research work, out of ten collections of *Auricularia*, isolation was successful in four, and one isolate was obtained from *Tremella* sp. The isolation of *Auricularia* is more difficult than that of other mushrooms due to the gelatinous nature of fruiting body which hinders the extraction of internal tissues and it poses contamination problem also. Evaluation of mycelial growth of these five isolates of jelly mushrooms (four *Auricularia* and one *Tremella*) was made to identify the fast-growing isolates. Accordingly, the *Auricularia* isolates - A1 obtained from College of Agriculture, Vellayani, recorded the highest growth of 8.90 cm on PDA followed by *Auricularia* isolate - A2 (8.45 cm) obtained from Neyyatinkara. The time taken by these two isolates for attaining maximum growth was also very short (10.25 days for A1 and 11.25 days for A2 respectively) compared to the remaining two *Auricularia* isolates obtained from Vellayani, Kattakada and also that of *Tremella* sp. obtained from Vellayani. The morphological characteristics of A1 and A2 also showed variation. The basidiocarps of A1 were dark brown coloured with rubbery texture whereas A2 exhibited light brown colouration with soft and velvety texture. Based on the morphological and cultural characteristics, these native isolates were temporarily identified as *Auricularia* sp. /*Tremella* sp. However, the identification was confirmed as *Auricularia polytricha* (accession no for A1 was DMRO-825 and DMRO- 826 for A2) at DMR, Solan. Vidyaresmi (2008) reported a native strain of *Auricularia* sp. with maximum diameter growth of 9.0 cm on the PDA medium after seven days of inoculation. On the contrary, Kalaw *et al.* (2016) observed a very slow mycelial growth of *A. polytricha* (2.32 cm) on PDA medium after seven days of incubation period, but the growth was very thick.

Musngi *et al.* (2005) characterized thirteen different strains of *A. polytricha* based on the colour, shape and morphology of basidiocarp as well as hair and hymenial characteristics. Palapala *et al.* (2006) reported the occurrence of three native strains of *A. auricula* in Kakamega Forest in Western Kenya and they were

morphologically different in colour and shape. Du *et al.* (2011) classified the 24 wild and cultivated strains of *A. polytricha* into six groups based on the similarity coefficient value and they also reported that genetic diversity of wild strains was higher than that of the cultivated strains. Onyango *et al.* (2011a) classified nine different strains of *A. auricula* based on the colour and they included yellow brown, brown and dark brown strains. The strain differentiation was also carried out based on the characteristics of mycelial colonies. Onyango *et al.* (2016) identified different strains of *Auricularia* spp. and were grouped into two based on the morphology and the strains from group one had long visible stipes with black colour, whereas group two strains had shorter stipes with brown colour and both the strains differed in internal hyphal stratifications.

5.4. PHYSIOLOGICAL STUDIES

Mushrooms derive their food from the substrate upon which they grow and in order to culture them, the essential nutrients needed for the growth and development have to be supplemented in the media. In the present study, the growth of *Auricularia polytricha* isolates (A1 and A2) was assessed by growing on different media and providing different temperature, pH and light conditions.

To find out the best media for the growth of *Auricularia polytricha* (A1 and A2), different media like potato dextrose agar, malt extract agar, oat meal agar and carrot extract agar were tested. Among these, the maximum mycelial growth in minimum days with thick and cottony mycelial growth was recorded for malt extract agar followed by potato dextrose agar medium (Figure 1). Primordial formation was absent in the media tried. Similar observations were made by Upadhyay (1999) who reported that malt extract agar medium supported good growth of *A. mesenterica* on which white coloured mycelial growth of the fungus was observed. Garasiya *et al.* (2007) also reported better growth of *A. polytricha* on malt extract agar medium.

Studies conducted by Tabata and Ogura (2003) indicated the maximum growth of *A. polytricha* on potato sucrose agar medium supplemented with

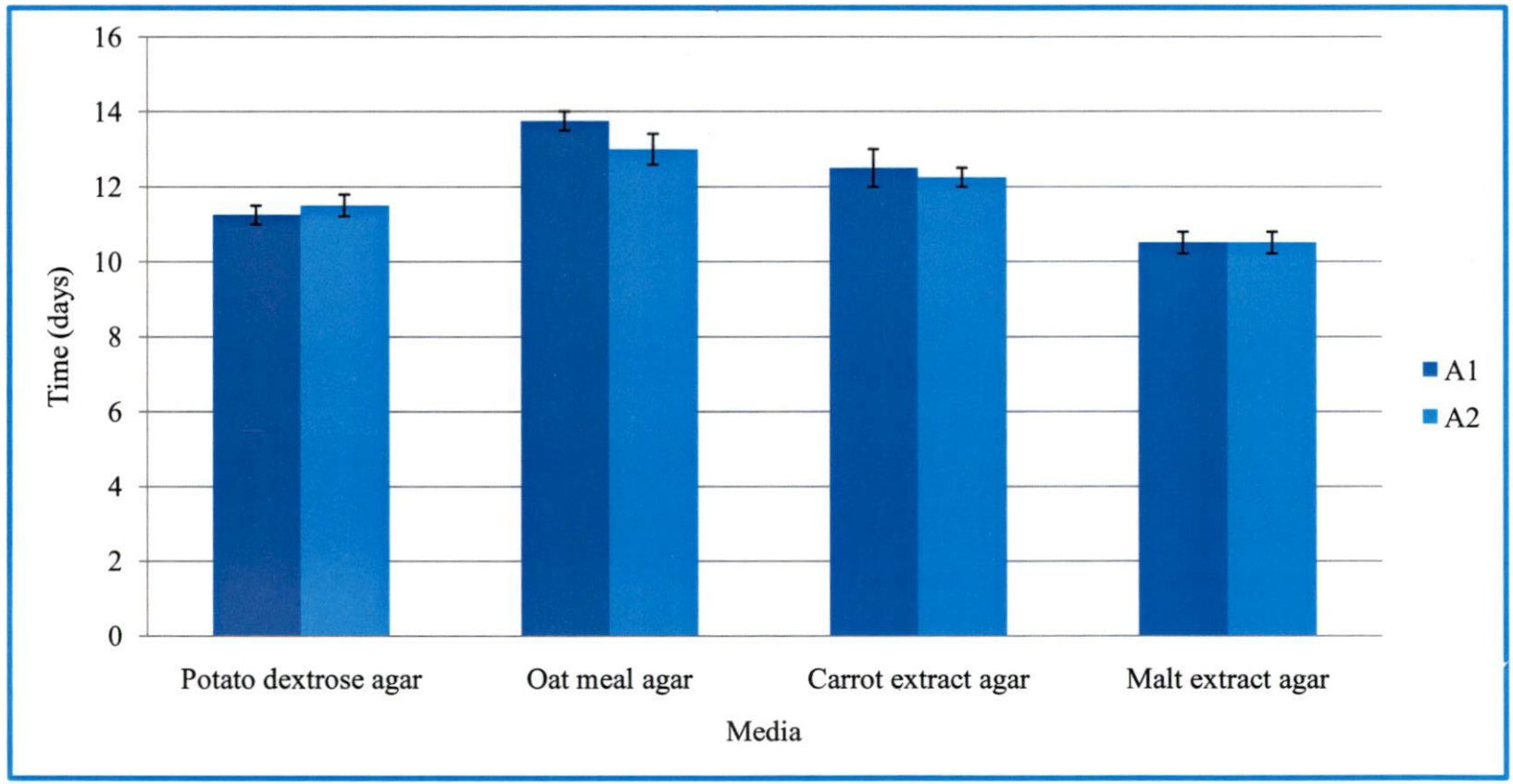


Figure 1. Influence of different media on mycelial growth of *A. polytricha* - A1 and A2

calcium phosphate, calcium sulphate, magnesium sulphate and magnesium chloride. The optimum growth of *A. polytricha* was recorded on three media namely czapeck's, malt extract and potato dextrose agar by Kapoor *et al.* (2011).

Shim *et al.* (2003) reported the suitability of PDA for other mushrooms including *Macrolepiota procera* where the mycelial growth was considerably favoured, but inhibited *Paecilomyces fumosoroseus.* However, Mbaluto (2015) recorded that MEA was the most favourable medium for mycelial establishment of *Macrolepiota* spp. Hur *et al.* (2008) studied the effects of different culture media on the growth of *Phellinus* spp. and recorded significant results on PDA. These contradictory results indicated that taxonomically distinct fungal groups require different nutritional requirement. Kibar and Peksen (2011) disclosed that the effect of culture media on mycelial growth differed significantly according to the mushroom species. Although there was a wide range of mycelial variations on the growth in four different media, it was observed that the mycelial densities of the two isolates A1 and A2 were compact and more on the MEA and PDA media tested. Hence, based on the above results the use of MEA was established as the best medium, and hence, used for further physiological studies.

Temperature plays a vital role in physiological activities of mushroom fungus and each mushroom prefers an optimum temperature for its growth and reproduction. An experiment was carried out to assess the efficacy of different temperature for the growth of isolates on MEA. Out of the three temperatures tested on malt extract agar medium, 25 ^{0}C was found to be the best temperature for the growth of *Auricularia polytricha* - A1 and A2 (9.0 and 8.84 cm) after nine days of inoculation (Figure 2) followed by growth at 30 ^{0}C while the least performance was noted at 20^{0} C. The mycelial growth was thick and cottony at 25 ^{0}C.

Xu and Yun (2003) also made similar observations and reported that the optimal temperature for mycelial growth of *A. polytricha* was found to be 25 ^{0}C. Jonathan *et al.* (2012) explained that *A. polytricha* can be cultivated in tropical

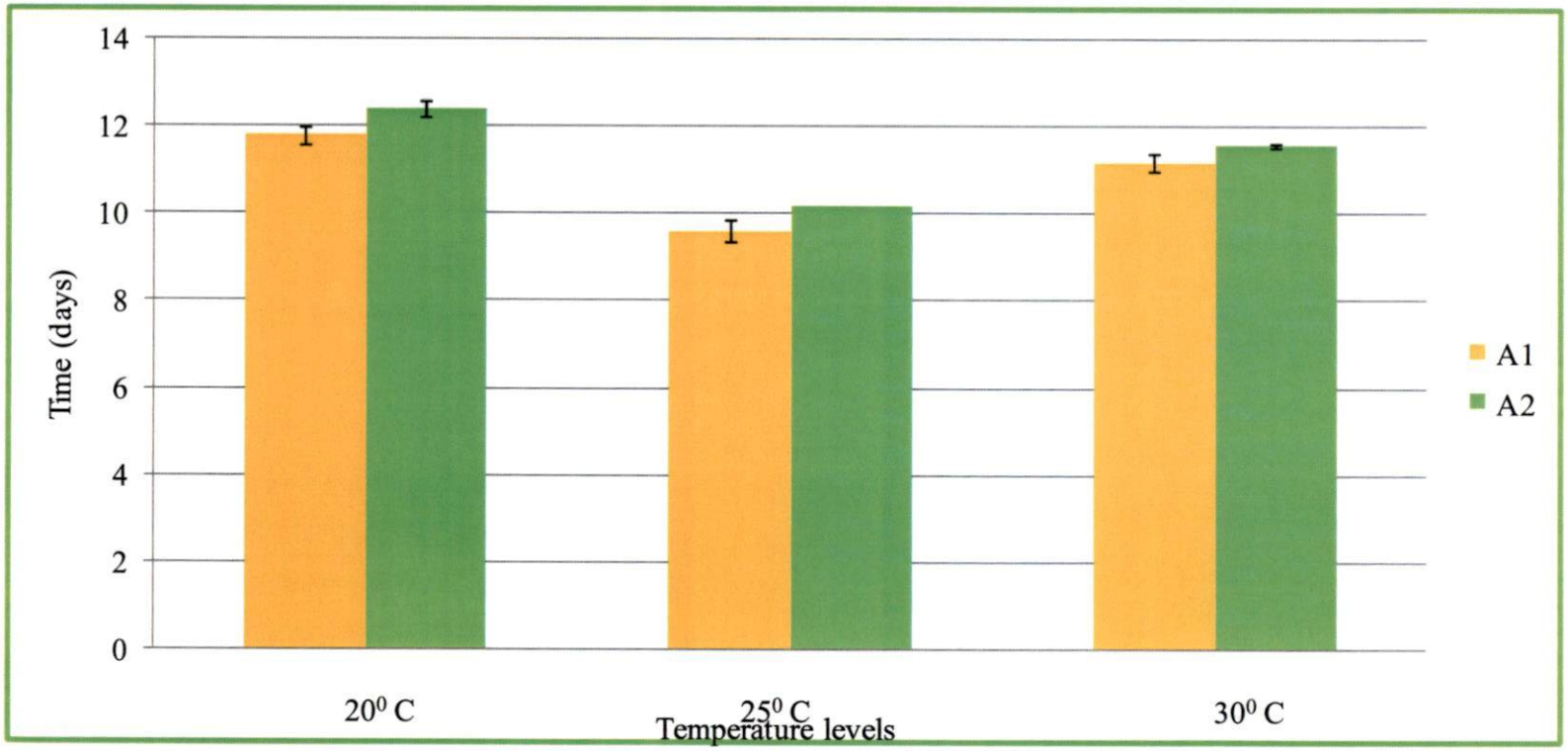


Figure 2. Influence of different temperature on mycelial growth of *A . polytricha* - A1 and A2

regions because its mycelium can grow at temperatures ranging from 10 to 40 ^{0}C. Similarly, Jing *et al.* (2014) recorded the optimum temperature levels for the growth of *A. polytricha, Auricularia* sp. and *A. delicata* as 25 ^{0}C and for *A. auricula* as 30 ^{0}C. For many other mushrooms also a temperature of 25 ^{0}C was found optimum. Likewise, Hur *et al.* (2008) worked on *Phellinus* spp. and reported that 25 ^{0}C was the most suitable temperature for mycelial development. Klomklung *et al.* (2014) reported that mushroom mycelia can grow at temperatures ranging from 20-30 ^{0}C, with optimal growth temperatures of 30 °C and 25 ^{0}C for *Lentinus* and *Pleurotus* species, respectively. The mycelial growth of *Macrolepiota* accessions was optimum at 25-30 ^{0}C and appeared to be suppressed at temperature above 30 ^{0}C (Mbaluto, 2015). Lishma (2015) reported the maximum mycelial growth of *Agaricus* spp. at 25 ^{0}C. Similarly, Sumi (2016) also recorded 25 ^{0}C as the best for mycelial growth of *H. ulmarius* wherein the complete colonisation was obtained within eight days. Usually many mushrooms require a temperature of 25 ^{0}C for mycelial development. The inhibition of growth at higher temperature may be attributed to the enzyme inactivation caused by the denaturation of important enzymes which catalysed fungal metabolic processes (Kibar and Peksan, 2011).

Hydrogen ion concentration of the medium has a profound effect upon the rate, amount of growth and many other life processes of the fungus, and different types of macrofungi prefer different pH conditions (Kibar and Peksen, 2011). Hence an attempt was made in the present study to find out the suitable pH for the mycelial growth of the *Auricularia*, and four different hydrogen ion concentrations *viz.*, pH 5, 6, 7 and 8 were evaluated. The study indicated that both the isolates preferred neutral pH for their maximum growth (9.0 cm and 8.95 cm) within the minimum period (9.50 days) for both A1 and A2 (Figure 3) followed by pH 6 (11.50 and 10.50 days). Comparatively lower mycelial growth was recorded in alkaline pH 8 and acidic pH 5. Similar observations were made by Ma and Luo (1992) who reported vigorous mycelial growth of *Auricularia* spp. in substrate with pH ranging from 5.5 to 6.5 while, at pH values below 5.0 and

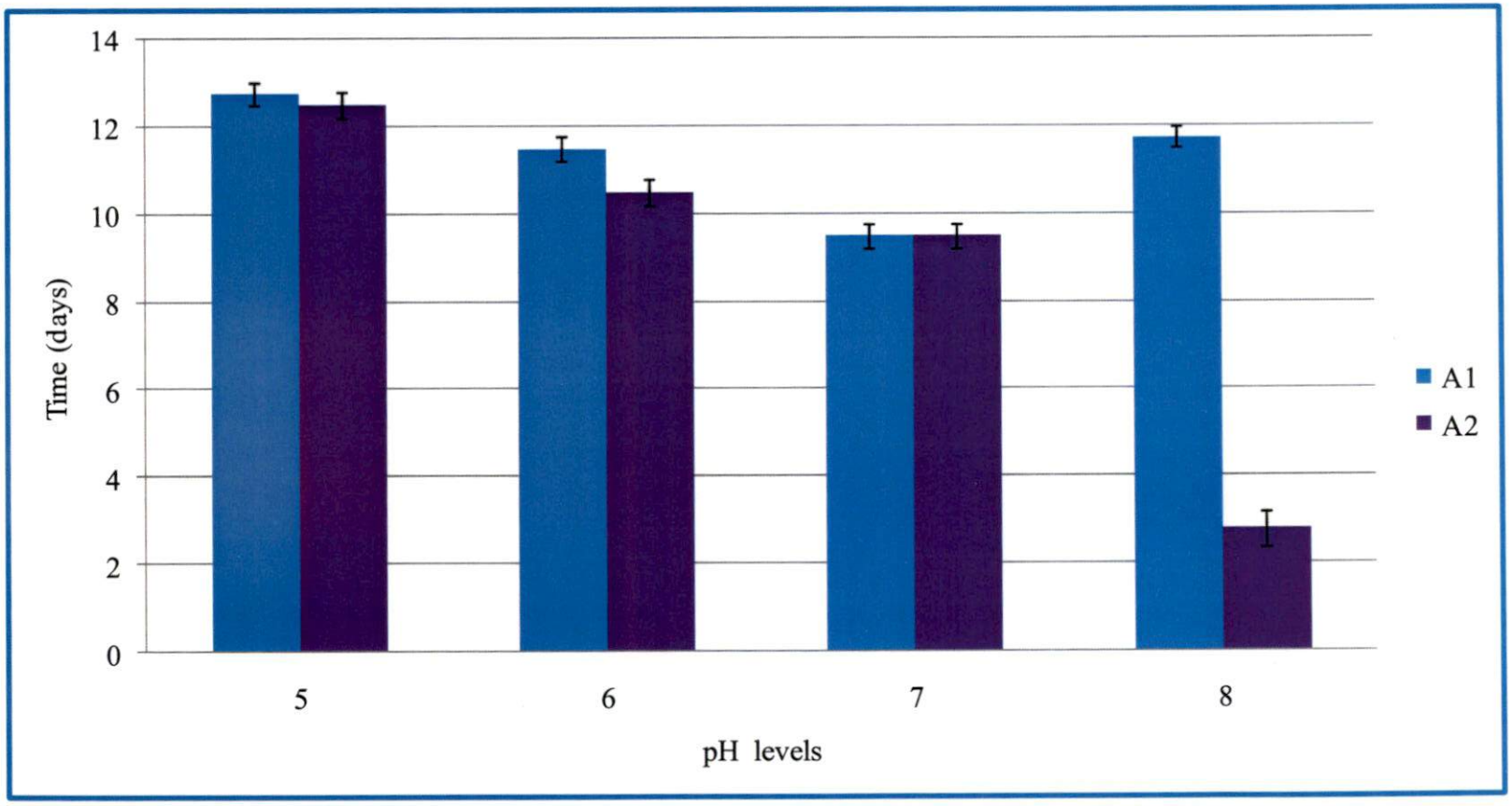


Figure 3. Influence of different pH on mycelial growth of *A. polytricha* - A1 and A2

above 7.0, the mycelial growth rate was markedly reduced. The decreased mycelial growth in acidic pH range is due to the alteration in the metabolic activities and ability to utilize certain substances as reported by Chang and Miles (2004) and due to the toxicity of very acidic pH to the hyphae as reported by Ibekwe *et al.* (2008). Jeong *et al.* (2004) and Jonathan *et al.* (2009) reported that pH 6-6.5 was optimum for inducing mycelial growth of *A. auricula* and *A. polytricha* while no biomass was produced at pH values 4.0. Contradictorily, Kalaw *et al.* (2016) recorded the maximum mycelial growth of *A. polytricha* at pH 7.5 (2.32 cm) seven days after incubation.

The suitability of neutral pH for other mushroom species namely *Macrolepiota procera*, *Ganoderma lucidum*, *Schizophyllum commune* and *Macrolepiota* spp. was reported earlier by many researchers (Shim *et al.* 2005; Jayasinghe *et al.* 2008; Imtiaj and Rahman, 2008; Lai *et al.* 2011; Mbaluto, 2015).

Results of the present studies collaborate with many of the previous studies that the hydrogen ion concentration near to neutrality favoured the mycelial growth of A1 and A2 isolates of *A. polytricha* and acidic conditions (pH 5) showed slight inhibition and even it took longer time to complete the thin and cottony mycelial growth compared to the thick and cottony growth obtained in pH 6,7 and 8.

The growth of A1 and A2 was assessed under light and dark conditions in MEA and the results indicated that maximum growth was favoured at light conditions (8.82 cm and 8.93 cm) in a shorter period of time than in the dark conditions. There was obvious difference in nature of mycelial growth also *i.e.*, both A1 and A2 produced thick white cottony mycelium in the presence of light in contrast to the thin cottony growth produced under dark conditions. These observations are in conformation with the study conducted by Kalaw *et al.* (2016) who reported that the maximum radial growth of *A. polytricha* obtained from light conditions (2.42 cm) than in the dark conditions (2.39 cm). Similar observations were also made by Vidyaresmi (2008), who recorded the maximum mycelial

biomass of *Auricularia* spp. in light conditions (0.95 g) than in the dark conditions (0.82 g). In contradictory to these results, Dulay *et al.* (2012) reported the maximum mycelial growth of *Lentinus tigrinus* under dark conditions (86.67 mm) when compared to light conditions (83.33 mm) three days after inoculation and Sumi (2016) obtained maximum mycelial growth of *Hypsizygus ulmarius* under dark conditions compared to light conditions.

5.5. SPAWN PRODUCTION

Spawn production is a fermentation process in which mycelium of the mushroom will be increased by colonizing through a solid organic matrix under controlled environmental conditions. In usual cases, organic matrix include sterilised grain namely sorghum, millet, rye or wheat as reported by Sinden (1934) and Marcel (2004). The purpose of the grain spawn is to promote uniform growth of mycelium and to support the mycelium to a state of vigour in a way that it will rapidly colonise the selected growth substrate for sporocarp production. The success or failure of mushroom production mainly depends on the accessibility of superior character of spawn. The yield and quality of spawn of the cultivated mushroom is mainly influenced by the genetic constitution of the strain as well as technology used for spawn production which also included the quality of substrates (Kumar, 1995).

In order to find out the best substrate for spawn production of two isolates in the present study, six different substrates like paddy grains, wheat, sorghum, ragi, sawdust of hardwood trees (teak) and rubber saw dust were evaluated after adding calcium carbonate (30 g kg^{-1} grains) to monitor the pH and also to avoid the stickiness. The quality was determined in terms of time taken for spawn run, nature of mycelial growth, presence of contaminants and keeping quality.

From the result it was found that paddy grain was the best medium for preparation of spawn as it required minimum time for complete spawn run (16.75 days and 16.50 days) for A1 (Figure 4) and A2 (Figure 5) respectively and produced thick and fluffy mycelia. The rapid mycelial growth on grain observed

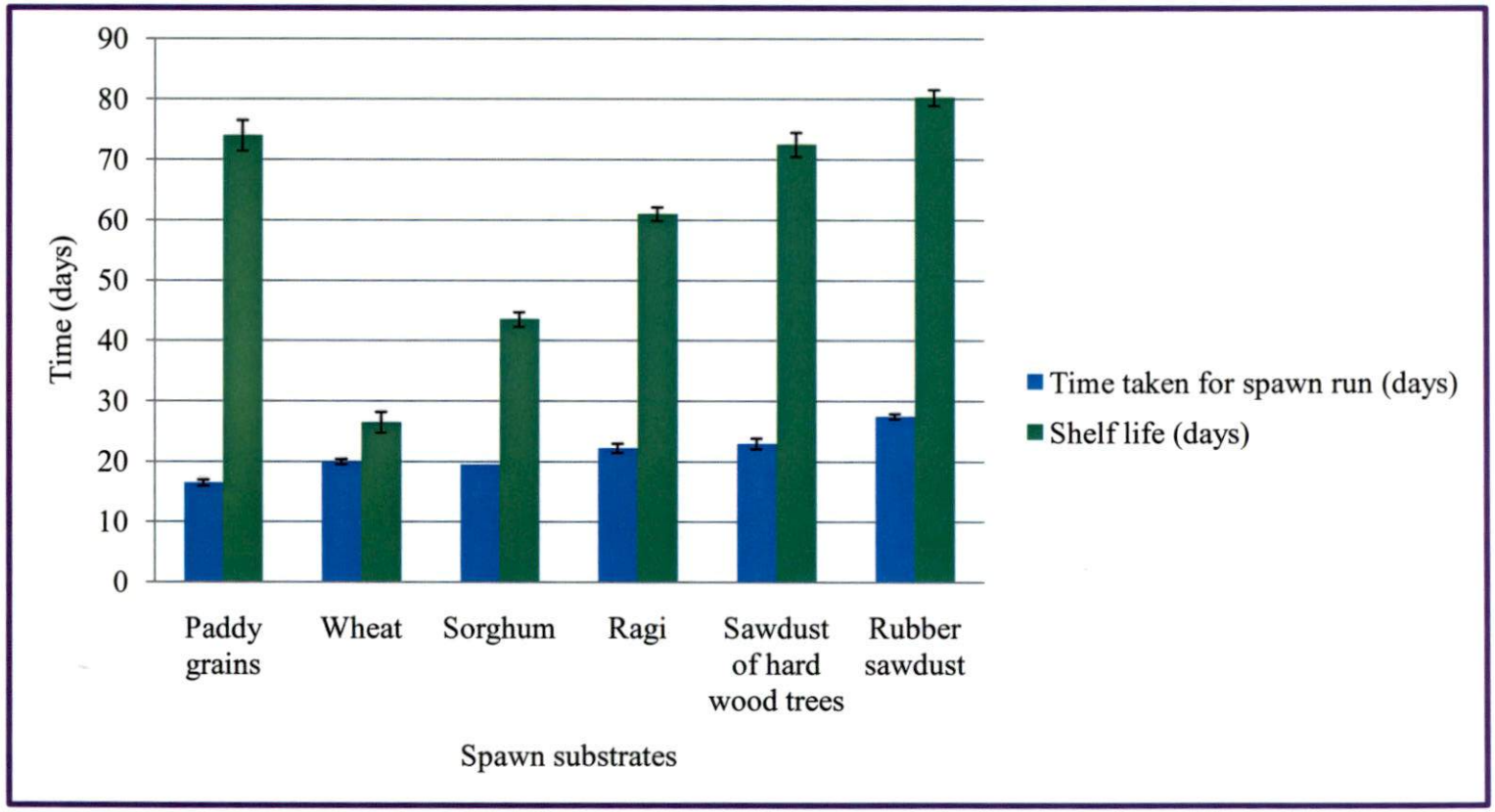


Figure 4. Influence of different substrates on spawn production of *A. polytricha* -A1

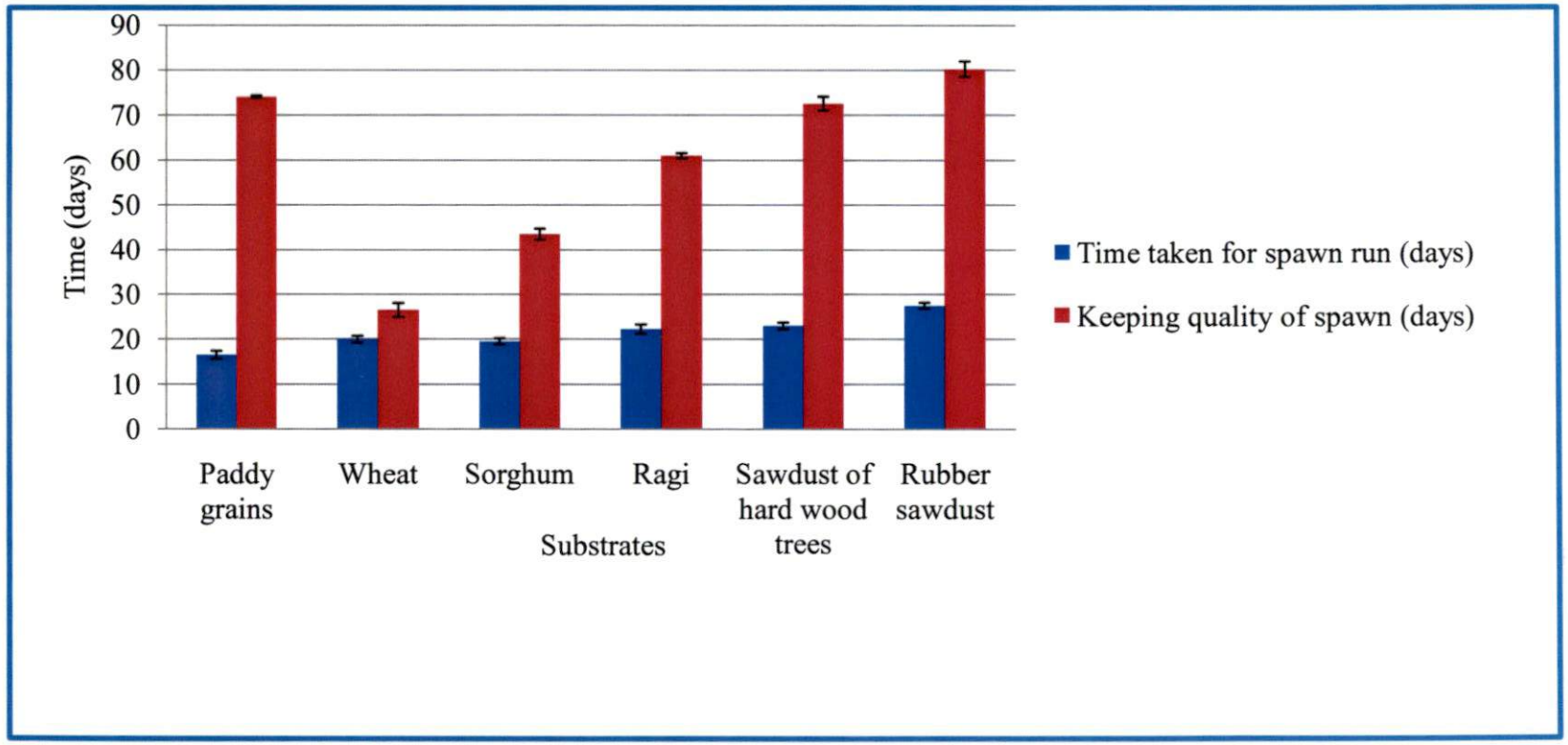


Figure 5. Influence of different substrates for spawn production of *A. polytricha* - A2

in this study may be attributed to a greater food reservoir and important nutrient support of paddy grains that eventually distributed equally for the mushroom inoculant so that each individual grain become coated with the mycelium turning it into mycelial capsule. The other factors that may have influenced the rate of mycelial growth was vigour, nature of the inoculants used and aeration of the grains.

Stamets (2000) recommended dense and thick mycelia to be used for inoculation of grains for spawn production. High rates of colonization may be attributed to the presence of nutritional ingredients such as carbon, nitrogen, lipids and minerals (Beyer and Wilkinson, 2002; Narain *et al.* 2008). The above observations were similar to studies conducted by Vidyaresmi (2008) who reported that in paddy grains *Auricularia* spp. took shorter period of 18 days to complete mycelial run whereas, Onyango *et al.* (2011a) reported that the spawn production of *Auricularia* using millet and sorghum grains helped in successful colonization of these grains by mycelia to produce high quality spawn. Devi *et al.* (2015a) recorded sorghum + wheat bran (3:1) as the best one compared to all the other treatments used and this substrate combination was able to complete mycelial run of *Auricularia* in a minimum period of 36 days.

In case of other mushroom species *viz.*, *Pleurotus florida*, *P. djamor*, *P. citrinopileatus* and *P. fossulatus*, sorghum was found to be the best for sporophore development, total yield and number of mushrooms (Geetha *et al.*, 2002).

In the same study, contaminant fungi *viz.*, *Aspergillus* sp., *Rhizopus* sp. and *Trichoderma* spp. were observed in almost all the substrates used and the per cent contamination was lower in sawdust of hard wood trees (24 % and 28 %). The maximum keeping quality was obtained in rubber sawdust spawn for the two isolates A1 and A2 (82.50 and 80.25 days) followed by paddy grain. Rubber sawdust was the only substrate that produced primordia as well as fruiting body in the spawn. The result obtained in the present study is in accordance with the report of Thiribhuvanamala *et al.* (2005) who mentioned the suitability of sawdust

for the production of *A. polytricha.* They also reported that a combination of 80 % sawdust, 10 % rice bran and 10 % wheat bran is ideal for maximum mycelial growth in a period of 14 days, whereas sawdust alone attained very sparse growth of mycelia in a maximum period of 17 days. Similar reports were also made by Kwon (2004), Vidyaresmi (2008) and Deepa (2016).

5.6. SUBMERGED CULTURING

Submerged culture production of mushroom is a novel biotechnological process for obtaining pharmaceutical substances having anticancer, antiviral, immunomodulating and antisclerotic action from fungal biomass (Lomberh *et al.*, 2002). Attempts were made for the submerged culture production of A1 and A2 isolates of *A. polytricha* in three different liquid media at optimum temperature of 25 ^{0}C and rotary action of 120 rpm for evaluating the production of mycelial pellets. Among these, malt extract broth supported the highest weight of dry mycelial pellets of 1.05 g for A1 and 1.01 g for A2 (Figure 6). Similar observation in *Auricularia auricula* was made by Devi *et al.* (2015b) which produced a very good biomass in malt extract broth.

The present study was in accordance with the higher biomass production of *A. polytricha* as reported by Yang *et al.* (2002) who noticed that the yield of *A. polytricha* was maximum at 20 ^{0}C, whereas for exopolysaccharide 30 ^{0}C was found optimum. Jeong *et al.* (2004) reported maximum production of both mycelia (10.5 g^{-1}) and exo-polysccharide (0.5 g^{-1}) of *Auricularia* spp. at 25 ^{0}C.

Wu *et al.* (2006) stated that *A. auricula* required a temperature range of 22 to 34 ^{0}C and 28 ^{0}C was found optimum for both mycelial growth and exopolysaccharide production under submerged conditions. Cho *et al.* (2006) cultivated *T. fuciformis* in shaken flasks at various temperatures (25, 28, 31 and 34 ^{0}C) and the higher temperature (34 ^{0}C) showed lesser dry weight. Jonathan *et al.* (2009) reported that the mushroom *A. polytricha* produced the best mycelial biomass (340 mg cm^{-3}) at 25 ^{0}C in shaking condition.

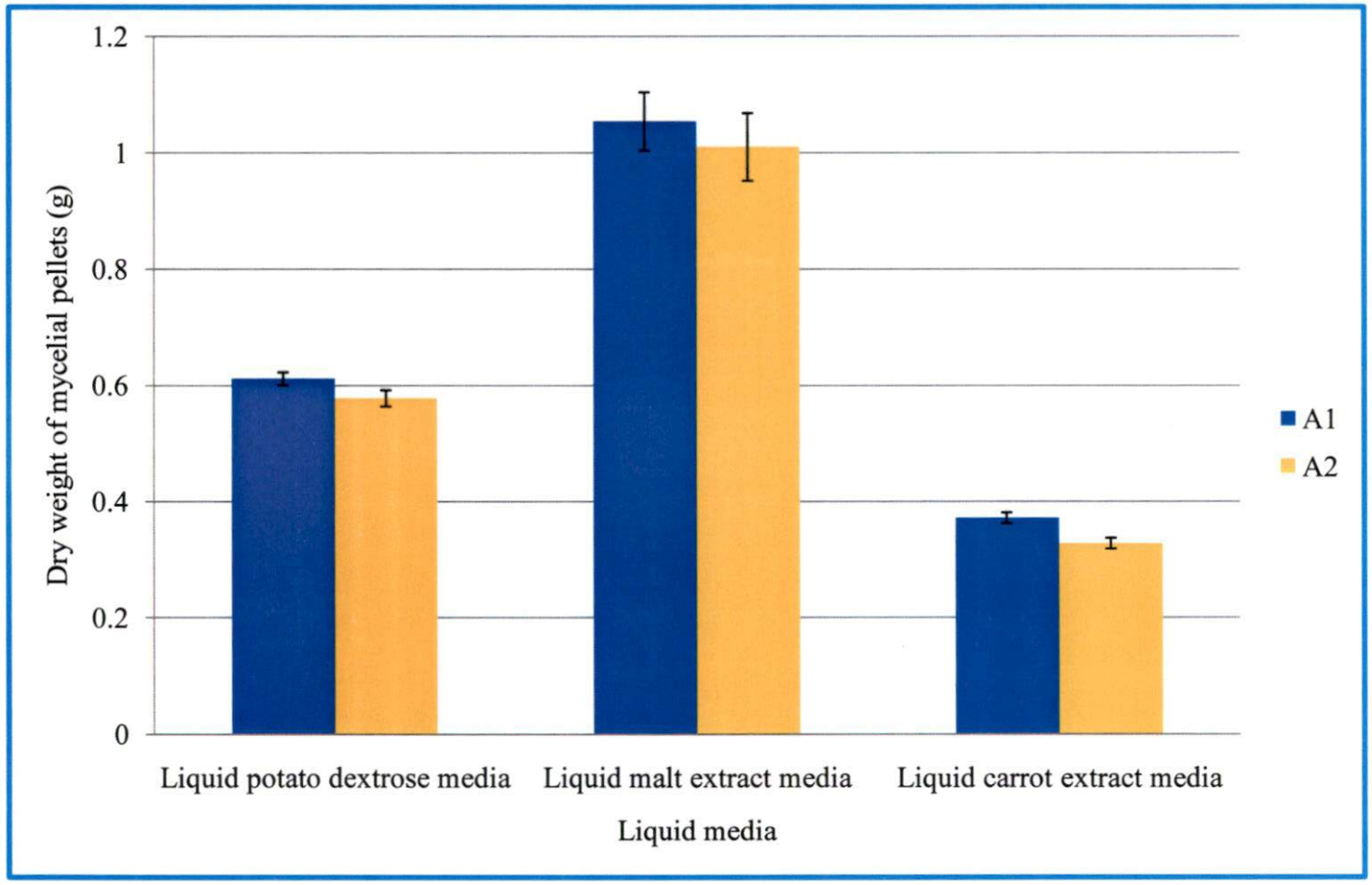


Figure 6. Evaluation of different liquid media on submerged culture production of *A. polytricha* – A1 and A2

Mycelial pellets produced in the present study were small, medium to big sized and light creamy to white coloured. Maziero *et al.* (1999) reported that the pellets of *A. fuscosuccinea*, had a gelatinous consistency and the culture filtrate was very clear.

5.7. CULTIVATION

The cultivation of *Auricularia* was first reported in China during 600 A.D. *A. auricula-judae* and *A. fuscosuccinea* were tried for cultivation for the first time (Lou, 1978; Quimio, 1979). According to Chang and Miles (2004) and Nilanjana *et al.* (2001), the ability of a mushroom species to colonize a given substrate depends on the nutritional status of the substrate, the ability of the mushroom to produce lignolytic enzymes that can break down a wide range of plant matter, cultural practices and the environmental conditions during cultivation. Carrera *et al.* (2002) reported that wood ear mushroom has the capacity to grow on a wide variety of agriculture wastes containing cellulose, hemicelluloses and lignin due to their lignolytic enzymes that are necessary for degradation of such substrates.

In the present cultivation trials, different substrates *viz.*, paddy straw, sawdust of softwood trees, rubber sawdust, banana pseudostem, sugarcane bagasse and neopeat were evaluated for the fruiting body production of *A. polytricha.* Observations such as time taken for complete spawn run, time taken for pinhead formation, time taken for first flush, total crop period, average weight of sporocarp, number of sporocarps, total yield from three harvests and biological efficiency were recorded. Mandeel *et al.* (2005) reported that biological efficiency was calculated from fresh weight of fruiting bodies divided by dry weight of substrates and expressed in percentage.

Substrates were chemically sterilized by formalin (500 ppm) and bavistin (75 ppm) along with steam sterilization. Similar experiment was conducted by Vijay and Sohi (1987) who reported chemical pasteurisation using formalin (500

ppm) and bavistin (75 ppm) for 18 h as the most effective method of sterilization of substrates for mushroom production.

Among the different substrates tried paddy straw completed spawn run with minimum time (27.25 and 27.75 days) for A1 (Figure 7) and A2 (Figure 8) and it also showed minimum time for pinhead formation. Paddy straw, a rich source of cellulose favoured mycelial growth of different types of mushrooms as reported by Suharban (1987). Nguyen (2004) reported that the production of mushrooms was proportional to the amount of cellulose present in the substrate. Chang (2006) attributed differential mycelial growth rates in mushrooms to requirements for higher concentrations of simpler carbohydrates such as cellulose than lignin. Narain *et al.* (2008) reported that mycelial growth and primordial development is dependent on the nutritional content of the substrate, especially the C:N ratio which is attained by using the right proportions of substrates. The increased level of nutrient available at higher rates would provide more energy for mycelial growth and primordial formation (Yang *et al.*, 2013). In the present study, even though paddy straw was found to be the best substrate for spawn run, the fruiting bodies produced were very low and only rudimentary primordia were produced. The proper development of fruit bodies in to mushrooms was absent, which may be due to the early decomposition and utilisation of substrate by the mushroom fungus for the mycelial growth. Similar findings were reported by Deepa (2016) during the cultivation of *Lentinula edodes*.

According to Stamets (1993), the ideal moisture content of the substrate should be between 60 and 75 per cent. Philippoussis *et al.* (2001) and Chang (2006) concluded that the duration from spawn running to pinning of mushrooms is highly dependent on free circulation of moisture and air in the substrate and another factor that influences rapid pinning is the availability of free simple carbohydrates in the substrate.

Among all the substrates rubber sawdust was found to be the best and the only substrate to produce fruiting bodies for both A1 and A2. It recorded a

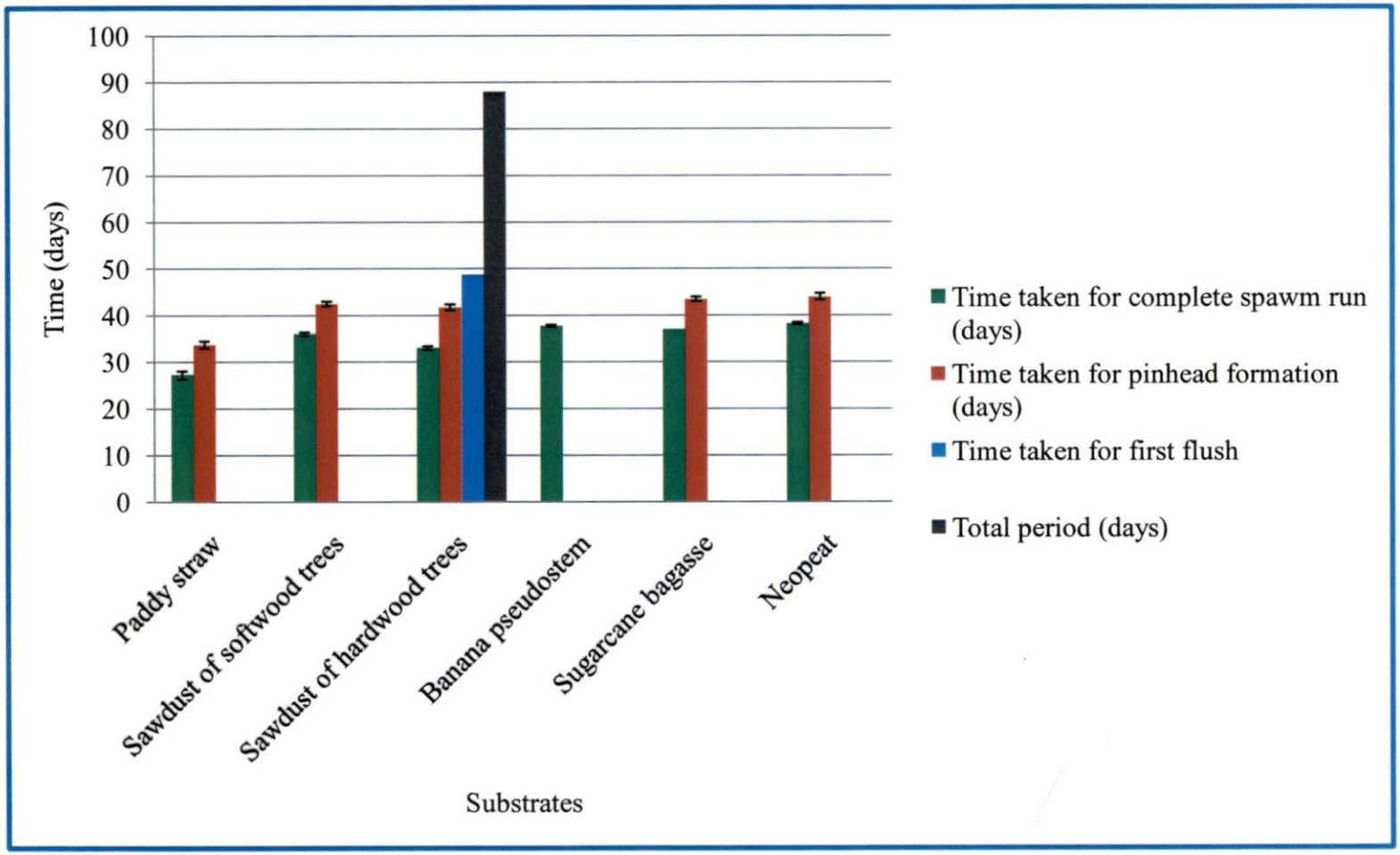


Figure 7. Influence of different substrates on growth stages of *A. polytricha*- A1

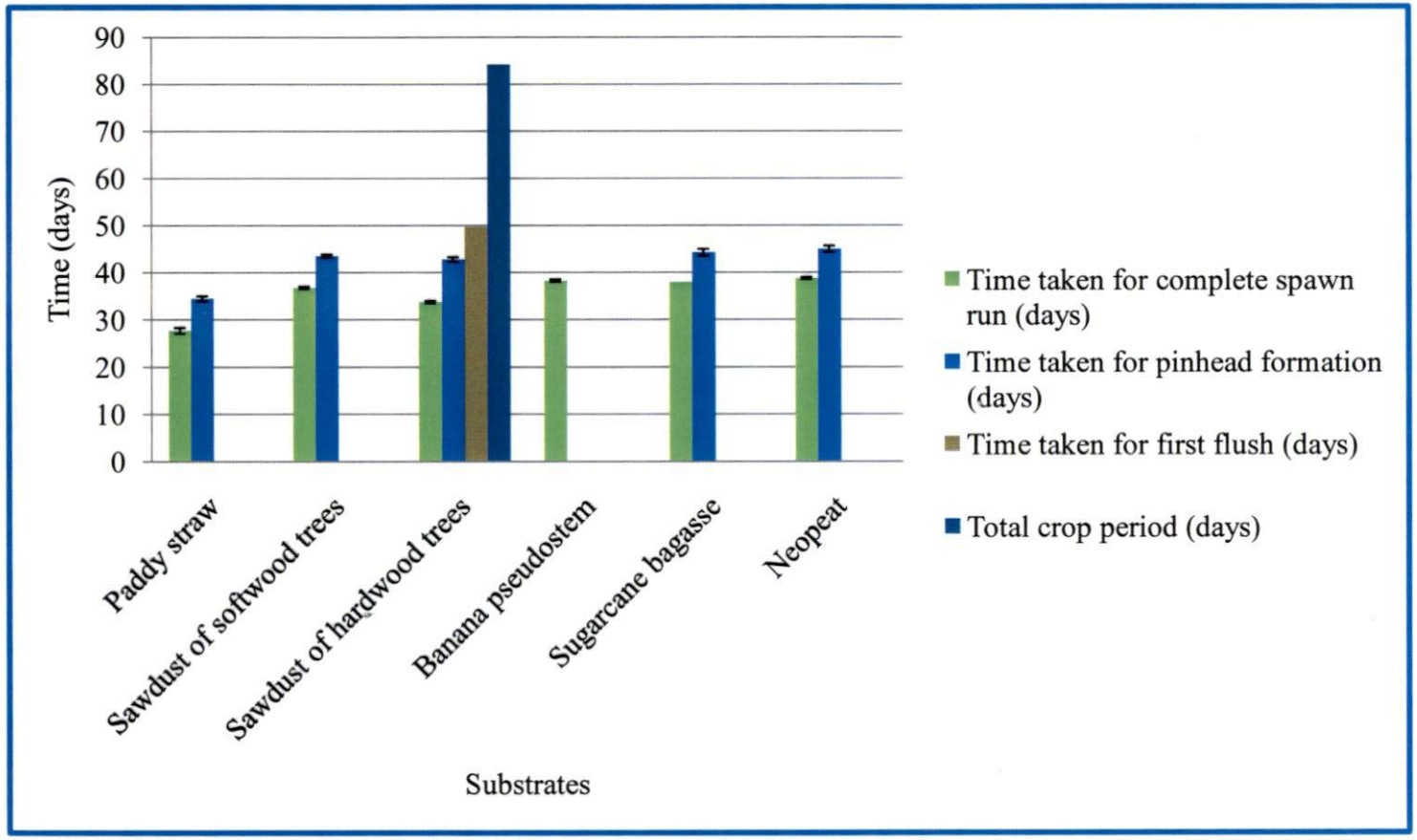


Figure 8. Influence of different substrates on growth stages of *A. polytricha* – A2

biological efficiency of 50.6 % and 48.6 % respectively from eight harvests in the total cropping period of 88.0 and 84.25 days. The nutrient contents of rubber sawdust is higher than that of other sawdust and it contained 1.68 % of N, 0.48 % of P, 1.18 % of K, 0.12 % of Ca and 0.04 % of Mg and this sawdust had particles of uniform size and structure which facilitated enrichment of substrate and more area for colonization by the mushroom fungus. Similar observations were made by Ohga (2000) who reported wood waste as an effective cultivation medium for *Auricularia polytricha*, which facilitated fast mycelial growth and fruit body formation. Nguyen (2004) reported that rubber sawdust is recognized to be very suitable for cultivation of *Pleurotus* spp., *Auricularia* spp., *Lentinula* spp. and *Ganoderma* spp.

The present results are in accordance with Vidyaresmi (2008) who reported that rubber sawdust when used as a substrate for the growth of *Auricularia* gave maximum yield of 180.67 g in three harvests. Irawati *et al.* (2012) reported that the highest fruiting body yield was recorded in the sawdust of *Falcataria moluccana.* Razak *et al.* (2013) recorded a maximum yield of *A. polytricha* (288.9 %) from the substrate combination of sawdust + empty fruit bunch of oil palm (50:50) along with ten per cent spent grain. Inyod *et al.* (2016) also reported sawdust as the most effective substrate for the production of the five different strains of *Macrocybe crassa* and the days taken for pinhead initiation was about 12 to 16 days after casing. Mathew *et al.* (1991) also reported the suitability of rubber sawdust for oyster mushroom cultivation. Sumi (2016) reported the suitability of rubber sawdust for the cultivation of *H. ulmarius.*

The mycelial colonisation as well as mushroom production was very low in sugarcane bagasse, which might be due to the presence of contaminants and competitor moulds attracted by the higher sugar content of the substrate. Nguyen (2004) reported that sugarcane bagasse contained 45-55 % cellulose, 20-25 % hemicelluloses and 18-24 % lignin and a rich source of xylan which favoured the

growth of competitive saprophytic fungi. The lowest productivity of oyster mushroom in sugarcane trash was also reported by Patil and Jadhav (1991).

Neopeat was the least effective substrate in terms of both growth as well as yield parameters in the present studies. Neopeat is the registered trade name of cocopeat. The cocopeat can hold great amounts of water, just like a sponge and is loaded with cellulose and lignin (Mason, 2003). Sherin (2003) reported that coirpith did not supported sporocarp production of both *Pleurotus florida* and *Calocybe indica*. Sumi (2016) also obtained similar results which is in accordance with the present study. The low yield may be due to high content of fibrous material, lignin and tannin in coirpith. Sherin (2003) reported that the low yield of mushrooms from cocopeat could be due to the high amount of hard lignocellulosic fibre, which the fungus is unable to digest and utilize for its growth. The study also reported that the structural features of cocopeat limit the sites for enzymatic attacks as the lignin surrounding the cells acts as a physical barrier. Coirpith being a rich source of lignin, the fungus must produce sufficient amount of enzymes like cellulose, laccase, polyphenol oxidase and ligninase to exploit the substrate.

The mushroom beds were kept in mushroom house where favourable climatic conditions were provided. Moisture plays an important role in the growth and development of *Auricularia* mushrooms. In the present trials, ice cold water was sprayed three to four times a day on the beds in order to maintain temperature (25 ±2 ^{0}C) and RH (> 80%) for inducing fruit body production. However, similar observations made by Razak *et al.* (2013) who reported that maintaining RH above 85 % in the experimental mushroom house by spraying water is a crucial step to promote good development of *A. polytricha* sporophores. Devi *et al.* (2015a) reported the optimum temprtature of 24±2 ^{0}C with a relative humidity of 80-85 % for spawn run, 20±2 ^{0}C for pinhead formation and 22±2 ^{0}C for fruiting body development of *A. polytricha*. Owaid *et al.* (2015b) reported that the temperature can be changed to 10-16 ^{0}C by cold shock to induce fructification of *Pleurotus ostreatus*. Sher *et al.* (2010) reported a positive relationship between

temperature and growth of mushroom mycelium *i.e.*, when temperature increased the mycelial growth also increased, which resulted in the shortening of spawn run time and they also reported that lower relative humidity delayed the formation of fruiting bodies from the opened bags. Bellettini *et al.* (2016) stated that the suitable environmental conditions for mycelial growth and subsequent fruiting are very diverse. Li (2015) reported that the optimum humidity during the spawn-running and mycelial stimulation was at a range of 60-75% and 85-97% respectively that enabled the satisfactory growth of *Pleurotus* spp. High humidity is highly congenial for pining and fruiting (Pandey *et al.*, 2008), which is also observed in the case of *Auriculaia polytricha.*

5.8. AMENDMENTS

Amendments are the nutritional supplements that regulate the yield as well as biological efficiency of the mushrooms. Isikhuemhen *et al.* (2000) and Okhuoya *et al.* (2005) have opined that supplementations of substrate improved the production, quality, flavour and shelf life of cultivated mushroom. Iqbal *et al.* (2005) and Chang (2008) reported the need for average amounts of supplements since the nitrogen component is usually required in moderate quantities by most mushrooms including the wood ears.

In the present studies five different amendments were evaluated for the fruiting body production of *A. polytricha* - A1 and A2 on the basis of both growth and yield parameters as in substrate studies. The amendements used were wheat bran, rice bran, cotton seed hull, neem cake and groundnut cake @ 2 and 4 % concentration with rubber sawdust as basal substrate. Wheat bran 2 % was the best amendment with minimum time taken for complete spawn run (32.33 and 34.0 days), pinhead formation (37.66 and 40.66 days) and first flush (44.33 and 48.33 days) (Figure 9 and 11). It may be due to high amount of aminoacids, enzymes and proteins that indirectly provided nitrogen to the fungus to support the maximum mycelial growth. The results are in accordance with study conducted by Oei (2003) and Onyango *et al.* (2011a) who confirmed the enhanced

performance of mycelia on availability of several amino acids and protease as well as transaminase enzyme activities on wheat bran. Shen and Royse (2001) reported that the quality of mushrooms was highly affected by the levels of brans in the substrates and that low concentrations can improve production. Uhart *et al.* (2008) analyzed the nutritional values of different supplements and concluded that wheat bran contained better quality nutrients that increased the rate of mycelial growth. Bran supplementation provided a protein rich medium which increased the rate of mycelial growth two fold (Onyango *et al.*, 2011a).

All the yield parameters were the highest in substrate amended with 2 % rice bran. It showed biological efficiency of 18.6 % and 15.9 % from three harvests (Figure 10) and 56.3 % and 52.0 % from eight harvests (Figure 12). This is in accordance with the work of Bhandal and Mehta (1986) who cultivated *A. polytricha* on fresh as well as composted wheat straw supplemented with rice bran and reported 60-80 % biological efficiency. Quimio (1982) reported that *Auricularia* spp. could be cultivated on sterilised sawdust mixtures and the most successful mixtures included rice bran (2-20 %) that provided the essential vitamins and minerals. Irawati *et al.* (2012) used commercial rice bran as a nutritive additive and mixed in a ratio of 8:1 for wood meal and rice bran to obtain maximum yield for *A. polytricha.* Similarly, Lau *et al.* (2014) reported the highest biological efficiency of *A. polytricha* (34.4 %) on rubber wood sawdust with rice bran (80:20). The present study also strongly recommends the use of rubber sawdust with 2 % rice bran amendment as an ideal medium for the cultivation of *A. polytricha.*

Kushwaha *et al.* (2006) reported that the yield performance of *A. polytricha* on rapeseed straw amended with 4 % wheat bran was found maximum *i.e.* 483.00 and 473.15 g per 500g dry substrate (96.6 % and 94.63 % biological efficiency) and the poor yield was recorded on paddy straw alone without any amendments. Kapoor *et al.* (2011) recorded the highest biological efficiency of *A. polytricha* of 73.6 % from wheat straw supplemented with 4 per cent wheat bran. Contradictorily, in the present study, even though good spawn run was noticed on

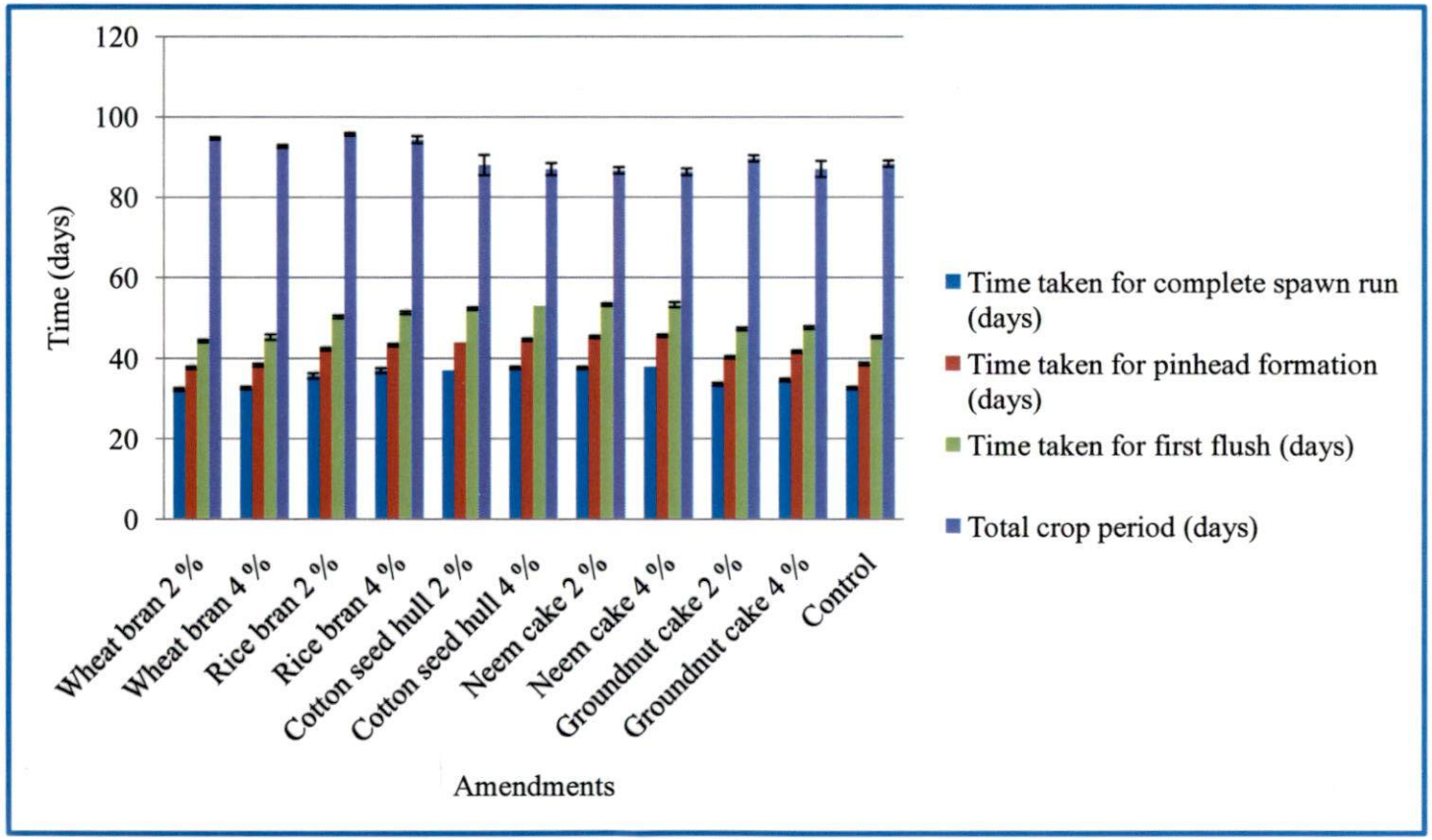


Figure 9 . Influence of different amendments on growth stages of *A. polytricha* –A1

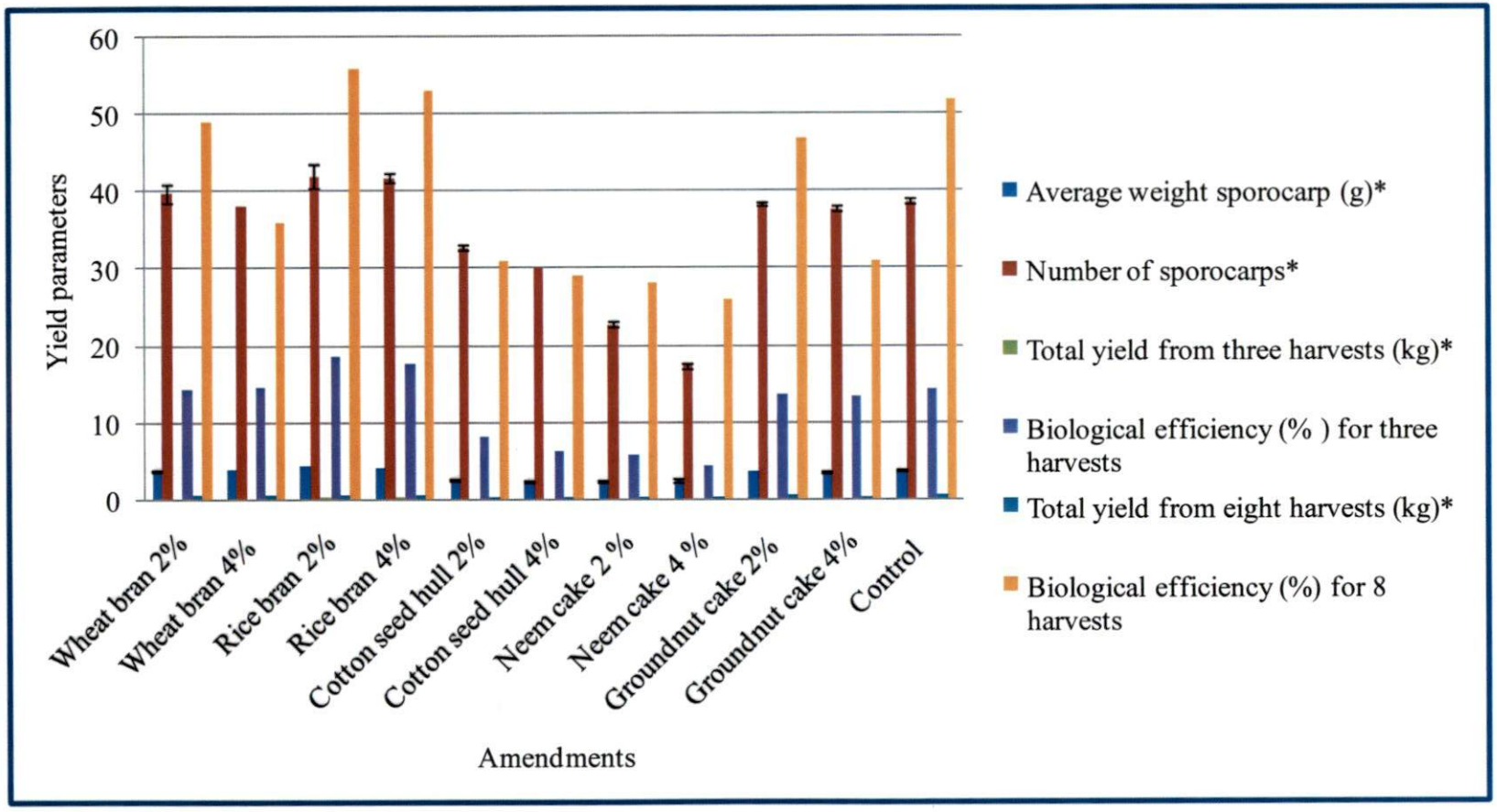


Figure 10. Influence of different amendments on the yield parameters of *A. polytricha* –A1

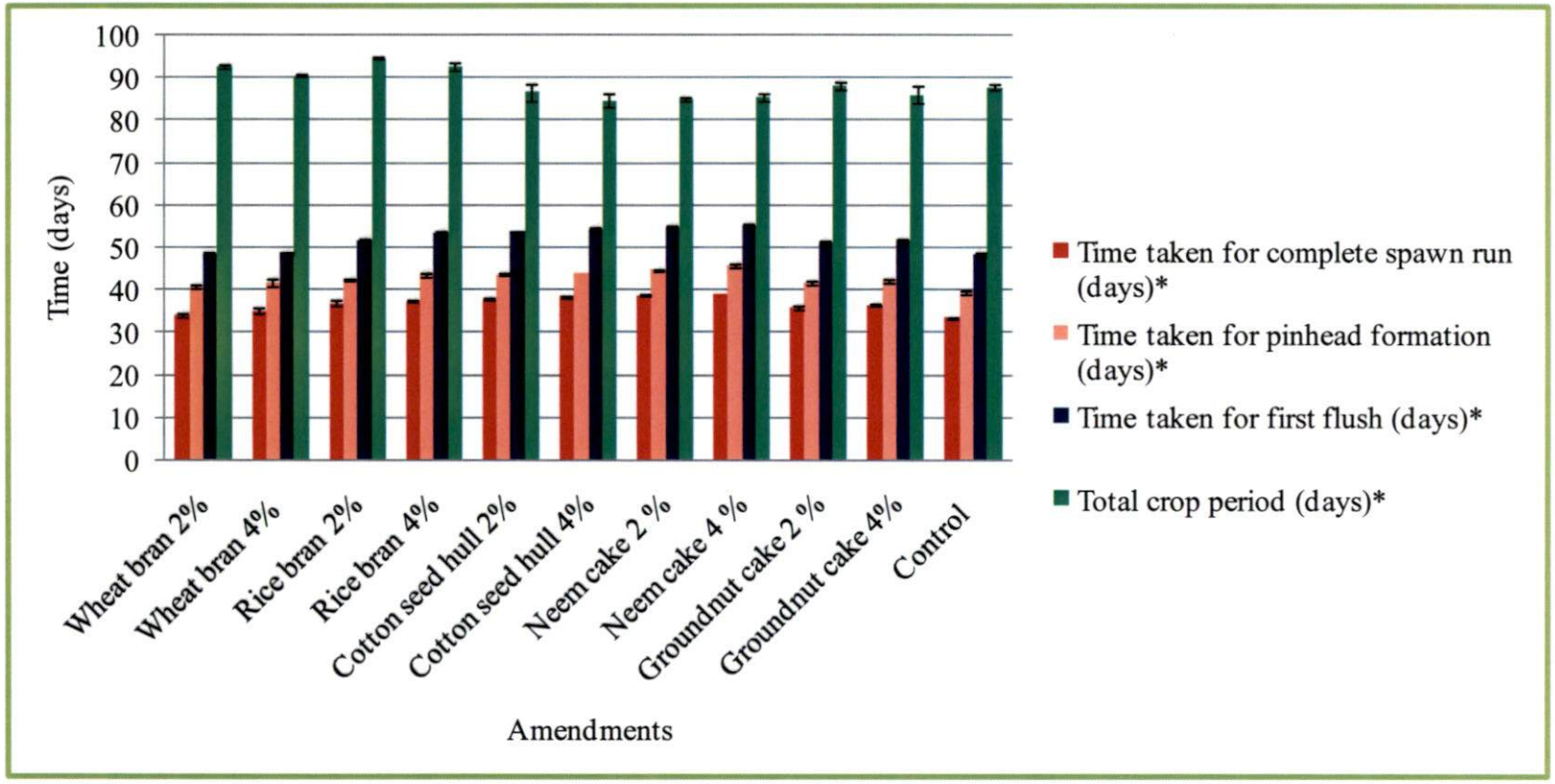


Figure 11. Influence of different amendments for growth stages of *A. polytricha* –A2

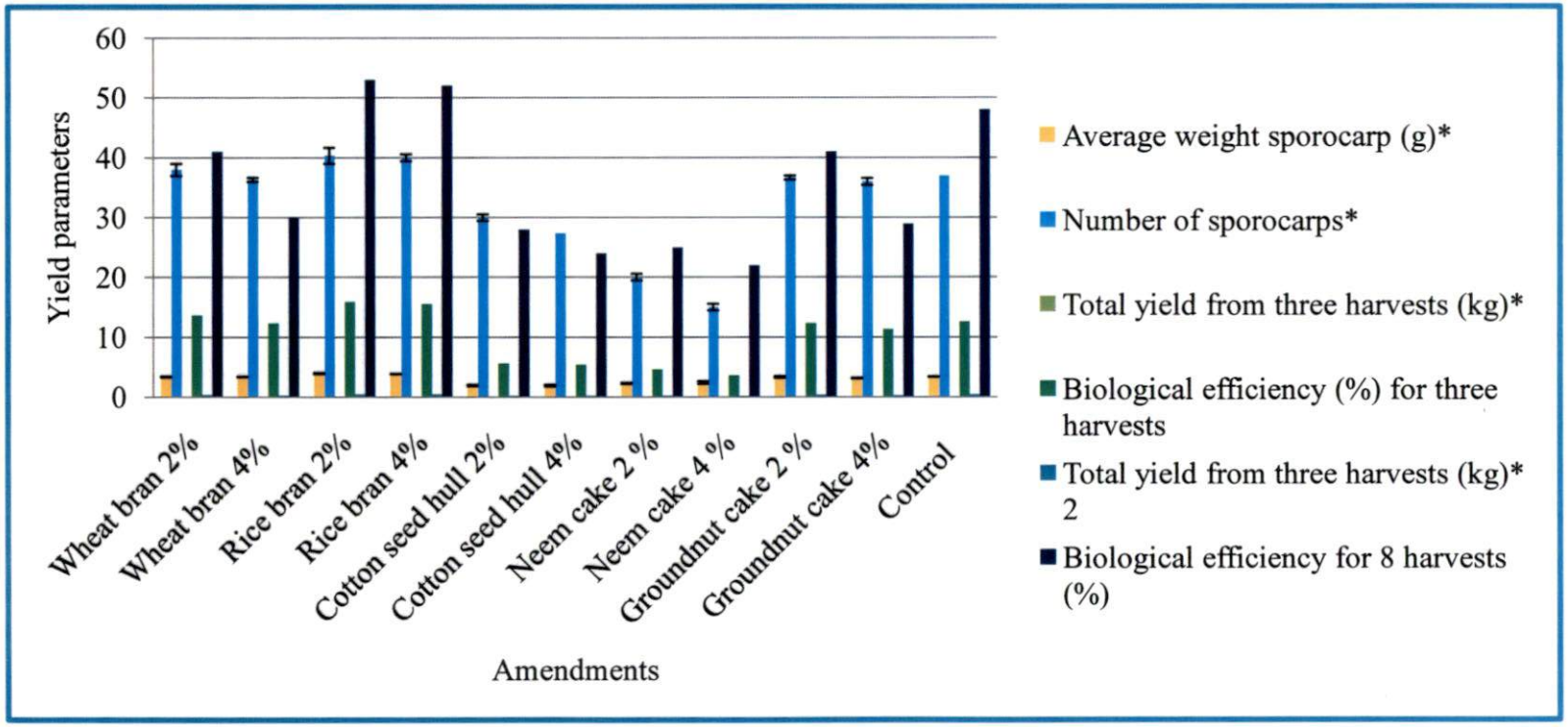


Figure 12. Influence of different amendments on the yield parameters of *A. polytricha* –A2

paddystraw beds, very few pinheads appeared and they did not develop in to sporocarps. Onyango *et al.* (2013) recorded the heaviest mushrooms as well as the highest fresh weight of 282 g from composted maize cobs at 10 % wheat bran level and absence of supplementation lowered the fresh weight with the least being 78 g. Devi *et al.* (2015a) recorded the highest yield of *A. polytricha* (155.0 g) from paddy straw + wheat bran (3:1) combination with a high biological efficiency of 62 %.

Upadhyay (1999) cultivated *A. mesenterica* on wheat straw with different supplements and reported 91-174 % B.E. and he gave contradictory results that *A. polytricha* yielded the best in wheat straw without any supplementation. However, Oei (2003) reported that excessive supplementation can increase the risk of contamination, anaerobiosis, antibiosis and subsequently lower yields. Fanadzo *et al.* (2010) reported that supplementation may cause a rise in substrate temperature, which is possibly due to faster metabolic activities triggered by extra nitrogen.

5.9. PROXIMATE CONSTITUENTS

The fast growing mushrooms have obtained a significant amount of interest recently with the realization that they are good source of delicious food with considearable amount of nutritional attributes and the determination of nutritional value needs analysis of proximate constituents. Chang and Miles (2004) reported that composition of a mushroom was affected by diversity in genetic frame work, environmental conditions and nature of the substratum. It was also influenced by the stage of development and the type of postharvest storage.

The chemical composition of mushroom depends upon the species as well as the substrate in which it grows and age of fructification (Manzi *et al.*, 2001). In general, chemical components contributing to large weight loss are incorporated into the fruiting bodies and partly emitted into the atmosphere as carbon dioxide

through mushroom respiration (Zhang *et al.*, 2002). Nutritional values and organoleptic properties of edible black fungus are attributed to its chemical composition.

In the present studies both A1 and A2 were evaluated for per cent content of moisture, carbohydrate, protein, fat, fibre, ash, total antioxidants, β-carotene, polyphenols and energy content. Kadnikova *et al.* (2015) reported that *Auricularia* spp. contained 3.6 g of ash, 12.5 g of protein, 1.7 g of fat per 100 g of dry matter and contained minerals such as Ca, K, Mg, Fe, Zn and low level of toxic elements like Pb and Cd. They also reported that chemical content proved this mushroom as a valuable raw material to produce low-calorie dietary food, as well as a good source of biologically active polysaccharides and essential aminoacids.

Moisture content considerably affects the nutritional value of any food item. It is the most variable component in mushrooms and is influenced by genotype, stage of growth, environmental conditions and postharvest storage before analysis (Rai, 1995) and they also observed that fruit bodies collected from an atmosphere with high humidity showed high moisture content and vice versa. The moisture content of A1 was found to be (90.12 %) on fresh weight basis which was high compared to that of A2 (89.91 %). Gbolagade *et al.* (2006) reported a higher moisture content of 97.1 % in *A. polytricha* and Goyal *et al.* (2010) reported 95.3 %. However, Kakon *et al.* (2012) reported a low moisture content of 87.1 % in *A. polytricha* on fresh weight basis. The present finding is in accordance with the report of Usha and Suguna (2014) who stated that the moisture content of *A. polytricha* was 90.6 %. Chang *et al.* (1981) and Ragunathan and Swaminathan (2003) reported similar results on the moisture content of fresh mushrooms as about 90 %.

Edible mushrooms are highly valued as a good source of carbohydrates and their contents usually ranged from 40.6 % to 53.3 % of dry weight as reported by Khanna *et al.* (1992) and Ragunathan *et al.* (1996). Carbohydrates of mushrooms

are not nutritionally important so far as calories are concerned but recently much interest has been noticed in the characterization of components in water-soluble polysaccharides obtained from mushrooms due to their ability to inhibit tumour growth (Chang and Miles, 2004).

Appreciable amount of carbohydrates was found in A1 (47.1 %) and A2 (48.8 %) on dry weight basis. The results are in accordance with the analysis conducted by Goyal *et al.* (2010) who reported that the carbohydrate content of *A. polytricha* was 58.4 % whereas, Kakon *et al.* (2012) reported 87.6 % carbohydrates in *A. polytricha.* However, Sylvester *et al.* (2014) reported the carbohydrate content as 42.82 % in *A. auricula,* which is in agreement with the present findings.

Kadnikova *et al.* (2015) explained that in *A. auricula,* carbohydrates were found to be the major nutrient, constituting 66.1 g/100 g of its dry matter and considerable proportion of the carbohydrate compounds were in the form of polysaccharides and these polysaccharides were represented by indigestible forms such as 38.8 % of uronic acids, 10.2 % of water-soluble polysaccharides (mannans and glucans), 7.4 % of pectin, 5.4 % of chitin and 4.3 % of cellulose, which are important for proper functioning of gastrointestinal tract.

In general, mushroom possesses high protein content which has been recognised by Food and Agriculture Organisation (Rai, 1995). The chitin nitrogen is responsible for high protein value (Crisan and Sands, 1978; Bano and Rajarathnam, 1982; Rai *et al.*, 1988). The protein content in mushroom is generally higher than those in green vegetables (Chang, 1980 and Jonathan, 2002). Chang and Miles (1989) reported that the protein content of edible mushrooms ranged from 19 to 35 % on dry weight basis. The present studies indicated that, A1 recorded a protein content of 18.06 % on dry weight basis compared to 20.75 % in A2. Goyal *et al.* (2010) obtained similar observations of protein content in *A. polytricha* as 22.6 %. Hung and Nhi (2012) indicated that the protein content of *Volvariella volvacea* as 36.5 %, *Pleurotus ostreatus* as 28.6

%, *Lentinus edodes* as 26.3 %, *Genoderma lucidum* as 13.3 % and *A. polytricha* as 7.2 %. Kakon *et al.* (2012) also reported that protein content of *A. polytricha* was 7.7 %. Sylvester *et al.* (2014) reported the protein content of *A. auricula* as 25.37 % and Usha and Suguna (2014) recorded that in *A. polytricha* as 36 %.

Crude fat in mushrooms represents all classes of lipid components. Soxhlet extraction apparatus was used for the estimation of fat content in mushroom powder. The fat content of A1 was estimated to be 0.76 % and that of A2 to be 0.56 % on dry weight basis. Almost similar observations were made by Kakon *et al.* (2012) who reported that the fat content of *A. polytricha* was 0.8 %. Sylvester *et al.* (2014) recorded the fat content as 1.12 % in *A. auricula,* while, Usha and Suguna (2014) reported a higher value of 3.40 % fat.

Studies conducted on the analysis of crude fibre content revealed that A1 had more fibre (17.69 %) than A2 (15.49 %) on dry weight basis. Similar observations were obtained from the studies conducted by Usha and Suguna (2014) and Sylvester *et al.* (2014) who reported that crude fibre content in *A. polytricha* was 17.85 to 22.35 %. A1 recorded an ash content of 5.5 % on dry weight basis and that of A2 was 5.3 %. The ash content in the present study is in accordance with study made by Gbolagade *et al.* (2006) who reported that the ash content of *A. polytricha* was 5.2 %. Johnsy *et al.* (2011) reported a higher value of 8.7 % ash in *Auricularia* sp. and Kakon *et al.* (2012) recorded 14.0 % on fresh weight basis. Sylvester *et al.* (2014) recorded the ash content of 18.2 % in *A. auricula* which was comparatively higher than the present studies. Usha and Suguna (2014) reported the ash content to be 5.2 in *A. polytricha* and 7.93 in *P. ostreatus.*

A1 recorded maximum total antioxidants of 116 µg and A2 recorded 74 µg. Hung and Nhi (2012) reported the antioxidant property of *A. polytricha* as 0.7 %. Emre *et al.* (2016) reported that *A. polytricha showed* 0.91 µmol Trolox of antioxidant activity. Beta-carotene is the principal compound present in a few mushrooms where they play a vital role in metabolism and bio-synthesis of other

bio-molecules (Ullah *et al.*, 2011). The β-carotene content was found to be the highest in A1 (0.178 µg) than that of A2 (0.150 µg). Robaszkiewicz (2010) reported that the *β*-carotene content varied considerably between the analyzed edible mushroom species and it ranged from 0.233 to 15.256 µg/g of dried fruiting bodies. The highest content was found in methanolic extracts of *Tricholoma equestre.*

Analysis of polyphenols was done by Folin-ciocalteu method and the results showed that the highest amount of polyphenols was present in A1 (9.53 µg) than that of A2 (6.89 µg). However, low content of polyphenols (2.90 %) was reported in *Auricularia auricula* by Boonsong *et al.* (2016). Sudheep and Sridhar (2014) reported that total phenolic content of *Agaricus abruptibulus* was 2.29 g and 2.98 g for *Termitomyces globulus.*

The analysis of energy value was done based on the contents of carbohydrate, protein and fat present in the sample. The energy value was more in A1 (251.49 Kcal) and less in A2 (264 Kcal). Kakon *et al.* (2012) reported a higher energy value of 347 Kcal for *A. polytricha*. *A. auricula-judae* recorded a calorific value of 1370 KJ/100 g of dry matter (Kadnikova, 2015). The energy value of *Agaricus abruptibulus* was 1433 KJ/100 g and that of *Termitomyces globulus* as 1413 KJ/100g as reported by Sudheep and Sridhar (2014).

5.10. SENSORY EVALUATION

Mushrooms are basically consumed for their texture, flavour and aroma. Mushrooms can solve world's food shortage problem since they occupy a place above vegetables and legumes (Boa, 2004). Das (1994) reported that the protein in mushroom is considered to be of high quality containing all the essential amino acids required for an adult individual. The amino acids - tryptophan and lysine - which are absent in vegetable protein are present in mushroom proteins. They are also excellent sources of minerals.

Seven different recipes were prepared in the present study by using both A1 and A2, and the recipes were pepper masala, pizza, pakoda, mushroom tomato sauce, soup, vegetable curry and bajji. The cooking quality and sensory evaluation were done based on nine point Hedonic rating scale. Among the different recipes tried, mushroom tomato sauce had maximum score for appearance (9.0), colour (8.9), flavor (8.9), texture (8.8), taste (9.0) aroma (8.8) and overall acceptability was (8.9 and 9.0) for both A1 and A2. Das (2011) prepared *Auricularia* wine and *Auricularia* fritters which proved to be excellent in taste and possessed good keeping quality. The deliciousness can be judged by colour, texture, flavour and taste and mushrooms can be used as ingredients to enhance taste and texture (Kumar and Barmanrai, 2007). Jaziya (2011) reported that inclusion of oyster mushroom was very acceptable to the respondents and it did not cause any difficulty in the acceptability of dishes prepared during the study.

Horticultural products have more keeping quality because they can be harvested before ripening, which is not applicable to mushrooms. Mushrooms are highly perishable commodities and hence, preservation has more importance to retain their freshness when there is a superfluity in the market.

The fresh samples of both A1 and A2 isolates of mushrooms remained fresh for 24 h under normal atmospheric conditions. Under refrigerated conditions (4 ^{0}C) the observations were recorded up to thirty days and it was concluded that mushrooms could be kept fresh for three days without any remarkable changes, and were acceptable for cooking. However, after thirty days it started to show tissue disintegration and complete liquefication. Minamide *et al.* (1985) reported that an increase in respiration and a decrease in contents of mannitol and trehalose occurred sharply in mushrooms kept at 20 ^{0}C, and the quality deteriorated rapidly, showing only one day of shelf life. The low temperature suppressed this deterioration, and the shelf life was extended to 15 days at 1 ^{0}C.

Rai and Arumuganathan (2008) reported that storage of mushrooms under low temperature is an excellent method for restricting deterioration of mushrooms for a certain period of time. The important changes occurring in harvested mushrooms include wilting, ripening, browning, liquefaction, loss of moisture and loss of texture, aroma and flavour. These changes are preceded by an increase in the rate of respiration which combined with other reactions and termination of the nutrient supply will activate a series of permanent reactions damaging the mushrooms (Cho *et al.*, 1982). The shelf life of mushroom can be increased by pre-treatments or storage at chilling temperature and depending on the species, the shelf life of mushrooms may vary from one day to two weeks. In the present study, the harvested mushrooms kept inside perforated polythene bags under refrigeration (4 ^{0}C) remained fresh up to three days.

Hammond and Nichols (1975) stated that the storage of mushrooms in plastic bags with perforations is vital to avoid suffocation of mushrooms. The influence of perforation is to found equilibrium between CO_2 and O_2 that reduces rate of respiration and also the moisture loss. It was noted that mushrooms were best kept at 8 to 10 ^{0}C in 100 gauge polyethylene bags of 30 μm thickness.

Similarly, Chopra *et al.* (1985) reported that button mushrooms could be kept fresh in polythene bags of 100 gauge thickness with 0.5 % ventilation under refrigerated conditions. Contradictory to this Saxena and Rai (1988) reported that mushrooms could be stored in non-perforated polypropylene bags of 100 gauge thickness and also mentioned about the adverse effects of over-ventilation of polythene packs. Dhar (1992) reported better shelf life of mushrooms in non-perforated polythene bags at low temperature compared to perforated bags at high temperature.

The shelf life of mushrooms is more in refrigerated condition than in ordinary condition. This may be due to lower temperature slowdowns the growth of micro organisms, reduces the amount of postharvest metabolic activities of the mushroom tissues and decreases the moisture loss (Rai and Arumuganathan,

2008). Shiitake mushrooms were stored up to 10 days in perforated polybags under normal condition and up to 30 days in refrigerated condition (Ramkumar *et al.*, 2010).

Sumi (2016) reported that *H. ulmarius* could be kept fresh for eight hours in normal atmospheric condition but *P. florida* for only six hours and under refrigerated conditions, they had five and three days of keeping quality respectively without any drastic changes. *L. edodes* had a shelf life of 14.66 days under refrigerated conditions whereas 4.33 days in normal room temperature (Deepa, 2016).

5.11. OUTDOOR CULTIVATION

During the present study, beds were hung on the trees where the proper cultivation conditions like shade, humidity, temperature and protection measurements were provided for outdoor cultivation by keeping indoor cultivation as control. Indoor cultivation conditions showed significant results for both growth as well as yield parameters compared to outdoor cultivation trials. It may be due to the mushrooms favoured by the appropriate temperature and humidity which was maintained artificially in the mushroom house in order to attain the maximum yield. Biological efficiency was highest in indoor method (14.7 % and 13.6 %) compared to outdoor cultivation (13.8 % and 12.3 %) for A1 and A2 respectively.

Krishnamoorthy *et al.* (2005) experimented a new approach for outdoor cultivation of paddy straw mushroom (*Volvariella volvacea*) and obtained an average yield of 1.734 kg/bed and a B.E. of 8.75 %. Prakasam *et al.* (2013) reported the maximum yield of *P. florida* (504 g), *P. djamor-roseus* (495), *P. eous* (462 g) and *P. platypus* (439 g) under outdoor cultivation. Tisdale (2006) utilised substrates from *Falcataria moluccana*, *Casuarina equisetifolia*, *Eucalyptus grandis*, *Psidium cattleianum* and *Trema orientalis* for the outdoor cultivation of *Pleurotus ostreatus* and reported that nitrogen fixing *C. equisetifolia*, *T. orientalis* and *F. moluccana* supported greater yield of 275.5, 272.4 and 268.8 g/bag

respectively than non-fixers. *P. cattleianum* (190.5 g/bag). However, the present study indicated the non-suitability of outdoor cultivation for *Auricularia polytricha* since it took more time for mushroom production, produced less yield and avoiding contamination as well as providing favourable climatic conditions proved strenuous.

5.12. YIELD PERFORMANCE OF OYSTER AND MILKY MUSHROOMS

Pleurotus florida and *Calocybe gambosa* are the most acceptable mushroom species and widely cultivated ones in Kerala. Hence, they were selected for comparative studies with A1 and A2 on paddy grains for spawn production as it was proved to be the best substrate under spawn studies and cultivation trials were conducted on the best substrate - rubber sawdust supplemented with rice bran 2 % for mushroom production.

Oyster mushroom took minimum days (14.75) to complete the spawn run compared to other mushrooms with a thick, white cottony growth of mycelia and it also took minimum time for spawn run, pinhead formation and first flush harvest (Figure 13) whereas, *A. polytricha*-A2 recorded maximum cropping period (88.50 days). Milky mushroom had the highest average weight of sporocarp (146.85 g) but the number of sporocarps was maximum in oyster mushroom (37.50). Biological efficiennncy was the highest in milky mushroom (67.0 %) followed by oyster mushroom (62.9 %) and four harvests were made, but *A. polyticha* continued to produce fruiting bodies up to eight harvests, which recorded a maximum biological efficiency of 52.8 % in A1 and 50.8 % in A2 (Figure 14). Irawati *et al.* (2012) collected fruiting bodies of *A. polytricha* eight times from the substrates made of *F. moluccana* and *Shorea* sp. and four times from the substrate made of *Tectona grandis*, whereas *P. cystidiosus* had only three flushes, and the highest yield was obtained at the first harvest, which gradually decreased in second and third harvests (Hoa *et al.*, 2015).

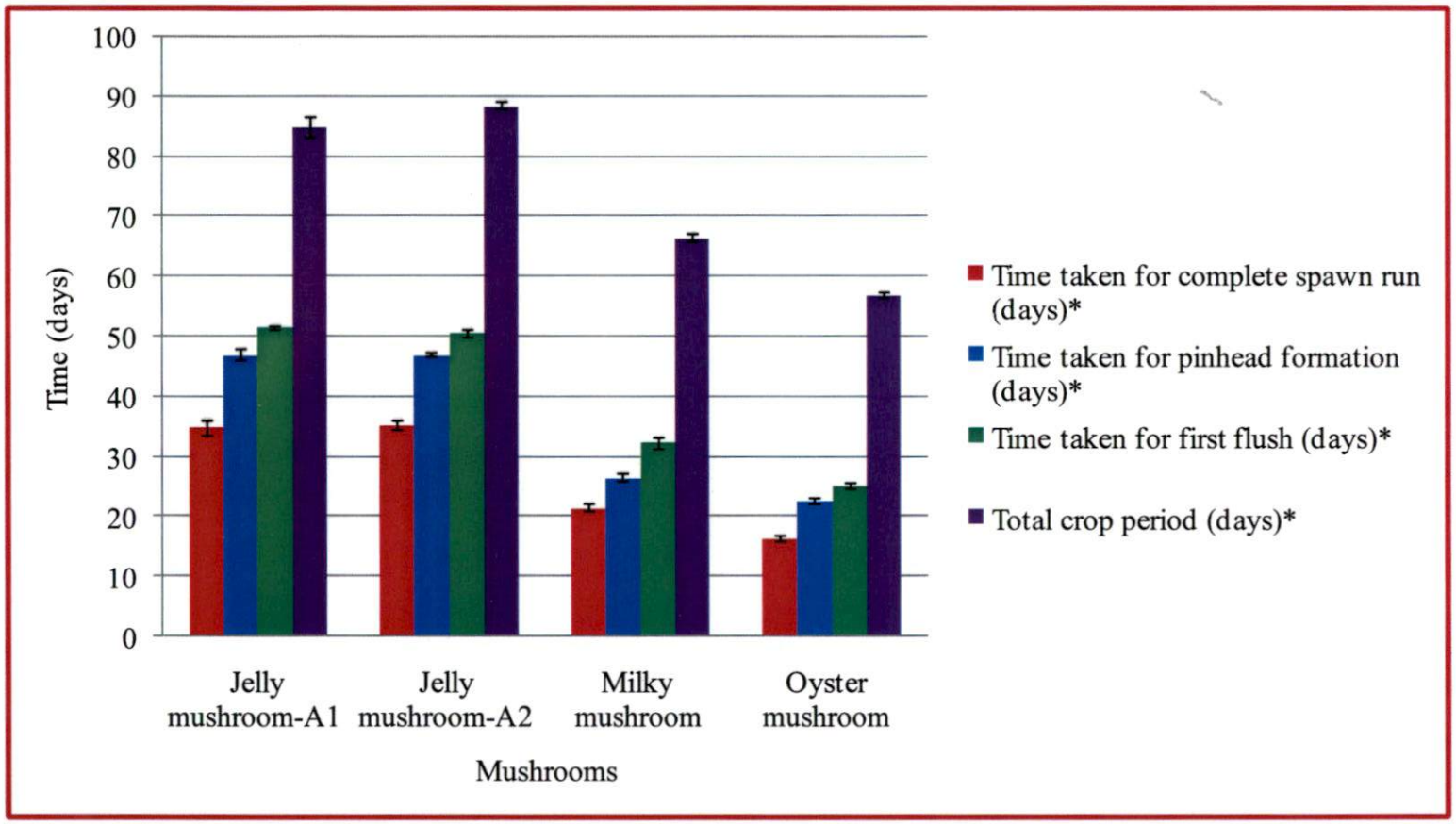


Figure 13. Comparison of growth parameters of Jelly mushrooms (*A. polytricha* -A1 and A2) with milky and oyster mushroom

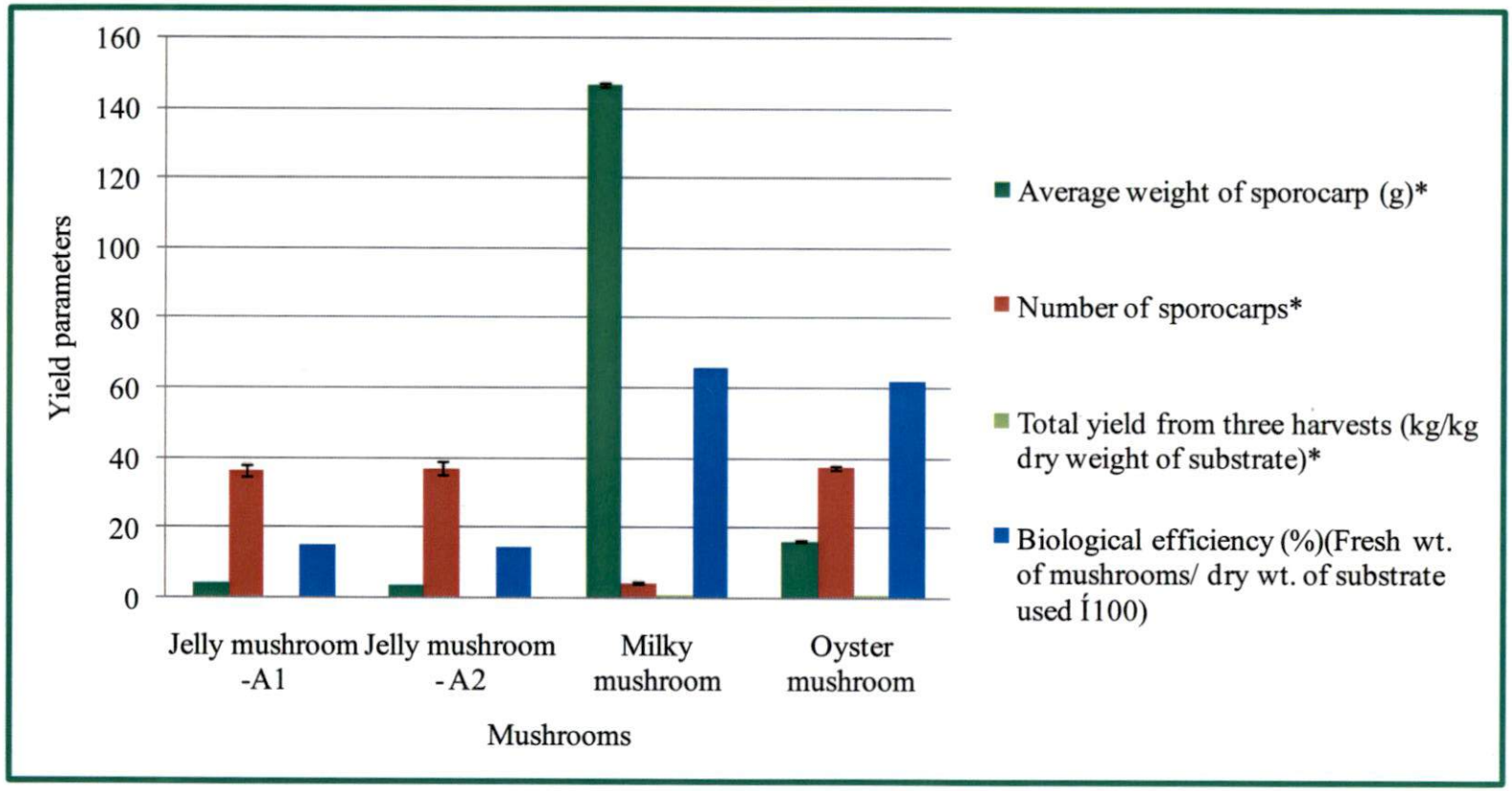


Figure 14. Comparison of yield parameters of Jelly mushrooms (*A. polytricha* -A1 and A2) with milky and oyster mushroom

5.13. DEVELOPMENTAL MORPHOLOGY

The mushroom development can be divided into two stages- the vegetative and the reproductive stage. The transition from vegetative to reproductive stage is principally controlled by environmental conditions *viz.*, light, CO_2 concentration, temperature and humidity.

The sporocarps took an average of six days from the day of pinhead formation to complete maturity. The pileus of pinheads formed on beds was creamish white coloured and changed to brown. The shape was changed from flattened or appressed to ear shaped and measured 40 mm diameter. At the time of pinhead formation, the pileus was circular with clear margin which became larger and irregular on maturity and the sporocarps were produced singly and very rarely in bunches. Abhymenial hair on the pileus was prominent and basidiospores were produced from the under surface of the pileus.

Luo (1993) reported that *Auricularia* mushrooms underwent five different stages of development starting from rice grain, coral, wood ear appearance, wood ear unfolding and maturation stage. The time for each stage was 1.5, 1.5, 2, 1.5, 2 days respectively at 24 ^{0}C and 95 % humidity. Onyango *et al.* (2011a) reported the four different shapes of *Auricularia* as ranging from cup-shaped to ear-shaped. According to them, if the fruiting body had less than 10 mm diameter, it was regarded as cup-shaped with soft texture, 11-20 mm as lobed with rubbery texture, 21-40 mm as flattened/appressed with leathery texture and >40 mm as ear-shaped with gelatinous texture.

5.14. LOG METHOD OF CULTIVATION

Poly bag method of cultivation for A1 and A2 showed supremacy over log method of cultivation by taking minimum time for primordial formation (42.50 and 44.25 days) and even total yield from three harvests was maximum in poly bag method of cultivation compared to log method of cultivation. The study concluded that maximum biological efficiency was obtained from polybag

method of cultivation compared to log method of cultivation. The reason may be because fungus required maximum time to degrade the hard substances in the logs whereas in poly bag or artificial log method, nutrients were readily available in sawdust for easy colonisation. Hence, it started to produce maximum primordia as well as fruiting bodies. The present study is in accordance with the research work conducted by Kwon (2004) who reported that log cultivation is not technically demanding and is relatively easy to carry out, but is seasonal and slow in comparison to growing mushrooms in sterilised sawdust mixtures, and hence, cannot meet demands for high productivity. He also stated that it is an open, non-sterile procedure and hence, contamination may occur easily compared to polybag method of cultivation.

5.15. PESTS AND DISEASES

The major insect pests and contaminants observed in the present studies were phorid flies (*Megaselia* spp.) springtails (*Seira* spp.), staphylinid beetles and mites. *Coprinus* spp., *Aspergillus* spp., *Penicillium* spp. and *Trichoderma* spp. were the major contaminants found in almost all the substrates. Zhang (1995) cultured 20 isolates of *Fusarium* from 16 *Auricularia polytricha* cultivated bags and it was confirmed that the causal agent was *Fusarium solani* based on its morphological characters, infection symptoms and pathogenicity. However, in the present study, no such observations were recorded. Sun and Bian (2012) and Peng *et al.* (2014) reported the new disease, slippery scar on the mycelium of *A. polytricha,* as caused by *Scytalidium lignicola.* Zhang *et al.* (2017) reported on white villous disease of *A. auricula-judae* from Northeast China and mentioned the typical symptoms of this disease as a white mycelial layer on the ventral side of sporocarp, as a result of which the morphology was changed causing a significant reduction in yield. Based on fungal morphology, ribosomal DNA internal transcribed spacer sequences, species-specific primers, pathogenicity of the mycelia and spores, the two pathogens were identified as *Fusarium equiseti* and *F. sporotrichioides*.

Zou (1993) reported a destructive mite pest, *Luciaphorus auriculariae* from China, which showed the typical symptoms of feeding on both the mycelia and sporophores of *A. polytricha* and *A. auricula* mushroom and caused a loss of 10-50 %. In the present study also, the infection by mites was found quite common in the beds approaching the final stages of mushroom harvests. Deepthi *et al.* (2004) stated that springtail, flies and beetles were found as the main pests, springtail being the dominant one in oyster mushroom and *Trichoderma* spp. and *Aspergillus* spp. were the common contaminants. Mignucci *et al.* (2000) reported that the springtails caused deterioration of *Pleurotus* basidiocarps. Complete reduction of yield in oyster mushrooms mainly during rainy season was caused by phorids as reported by Kumar and Sharma (2000). Kumar *et al.* (2001) reported that the damaging stage of the pest was the larvae, which fed extensively on mushroom mycelium and sporocarps. Kumar and Sharma (2001) reported that under laboratory conditions, *Megaselia* sp. transmitted *Trichoderma viride* to the extent of 100 per cent and they also reported that phorids were the vectors of mite.

Krishnamoorthy *et al.* (1991) reported for the first time the occurrence of phorid flies (*Megaselia* sp.) on oyster mushroom in India. Balakrishnan (1994) reported the occurrence of spring tails in the oyster mushroom beds of Kerala and he also described the occurrence of *Coprinus* spp. as a weed mould in oyster mushroom beds. Many important fungal species like *Trichoderma*, *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, *Chaetomium* and *Coprinus* were found continuously attacking the beds of oyster mushroom in Kerala (Das and Suharban, 1991). Several moulds like *Aspergillus* spp., *Pencillium* spp., *Rhizopus* spp., *Mucor* spp., and *Trichoderma* spp. were observed as frequent contaminants in production of spawn (Singh *et al.* 2002). Prakasam *et al.* (2002) reported that yield reduction of mushroom was due to *Aspergillus flavus*, *A. niger* and *T. harzianum*.

5.16. ANTI-CANCEROUS ACTIVITIES

Mushrooms can also solve most of the world's health problems because they are endowed with bioactive compounds that are of medicinal value (Chang and Miles, 2004). Due to their good nutritious and medicinal values, mushrooms are considered ideal for vulnerable groups in the society such as children, nursing mothers, the old and the sick especially those suffering from diabetes, heart diseases, cancer and HIV/AIDS.

The present study on the anti-cancerous activities of *A. polytricha* (A1) concluded that the fruiting bodies showed anti-cancerous activities against different types of cancer cell lines *viz.*, cervical, colon and liver cancer, and the activity was found to be dosage dependent. As the concentration of sample increased, the percentage viability of cancer cells was found to be decreasing. The detectable changes in the morphology of the cells like rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were observed as indicators of cytotoxicity.

Very similar observations were made by Yu *et al.* (2014) who performed the MTT assay to measure the growth inhibitory effect of *A. polytricha* polysaccharides on human lung cancer (A549 cells) and the results showed induced cytotoxicity in A549 cells in a dose-dependent manner because when the drug concentration increased over 25 g/mL, cell viability was markedly suppressed to less than 42.25% of the control after the 48 h treatment.

Wasser and Weis (1999) reported that *Auricularia* produced many different polysaccharides, which were found to stimulate the immune system in humans or in some cases caused the production of interferon and interleukins that stopped the proliferation of cancer cells. They also reported that polysaccharides from *Auricularia* were found to have cardiovascular, hypocholesterolemic, antiviral, antibacterial and antiparasitic effects. Similar observations were also made by Stamets (2000). However, the present study is very preliminary and requires further, elaborate research.

Summary

6. SUMMARY

Surveys were conducted in ten different locations of Thiruvananthapuram and Kollam districts of Kerala *viz.*, Vellayani, Venganoor, Vanchiyoor, Neyyattinkara, Kattakada, Ponmudi, Nedumangad, Palode, Arippa and Kulathupuzha to identify different strains of *Auricularia* and *Tremella* spp. Dead stumps and wood logs of mango, coconut, bottle brush tree, copper pod tree, yellow bells, pine tree, wattles/acacias, cashew, golden temple tree, rubber, teak wood, arecanut and drumstick were recognised as hosts for the occurrence of *Auricularia* spp. from the surveyed locations. *Tremella* spp. occurred only on Jack tree from Vellayani. All collected mushrooms were gregarious in habit and lignicolous in habitat.

The morphological characters *viz.*, pileus of the collected *Auricularia* mushrooms ranged from light brown to dark brown in colour, 2.6 to 3.8 cm in diameter, with varied shapes such as, ear shaped, spathulate and flabelliform, incurved margin and varied textures *viz.*, soft, velvety, leathery, tomentose and caespitose, whereas *Tremella* sp. had chrysanthemum like appearance with a diameter of 6.2 cm. All the collected jelly mushrooms had rudimentary stipe. The microscopic studies revealed that hyphae of the collected mushrooms were septate, branched, hyaline, aerial as well as submerged. Internal stratification of hyphae showed different zonations *viz.*, zona pilosa, zona compacta, zona subcompacta superiosis, zona laxa superiosis, medulla, zona laxa inferiosis, zona compacta inferiosis, hymenium and abhymenial hair. Spore print obtained was pure white in colour. The individual basidiospores were hyaline, sausage shaped and measured 10.8 × 3.2 to 12.8 × 3.4 µm in size.

Pure culturing of collected mushrooms was done on the PDA medium by standard tissue culturing technique. White and thick cottony growth was obtained after 14 to 15 days of incubation. The pure cultures were maintained and sub cultured. Two fast growing cultures were selected based on the time taken for the complete mycelial coverage in petri plates and nature of mycelial growth. Vellayani - 1 (*Auricularia* spp.) completed its 90 mm diameter growth in 10.25

days. The isolate recorded a thick cottony, fluffy white growth with irregular margin and a colony diameter of 8.9 cm. Neyyatinkara (*Auricularia* spp.) isolate took 11.25 days for completing 8.45 cm diameter growth. The colony growth of Neyyatinkara isolate was cottony with smooth margins. These two strains were selected for further studies and were designated as A1 and A2 respectively. The cultures were identified as *Auricularia polytricha* (Mont.) Sacc. and accession numbers were obtained from Directorate of Mushroom Research (DMR) at Solan, *viz.*, DMRO-825 for A1 and DMRO-826 for A2.

The cultural characteristics of *A. polytricha* - A1 and A2 were studied on four different media *viz.*, potato dextrose agar, oat meal agar, carrot extract agar and malt extract agar. Among them, malt extract agar took minimum time (10.50 days) to complete the mycelial growth with a diameter of 9.0 cm. Thick, cottony, fluffy growth with irregular margin was observed for A1 whereas A2 showed smooth margin with white coloured mycelia. Efficacy of three different temperature *viz.*, 20 ^{0}C, 25 ^{0}C and 30 ^{0}C on the growth of *A. polytricha*- A1 and A2 indicated that, a temperature of 25 ^{0}C was best followed by 30 ^{0}C and least performance by 20 ^{0}C. Maximum growth of 9.0 and 8.95 cm was obtained from A1 and A2, respectively, at a neutral pH of 7 followed by pH 6, slightly alkaline pH 8 and the least growth was seen at acidic pH 5 (8.45 and 8.42 cm). Light conditions recorded minimum time to complete the mycelial growth and maximum colony growth both for *A. polytricha*- A1 and A2.

Six substrates such as paddy grains, wheat, sorghum, ragi, sawdust of hardwood trees and rubber saw dust were evaluated for spawn production studies. The time taken for spawn run, nature of mycelial growth and presence of contaminants were taken into consideration. Among these substrates, paddy grain was selected as the best substrate for mycelial run of *A. polytricha* - A1 and A2 with minimum time of 16.75 and 16.50 days respectively. Maximum per cent contamination was found in wheat substrate (48 and 52 %). *Aspergillus* sp., *Rhizopus* sp. and *Trichoderma* spp. were the common contaminants observed in

almost all the substrates tried. The maximum shelf life was observed from rubber sawdust (82.50 and 80.25 days).

A. polytricha - A1 and A2 were subjected for submerged culture production for which three different liquid media were used namely malt extract broth, potato dextrose broth and carrot extract broth. Among the three media, malt extract broth supported the maximum dry weight of mycelial pellets (1.05 and 1.01g) for A1 and A2 respectively.

Cultivation studies were conducted, by utilizing six different substrates *viz.*, paddy straw, sawdust of softwood trees, sawdust of hardwood trees, banana pseudostem, sugarcane bagasse and neopeat for evaluating fruiting body production of *A. polytricha* - A1 and A2. Among the different substrates tried for mushroom production, paddy straw took minimum time of 27.25 and 27.75 days respectively for A1 and A2 for pinhead formation. The next best substrate found was rubber sawdust in terms of growth parameters. Neopeat and sugarcane bagasse recorded the least growth parameters. Beds prepared from rubber sawdust recorded the maximum number of sporocarps, weight of sporocarp, total yield and biological efficiency. However, flushes of mushrooms continued to produce upto eight harvests for a period of 88 and 84.25 days.

For amendment studies, five different amendments were used such as wheat bran, rice bran, cotton seed hull, neem cake and groundnut cake at two and four per cent concentration with rubber sawdust as basal substrate for the fruiting body production of *A. polytricha* – A1 and A2. The study indicated that, among the different amendments used, two per cent wheat bran was found to be the best with minimum time taken for complete spawn run (32.33 days) followed by four per cent wheat bran (32.66 days). Beds amended with four per cent neem cake took maximum number of days (38.0 days). In case of time taken for pinhead formation also, two per cent wheat bran was found to be the best (37.66 days). Two and four per cent neem cake took maximum number of days (45.33 and 45.66) for pinhead formation. After 44 days of bed preparation, beds amended with two per cent wheat bran produced the first flush of *A. polytricha* - A1. Total

crop period was the highest in two per cent rice bran (95.66 days). All the yield parameters like average weight of sporocarp (4.26 g), number of sporocarps (42.0) and total yield and biological efficiency from three harvests (0.186 kg and 18.6) were found to be the highest in beds amended with two per cent rice bran. In case of *A. polytricha* - A2, non-amended beds took 33.33 days for completing spawn run and 39.33 days for pinhead formation. Non-amended beds and beds amended with two per cent wheat bran took 48.33 days to produce the first flush of mushroom. Similar to A1, the total crop period, the average weight of sporocarp, number of sporocarps, total yield as well as biological efficiency was the highest in substrate amended with two per cent rice bran.

Assessment of proximate constituents indicated that, the moisture content of *A. polytricha* - A1 and A2 was 90.12 % and 89.91 % respectively on fresh weight basis. Other nutrient components were present in appreciable amount *viz.*, carbohydrates (47.1 % and 48.8 %), protein (18.06 % and 20.75 %), fat (0.76 % and 0.56 %), fibre (17.69 % and 15.49 %), ash (5.5 % and 5.3 %), total antioxidants (116 µg and 74 µg), β-carotene (0.178 µg and 0.150 µg), polyphenol (9.53 µg and 6.89 µg) and energy value (251.49 Kcal and 264 Kcal).

Sensory evaluation and cooking quality was done for *A. polytricha* - A1 and A2 with seven different recipes like pepper masala, pizza, pakoda, mushroom tomato sauce, soup, mushroom veg curry and bajji. The study revealed that, mushroom tomato sauce recorded the maximum score for appearance (9.0), colour (8.9), flavor (8.9), texture (8.8), taste (9.0) aroma (8.8) and overall acceptability (8.9 and 9.0) respectively, for A1 and A2. The keeping quality of *A. polytricha* - A1 and A2 in perforated polythene covers indicated that both the samples could be kept fresh for 24 h under ordinary condition and they started drying after one day. Mushrooms kept under refrigerated conditions (4 ^{0}C) were fresh, brown, without bad smell and watery appearance up to 72 h and they were found ideal for cooking. After five days, mushrooms changed morphologically as well as physiologically. After thirty days of refrigeration, mushrooms showed complete

liquefaction. The per cent reduction in fresh weight for A1 and A2 was 1.6 and 2.4, 24 h after keeping under refrigerated condition and three days after refrigeration, it was changed respectively to 4.8 and 5.6 per cent.

Outdoor cultivation studies were conducted along with indoor cultivation as control for both *A. polytricha* A1 and A2. The study revealed that indoor cultivation was comparatively better than outdoor cultivation, in terms of all the growth parameters *viz.*, days for complete spawn run, pinhead formation, time taken for first flush, total cropping period and yield parameters. Average weight of sporocarp, number of sporocarps, total yield from three harvests (0.147 kg and 14.7 %) and biological efficiency was also the highest in indoor cultivation for A1 and for A2 (0.136 kg and 13.6 %).

Comparative performance of *A. polytricha* – A1 and A2 with *Pleurotus florida* and *Calocybe gambosa* indicated that the supremacy of all the growth parameters was best in *P. florida* followed by *C. gambosa, A. polytricha* – A1 and A2, except the total cropping period. *A. polytricha*- A2 recorded the maximum cropping period (88.50 days). *C. gambosa* showed the highest average weight of sporocarp (146.85 g). Number of sporocarps was found maximum in *P. florida* (37.50). Total yield and biological efficiency was highest in *C. gambosa* (0.661 kg and 66.1 %), followed by *P. florida* (0.629 kg and 62.9 %), *A. polytricha* - A1 and A2 which recorded total yield of 0.528 kg and 0.508 kg and BE of 52.8 % and 50.8 % respectively. Major insect pests observed were phorid flies (*Megaselia* spp.), springtails (*Seira* spp.), staphylinid beetles and mites. *Coprinus* spp., *Aspergillus* spp., *Penicillium* spp. and *Trichoderma* spp. were the major contaminants found in the substrates.

The sporocarps took an average of six days from the day of pinhead formation to complete maturity. The pileus of the pinhead was creamish white in colour, 2 mm diameter with rubbery and soft texture. Full maturity with exact ear shape occurred after six days and measured 40 mm diameter with dark brown colouration.

Comparison between log method of cultivation and polybag method of cultivation indicated that polybag method of cultivation was found to be the best with maximum biological efficiency of 13.6 % and 13.0 % compared to log method, which showed only 3.7 % biological efficiency for A1 and 3.5 % for A2.

A. polytricha showed anti-cancerous activities against cervical cancer cells, colon cancer cells and liver cancer cells. The MTT assay revealed that, the activity was dosage dependent, as the concentration of sample was increased, the percentage viability of cancer cells was decreased. The detectable changes in the morphology of cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm were observed as indicators of cytotoxicity.

The present study revealed that *A. polytricha* can be cultivated successfully in Kerala by utilizing the locally available materials. Availability of rubber sawdust is not uncommon so it can be effectively utilized for the production of fruiting bodies along with two per cent rice bran since it grows well in tropical conditions. In order to enhance the productivity of mushrooms, weather parameters like temperature, aeration, relative humidity and light have to be managed properly. The purplish brown colour, ear shape and sliminess make this mushroom unpopular but its nutritional as well as medicinal attributes are the supplementary benefits of this mushroom. The anti-cancerous activities revealed that there is a great scope for utilizing this particular mushroom in medical field.

References

REFERENCES

Acton, Johnny, Sandler, and Nick. 2001. *Mushroom*. Kyle Cathie Publishers, USA, 160p.

Adebiyi, A. O. and Yakubu, H. O. 2016. A survey of mushrooms in two local government areas of Ekiti State, Nigeria. *Donnish J. Agric. Res.* 3(2): 013-016.

Afiukwa, C. A., Ugwu, Okechukwu, P. C., Ebenyi, L. N., Oketa, H. A., Idenyi, J. N., and Emmanuel, O. 2013. Phytochemical analysis of two wild edible mushrooms, *Auricularia polytricha* and *Pleurotus ostreatus* common in ohaukwu area of Ebonyi State, Nigeria. *Res. J. Pharma. Biol. Chem. Sci.* 4(2):1065- 1070.

Ahmed, S. A., Kadam, J. A., Mane, V. P., Patil, S.S., and Baig, M. M. M. 2009. Biological efficiency and nutritional contents of *Pleurotus florida* (Mont.) Singer cultivated on different agro-wastes. *Nat. Sci.* 7(1): 44-49.

Ainsworth, G. C. 1971. *Ainsworth and Bisby's Dictionary of the Fungi*. (6th Ed.). Common Wealth Mycological Institute Kew, Surrey, England, 663p.

Alam, N., Amin, 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*. *Mycol.* 36: 228-232.

Alananbeh, K. M., Bouqellah, N. A., and Kaff, N. S. A. 2014. Cultivation of oyster mushroom *Pleurotus ostreatus* on date-palm leaves mixed with other agro-wastes in Saudi Arabia. *Saudi J. Biol. Sci.* 21: 616–625.

Alexopoulos, C. J., Mims, C. M., and Blackwell, M. M. 1996. *Introductory Mycology*. (4th Ed.). John and Willey Publishers, New York, 880p.

Aminoff, D., Binkley, W. D., Schaffer, R., and Mowry, R. W. 1970. *Analytical Methods for Carbohydrates- Chemistry and Biochemistry.* Cambridge University Press, U. K., 765p.

Ares, G., Lareo, C., and Lema, P. 2007. Modified atmospheric packaging for the postharvest storage of mushrooms. *Fresh Produce* 1: 32-40.

Ayodele, S. M. and Akpaja, E. O. 2007. Yield evaluation of *Lentinus squarosulus* (Mont) Sing. on selected sawdust of economic tree species supplemented with 20 % oil palm fruit fibers. *Asian J. Plant Sci.* 6: 1098 -1102.

Back, C. G., Lee, C. Y., Seo, G. S., and Young, H. 2012. Characterization of species of *Cladobotryum* which cause cobweb disease in edible mushrooms grown in Korea. *Mycobiol.* 40(3):189-194.

Balakrishnan, B. 1994. Improvement on the technique for the cultivation and preservation of tropical species of mushrooms. Ph. D. thesis, Kerala Agricultural University, Thrissur, 217p.

Bandara, A. R., Chen, J., Karunarathna, S., Hyde, K. D., and Kakumyan, P. 2015. *Auricularia thailandica sp. nov.* (*Auriculariaceae*, *Auriculariales*) a widely distributed species from Southeastern Asia. *Phytotaxa* 208(2): 147–156.

Bano, Z. and Rajarathnam, S. 1982. *Pleurotus* mushroom as nutritious food. In: Chang, S. T. and Quimio, T. H. (eds), *Tropical Mushroom Biological Nature and Cultivation Methods*. The Chinese University Press, Hong Kong, pp. 363-382.

Barros, L., Ferreiraa, M., Queirósa, B., and Ferreiraa, I. C. F. R. 2007. Total phenols, ascorbic acid, βcarotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chem.* 103 (2): 413–419.

Baskaran, T. L., Sivaprakasam, K., and Kandaswamy, T. K. 1978. Compact bag method- A new method of increasing the yield of *Pleurotus sajor-caju*. *Indian J. Mushrooms* 4: 10-12.

Bellettini, M. B., Assumpc, F., Maieves, H. A., Teixeira, G. L., Suelen, A. V., Hornung, P. S., Maccari, A., and Ribani, R. H. 2016. Factors affecting mushroom *Pleurotus* spp. *Saudi J. Biol. Sci.* (in press).

Berkeley, M. J. 1856. Decades of Fungi LXILXII: Rio Negro fungi. *Hooker's J. Bot. Kew Garden miscellany* 8: 272–280.

Beyer, D. M. and Wilkinson, V. L. 2002. *Mushroom Science and Technology.* Pen State Department of Plant Pathology, Pennsylvania, 7p.

Bhandal, M. S. and Mehta, K. B. 1986. Cultivation of *Auricularia polytricha* in India. *Indian Phytopathol.* 39:159.

Boa, E. 2004. Wild edible fungi a global over view of their use and importance to people. Rome, Italy. *Non-Wood Forest Products, 17.* Food and agriculture organization, Rome, 157p.

Boonsong, S., Klaypradit, W., and Wilaipuna, P. 2016. Antioxidant activities of extracts from five edible mushrooms using different extractants. *Agric. Nat. Resour.* 50: 89-97.

Carrera, M. D., Bonialla, M., Martinez, W., Sobal, M., Aguilar, A., and Gonzalez, P. 2002. Characterization and cultivation of wild *Agaricus* species from Mexico. *Int. J. Appl. Mycol.* 13: 9-24.

Celestine, A. A., Augustine, O. O., Joseph, A. N., Okechukwu, U. P. C., Fredrick, A. U., and Emmanue, O. 2013. Proximate and mineral element compositions of five edible wild grown mushroom species in Abakaliki, Southeast Nigeria. *Res. J. Pharm. Biol. Chem. Sci.* 4(2): 1056-1063.

Cha, M. H., Heo, J. Y., Lee, C., Lo, Y. M., and Moon, B. 2014. Quality and sensory characterization of white jelly mushroom (*Tremella fuciformis*) as a meat substitute in pork patty formulation. *J. Food Processing Preservation* 38: 2014–2019.

Chan, H. K. M., 1981. Consumption of edible mushrooms in Hong Kong. *Mushrooms News lett. Tropic.* 1: 5-10.

Chandra, P., Neelam, K., Usha, B., and Kailash, R. P. Y. 2013. Cultivation of *Pleurotus sajor-caju* using different agricultural residues. *Int. J. Agric. Policy Res.* 1(2): 019-023.

Chang, S. T., Lau, D. W., and Cho, K. Y. 1981. The cultivation and nutritional value of *Pleurotus sajor-caju*. *Eur. J. Appl. Microbiol. Biotechnol.* 12: 58-62.

Chang, S. T. 1980. Mushrooms as human food. *Bio Sci.* 30: 399-401.

Chang, S. T. 2006. The world mushroom industry: Trends and technological development. *Int. J. Med. Mushroom* 8: 297-314.

Chang, S. T. 2008. *Overview of Mushroom Cultivation and Utilization as Functional Foods Mushrooms as Functional Foods*. John Wiley and Sons Publishers, New York, pp. 1-34.

Chang, S. T. and Miles, P. G. 1989. *Edible Mushrooms and Their Cultivation.* CRC Press, Boca Raton. 345p.

Chang, S. T. and Miles, P. G. 2004. *Mushrooms: Cultivation, Nutritional Value, Medicinal Effect and Environmental Impact.* CRC press, Boca Raton, Florida. 451p.

Chang, S. T. 2001. *Mushrooms and mushroom cultivation.* John Wiley and Sons, Chichester.Avialable:http://www.els.netdoi:10.1038/npg.els.0000370 html.[20 JUN 2001].

Chang, S. T. and Miles, P. G. 2008. *Mushrooms: Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact* (2nd ED.) CRC Press, Boca Raton, USA. 451p.

Chang, S. T. and Quimio, T. H. 1982. *Tropical Mushrooms: Biological Nature and Cultivation Methods.* The Chinese University Press, Hong Kong, 370p.

Chang, S.T., Lau, O.W., and Cho, K.Y. 1981. The cultivation and nutritional value of *Pleurotus sajor-caju. Eur. J. Appl. Microbiol. Biotechnol.* 12: 58-62.

Cheng, S. and Tu, C. C. 1978. *Auricularia* spp, In: Chang, S. T. and Hayes, W. A. (eds), *The Biology and Cultivation of Edible Mushrooms*. Academic Press, New York, pp. 605- 625.

Cheung, P. C. K., 1998. Plasma and hepatic cholesterol levels and feacal neutral sterol excretion are altered in hamsters fed straw mushroom diet. *J. Nut.* 128: 1512-1516.

Cho, E. J., Oh, J. Y., Chang, H. Y., and Yun, J. W. 2006. Production of exopolysaccharides by submerged mycelia culture of a mushroom *Tremella fuciformis. J. Biotechnol.* 127(1): 129-140.

Cho, K. Y., Young, K. H., and Chang, S. T. 1982. Preservation of cultivated mushroom. In: Chang, S. T. and Quimio, T. H. (eds), *Biological Nature and Cultivation Methods of Tropical Mushrooms*. The Chinese University Press, Hong Kong. 39p.

Chopra, S. K., Naquash, G. S., and Chadha, T. R. 1985. Effect of pre-harvest sprays of honey, citric acid and *Euphorbia royleana* latex on the shelf life of button mushrooms (*A. bisporus*). In: *National Symposium on Production and Conversation Forestry*. 12-13 April, Solan (HP), 32p.

Choudhury, M. P. and Sarma, T. C. 2014. Studies on ear fungus- *Auricularia* from the woodland of Nameri national park, Sonitpur district, Assam. *Int. J. Interdisciplinary Multidisciplinary Stud.* 1(5): 262-265.

Colak, A., Faiz, O., and Sesli, E. 2009. Nutrition composition of some wild edible mushrooms, *Turkish J. Biochem.* 34: 25-31.

Confortin, F. G., Marchetto, R., Bettin, F., Camassola, M., Salvador, M., and Dillon, A. J. P. 2008. Production of *Pleurotus sajor-caju* strain PS-2001

biomass in submerged culture. *J. Indian Microbiol. Biotechnol.* 35:1149-1155.

Crisan, E. V. and Sands, A. 1978. Nutritional value of edible mushrooms. In: Chang, S. T. and Hayes, W. (eds), *The Biology and Cultivation of Edible Mushrooms*. Academic Press, New York, pp.137-168.

Das, L. 1992. Cooking mushrooms with Indian Spices. *Indian Spices* 29 (3): 2.

Das, L. 1994. Nutritional and medicinal aspects of Mushrooms. *Mushroom* 94: 22- 23.

Das, L. 2011. Cooking god's own food in god's own country – the easy way. *Proceedings of the 7th International Conference on Mushroom Biology and Mushroom Products (ICMBMP7), 4-7 October 2011.* Arcachon, France. World Society for Mushroom Biology and Mushroom Products - and INRA, pp. 202-209.

Das, L. and Suharban, M. 1991. Fungal parasites of oyster mushroom in Kerala. In: Nair, M. C., Balakrishnan, S. and Gokulapalan, C (eds), Proceedings of National symposium on mushrooms. Kerala Agricultural University, Thrissur, pp. 253-254.

De, S. K. 1965. *Practical Agricultural Chemistry*. Narayan Publishing Home, Allahabad, India, 110p.

Deacon, J. 2005. *Fungal Biology.* Blackwell Publishing, Cambridge, 384p.

Deepa, R. C. V. 2016. Strain evaluation and production technology of shiitake mushroom (*Lentinula edodes* (Berk.) Pegler). Ph. D thesis, Kerala Agricultural University, Thrissur, 216p.

Deepthi, S., Suharban, M., Geetha, D., and Sudharma, K. 2004. Pests infesting oyster mushroom in Kerala and the seasonality of their occurrence. *Mushroom Res.* 13 (2): 76-81.

Devi, A. P., Veeralakshmi, S., Prakasam, V., and Thiribhuvanamala, G. 2015a. Induction of different lignocellulolytic enzymes in the process of degradation of agricultural waste substrates by the Jew's ear mushroom. *Int. J. Biotechnol. Res.* 3(2): 28-34.

Devi, A. P., Veeralakshmi, S., Prakasam, V., and Vinothini, M. 2013. Saw dust and wheat bran substrates for the cultivation of new wood ear mushroom (*Auricularia polytricha* (Mont.) Sacc.). *Am. Eur. J. Agric. Environ. Sci.* 13: 1647-1649.

Devi, M. B. Singh, S. M., and Singh, N. I. 2015b. Biomass production in *Auricularia* spp. (Jew's ear) collected from Manipur. *India Inter. J. Curr. Microbiol. Appl. Sci.* 4(6): 985-989.

Dhakad, P. K., Chandra, R. M. K., Yadav., and Patar, U. R. 2015. Comparative study on growth parameters and yield potential of five strains of milky mushroom (*Calocybe indica*). *J. Pure Appl. Microbiol.* 9(3): 23-33.

Dhar, B. L. 1992. Post harvest storage of white button mushroom *Agaricus bitorquis*. *Mushroom Res.* 1: 127-130.

Dhar, B. L. 2014. Changing global scenario in mushroom industry [abstract]. In: *Abstracts, 8th International Conference on Mushroom Biology and Mushroom Products*; 19-22, November, 2014, New Delhi, India. World Society of Mushroom Biology and Mushroom Products ICAR-Directorate of Mushroom Research, Solan, Mushroom Society of India, Solan, p.602. Abstract No. VII-0-8.

Dinesh, M. D, Athira, P. S., Ajma, N, Abhisha, N. C, and Ashitha, C. 2016. Anti-cancer activity (Oral) of betel leaf extract by *in- vitro*. *Int. J. Life. Sci. Scientific Res.* 2(4): 412-414.

Du, P., Cui, B. K., and Dai, Y. C. 2011. Genetic diversity of wild *Auricularia polytricha* in Yunnan province of South-Western China revealed by sequence-related amplified polymorphism (SRAP) analysis. *J. Med. Plants Res.* 5(8): 1374-1381.

Dulay, R. M. R., Kalaw, S. P., Reyes, R. G., Cabrera, E. C., and Alfonso, N. F. 2012. Optimization of culture conditions for mycelial growth and basidiocarp production of *Lentinus tigrinus* (Bull.) Fr., A new record of domesticated wild edible mushroom in the Philippines. *Philipp Agric Sci.* 95(3): 278–285.

Ekpo, E. N., Olasupo, O. O., and Eriavbe, M. A. 2009. Wheat bran: A supplement for mushrooms cultivation in Nigeria. *Women Nat. Resour.* Available: http://www.webpages.uidaho.edu/winr/Ekpo.htm.

Emre, A., Gamze., Gulcin, A. A., Menderes, S., and Cevher, S. C. 2016. An edible mushroom with medicinal significance; *Auricularia Polytricha. Hittite J. Sci. Eng.* 3 (2): 111-116.

Ergonul, P. G., Akata, I., Kalyoncu, F., and Ergonul, B. 2013. Fatty acid compositions of six wild edible mushroom species. *Sci. World J.* vol. 2013. Available: http://dx.doi.org/10.1155/2013/163964.

Fakkiravva, R. S. 2014. Evaluation of growth of pink oyster (*Pleurotus eous)* and milky (*Calocybe indica*) mushroom with addition of secondary-micronutrients to substrate . M. Sc. (Ag) thesis, University of Agricultural Sciences Bengaluru, 71p.

Fanadzo, M., Zireva, D. T., Dube, E., and Mashingaidze, A. B. 2010. Evaluation of various substrates and supplements for biological efficiency of *Pleurotus sajor-caju* and *Pleurotus ostreatus*. *Afr. J. Biotechnol.* 9: 2756-2761.

Fernandes, A., Antonio, A. L., Oliveira, M. B. P. P., Martins, A., and Ferreira. 2012. Effect of gamma and electron beam irradiation on the physico-chemical and nutritional properties of mushroom: a review. *Food Chem.* 135 (2): 641-650.

*Fries, E. 1823. *Systema Mycologicum.* Richmond Publishers, London, 221p.

Garasiya, N., Sharma, S. S., Gour, H. N., and Singh, R. 2007. Cultivation of jelly mushroom (*Auricularia polytricha* (Mont.) Sacc.) in Rajasthan. *Indian J. Mycol. Pl. Pathol.* 37 (2): 332-335.

Gateri, M. W., Ndung, U. B., Muriuki, A.W., Rauwl, V., and Kabacia, S. 2014. Collection, identification and morphological characterization of indigenous mushrooms in coastal Kenya. *Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Products (ICMBMP8),* 20-22 November 2014, New Delhi. World Society for Mushroom Biology and Mushroom Products, Directorate of Mushroom Research and Mushroom Society of India, pp. 17-23.

Gbolagade, J. S., Ajayi, A., Oku, I., and Wankasi, D. 2006. Nutritive value of common wild edible mushrooms from southern Nigeria. *Glob. J. Biotechnol. Biochem.* 1(1):16-21.

Gea, F. H., Carrasco, J., Dianez, F., Santos, M., and Navarro, M. J. 2014. Control of dry bubble disease (*Lecanicillium fungicola*) in button mushroom (*Agaricus bisporus*) by spent mushroom substrate tea. *European J. Plant Pathol.* 138(4): 711-720.

Geetha, D. 1993. Studies on oyster mushroom (*Pleurotus* spp.). Ph. D thesis, Agricultural College and Research Institute, Madurai, 253p.

Geetha, D. 2011. *Collection, Identification, Cultivation and Population of Edible/ Medicinal Mushrooms of Western Ghats of Kerala.* WGDP Report, Kerala, 69p.

Geetha, D., Sivaprakasam, K., and Seetharaman, K. 2002. Spawn production potential of *Pleurotus* spp. on different substrates. *Proceedings of 3rd Indian Mushroom Conference*, 6-7 March 2002, Mushroom Society of India. Tamil Nadu, India, p.88.

*Gerarde, J. 1597. *The Herball.* John Norton, London, 1385p.

Gogoi, Y. and Sarma, T. C. 2012. An ethnomycological survey in some areas of Dhemaji district (Assam). *Ecoscan* 1: 403-407.

Goyal, A., Dhanda, S., Kapoor, S., and Sodhi, H. S. 2010. Nutritional profile of vegetative mycelium of *Auricularia polytricha* (Mont.) Sacc. Ponnam. *Mushroom Res.* 19 (1): 1-8.

Guerrero, R. T. 1971. On the real nature of the setae in *Tremella fuciformis*. *Mycologia* 63 (4): 920–924.

Gupta, D., Sharma, M., Suman, B. C., and Jarial, R. S. 2011. Comparative study on cultivation and performance of oyster mushroom (*Pleurotus ostreatus*) on different substrates. *Indian J. Mush.* 29 (1): 21-26.

Hadwan, H. A., Jaboury, A. M. H., and Hassan, A. O. 1997. Suitability of different substrate and amendments on the cultivation of oyster mushroom. Collection of thesis materials, S and T, Development, Environment and Resources. Proceeding of 1996 FUZHOU International Symposium on the Development of Juncau Industry, pp. 215-221.

Hammond, J. B. W. and Nichols, R. 1975. Changes in respiration and soluble carbohydrates during the postharvest storage of mushrooms (*Agaricus bisporus*). *J. Sci.Food Agric.* 26: 835-842.

Harding and Patrick. 2008. *Mushroom Miscellany*. Harper Collins Publishers, London, 208p.

Haryati, J. R. and Azrianingsih, R. 2012. Ethno-edible mushroom of *Pleurotus* sp., *Clytocybe nebularis* and *Auricularia auricula* in Ranupani Village, East Java. *J. Trop. Life Sci.* 2(2): 40-43.

Hassan, F. R. H., Ghada, M. Medany., and Kady, E. A. T. M. 2012. Mycelial Biomass Production of Enoke Mushroom (*Flammulina velutipes*) by Submerged Culture. *Aust. J. Basic Appl. Sci.* 6(7): 603-610.

Haxo, F. 1950. Carotenoids of the mushroom *Cantharellus cinnabarinus*. *Bot. Gaz.* 112 (2): 228-232.

Hemmes, D. E. and Desjardin, D. E. 2004. Mushrooms of Hawaii. An identification guide. Ten Speed Press, Berkeley. 224p.

Hoa, H. T., Wang, C. L., and Wang, C. H. 2015. The effects of different substrates on the growth, yield, and nutritional composition of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). *Mycobiol.* 43(4): 423–434.

Hung, P. V. and Nhi, N. N. Y. 2012. Nutritional composition and antioxidant capacity of several edible mushrooms grown in the Southern Vietnam. *Int. Food Res. J.* 19(2): 611 -613.

Hur, H., Imtiaj, A., Lee, M. W., and Lee, T. S. 2008. Suitable conditions for mycelial growth of *Phellinus* spp. *Mycobiol.* 36 (3): 152-156.

Ibekwe, V. I., Azubuike, P. I., Ezeji, E. U., and Chinakwe, E. C. 2008. Effects of nutrient sources and environmental factors on the cultivation and yield of oyster mushroom (*Pleurotus ostreatus*). *Pakist. J. Nutr.* 7(2): 349-351.

Imtiaj, A. and Rahman S. A. 2008. Economic viability of mushrooms cultivation to poverty reduction in Bangladesh. *Trop. Sub trop. Agric. ecosyst.* 8: 93-99.

Ingold, C. T. 1982. Basidiospore germination and conidium development in *Auricularia*. *Trans. Br. Mycological Soc.* 78: 161–166.

Inyod, T., Sassanarakit, S., Payapanon, A., and Keawsompong, S. 2016. Selection of *Macrocybe crassa* mushroom for commercial production. *Agric. Nat. Resour.* 50: 186-191.

Iqbal, S. M., Rauf, C. A., and Sheik, M. I. 2005. Yield performance of oyster mushroom on different substrates. *Int. J. Agri. Biol.* 7: 900-903.

Irawati, D., Hayashi, C., Takashima, Y., Wedatama, S., Ishiguri, F., Iizuka, K., Yoshizawa, N., and Yokota, S. 2012. Cultivation of the edible mushroom *Auricularia polytricha* using saw dust based substrate made of three Indonesian commercial plantation species, *Falcataria moluccana*, *Shorea* spp., and *Tectona grandis*. *Mycologia aplicada Int.* 24: 33-41.

Isikhuemhen, O. S., Nemd, T., and Vilgahus, R. 2000. Cultivation studies in wild and hybrid strain of *Pleurotus tuberrigium* (Fr) Sing on wheat straw substrate. *World J. Microbiol. Biotechnol.* 16: 431- 435.

Jackson, M. L. 1973. *Soil Chemical Analysis* (2nd Ed.). Prentice Hall of India, New Delhi, 498p.

Jadhav, A. C., Bhalerao, V. K., and Shinde, D. B. 2017. Cultivation and yield evaluation of wild *Pleurotus* spp. from Western Ghats of Maharashtra [abstract]. In: *Abstracts, National Symposium on Mushrooms Trends and Innovations in Mushroom Sciences*; 27-28, April, 2017, Solan, India. Mushroom Society of India and ICAR- Directorate of Mushroom Research, Solan, p. 84. Abstract No. V-O-8.

Jagadish, L. K., Venkata, V., Shenbhagaraman, R., and Kaviyarasan, V. 2009. Comparitive study on the antioxidant, anticancer and antimicrobial property of *Agaricus bisporus* (J. E. Lange) Imbach before and after boiling. *Afr. J. Biotechnol.* 8(4): 654 - 661.

Jayasinghe, C., Imtiaj, A., Hur, H., Lee, G. W., Lee, T. S., and Lee, U. Y. 2008. Favorable culture conditions for mycelial growth of Korean wild strains in *Ganoderma lucidum*. *Mycobiol.* 36 (1): 28-33.

Jaziya, S. 2011. Evaluation of nutritional quality and health benefits of oyster mushroom. M.Sc.(Ag) thesis, Kerala Agricultural University, Thrissur, 98p.

Jellinick, G. 1985. *Sensory Evaluation of Food -Theory and Practice*. Ellis Horwood Ltd., Chichester, England, 240p.

Jeong, S. C., Cho, S. P., Yang, B. K., Gu, Y. A., Jang, J. H., Huh, T. L., and Song, C. H. 2004. Production of an anti-complement exo-polymer produced by *Auricularia auricula-judae* in submerged culture. *Biotechnol. Lett.* 26: 923–927.

Jianjung, Z. 1991. Bottle cultivation techniques of *Auricularia auricula* with straw won the Golden Medal in Thailand. *Edible Fungi China.* 10(2): 48.

Jing, W., Fang, J. Y., Xiao, W., Ming, F., and Ying, C. 2014. Study on the cultivation characteristics of four wild species of *Auricularia. Edible Fungi China*. 33(2): 15-19.

Johnsy, G., Davidson S., Dinesh, M. G., and Kaviyarasan, V. 2011. Nutritive value of edible wild mushrooms collected from the Western Ghats of Kanyakumari District. *Botany Res. Int.* 4(4): 69 - 74.

Jonathan, S. G., Bawo, D. D. S., Adejoye, D. O., and Briyai, O. F. 2009. Studies on biomass production in *Auricularia polytricha* collected from Wilberforce Island, Bayelsa State, Nigeria. *Am. J. Appl. Sci.* 6 (1): 182-186.

Jonathan, S. G., 2002. Vegetable growth requirements and antimicrobial activities of some higher fungi in Nigeria Ph.D. thesis, University of Ibadan. 252p.

Jonathan, S. G., Okorie, A. N., Garuba, E. O., and Babayemi, O. J. 2012. Bioconversion of sorghum stalk and rice straw into value added ruminant feed using *Pleurotus pulmonarius*. *Nat. Sci.* 10: 10-16.

Kadnikova, I. A., Costa, R., Kalenik, T. K., Guruleva, O. N., and Yanguo, S. 2015. Chemical composition and nutritional value of the mushroom *Auricularia auricula-judae*. *J. Food Nutr. Res.* 3(8): 478-482.

Kakon, A. J., Choudhury, M. B. K., and Saha, S. 2012. Mushroom is an ideal food supplement. *J. Dhaka Natl. Med. Coll. Hos.* 18 (1): 58-62.

Kalaw, S. P., Alfonso, D. O., Dulay, R. M. R., Leon, D., Undan, A. M., Undan, J. R., and Reyes, R. G. 2016. Optimization of culture conditions for secondary mycelial growth of wild macrofungi from selected areas in Central Luzon, Philippines. *Curr. Res. Environ. Appl. Mycol.* 6(4): 277-287.

Kaneda, T. and Tokuda, S. 1966. Effect of various mushroom preparations on cholesterol levels in rats. *J. Nutr.* 90: 371-376.

Kapoor, S., Kaur, A., Sodhi, H. S., and Khanna, P. K. 2011. Physiology and cultivation of jew's ear mushroom *Auricularia polytricha* (Mont.) Sacc. *Indian J. Ecol.* 38(1):103-106.

Khan, A., Amin, R. S. M., Nazim, U. M., Tania, M., and Alam, N. 2008. Comparative study of the nutritional composition of oyster mushrooms cultivated in Bangladesh. *Bangladesh J. Mushroom* 2(1): 9-14.

Khanna, P. K., Bhandari, R., and Soni, G. L. 1992. Evaluation of *Pleurotus* spp. for growth, nutritive value and antifungal activity. *Indian J. Microbiol.* 32: 197-200.

Kibar, B. and Peksen, A. 2011. Nutritional and environmental requirements for vegetative growth of edible ectomycorrhizal mushroom *Tricholoma terreum. Agric.* 98(4): 409–414.

Kim, K. M., Ko, J. A., Lee, J. S., Park, H. J., and Hanna, M. A. 2006. Effect of modified atmospheric packaging on the shelf life of coated, whole and sliced mushrooms. *Food Sci. Technol.* 39(4): 365-372.

Kinjo, K. and Kondo, K. 1979. Study on the cultivation culture media of basidiomycetes. III. with reference to cultural characteristics of *Auricularia polytricha* (Mont.) Sacc. *Mokuzai Gakkaishi.* 25: 799- 803.

Kirk, P. M. 2015. Species 2000 and ITIS catalogue of life. *Mycologia* 79(6): 847-856.

Klomklung, N., Karunarathna, S. C., Hyde, K. D., and Chukeatirote, E. 2014. Optimal conditions of mycelia growth of three wild edible mushrooms from northern Thailand. *Acta. Biol. Szeged.* 58(1): 39-43.

Kounsar, S., Shah, S., Munshi, N. A., Shah, M. D., and Sheikh, P. A. 2017. Evaluation of bacterial antagonists against *Mycogone perniciosa* causing wet bubble disease of white button mushroom (*Agaricus bisporus*) in Kashmir [abstract]. In: *Abstracts, National Symposium on Mushrooms Trends and Innovations in Mushroom Sciencs*; 27-28, April, 2017, Solan, India. Mushroom Society of India and ICAR- Directorate of Mushroom Research, Solan, p. 79. Abstract No. V-l-5.

Koutrotsios, G., Mountzouris, K. C., Chatzipavlidis, I., and Zervakis, G. I. 2014. Bioconversion of lignocellulosic residues by *Agrocybe cylindracea* and

Pleurotus ostreatus mushroom fungi -Assessment of their effect on the final product and spent substrate properties. *Food Chem.* 161:127–135.

Krishnamoorthy, A. S., Thiribhuvanamala, G., Shanthi, K., and Marimuttu, T. 2005. Outdoor cultivation of paddy straw mushroom as inter-crop in maize field . *Mushroom Res.* 14 (1): 9-12.

Krishnamoorthy, A. S., Habeebilah, B., and Marimuthu, T. 1991. *Glimpses of Mushroom Research* (eds), Marimuthu, T., Krishnamoorthy, A. S. and Jeyrajan, R.). Tamil Nadu Agricultural University, Coimbatore, pp. 27-29.

Kumar, K. and Barmanrai, A. 2007. Studies on drying characteristics of white button mushroom dried by different drying techniques. *Mushroom Res.* 16(1): 37-40.

Kumar, R., Singh, G., Mishra, P., and Singh, R. 2012. Effect of different organic supplements and casing mixtures on yield of two strains of milky mushroom (*Calocybe indica*). *Indian Phytopathol.* 65(4) : 399-403.

Kumar, R., Singh, G., Pandey, P., and Mishra, P. 2011. Cutural, physiological characteristics and yield attributes of strains of milky mushroom (*Calocybe indica*). *J. Mycol. Plant Pathol.* 41(1): 67-71.

Kumar, S. 1995. Spawn production technology. In: Chadha, K. L. and Sharma, S. R. (eds), *Advances in horticulture Vol.13-Mushrooms*. Malhotra Publishing House, New Delhi, pp.109-114.

Kumar, S. and Sharma, S. R. 2000. Phorids affecting mushroom production and their management. *Mushroom Res.* 9 (2): 55-69.

Kumar, S. and Sharma, S. R. 2001. Studies on seasonal abundance of mushroom pests. *Mushroom Res.* 10 (2): 121-123.

Kumar, S., Sharma, S. R., and Gautam, Y. 2001. Scirids affecting mushroom production and their management. *Indian J. Mushrooms* 19 (182): 62-69.

Kumar, S., Sharma, V. P., and Kamal, S. 2017. Holistic approaches for management of pest and diseases in mushrooms [abstract]. In: *Abstracts, National Symposium on Mushrooms Trends and Innovations in Mushroom Sciences*; 27-28, April, 2017, Solan, India. Mushroom Society of India and ICAR- Directorate of Mushroom Research, Solan, p.78. Abstract No. V-l-5.

Kumari, B., Upadhyay, R. C., and Atri, N. S. 2013. *Auricularia olivaceus*: a new species from North India. Mycosphere 4 (1): 133-138.

Kushwaha, K. P. S., Bhatt, P., and Singh, R. P. 2006. Evaluation of different substrate for yield performance of *Auricularia polytricha* a medicinal mushroom. *Int. J. agric. Sci.* 2(2): 389-391.

Kwon, H. 2004. *Oyster Mushroom Cultivation.* Mushworld, Republic of Korea, pp. 144-159.

Lai, W. H., Murni, M. J. S., Fauzi, D., Mazni, O. A., and Saleh, N. M. 2011. Optimal culture conditions for mycelial growth of *Lignosus rhinoceros*. *Mycobiol.* 39(2): 92-95.

Lau, H. L., Wong, S. K., Bong, C. F. J., and Rabu, A. 2014. Suitability of oil palm empty fruit bunch and sago waste for *Auricularia polytricha* cultivation. *Asian J. Plant Sci.* 13(3): 111-119.

Lazo, C. R. M., Kalaw, S. P., and Leon, D. A. M. 2015. Ethnomycological survey of macrofungi utilized by Gaddang communities in Nueva Vizcaya, Philippines. *Curr. Res. Environ. Appl. Mycol.* 5(3): 256-262.

Lees, R. 1975. *Food Analysis – Analytic and Quality Control Methods for the Food Manufacturers and Buyers* (3rd Ed.). Leonard Hill Books, London, 91p.

Leon, D. A. M., Reyes, R. G., and Tee, D. C. 2012. An ethnomycological survey of macrofungi utilized by Aeta communities in Central Luzon, Philippines. *Mycosphere* 3(2): 251-259.

Li, B., Li, W., Chen, X., Jiang, M., and Dong, M. 2012. *In vitro* antibiofilm activity of the melanin from *Auricularia auricula*, an edible jelly mushroom. *An. Microbiol.* 62(4): 1523-1530.

Li, W., Li, X., Yang, Y., Zhou, F., Liu, L., and Zhou, S. 2015. Effects of different carbon sources and C/N values on nonvolatile taste components of *Pleurotus eryngii*. *Int. J. Food Sci. Technol.* 50: 2360-2366.

Li, Y. 2012. Present development situation and tendency of edible mushroom industry in China. *Mushroom Sci.* 18: 3-9.

Liao, W. C., Hsueh, C. Y., and Chan, C. F. 2014. Antioxidative activity, moisture retention, film formation, and viscosity stability of *Auricularia fuscosuccinea*, white strain water extract. *Biosci. Biotechnol. Biochem.* 78(6): 1029-1036.

Lilly, V. G. and Barnett, H. L. 1951. *Physiology of the fungi*. Mcgraw Hill Book Company Inc., New York. 439p.

Ling, L. I., Junan, D., Luo, L. I., Li, Z., Xiang-li, W., and Gui-hua, Z. 2005. Preliminary studies on the early quality identification of *Auricularia auriculae*. *J. For. Res.* 16(1):61-64.

*Linnaeus, C. 1753. *Species Plantarum*. Laurentius Salvius, Holmiae, 1157p.

Lishma, N. P. 2015. Standardization of techniques for cultivation of button mushroom (*Agaricus* spp.) in Kerala. M.Sc.(Ag) thesis, Kerala Agricultural University, Thrissur, 120p.

Liu, G. Y. 2005. A new method for tissue separation of edible fungus (*Auricularia auricula-judae*). *Guizhou Agric. Sci.* 1:73.

Lo, Y. G., Lin, S. Y., Ulzijarga, E., Chen, S. Y., Chien, R. C., Tzou, Y. J., and Mau, J. L. 2012. Comparative study of contents of several bioactive components in fruiting bodies and mycelia of culinary-medicinal mushrooms. *Int. J. Med. Mushrooms* 14: 357–363.

Lomberh, M. L., Solomko, E. F., Buchalo, A. S., and Kirchhoff, B. 2002. Studies of medicinal mushrooms in submerged cultures. In: Sanchez (eds) Mushroom biology and mushroom products. The 4th international conference on mushroom breeding and mushroom products. pp. 367-377.

Looney, B. P., Birkebak, J. M., and Matheny, P. B. 2013. Systematics of the genus *Auricularia* with an emphasis on species from the Southeastern United States. *N. American Fungi* 8(6): 1-25.

Lopez, C. N. and Vazquez, J. E. S. 1997. Mycelial growth of *Pleurotus* and *Auricularia* in agroindustrial effluents. *Micol. Neotrop. Apl.* 10: 47-56.

Lou, L. H. 1978. *The Cultivation and Processing of Edible Mushrooms*. Peking Agricultural University, Peking, China, pp. 96-106.

Lowy, B. 1952. The genus *Auricularia*. *Mycologia* 44 (5): 656–692.

Lu, J. V. and Tang, A. V. 1986. Cellulolytic enzymes and antibacterial activity of *Auricularia polytricha*. *J. Food Sci.* 51(3): 668-669.

Luo, X. C. 1993. Biology of artificial log cultivation of *Auricularia* mushrooms. In. *Mushroom Biology and Mushroom Products*, Chinese University Press, China, pp. 129-132.

Ma, H., Xu, X., and Feng, L. 2014. Responses of antioxidant defenses and membrane damage to drought stress in fruit bodies of *Auricularia auricula-judae*. *World J. Microbiol. Biotechnol.* 30:119-124.

Ma, W. Z. and Luo, X. C. 1992. Nutritional conditions of basidial spore germination and mycelia growth in *Auricularia auricula*. *Proceedings of 2nd China-Japan International Congress of Mycology*, 107p.

Mahmud, A. A., Herliyana, E. N., and Mansur, I. 2013. Analysis of ear mushroom (*Auricularia* sp.) cultivation using the cutting waste of forest tree species. *Proccedings of the 4th International Conference on sustainable future for human security*, 19-21 October 2013, Japan. Indian student association Kyoto, pp.189-192.

Mandeel, Q. A., Laith, A. A. A., and Mohamed, S. A. 2005. Cultivation of oyster mushrooms (*Pleurotus* spp.) on various lignocellulosic wastes. *World J. Microbiol. Biotechnol.* 21: 601-607.

Manzi, P. and Pizzoferrato L. 2000. Beta-glucans in edible mushrooms. *Food chem.* 68(3): 315-318.

Manzi, P., Aguzzi, A., and Pizzoferrato, L., 2001. Nutritional value of mushrooms widely consumed in Italy, *Food Chem.* 73: 321- 325.

Marcel, D. 2004. Fungal biotechnology in agricultural, food and environmental production of edible fungi. In: Rai, R. D. (eds) *Production of edible fungi.* National Research Centre for Mushroom, Solan, pp.1-14.

Mason, J. (ed.). 2003. Understanding the products used in sustainable agriculture. *Sustainable Agriculture* (2nd Ed.). Landlinks Press, Australia, 191p.

Mathew, J., Kothandaraman, R., and Joseph, K. 1991. Cultivation of oyster mushroom on rubber processing factory waste- A possible solid waste utilization method. In: Nair, M. C., Balakrishnan, S., and Gokulapalan, C. (eds), *Indian Mushrooms.* Proceedings of the National Symposium on Mushrooms, Kerala Agricultural University, Thrissur, pp. 97-99.

Mattila, P., Vaananen, S. P., Konko, K., Aro, H., and Jalava, T. 2002. Basic composition and amino acid contents of mushrooms cultivated in Finland, *J. Agric. Food Chem.* 50: 6419-6422.

Maziero, R., Cavazzoni, and Bononi, V. L. R. 1999. Screening of basidiomycetes for the production of exopolysaccharide and biomass in submerged culture. *Rev. Microbiol.* 30 (1): 1-8.

Mbaluto, C. M. 2015. Characterisation and development of propagation spawns for selected wild edible mushrooms from aberdare national forest, Kenya. M. Sc thesis, Pure and Applied Sciences of Kenyatta University, Kenya.132p.

Mehta, B. K. and Jandaik, C. L. 1989. Storage and dehydration studies of fresh fruit bodies of dhingri mushroom (*Pleurotus sapidus*). *Indian J. Mushrooms* 15: 17-22.

Meng, X., Liang, H., and Luo, L. 2016. Antitumor polysaccharides from mushrooms: a review on the structural characteristics, antitumor mechanisms and immunomodulating activities. *Carbohyd. Res.* 424(7): 30-41.

*Micheli, P. 1728. *Nova Plantarum Genera*. Bernard Paperini, Florence, 124p.

Mignucci, J. S., Baco, H. C., Vargas, R, L., Betancourt, C., and Alameda, M. 2000. Diseases and pests research on oyster mushrooms (*Pleurotus* spp.) in Puerto Rico. *Int. J. Mushroom Sci.* 3 (1): 21-26.

Minamide, Iwata, T., Habu, T., and Toshio. 1985. Studies on physiological, chemical properties and post-harvest keeping quality of mushrooms : IX. Changes in soluble carbohydrates and keeping the freshness of mushrooms (*Agaricus bisporus* Sing.) after Harvest. *Bull. Univ. Osaka Prefecture. Ser. Agric. Biol.* 37: 5-12.

Mohanan, C. 2011. Macrofungi of Kerala. Ph. D thesis, Kerala Forest Research Institute, Thrissur, Kerala, 597p.

Mota, W. F., Finger, F. L., Cecon, P. R., Silva, D. J. H., Correa, P. C., Firme, L. P., and Neves, L. L. M. 2006. Shelf life of four cultivars of okra covered with PVC film at room temperature. *Hortic. Brasileira* 24: 255-258.

Murugkar, A. D. and Subbulakshmi, G. 2005. Nutritional value of edible wild mushrooms collected from the Khasi hills of Meghalaya. *Food Chem.* 89: 599-603.

Musngi, R. B., Abella, E. A., Lalap, A. L., and Reyes, R. G. 2005. Four species of wild *Auricularia* in Central Luzon, Philippines as sources of cell lines for researchers and mushroom growers. *J. Agric. Technol.* 1(2): 279-299.

Nair, M. C. 1990. *Mushrooms. Technical Bulletin- 17*. Kerala Agricultural University, Thrissur, 137p.

Narain, R., Sahu, R. K., Kumar, S., Garg, S. K., Singh, C. S., and Kanaujia, R. S. 2008. Influence of different nitrogen rich supplements during cultivation of *Pleurotus florida* on corn cobs substrate. *Environ.* 13: 67 -71.

Navathe, S., Borkar, P. J., and Kadam, J. J. 2014. Cultivation of *Calocybe indica* (P and C) in Konkan Region of Maharashtra, India. *World J. Agric. Res.* 2 (4): 187-191.

Nguyen, T. B. 2004. Oyster mushrooms - rubber tree sawdust. 2004. In: *Mushroom Grower's Handbook 1*. pp.116-119.

Nilanjana, D., Mahapatra, S. C., and Chattopadhyay, R. N. 2001. Effect of hormones on the yield of oyster mushroom, *Pleurotus florida. Indian J. Mushrooms* 19(1): 39-40.

Nwordu, M. E., Isu, R. N., and Ogbadu, G. H. 2013. Catalogue and identification of some wild edible macro fungi in Nigeria. *Int. J. Food Sci.* 2(1): 1-15.

Oei, P. 2003. *Mushrooms Cultivation. Appropriate Technology for mushrooms growers* (3rd Ed.). Bachhuys Publishers, Netherlands, 426p.

Ohga, S. 2000. Influence of wood species on the sawdust-based cultivation of *Pleurotus abalonus* and *Pleurotus eryngii. J. Wood Sci.* 46: 175-179.

Okhuoya, J. A., Akpaja, E. O., and Abbot, O. 2005. Cultivation of *Lentinus squarrosulus* (Mont) Sing on sawdust of selected tropical tree species. *Int. J. Med. Mushroom* 7: 213-218.

Okwulehie, I. C. and Odunze, E. I. 2004. Evaluation of the nutritional value of some tropical edible mushrooms. *J. Sustain. Agric. Environ.* 6(2): 157-162.

Ondo, J. P., Obame, L. C., Andzibarhe, T., Nsi, G. A, Nsi, E. E., and Lebibi, J. 2013. Phytochemical screening, total phénolic content and antiradical activity of *Aspleniuma fricanum* (Aspleniaceae) and fruit of *Megaphrinium macrostachyum* (Marantaceae). *Appl. Pharma. Sci.* 3(8): 92-96.

Onyango, B. O., Mbaluto, C. A., Mutuku, C. S., and Otieno, D. O. 2016. Molecular characterization of wood ear mushrooms (*Auricularia* sp.) from Kakamega Forest in Western Kenya. *Curr. Res. Environ. Appl. Mycol.* 6 (1): 51-60.

Onyango, B. O., Otieno, C. A., and Palapala V. A. 2013. Effect of wheat bran supplementation with fresh and composted agricultural wastes on the growth of Kenyan native wood ear mushrooms [*Auricularia auricula* (L. ex Hook.) Underw.]. *Afr. J. Biotechnol.* 12: 2692-2698.

Onyango, B. O., Palapala, V. A., Arama, P. F., Wagai, S.O., and Gichimu, B. M. 2011a. Morphological characterization of Kenyan native wood ear mushroom (*Auricularia auricula* (L. ex Hook.) Underw.) and the effect of supplemented millet and sorghum grains in spawn production. *Agric. Biol. J. N. Am.* 2(3): 407-414.

Onyango, B. O., Palapala, V. A., Arama, P. F., Wagai, S.O., and Gichimu, B. M. 2011b. Suitability of selected supplemented substrates for cultivation of Kenyan native wood ear mushroom (*Auricularia auricula*). *Am. J. Food Technol.* 6(5): 395-403.

Osemwegie, O. O., Eriyamremu, G. E., and Abdulmalik, J. 2006. A survey of macrofungi in Edo/Delta region of Nigeria, their morphology and uses. *Glob. J. Pure Appl. Sci.* 12 (2): 149- 157.

Owaid, M. N., Abed, I. A., Saleem, S. S., and Saeedi, A. 2015a. Using of date palm fiber mixed with other lignocelluloses toward *Pleurotus ostreatus* (Higher Basidiomycetes) cultivation. *Emirates J. Food Agric.* 27(7): 556-561.

Owaid, M. N., Salem, S. S., Saeedi, A., Sabaratnam, V., Assaffii, I. A. A., and Raman, J. 2015b. Growth performance and cultivation of four oyster mushroom Species on Sawdust and Rice Bran Substrates. *J. Ad. Biotechnol.* 4(1): 421-429.

Packialakshmi, Sudha, G., and Charumathy, M. 2016. Bioactive constituents and antioxidant efficacy of *Auricularia polytricha. Asian J. Pharma. Clin. Res.* 9 (1): 125-129.

Palapala, V., Miheso F. P. and Nandi, O. 2006. *Cultivation potential of indigenous species of African wood ear mushrooms. (Lecture notes)* at Masinde Muliro University, Kenya: pp 1-21.

Pandey, A., Soccol, C. R., and Larroche, C., 2008. *Current Developments in Solid-State Fermentation.* Asiatech Publishers, New Delhi, 517p.

Patil, B. D. and Jadhav, S. W. 1991. Yield performance of *Pleurotus sajor-caju* on various substrates. In: Nair, M. C., Balakrishnan, S., and Gokulapalan, C. (eds), *Indian Mushrooms.* Proceedings of the National Symposium on Mushrooms, Kerala Agricultural University, Thrissur, pp. 84-86.

Pegler, D. N. 2001. Useful fungi of the world: mu-erh and silver ears. *Mycologist* 15(1): 19-20.

Peng, W., He, X., Wang, Y., Zhang, Y., Ye, X., Jia, D., Guo, Y., Gan, B., Zheng, C., Yang, Z., and Sun, Q. 2014. A new species of Scytalidium causing slippery scar on cultivated *Auricularia polytricha* in China. FEMS. *Microbiol. Lett.* 359(1):72-80.

Petcharat, V. and Tongwised, A. 2003. Effect of oil Palm Kernel Meal on Yield of Hed Hu Hnu (*Auricularia polytricha* (Mont.) Sacc.) and Hed Khon Khao (*Lentinus squarrosulus* Mont.). *Songklanakarin J. Sci. Technol.* 25(5): 589-594.

Philippoussis, A., Zervakis, G., and Diamantopoulou, P. 2001. Potential for the cultivation of exotic mushroom species by exploitation of Mediterranean agricultural wastes. In: Van Griensven, L. J. L. D. (eds). *Science and Cultivation of Edible Fungi.* Balkema, Rotterdam, pp.523-530.

Phillips and Roger. 1981. *Mushrooms and Other Fungi of Great Britain and Europe.* Pan Books, London, 262p.

Pizzoferrato, L., Manzi, P., Bertocchi, F., Fanelli, C., Rotilio, G., and Paci, M. 2000. Solid-state CCPMASNMR spectroscopy of mushrooms gives directly the radio between proteins and polysaccharides. *J. Agric. Food Chem.* 48(11): 5484-5488.

Prakasam, V., Kumar, V.D., and Sangeetha, G. 2002. Effect of fungal contaminants on the growth of *Pleurotus florida* (abstract). In: Abstracts, III Mushroom Conference; 6-7 March, 2002, Coimbatore. Tamil Nadu Agricultural University, Coimbatore.101p.

Prakasam, V., Thiribhuvanamala, G., and Devi, P. A. 2013. Open field cultivation of oyster mushroom in bamboo plantations. *Mushroom Res.* 22 (1): 53-57.

Prasad, S., Rathore, H, Sharma, S., and Yadav, A. S. 2015. Medicinal mushrooms as a source of novel functional food. *Int. J. Food Sci. Nutr. Diet.* 04(5): 221-225.

Prieto, P., Pineda, M., and Anguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of Vitamin E. *Anal. Biochem.* 269(2): 337-341.

Pushpa, H. and Purushothama, K. B. 2010. Nutritional analysis wild and cultivated edible medicinal mushrooms. *World J. Dairy Food Sci.* 5: 140-144.

Quimio, T. H. 1979. Taxonomic consideration of Auriculariales imported into the Philippines. *Philipp. J. Biol.* 6: 69-72.

Quimio, T. H. 1982. Physiological considerations of *Auricularia* spp. In: Chang, S. T. and Quimio, T. H. *Tropical Mushrooms - Biological Nature and Cultivation Methods* (eds), The Chinese University Press, Hong Kong, pp. 397-408.

Raghuramulu, N., Nair, M. K., and Kalyanasundaram, S. 1983. *A Manual of Laboratory Technique.* National Institute of Nutrition, I. C. M. R., 359p.

Ragunathan R and Swaminathan K. 2003. Nutritional status of *Pleurotus* spp. grown on various agro-wastes. *Food Chem.* 80: 371-375.

Ragunathan R., Gurusamy R and Palaniswamy M. 1996. Cultivation of *Pleurotus* spp. on various agro-residues. *Food Chem.* 55: 139-144.

Rai, R. D. 1995. Nutritional and medicinal values of mushrooms. In: Chadha, K. L. and Sharma, S. R. (eds), *Advances in horticulture Vol.13-Mushrooms*. Malhotra Publishing House, New Delhi, pp. 537-551.

Rai, R. D. and Arumuganathan, T. 2008. *Postharvest technology of mushrooms Technical Bulletin*. National Research Centre for Mushroom, Solan, 13p.

Rai, R. D., Saxena, S., Upadhyay, R. C., and Sohi, H. S. 1988. Comparative nutritional value of various *Pleurotus* spp. grown under identical conditions. *Mushroom. J. Tropics* 8: 93-98.

Ramkumar, L., Thirunavukkarasu, P., and Ramanathan, T. 2010. Development of improved technology for commercial production and preservation of Shiitake mushroom (*Lentinula edodes*). *Am. Eurasian J. Agric. Environ. Sci.* 7(4): 433-439.

Rangaswamy, G. and Mahadevan, A. 2008. *Diseases of Crop Plants in India*. PHI Learning Private Limited, New Delhi, pp.117-129.

Razak, D. L. A. 2013. Cultivation of *Auricularia polytricha* Mont. Sacc (Black jelly mushroom) using oil palm wastes. M. Sc. thesis. University of Malaya, Kualalumpur, 137p.

Razak, D. L. A., Abdullah, N., Johari, N. M. K., and Sabaratnam, V. 2013. Comparative study of mycelia growth and sporophore yield of *Auricularia polytricha* (Mont.) Sacc on selected palm oil wastes as fruiting substrate. *Appl. Microbiol. Biotechnol.* 97: 3207 - 3213.

Regula, J. and Siwulski, M. 2007. Dried shiitake (*Lentinula edodes*) and oyster (*Pleurotus ostreatus*) mushrooms as a good source of nutrient, *Acta Scientiarum Polonorum. Technologia Alimentaria* (6): 135-142.

Reza, A., Choi, M., Damte, D., Jo, W., Lee, S., Lee, J., and Park, S. 2011. Comparative antitumor activity of different solvent fractions from an *Auricularia auricula-judae* ethanol extract in P388D1 and sarcoma 180 Cells. *Toxicol. Res.* 27(2): 77–83.

Robaszkiewicz, A., Bartosz, G., Ławrynowicz, M., and Soszyńsk, M. 2010. The Role of Polyphenols, β-Carotene, and lycopene in the antioxidative action of the extracts of dried, edible mushrooms. *J. Nutr. Metab.* Available: http://dx.doi.org/10.1155/2010/173274

Roberts P. 2001. Heterobasidiomycetes from Korup National Park, Cameroon. *Kew Bull.* 56(1): 163-187.

Roberts, P. and Meijer, D. 1997. Macromycetes from the state of Parana, Brazil. 6. Sirobasidiaceae & Tremellaceae. *Mycotaxon* 64: 261-283.

Royse, D. J. 1997. Specialty Mushrooms and their Cultivation. *Horticulture Review.* 19: 59-97.

Royse, D. J. 2014. A global perspective on the high five: Agaricus, Pleurotus, Lentinula, Auricularia & Flammulina. *Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Products (ICMBMP8)*, 20-22 November 2014, New Delhi. World Society for Mushroom Biology and Mushroom Products, Directorate of Mushroom Research and Mushroom Society of India, pp. 1-6.

Royse, D. J. and Vazquez, S. J. E. 2001. Influence of wood chip particle size used in substrate on biological efficiency and post-soak log weights of shiitake. *Mushroom Sci.* 15: 367-373.

Royse, D. J., Fales, S. L., and Karunananda, K. 1991. Influence of formaldehydetreated soyabean and commercial nutrient supplementation on mushroom (*Pleurotus sajorcaju*) yield and *in vitro* dry matter digestibility of spent substrate. *Appl. Microbiol. Biotechnol.* 36: 425- 429.

Sanchez, C. 2010. Cultivation of *Pleurotus ostreatus* and other edible mushrooms. *Appl. Microbiol. Biotechnol.* 85:1321–1337.

Sanmee, R., Dell, B., Lumyong, P., Izumori, K., and Lumyong, S. 2003. Nutritive value of popular wild edible mushrooms from northern Thailand. *Food Chem.* 84: 527-532.

Sarma, T. C., Sarma, I., and Patiri, B. N. 2010. Wild edible mushrooms used by some ethnic tribes of western Assam. *Bioscan* 3: 613-625.

Saxena, S. and Rai, R. D. 1988. Storage of button mushrooms (*Agaricus bisporus*)-the effect of temperature, perforation of packs and pre-treatment with potassium metabisulphite. *Mushroom J. Tropics* 8: 15-22.

Schenck, S. and Dudley, N. S. 1999. *Wood Ear (Pepeiao) Production in Forest Understory*. Hawaii Agriculture Research Center, Hawaii, pp. 102- 106.

*Schroeter, J. 1889. *Pilze in Kryptogramen -Flora von Schlesien*. Kern's Verlag, Breslau, 386p.

Shamsi, Y. M. A. 2016. Biological efficiency of recycling organic waste with edible fungi. United Arab Emirates University, Arab, 76p.

Sharma, S. S., Doshi, A., and Trivedi, A. 1992. Edible fleshy fungi from Rajasthan. *Mushroom. Res*. 1:141.

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

Sharma, S., Rathore, H., Prasad, S., and Singh, A. 2017. Utilization of nitrogen rich supplements for quality and quantity improvement of *Lentinula edodes* and *Calocybe indica* and its propagation in villages of Haryana. [abstract]. In: *Abstracts, National Symposium on Mushrooms Trends and Innovations in Mushroom Sciences*; 27-28, April, 2017, Solan, India. Mushroom Society of India and ICAR- Directorate of Mushroom Research, Solan, p. 84. Abstract No. V-O-3.

Sharma, V. P., Kumar, S., and Sharma, S. R. 2005. Studies on chemical sterilization of substrate for the cultivation of some specialty mushrooms. *Mushroom Res.* 14(1): 41-45.

Sharma, V. P., Kumar, S., and Singh, R. 2012. Effect of moisture level on the linear growth and different casing soil treatment methods on the productivity of *Calocybe indica*. *Indian J. Mush.* 30(1): 27-29.

Shashireka, M. N., Rajrathnan, S., and Bano, Z. 2005. Effects of supplementing rice bran substrate with cotton seeds on analytical characterization of mushroom *Pleurotus florida*. *Food Chem.* 92: 255 - 259.

Shen, Q. and Royse, D. 2001. Effect of nutrient supplement on biological efficiency, quality and crop cycle time on maitake (*Griofola frondosa*). *Appl. Microbiol. Biotechnol.* 57: 74-78.

Sher, H., Yemeni, M. A., Bahkali, A. H. A., and Sher, H. 2010. Effect of environmental factors on the yield of selected mushroom species growing in two different agro ecological zones of Pakistan. *Saudi J. Biol. Sci.* 17: 321–326.

Sherin, A. S. 2003. Utilization of fungi for composting and mushroom production on coirpith. M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 90p.

Shim, S. M., Lee, K. R., Kim, S. H., Im, K. H., Kim, J. W., Lee, U. Y., Shim, J. O., Lee, M. W., and Lee, T. S. 2003. The optimal culture conditions affecting the mycelial growth and fruiting body formation of *Paecilomyces fumosoroseus*. *Mycobiol.* 31(4): 214-220.

Shim, S. M., Oh, Y. H., Lee, K. R., Kim, S. H., Im, K. H., Kim, J. W., Lee, U. Y., Shim, J. O., Shim, M. J., Lee, M. W., Ro, H. S., Lee, H. S., and Lee, T. S. 2005. The characteristics of cultural conditions for the mycelia growth of *Macrolepiota procera*. *Mycobiol.* 33(1): 15-18.

Sibounnavong, P., Cynthia.C. D., Kalaw, S. P, Reyes, R. G., and Soytong, K. 2008. Some species of macrofungi at Puncan, Carranglan, Nueva Ecija in the Philippines. *J. Agric. Technol.* 4(2): 105-115.

Sinden, J. W. 1934. Mushroom spawn and methods of making the same. US Patent 2: 844-861.

Singh, A., Singh, S., and Upadhaya, R. C. 2002. Management of contaminants in spawn production [abstract]. In: *Abstracts, Third Mushroom Conference*; 6-7, March, 2002, Coimbatore. Tamil Nadu Agricultural University, Coimbatore, 49p.

Singh, V. P., Srivastava, S., Rastogi, J., Singh, P., Abhilasha, I., and Gupta, P. 2014. *In vitro* evaluation of carbendazim 50 % WP on green mould disease of some *Pleurotus* spp. *Mushroom Res.* 23(1): 93-97.

Smith, A. H. 1964. *The mushroom hunter field guide revised and enlarged.* Ann Arbor. University of Michigan Press, Michigan. 264p.

Smith, D. and Onions, A. H. S. 1983. *The Preservation and Maintenance of Living Fungi.* Kew: CAB Commonwealth Mycological Institute. U.K., 51p.

Sobal, M., Carrera, M. D., Morales, P., and Roussos, S. 2007. Classical characterization of mushroom genetic resource from temperate and tropical regions of Mexico. *Mycologia Aplicada Int.* 19:15-23.

Sohi, H. S. and Upadhyay, R. C. 1990. Natural occurrence of different species of *Auricularia* in Himachal Pradesh. *Mushroom J. Trop.*10: 47-51.

Song, G. and Du, Q. 2012. Structure characterization and antitumor activity of an α βglucanpolysaccharide from *Auricularia polytricha. Food Res. Int.* 45(1): 381-387.

Srivastava, R. P. and Kumar, S. 2002. *Fruit and Vegetable Preservation: Principles and Practices.* Motilal Banarsidass Publishers. New Delhi, India. 474p.

Stamets, P. 1993. *Growing gourmet and medicinal mushrooms.* Ten Speed Press, Berkeley, 56p.

Stamets, P. 2000. *Growing gourmet and medicinal mushrooms.* (3rd Ed.). Ten Speed press, Berkeley-Toronto, 339p.

Sterry, Paul, Hughes, and Barry. 2009. *Complete Guide to British Mushrooms and Toadstools*. Harper Collins Publishers, London, 290p.

Subramanian, K. and Shanmugasundaram. K. 2015. Optimization of casing process for enhanced bioefficiency of *Calocybe indica*, an indigenous tropical edible mushroom. *Internat. J. Recent Sci. Res.* 6 (2): 2594-2598.

Sudheep, N. M. and Sridhar, K. R. 2014. Nutritional composition of two wild mushrooms consumed by the tribals of the Western Ghats of India. *Mycol.* 5(2): 64–72.

Suharban, M. 1987. Monographic studies on edible species of *Pleurotus* and standardisation of the techniques for large scale cultivation. Ph.D thesis, Kerala Agricultural University, Thrissur, 195p.

Sumi, I. 2016. Physiological and cultural studies on blue oyster mushroom (*Hypsizygus ulmarius* (Bull.:Fr.) Redhead). M. Sc.(Ag) thesis, Kerala Agricultural University, Thrissur, 102p.

Sun, J. and Bian, Y. 2012. Slippery scar a new mushroom disease in *Auricularia polytricha. Microbiol.* 40: 129-133.

Sylvester, E. C., Ezejiofor, A. J., and Nnedinma, E. A. 2014. Survey and proximate analysis of edible mushrooms in Enugu State, Nigeria. *Ann. Exp. Bio.* 2 (3): 52-57.

Tabata, T. and Ogura, T. 2003. Absorption of Calcium and Magnesium to the fruit body of Aragekikurage (*Auricularia polytricha* (Mont.) Sacc.) from sawdust culture media supplemented with Calcium and Magnesium Salts. *Food Sci. Technol. Res.* 9 (3): 250-253.

Thiribhuvanamala, G., Krishnamoorthy, A. S., Shanthi, K., and Marimuthu, T. 2005. Development of suitable substrates for spawn and mushroom production of *Lentinula edodes* and *Auricularia polytricha. Madras Agric. J.* 92 (4): 344-348.

Tisdale, T. E., Miyasaka, S. C., and Hemmes, D. E. 2006. Cultivation of the oyster mushroom (*Pleurotus ostreatus*) on wood substrates in Hawaii. *World J. Microbiol. Biotechnol.* 22:201.

Toma, F. M., Ismael, H. M. and Abdulla, N. Q. F. 2013. Survey and identification of mushrooms in Erbil Governorate. *Res. J. Environ. Earth Sci.* 5(5): 262-266.

Toshinungla, A. O., Deb, C. R., and Khruomo, N. 2016. Wild edible mushrooms of Nagaland, India: a potential food. *Resour. J. Exp. Biol. Agric. Sci.* 4(1): 59-65.

Tu, Y., Sun, Y., Tian, Y., Xie, M., and Chen, J. 2009. Physicochemical characterisation and antioxidant activity of melanin from the muscles of Taihe Black-bone silky fowl (*Gallus gallus*). *Food Chem.* 114: 1345-1350.

Tuite, J. 1969. *Plant Pathological Methods: Fungi and Bacteria.* Burgess Publishing Co. Minneapolis, U.S.A., 239p.

Uhart, M., Piscera, J. M., and Albert, E. 2008. Utilization of new naturally occurring strains and supplementation to improve biological efficiency of the edible mushroom *Agrocybe cylindracea*. *J. Indian Microbiol. Biotechnol.* 35: 595 -602.

Ukoima, H. N., Ogbonnaya, L. O., Arikpo, G. E., and Ikpe, F. N. 2009. Cultural studies of mycelia of *Volvariella volvacea*, *Pleurotus tuberregium* and *Pleurotus sajor-caju* on different culture media. *Pakistan J. Nutr.* 8(7): 1052-1054.

Ullah, N., Khan, A., Khan, F. A., Khurram, M., Hussan, M., Khayam, S. M. U., Amin, M., and Hussain, J. 2011. Composition and isolation of beta-carotene from different vegetables and their effect on human serum retinal level. *Middle E. J. Sci. Res.* 9 (4): 496-502.

Upadhyay, R. C. 1999. Studies on cultivation of *Auricularia mesenterica* Pers. *Mushroom Res.* 8(2): 43-45.

Usha, S. and Suguna, V. 2014. Investigation on the nutritional value of edible mushrooms *viz., Auricularia polytricha* and *Pleurotus ostreatus. Asian J. Sci. Technol.* 5 (8): 497-500.

Vidyaresmi, C. V. 2008. Biology and cultivation of *Auricularia* spp. M. Sc.(Ag) thesis, Kerala Agricultural University, Thrissur, 86p.

Vijay, B. and Sohi, H. S. 1987. Cultivation of oyster mushroom, *Pleurotus sajor-caju*, on chemically sterilized wheat straw. *Mushroom J. Tropics* 7: 67-75.

Vijaykumar, G., Priya, G., and Kotrige, G. 2014. Selection of different substrates for the cultivation of milky mushroom (*Calocybe indica*). *Indian J. Tradit. Knowl.* 13(2): 434-436.

Wang, D., Sakoda, A., and Susuki, M. 2001. Biological efficiency and nutritional value of *Pleurotus ostreatus* cultivated on spent beer grain. *Bioresour. Technol.* 78: 293-300.

Wang, J., Li, X., and Song, G. 2013. An efficient separation method of polysaccharides: Preparation of an antitumor polysaccharide APS-2 from *Auricularia polytricha* by Radial Flow Chromatography. *Chromatographia* 76: 629–633.

Wang, R., Cao, H., and Zhang, J. 2015. Scientific Explorations of the Snow Fungus (*Tremella fuciformis* Berk.) in Republican China. *Indian J. Hist. Sci.* 50(2): 340-344.

Wasser, S. P. and Weis, A. L. 1999. Therapeutic effects of substances occurring in higher basidiomycetes mushrooms: A modern perspective. *Crit. Rev. Immunol.* 19: 65-96.

Weber, R. W. S. and Webster, J. 2006. Teaching techniques for mycology: 24. Patterns of basidiospore germination in *Auricularia* (Heterobasidiomycetes). *Mycologist* 20: 105-108.

Well, K. 1984. The Jelly fungi, then and now. *Mycologia* 86: 18-48.

Wong, G. and Wells, K. 1987. Comparative morphology, compatibility and infertility of *Auricularia cornea*, *A. polytricha* and *A. tenuis*. *Mycologia* 79:847-856.

Wong, G. J. 1993. Mating and fruiting studies of *Auricularia delicata* and *A. fuscosuccinea*. *Mycologia* 85: 187-194.

Wong, G. J. and Wells, K. 1987. Comparative morphology, compatibility and infertility of *Auricularia cornea, A. polytricha and A. tenuis*. *Mycologia* 79:847-856.

Wong, George, J., Wells, K., and Bandoni, R. J. 1985. Interfertility and comparative morphological studies of *Tremella mesenterica*. *Mycologia*. 77(1): 36- 49.

Wu, J., Ding, Z. Y., and Zhang, K. C. 2006. Improvement of exopolysaccharide production by macrofungus *Auricularia auricula* in submerged culture. *Enzyme Microbiol. Technol.* 39 (4): 743–749.

Xu, C. P. and Yun, J. W. 2003. Optimization of submerged culture conditions for mycelial growth and exobiopolymer production by *Auricularia polytricha* (wood ears fungus) using the methods of uniform design and regression analysis. *Biotechnol. Appl. Biochem.* 38(2):19-39.

Xu, P., Ding, Z. Y., Qian, Z., Zhao, C. X., and Zhang, K. C. 2008. Improved production of mycelial biomass and ganoderic acid by submerged culture of *Ganoderma lucidum* SB97 using complex media. *Enzyme Microbiol. Technol.* 42(4): 325-331.

Yagi, F. and Tadera, K. 1988. Purification and characteriazation of lectin from *Auricularia polytricha*. *Agric. Biol. Chem.* 52: 2077-2079.

Yang, B. K., Ha, J. Y., Jeong, S. C., Jeon, Y. J., Ra, K. S., Das, S., Yun, J. W., and Song, C. H. 2002. Hypolipidemic effect of an exo-biopolymer produced from submerged mycelial culture of *Auricularia polytricha* in rats. *Biotechnol. Lett.* 24: 1319-1325.

Yang, W. J., Guo, F. L., and Wan, Z. J. 2013. Yield and size of oyster mushroom grown on rice / wheat straw basal substrate supplemented with cotton seed hull. *Saudi J. Biol. Sci.* 20 (4): 33-38.

Yanjie, L., Yutaka, I., Takaaki, S., Hiroaki, K., and Xiaoli, Q. 2013. Effect of high oxygen packaging on respiratory physiology and sensorial qualities of fresh Shiitake mushrooms (*Lentinus edodes*). *J. Food Res.* 2(6): 89-96.

Young, Tony, Smith, and Kay. 2005. *A Field Guide to the Fungi of Australia.* University of New South Wales Press, Australia, 240p.

Yu, J., Sun, R., Zhaoc, Z., and Wang, Y. 2014. *Auricularia polytricha* polysaccharides induce cell cycle arrest and apoptosis in human lung cancer A549 cells. *Int. J. Biol. Macromolecules* 68: 67-71.

Yu, M. Y., Ma, B., Luo, X., Zheng, L. Y., Xu, X. Y., and Yang, Z. R. 2008. Molecular diversity of *Auricularia polytricha* revealed by inter-simple sequence repeat and sequence-related amplified polymorphism markers. *Curr. Microbiol.* 56: 240-245.

Zervakis, G., Philippoussis, A., Ioannidou, S., and Diamantopoulou, P. 2001. Mycelium growth kinetics and optimal temperature conditions for the cultivation of edible mushroom species on lignocellulosic substrates. *Folia Microbiol.* 46(3): 231- 234.

Zhang, H. X. L. 1995. Study of Fusarium pathogen on *Auricularia polytricha* cultivated with plastic bag in Sichuan Province. *Acta edulis fungi* 22: 44-47.

Zhang, J. E., Kong, X. H., Zhang, P. Q., Liu, J. N., Ma, Y. P., Dai, X. D., Zan, H. Z., and Yu, L. P. 2017. Identification of a new fungal pathogen causing white villous disease on the fruiting body of the culinary-medicinal Mushroom *Auricularia auricula-judae* (Agaricomycetes) in China. *Int. J. Med. Mushrooms.* 19(2): 155-161.

Zhang, M., Xiao, G., Thring, R. W., Chen, W., Zhou, H., and Yang, H. 2015. Production and characterization of melanin by submerged culture of culinary and medicinal fungi *Auricularia auricula. Appl. Biochem. Biotechnol.* 176(1): 253-266.

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

Zou, P., Gao, J. R., and Ma, E. P. 1993. Preliminary studies on the biology of the pest mite *Luciaphorus auriculariae* (Acari: Pygmephoridae) infesting Jew's ear mushroom *Auricularia polytricha* in China. *Exp. Appl. Acarology*. 17(3): 225-232.

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* - *Originals not seen*

Characterization and exploitation of jelly mushrooms (*Auricularia* spp. / *Tremella* spp.)

by

PRIYA R. U.

(2014-21-133)

Abstract of the thesis

Submitted in partial fulfilment of the

requirements for the degree of

DOCTOR OF PHILOSOPHY IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695522

KERALA, INDIA

2017

ABSTRACT

The present study entitled "Characterization and exploitation of jelly mushrooms (*Auricularia* spp./ *Tremella* spp.)" was carried out in College of Agriculture, Vellayani during 2014-2017, with the objective of standardization of techniques for production of jelly mushrooms (*Auricularia* spp./ *Tremella* spp.) in agricultural wastes and to study their morphological, physiological and cultural characteristics as well as nutritional and organoleptic qualities.

Survey was conducted in ten different locations of Thiruvananthapuram and Kollam districts of Kerala during 2014-2016. Sporocarps of *Auricularia* spp. and *Tremella* spp. were collected from tree stumps, predominantly Mango, Coconut, Drumstick, Teak wood and Rubber. All the mushrooms collected from all the locations were gregarious in nature and lignicolous in habitat. Morphological studies of jelly mushrooms showed that the sporocarps were light brown to dark brown in colour with incurved margin, ear shaped, soft and velvety in texture and devoid of stipe. The internal stratification of hyphae showed eight different zonations. Basidiospores were hyaline, oval and sub cylindrical to cylindrical shaped.

Two fast growing isolates selected based on the time taken for complete mycelial growth and nature of mycelial growth, designated as A1 and A2 were sent to Directorate of Mushroom Research, Solan for identification. These were identified as *Auricularia polytricha* (Mont.) Sacc. (Accession number of A1 was DMRO-825 and A2, DMRO-826).

The maximum mycelial growth was recorded on malt extract agar medium and a temperature of 25^0C, pH 7 and light conditions were found favourable for mycelial growth. Evaluation of different substrates for spawn production revealed that paddy grain was the best substrate followed by sorghum. Rubber sawdust spawn recorded maximum keeping quality. Malt extract broth was found to be the best for submerged culture production of both A1 and A2.

Evaluation of different substrates for mushroom production revealed that rubber sawdust was the best substrate for cultivation which recorded maximum Biological Efficiency (BE) of 14.8% for A1 and 12.2% for A2. The minimum time for spawn run was taken by paddy straw and the maximum was taken by neopeat. Major insect pests observed were *Megaselia* sp., *Seira* sp. and *Staphylinus* sp. The competitor moulds observed were *Coprinus* sp, *Aspergillus* spp., *Penicillium* sp. and *Trichoderma* sp. Among the different amendments, wheat bran (2.0 and 4.0 %) and groundnut cake (2.0%) were found to be the best for enhancing the growth parameters whereas, rice bran (2.0% and 4.0%) was the best for increasing yield parameters.

Analysis for the proximate constituents in *A. polytricha* (A1 and A2) revealed that it contained appreciable amount of carbohydrate (47.1 and 48.8%), protein (18.06 and 20.75%), polyphenols (9.53 and µg), fibre (17.69 and 15.49%), total anti oxidants (116 and 74µg), β carotene (0.178 and 0.150 µg) and the energy value was 251.49 and 264 respectively. Sensory evaluation of mushroom products made from *A. polytricha* (A1 and A2) indicated that mushroom tomato sauce scored maximum for overall acceptability. Under refrigeration (4^0C) in perforated poly propylene covers mushrooms could be kept fresh for three days.

Indoor cultivation of both A1 and A2 showed significant results for growth parameters as well as yield parameters compared to outdoor cultivation. Comparative performance of *A. polytricha* with two ruling mushrooms of Kerala namely oyster mushroom (*Pleurotus florida* (Mont.) Singer.) and milky mushroom (*Calocybe gambosa* (Fr.) Donk.) indicated that oyster mushroom took minimum days for spawn run, pinhead formation and first flush compared to milky mushroom and *A. polytricha*. Milky mushroom recorded highest BE (66.1%) compared to others.

Studies on medicinal activities of *A. polytricha* indicated that it possessed anticancerous activities for cervical, colon and liver cancer cell lines and higher activity was recorded in 100 µg/ml concentration.

Based on the results of present investigation, *A. polytricha* can be cultivated successfully in tropical areas on locally available materials. Paddy grain was the

most suitable substrate for spawn production and Rubber sawdust amended with 2% rice bran, the most suitable growing medium. Mushrooms possessed significant nutritional and medicinal activities. The findings of the above investigations recommend the adoption of a suitable cultivation package for jelly mushrooms (*A. polytricha*), a highly prized mushroom.

Appendices

APPENDIX-I

Data sheet

Collection no Date of collection

Collected

1. General

   Locality

   Habitat

   Any other details

2. Pileus

   Colour : ..... Diameter : ..

   Shape : Immature..... Mature :.....

   Texture : Soft, brittle, fleshy, coriaceous, membranous

   Surfac : Dry, moist, greasy, smooth, downy, velvety, shaggy, peeling out easily or not.

   Margin : Regular, wavy, smooth, rough, furrowed, incurved or not/ striate or not.

   Veil : Present/ absent ..... Colour..... Abundant.....

   Scarce..... Appendiculate/ membranous

3. Gills

   Colour :

   Arrangement : Remote/ free/adnate/ adnexed/ sinuate/ crowded/ distant, easily seperable from pilear tissue or not.

   Consistency : Pliable/ brittle/ waxy/ fleshy

   Size : cm

   Gill edge : Special features if any

4. Stipe

   Position : Central / eccentric/ sessile

APPENDIX-I (Continued)

Colour :

Size : Length..... Diameter.....

Consistency : Fleshy/ leathery/ woody

Surface characters : Fibrillose/ dry/ viscid pubscent/ squamose/ glabrous

Annulus : Present/ absent Size..... Single/ double

Membranous/ filamentous

Position : Apical, medical, basal

Volva : Present/ absent..... Shape..... Colour.....

Texture : Fleshy/ tough/ papery

5. Flesh

When wet When dry

Colour in Pileus

Colour in Stipe

Change in colour when exposed to air

6. Others

Presence of abnormal liquid/ milky fluid/others

Before cutting/ after cutting

Any other character

APPENDIX-II

COMPOSITION OF STAIN USED

1. **Lactophenol- Cotton blue**

Annhydrous lactophenol	- 67.0 ml
Distilled water	- 20.0 ml
Cotton blue	- 0.1 g

Anhydrous lactophenol prepared by dissolving 20 g phenol in 16 ml lactic acid in 3 ml glycerol.

APPENDIX - III

Composition of different media

a) Potato Dextrose Agar (PDA)

Potato	:	200 g
Dextrose	:	20 g
Agar-agar	:	20 g
Distilled water	:	1 litre

b) Malt Extract Agar (MEA)

Malt extract	:	25 g
Agar-agar	:	20 g
Distilled water	:	1 litre

c) Oat Meal Agar (OMA)

Oat flakes	:	30 g
Agar-agar	:	20 g
Distilled water	:	1 litre

d) Carrot Extract Agar (CEA)

Grated carrot	:	20 g
Agar-agar	:	20 g
Distilled water	:	1 litre

APPENDIX- IV

Particulars	Score	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability
Like Extremely	9						
Like Very Much	8						
Like Moderately	7						
Like Slightly	6						
Neither Like nor Dislike	5						
Dislike Slightly	4						
Dislike moderately	3						
Dislike Very Much	2						
Dislike Extremely	1						

Hedonic rating scale for the evaluation of mushroom recipe

APPENDIX- V

RECIPE 1. PEPPER MASALA

Ingredients		Amount
Mushroom	-	100g (Sliced)
Onion	-	10g (Big)
Green chilli	-	5g (Medium)
Tomato	-	20g (Medium)
Coconut oil	-	1 tsp
Pepper powder	-	1/8 tsp
Turmeric powder	-	a pinch
Red chilli powder	-	a pinch
Salt	-	to taste
Curry leaves	-	6-8 leaves
Mustard	-	Few seeds
Cumin powder	-	A pinch

Procedure:

Oil was heated in a pan, mustard, cumin powder and curry leaves added, sauted for 1one minute, sliced abig onion, green chilli and tomato were added and fried. Mushroom slices, salt and other ingredients added later. Then sauted thoroughly and cooked by covering the pan. Served hot.

APPENDIX-V (Continued)

RECIPE 2. PIZZA

Ingredients		**Amount**
Mushroom	-	100 g (Sliced)
Tomato	-	5-6 (Round cuttings)
Green chilli	-	6-8 (Round cuttings)
Onion	-	5-6 (Round cuttings)
Refined wheat flour	-	100g (Base material)
Cheese	-	50 g
Yeast	-	2.5 g
Olive oil	-	2 ml
Sugar	-	5 g
Water	-	65 ml
Salt	-	2.5 g

Procedure:

Yeast was fermented in a little amount of water. It was mixed with wheat flour, salt, sugar and olive oil. Kneaded and proofed for 15 minutes. Rolled and baked in an oven until the dough turned slightly brown and slightly hard on the edges. Once it cooled down, toppings such as cheese, slices of tomatoes, chillies, onions and mushrooms were added. The toppings along with the dough were baked again for a few more minutes until cooked well.

APPENDIX-V (Continued)

RECIPE 3. PAKODA

Ingredients		Amount
Mushroom	-	100 g (Sliced)
Gran flour	-	150 g
Ginger and Garlic paste	-	1 tbs each
Red chilli powder	-	A pinch
Cumin powder	-	A pinch
Baking soda	-	A pinch
Onion	-	1 (Chopped)
Cillies	-	3-4 (Chopped)
Salt	-	To taste
Cooking Oil	-	250 g
Water	-	As required to make paste form

Procedure:

All the ingredients, except oil, were mixed well to form a thin paste. Allowed to settle for 15 minutes. Oil was heated in a pan. The mixture was made into balls, which was deep fried in the oil. Pakodas were served hot.

APPENDIX-V (Continued)

RECIPE 4. MUSHROOM TOMATO SAUCE

Ingredients		Amount
Mushroom	-	250 g (Finely sliced)
Tomatoes	-	250 g (Finely chopped)
Onion	-	1 (Finely chopped)
Garlic	-	1 clove (Crushed)
Green chillies	-	2 (Finely chopped)
Ginger	-	50 g (Grated)
Cooking oil	-	25 g
Salt and Pepper	-	To taste
Water	-	100 ml
Pepper powder	-	A pinch
Sugar	-	Half spoon

Procedure:

Chopped tomatoes were boiled in 50 ml of water to make thick paste. Garlic and ginger added to it and placed in pressure cooker, after three whistles the material was sieved. Oil was heated in a heavy pan then gently fried chopped onions and chillies until the colour changed to light brown after that the chopped mushrooms were added and cooked for five minutes. Little amount of water, salt, pepper powder and sugar were added later and boiled for ten minutes until to reached creamy appearance.

APPENDIX-V (Continued)

RECIPE 5. SOUP

Ingredients		Amount
Mushroom	-	250 g (Cut into small pieces)
Corn flour	-	20 g
Milk	-	2 Cup
Butter	-	20 g
Ginger	-	15 g
Garlic	-	15 g
Sugar and Salt	-	To taste
Pepper powder	-	To taste

Procedure:

Mushroom was boiled with grated ginger and garlic in three cups of water. After cooling the mixture was grinded and sieved. Corn flour was added in milk, butter was placed on heavy pan, slightly heated and then sieved material along with milk mixture was added and boiled for ten minutes until it became thick. Finally, sugar, salt and pepper powder were added. Seasoned with sliced mushrooms and fresh cream and served hot.

APPENDIX-V (Continued)

RECIPE 6. MUSHROOM VEG CURRY

Ingredients		Amount
Mushroom	-	250 g (Cut into quarters)
Onion	-	2 (Quartered and separated into petals)
Garlic	-	5 cloves
Capsicum	-	100 g (Cut into chunks)
Cabbage	-	100 g (Shredded)
Beans	-	50 g (Cut into thin lengths)
Tomato	-	1
Cooking oil	-	2 tbs
Salt and Red chilli powder	-	To taste
Turmeric powder	-	A pinch
Cumin powder	-	A pinch
Curry leaves	-	6-8 leaves
Coconut powder	-	2 tbs

Procedure:

Oil was heated in a pan, garlic, onion, beans and capsicum were added and then cooked for two minutes. Cabbage added and cooked quickly, tossed for three minutes untill all the vegetables softened slightly and little crisp. When cabbage started wilting, mushrooms were added and kept tossing for another two minutes. Onion, tomato, chillies, pepper powder and coconut powder were grained into a fine paste. Again, the oil was heated in a pan; mustard, cumin powder and curry leaves were added and sauted. The grinded mixture was added along with vegetable and

mushroom mix. Water was added along with salt, turmeric and red chilli powder. Boiled for 10 to 15 minutes. Served with rice.

APPENDIX-V (Continued)

RECIPE 7. BAJJI

Ingredients		**Amount**
Mushroom	-	100 g (Medium sized)
Gran flour	-	150 g
Ginger and Garlic paste	-	1 tbs each
Red chilli powder	-	A pinch
Cumin powder	-	A pinch
Baking soda	-	A pinch
Salt	-	To taste
Cooking Oil	-	250 g
Water	-	As required to make paste form

Procedure:

Washed mushrooms were boiled with a pinch of salt and drained out the excess water and the mushroom were dried for ten minutes by spreading on a dry paper and then mushrooms squeezed properly to remove water content. All the above ingredients were mixed properly to make a thin paste. Mushrooms were dipped in the mixture, deep fried in the hot oil and served fresh.

APPENDIX-VI

Month	Weather parameters				
	T(^{0} C)		RH (%)	No. of rainy days	Total rainfall (mm)
	Mean day	Mean night			
January	32.32	22.67	92.0	1	0.4
February	32.77	23.02	92.55	9	52.1
March	34.3	24.2	90.27	7	61.2
April	35.32	26.25	90.97	5	18.8
May	34.0	24.52	91.4	13	275.8
June	31.35	24.4	94.6	22	442
July	31.65	24.65	93.0	15	110
August	31.75	24.8	90.92	7	33.4
September	31.82	24.57	91.72	2	2.8
October	31.72	24.25	90.15	1	12
November	31.95	24.2	92.77	4	36.2
December	32.0	23.4	92.1	2	7.8