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# 8<sup>th</sup> International Conference on Mushroom Biology and Mushroom Products

19-22 November 2014, New Delhi, India

## AbstrActs

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**World Society of Mushroom Biology and Mushroom Products  
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# Preface

The 8<sup>th</sup> International Conference on Mushroom Biology and Mushroom Products is a continuum of the conferences organized by World Society of Mushroom Biology and Mushroom Products (WSMBMP) since 1993 after every three years in one or other part of the world. The 7<sup>th</sup> conference was held in Arcachon, France in October 2011 where we agreed to hold the 8<sup>th</sup> conference in India. Considering the changes taking place all over the globe and impetus towards growth of mushroom research and development in India, this was an apt decision and the interactions during the event will help all of us to promote the research in various facets of mushroom biology and mushroom products.

We thank our colleagues from all parts of the world for their overwhelming response to the call for presentations and participation in 8<sup>th</sup> International Conference on Mushroom Biology and Mushroom Products jointly organized by WSMBMP, ICAR-Directorate of Mushroom Research, Solan (ICAR-DMR) and Mushroom Society of India (MSI) at New Delhi from 19-22 November 2014.

In this compilation of abstracts, there are more than 200 contributions including 14 keynote presentations, 70 oral and about 140 poster presentations on various facets of mushroom biology and mushroom products in addition to a theme lecture by Dr. Daniel Royse on global perspective on the five important mushrooms. The contributions have been grouped into 10 sessions that are: (i) Biodiversity and taxonomy, (ii) Genomics, genetics and breeding, (iii) Bioinformatics and nanotechnology, (iv) Biology, biochemistry, physiology and development, (v) Waste conversion & utilization, substrates, casing and crop management, (vi) Myco-molecules, medicinal, nutritional and nutraceutical properties, (vii) Mycorrhizal, entomopathic and other novel mushrooms, (viii) Pests and diseases, (ix) Value addition and mushroom products and (x) Economics, social, IT and marketing issues. A session on Scientist-farmer-Industry interface has also been included in the programme.

I would like to thank all members of Scientific Advisory Committee and Organizing Committee for their help and support. We thank Dr. S. Ayyappan, Secretary, DARE & DG-ICAR and Dr. N.K. Krishna Kumar, DDG (HS), ICAR for their guidance. I particularly thank my colleagues at ICAR-DMR who have been working for this conference since the inception of the concept to organize the conference in India. The support from Sh Arvind Kaushal, Secretary ICAR for providing facilities at NASC Complex, New Delhi is acknowledged. We thank the exhibitors for showcasing their products and making the conference more meaningful.

We thank Indian Council of Agricultural Research and Department of Agriculture & Cooperation, Government of India for their financial support for this conference. We also thank Haryana Agro-Industries Corporation, Chandigarh for their support in the field visit. Our thanks are also due to Dr. P.S. Ahuja, Director General, CSIR, Dr. Ravinder Kaur, Director IARI, Dr. U.C. Sud, Director IASRI, Dr. R.C. Budhani, Director National Physical Laboratory (CSIR-NPL) and Dr. Rajesh Luthra, Head, Human Resource Development Group, New Delhi for their support. Thanks are also due to Mrs. Sunila Thakur for her secretarial support.



(Manjit Singh)  
Chairman, Steering Committee 8<sup>th</sup> ICMBMP



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## Theme Lecture

### A global perspective on the high five: *Agaricus*, *Pleurotus*, *Lentinula*, *Auricularia* & *Flammulina*

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World production and consumption of mushrooms has increased at a rapid rate, especially since the mid 1990s. Not only has production and consumption increased as the world's population has increased, but *per capita* consumption of mushrooms has increased as well. Over a 15-year period (1997 to 2012), *per capita* consumption of mushrooms increased from about 1 kg/year to over 4 kg/year. China is the main producer and consumer of mushrooms. The demand for mushrooms has been phenomenal; production to meet the growing demand is a performance seldom duplicated in agriculture today.

In 2012, nearly all consumption of mushrooms in China, the EU and India was supplied from domestic sources. Nearly all mushroom consumption in Russia was supplied from imports while consumption in the United States, Canada, Japan and Australia was supplied mostly by domestic sources but also by substantial amounts of imports.

Five main genera constitute ca. 85% of the world's mushroom supply. *Agaricus* (primarily *A. bisporus* with some *A. brasilensis*) is the major genus, contributing about 30% of the world's cultivated mushrooms. *Pleurotus*, a close second, with 5 to 6 cultivated species, constitutes about 27% of the world's output while *Lentinula edodes* (shiitake), contributes ca. 17%. The other two genera, *Auricularia* and *Flammulina* are responsible for 6% and 5% of the volume, respectively.

Production of *A. bisporus* has continued to increase worldwide especially since the 1950s. Beginning in about 1998, China became the world's leading producer of this species. Oyster mushroom production has increased at a rapid rate worldwide in the last few years. From 1997 to 2010, *Pleurotus* spp. production increased from 876 to 6,288 tonnes (618%). Approximately 25% of China's mushroom production in 2010 was from two species of *Pleurotus*: *P. ostreatus* and *P. cornucopiae*. In the last five years or so, however, substantial increases in production of *P. eryngii* and *P. nebrodensis* have occurred. Until the late 1980s, Japan was the world's main producer of *L. edodes* (shiitake). Using sawdust-based techniques that reduce crop cycle time and increases production efficiency, however, China became the major producer of shiitake by 1990. Japan once dominated production of *F. velutipes* (enoki) – until the mid 1990s when China equaled then surpassed Japan – a similar pattern to shiitake except for the magnitude. Since mushroom production is a relatively labor intensive industry, mushroom expansion is expected to increase at a faster rate in countries with lower labor costs. In industrialized countries, greater use of mechanized systems and bulk handling of materials for preparation of substrate is expected.



# **Session-I**

## **Biodiversity and Taxonomy**





## Keynote Presentation

### I-K-1. The mushroom biodiversity in India-prospects and potential

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Mushrooms are an important and integral component of the ecosystem. Status of Indian Agaricales was reviewed first by Sathe and Rahalkar (1) making 1825 as the base and then by Manjula (2), providing a very exhaustive list of Agaricoid fungi from India and Nepal. This list has been updated by Natarajan *et al* (3). The systematics of Agaricales can be divided into three phases: Phase I (1825-1899), Phase –II (1900-1969) and Phase-III (1970-onwards). The following groups became active in phase III; Natarajan and his group in South India, Sathe and his Co Workers, in South West India, Kapoor and associates in and around Delhi, Rawala and his students, Saini and Atri and their students in North India, Lakhanpal and his co-worker, Kaul and his associates and Upadhyay *et. al* at DMR Solan in the Himalayan region.

Lakhanpal (4) reviewed the exploratory work on the Himalayan agarics. Atri and Saini (5) reviewed the work on Russulaceous fungi the world over and sieved the Indian contribution. Gupta *et al.* also (6) reviewed the Indian work on the Agarics. From South Indian region, excluding Kerala, Natarajan (7) reported 457 species of agarics spread over 76 genera. Work on mushrooms from Kerala has been reviewed by Bhawani Devi (8). Patil *et al* (9) listed 212 species of Agarics spread over 63 genera from Maharashtra. Verma *et al.* (10) listed 95 additional species of mushrooms.

The wild mushrooms seem to have been traditionally consumed by man since early times, but these were then probably considered food in wilderness, which now has come to occupy a very popular place in the modern dietetic regimen because of its nutritive value. Mushrooms are seasonal fungi, which occupy diverse niches in nature in the forest ecosystem they predominantly occur during the rainy season and also during spring when the snow melts but in lesser numbers.

A family wise exploration which began with family Boletaceae in 1976 culminated with exhaustive survey of 15 families (*sensu* Singer, 1986) [11] with an interlude of multi faceted investigation on Morels and some members of traditional Ascomycetes. They now stand investigated for systematics ecology, physiology mycorrhizal relationship nutritional and nutraceutical aspects, development of cultivation technology and more importantly ethno-mycological studies. The investigations include the award of more than a dozen M.Phil and Ph.D degrees and family wise monographic treatment of Boletaceae, Amanitaceae, Russulaceae and Morels. Use of mushroom species in the synthesis of mycorrhiza with different conifers has been a challenging and equally rewarding experience in amelioration of the environment.

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## Oral Presentations

### I-O-1. Collection, identification and morphological characterisation of indigenous mushrooms from coastal Kenya

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Mushrooms are consumed all over the world as part of the regular diet. They are consumed for their ample nutrition, medicinal value and enticing flavour. In Kenya, both cultivated and wild mushrooms are consumed, the latter forming an integral part of a long standing cultural practice. However, many people shy off from such wild sources due to fear of poisoning. That notwithstanding, these wild resources risk extinction due to climate change, over exploitation and wanton destruction of their natural habitat. To improve on their utilization, a survey was conducted in Arabuko Sokoke and Kaya Teleza Forest Shrines of coastal Kenya to document the edible species which are well known to the communities who consume them but completely unknown to science. An attempt was made to identify and characterize the mushrooms using habitat, morphological features and any phenotypic features easily identifiable. The collections made included several species categorized as edible hence, a food source, poisonous or ornamental. The edible included *Ganoderma* spp, *Cantherellus* spp, *Agaricus* spp, *Pleurotus* spp, *Russula* spp, *Auricularia* spp and *Termitomycetes* spp; poisonous species included the deadly *Amanita* spp, *Lactarius* spp and stink horn spp while ornamental included the beautiful ringed *Microporous* spp. The survey revealed a rich diversity of indigenous mushrooms which could be of economic importance especially for food security if well exploited. The information obtained can be used as a baseline for future studies including fungal genetic diversity, future trends associated with climate change, indigenous knowledge application to current usage of mushrooms and species likely to go for domestication. The information can also be used to improve the management strategy on sustainable utilization of edible mushroom resources from the forests. However, further studies using modern methods of characterization involving molecular tools are recommended.

### I-O-2. Diversity of resupinate, non-poroid agaricomycetous fungi in the Himalaya and adjoining areas

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Resupinate, non-poroid agaricomycetous fungi are generally lignicolous with unilateral and gymnocarpic hymenium and belong to class *Agaricomycetes* (phylum *Basidiomycota*, subphylum *Agaricomycotina*). These fungi have been assigned to two subclasses *Agaricomycetidae* (orders *Agaricales*, *Atheliales*, *Boletales*) and *Agaricomycetes incertae sedis* (orders *Auriculariales*, *Cantharellales*, *Corticiales*, *Gloeophyllales*, *Hymenochaetales*, *Polyporales*, *Russulales*, *Sebacinales*, *Thelephorales* and *Trechisporales*). The diversity study of these fungi is an outcome of extensive work of almost three and a half decades in the Eastern and the North Western Himalaya and adjoining areas covering a distance of about 2500 km from West to East, with 100-400 km average width along the entire longitudinal extension and lot of variation in altitude gradients. Based on macroscopic and microscopic observations of more than 4150 collections, using standard techniques, more than 400 taxa have been identified, which belong to more than 105 genera of the different families and orders. These include seven genera

(*Confertextum*, *Cordochaete*, *Dendrophlebia*, *Hallenbergia*, *Radulopsis*, *Repetobasidiopsis* and *Trimitiella*), two sub genera (*Stereum* subgen. *Acanthostereum*, *Stereum* subgen. *Aculeatostereum*), as many as 60 species (*Aleurodiscus himalaicus*, *A. indicus*, *Athelopsis parvispora*, *Byssocorticium microsporum*, *Candelabrochaete himalayana*, *Ceraceomyces bizonatus*, *Clavulicium hallenbergii*, *Confertextum macrosporum*, *C. microsporum*, *Conohypha grandispora*, *Corticium mussooriensis*, *Cristinia tubulicystidiata*, *Cylindrobasidium indicus*, *Dendrophlebia crassispota*, *Flavophlebia sphaerospora*, *Fibulomyces cystoideus*, *Hallenbergia singularisa*, *Hyphoderma bicytidiata*, *H. clarusproprietat*, *H. densustextum*, *H. hallenbergii*, *H. parvisporum*, *H. sikkimia*, *H. sporulosum*, *H. subglobosum*, *Hyphodontia caulicystidiata*, *H. dhingrae*, *Leptosporomyces singularis*, *Leptocorticium indicum*, *Leucogyrophana thimphina*, *Paullicorticium indicum*, *Peniophora hallenbergii*, *Phlebia crassisubiculata*, *P. interjacenoides*, *P. microspora*, *P. kamengii*, *P. singularisa*, *P. thindii*, *Phlebiopsis darjeelingensis*, *P. himalayensis*, *P. mussooriensis*, *Radulodon indicus*, *R. acaciae*, *Radulopsis cystidiata*, *Repetobasidiopsis grandisporus*, *R. macrospora*, *Scytinostroma pulverulentum*, *S. renisporum*, *Sistotremastrum roseum*, *Sistotrema angustispora*, *Stereum peculiare*, *Tomentella garhwaliana*, *T. kalatopii*, *T. unicus*, *Trimitiella indica*, *Vararia himalayana*, *V. indica*, *V. longicystidiata*, *Xylodon mussooriensis*, *X. subglobosus*) and 9 varieties (*Amphinema byssoides* var. *macrospores*, *Botryobasidium subcoronatum* var. *crassispota*, *Ceraceomyces sublaevis* var. *grandisporus*, *Conohypha albocrema* var. *angustisporum*, *Hyphoderma roseocrema* var. *minutisporum*, *H. setigerum* var. *bicytidium*, *Scytinostroma phaeosarcum* var. *angustispora*, *Tomentella cladii* var. *grandii*, *Tubulicium vermifer* var. *hexasterigmatum*) are new to science and more than 193 new reports from the Himalaya.

### I-O-3. Conservation and characterization of *Pleurotus* variability of India

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*Pleurotus* species (oyster mushrooms) have wide adaptability to environment and growing substrate. They have excellent nutritional and medicinal properties and can play a pivotal role in income generation and nutrition enhancement especially for the vegetarian population. India, due to its varied geography and forest types is a rich source of mushroom diversity which awaits conservation and domestication. The present investigation was undertaken to conserve and characterize the variability of *Pleurotus* species in India. The forest areas of Western Ghats of Shimoga (Karnataka), Balaghat (Madhya Pradesh), forest areas of Madurai (Tamil Nadu) and Mysore plateau of Deccan plateau region (Karnataka) were explored. Five *Pleurotus* isolates were documented. The species/isolates were cultured, purified, validated and conserved. They were characterized for cultural, morphological, nutritional and agronomical characters. The isolates from Western Ghats, Madhya Pradesh and Madurai showed a broad temperature tolerance between 15-35 °C, optimum being 25-30 °C. *P. cystidiosus* collected from Bangaluru showed a narrower temperature tolerance between 20-35 °C, optimum being 25-30 °C. The pH range for *P. cystidiosus* was 3-10, *P. djamor* 3.5-10, pink and white *Pleurotus* isolate from Balaghat 3.5-9.0 and Madurai isolate 4-10. All the isolates could be grown on commonly used media viz. malt extract agar and potato dextrose agar medium. *P. cystidiosus* produced characteristic black coremia (asexual spores) in culture medium as well as during spawn running. Sporophores of *Pleurotus djamor* from Western Ghats were pink, darker gills, almost sessile, stipe in some sporophores tough, eccentric, and stub like. Pink oyster isolate from Balaghat showed pink sporophores with eccentric to central, cylindrical, distinct stipe, and deeply lobed sporophore margin which gave it a flower like appearance. The white *Pleurotus* isolate from the same region showed eccentric to central but longer and thicker stipe, attenuated inflow and strongly decurrent becoming funnel shaped on maturity. Madurai isolate had white sporophores with long slightly hairy stipe, eccentric to sub lateral. *P. cystidiosus* showed large, thick, fleshy sporophores, initially black becoming

light brown on maturity. Stipe was short, tough, gills thick, producing white spore print. Agronomical trials of *P. djamor* (Western Ghats) and pink *Pleurotus* isolate (Balaghat) showed short crop cycle of 20 and 21 days (single flush) to 32 and 34 days (2 flush) crop. White *Pleurotus* isolate (Balaghat) showed 27 days (single flush) and 64 days (2 flush) crop. Madurai isolate showed 37 days (single flush) and 50 days (2 flush) crop and *P. cystidiosus* showed 44 days (single flush) and 92 days (2 flush) crop. *P. djamor* (western Ghats) and pink *Pleurotus* isolate (Balaghat) showed synchronous cropping pattern whereas *P. cystidiosus* showed highly staggered crop. Pink *Pleurotus* isolate (Balaghat) showed highest protein. Highest crude fiber and lowest fat was in *P. djamor* (Western Ghats). Both isolates of Balaghat region showed high iron and zinc content. Highest niacin was in white isolate from Balaghat and *P. cystidiosus*. The white isolate from Balaghat showed high pantothenic acid too.

#### I-O-4. Medicinal mushrooms in Italy and their *ex situ* conservation through culture collection

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Italy, though a small country, is characterized by a variety of habitats, from the Alpine to the Mediterranean environments; exhibiting, many fungal species [1, 2] including mushrooms that are collected by mycophiles for culinary uses. In the last decade, interest in medicinal mushrooms has been increasing as almost all the better-known species having officinal properties grow in the Italian areas. These fungal species need to be protected from over exploitation and hence, *ex situ* conservation is suggested [3, 4]. Culture collections play a key role in preservation and maintenance of fungal genetic resources and are an important tool to get biological material for application purposes [5]. Aims of the present work were: a) to survey selected areas for wood-inhabiting basidioma; b) to identify the species through morphological characteristics; c) to isolate the mycelia in pure culture; d) to confirm the strain identification by molecular analysis. Wood-inhabiting fungi were collected from Italian Alps, Appenines, wood plains and Mediterranean areas. Particular attention has been paid towards “polyporoid” fungi and Corticiaceae as well [6, 7]. Pure culture isolation was carried out from fruiting bodies under sterile conditions. The culture collection is conserved at the Mycological Laboratory of Pavia University and is linked to a database with ecological information. In a few years, about 150 species were isolated, among them some rare and precious *taxa* such as *Laricifomes officinalis*, *Lenzites warnieri* and *Perenniporia meridionalis*. It is well known that medicinal mushroom bioactivities could depend on different characteristics such as the genetic profile, the geographical provenance and the substrate [8]. Therefore, Italy together with many other countries could become a resource of different ecotypes with different properties to be investigated.

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### I-O-5. Family *Pluteaceae* in North West India

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Family *Pluteaceae* the “pink spored family” falls under order Agaricales. It includes four genera spread over 346 species. Presently, 30 species falling under three genera of this family, *Pluteus*, *Volvariella* and *Volvopluteus*, have been collected from various localities of North West India. Of these 7 species and 4 varieties are presented as new to science while 11 are recorded for the first time from India. The genus *Pluteus* is defined by the presence of bilateral convergent trama and carpophore stipe lacking both annulus and volva, while genus *Volvariella* has bilateral convergent trama with the stipe lacking an annulus, the genus *Volvopluteus* is a newly constructed genus on the basis of spore size more than 11 µm and stipe with volva and no annulus. The pluteoid mushrooms occur in abundance in the tropical region, they come up early in the monsoon season, mostly they are terrestrial, few are folicolous and lignicolous, only genus *Volvariella* was collected from coprophilous habitat. In the study area *Volvariella bombycina*, *V. diplasia* and *Volvopluteus gloiocephalus* are the other commonly hunted mushrooms from the wild for human consumption. These species are also being cultivated commercially. Their wild relatives are of common occurrence in North Western India. From the surveys it has become apparent that there is enough wild germplasm for utilization in strain improvement programme in paddy straw mushroom in study area. Various available wild species of *Volvariella*, namely *V. bakeri*, *V. terastia*, *V. taylorii* and *V. cubensis*, etc. possesses acceptable agronomic features with possibilities of introduction into cultivated commercial strains through breeding experiments.

### I-O-6. Diversity of macrofungal communities in Chikmagalur district of Western Ghats, India

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The present study is an effort to expand the baseline database for macro-fungal documentation, diversity and its distribution in the Western Ghats of Chikmagalur district for preparing an inventory. Twenty transects each measuring 50 x 20 m were laid in sampling stations of Chikmagalur district. The study sites were selected randomly and macro-fungi were collected during 2007 to 2011. A total of 6,950 sporomas were recollected and maximum productivity was recorded during 2007 and lowest in 2011 with 905 individuals. The relationship between macro fungal species richness and sampling plots was analysed using linear regression. Dacrymycetaceae, Ganodermataceae, Polyporaceae and Russulaceae were found to be the most dominant families during study. *Armillaria*, *Calocera*, *Ganoderma*, *Panaeolus*, *Polyporus*, *Psathyrella* and *Russula* were the most dominating genera during five years study (2007-11). *Caloceraviscosa*, *Ganoderma carnosum*, *Panaeolus fimicola*, *Psathyrella candolleana*, *Russula atropurpurea*, *Scutellinia erinaceus*, *Termitomyces tylerianus* and *Trametes hirsuta* were the most predominating species. The Shannon and Simpson diversity indices were found to be highest during 2007 ( $H' = 4.35$ ;  $D = 0.031$ ), respectively.

### I-O-7. Diversity of poroid mushrooms in Punjab: family Hymenochaetaceae

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Punjab, the 'land of five rivers' is relatively a small state of India occupying an area of 50, 362 km<sup>2</sup>. It is located in the North Western part of India between 29° 30' and 32° 32' North latitudes and 73° 55' and 76° 50' East longitudes. It is predominantly an agriculture state and has only 6.12% of the total geographical area under forest cover. Depending upon the altitude and climate the main forest types as per are Northern dry mixed deciduous forests, dry deciduous scrub forests, khair, sissoo forests, Shiwalik chir pine forests and dry deciduous bamboo forests. All these forests types along with the strip plantation, offer a favourable environment for the growth of poroid fungi particularly during the monsoon season. Family Hymenochaetaceae (Agaricomycetes, Hymenochaetales) is characteristic in having resupinate topileate, smooth to poroid basidiocarps, xanthochroic tissue, hyphae without clamps, presence/absence of setae, 2-4 sterigmate basidia and thin to thick-walled basidiospores. Several taxa of the family are reported to be implicated in many diseases of broad-leaf and coniferous trees, causing various types of rots and diseases. A large number of the species have medicinal and nutritional importance. The present paper deals with the diversity of poroid members of this family in Punjab. Fifteen species belonging to three genera i.e. *Fuscoporia* (*F. gilva* and *F. rhabarbarina*), *Inonotus* (*I. dryadeus*, *I. patouillardii* and *I. rheades*) and *Phellinus* (*P. fastuosus*, *P. badius*, *P. xeranticus*, *P. grenadensis*, *P. rimosus*, *P. pectinata*, *P. melleoporus*, *P. purpureogilvus*, *P. robustus* and *P. conchatus*) are being described. Ten species are being described for the first time from the study area.

### I-O-8. Wild edible and medicinal mushroom species of Sudan

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African forests offer variety of mushroom species which have remained largely untapped. Long periods of warm weather and humidity provide excellent conditions for the growth of mushrooms. There are several species, with different habitats like decaying roots, dead wood, and termite mounds or directly on cultivated land. These can play a pivotal role in transforming livelihood security of local farmers in Africa. The potential of wild mushrooms is enormous but it requires sensitization of people, creating awareness and undertaking research on use of African mushrooms as food.

In Sudan, mushroom consumption is primarily around cities and fresh and canned white button mushroom are imported from Egypt, China and Indonesia. The traditional knowledge about wild species of mushrooms is not well documented and is gradually diminishing due to some taboos present.

The present study was conducted in Khartoum and Sinnar States during rainy seasons of 2012-2013 to survey and identify wild mushrooms and to analyse key nutritional components of some selected edible and medicinal mushroom. The survey was documented with photos, Interviews and notes. Identification of species was done following different key references and manuals. Some selected edible and medicinal species were chemically analysed for moisture content, protein, fat, carbohydrate, ash, fibre and minerals using standard techniques.

In Sudan, some tribes traditionally consume mushrooms while others do not. Mushrooms are known by different names in Sudan; In Northern Sudan mushroom is known as "Wad Al Werda" meaning which causes fever; in other parts of Northern Sudan such as "Dongolla", people tend to eat mushroom and name it as "Goroo". Some Nilotic tribes sell wild mushrooms as "Barnoog", i.e. without growth, which indicates that they may not have definite tribe or are of mixed origin. Some tribes call mushroom as "Gowangy", and eat it after drying it as porridge. In areas of central and Eastern Sudan, some people consume mushroom and call it as "Al-Afan" or Abo- Elefeen or "Afan Al-Watta" meaning a "mould". Interviews with locals in areas around Sinner State revealed that some people eat wild mushroom and call

it “Abo-Zomo” and “Laham Al Watta” which means the flesh of the land. Other name around Sinnar State is “Lahm el fertit” referring to it as a food for pigs.

Fourteen wild species have been found in Khartoum and Sinnar States. In Khartoum State three edible or of medicinal value namely *Agaricus bisporus*, *Ganoderma lucidum* and *Podaxis pistillaris* are reported. In Ronga forest in El Suki locality-Sinner state two edible: (*Agaricus bisporus* and *Pluteus umbrosus*); and three of medicinal value (*Ganoderma lucidum*, *Podaxis pistillaris* and *Grifola frondosa*), *Calvatia cyathiformis* whereas, with dual properties was reported. Other wild species identified were *Agaricus placomyces*, *Aleuria aurantia*, *Abortiporus biennis* and *Pisolithus tinctorius* in Khartoum state, *Lentinus tigrinus* and *Entoloma cephalotrichum* in Sinnar state. *Coprinus disseminatus* and *Mutinus ravenelii* have been found in both Khartoum and Sinnar states.

Analysis of key nutritional attributes revealed moisture content of 33%-90.9% on fresh weight basis. Other attributes were estimated on dry weight basis recording 9.8%-40.47% of protein, 3.05%-24% of crude fat, 6.9%-40.7% of carbohydrate, 0.04%-29.0% crude fiber, 2.3%-26% of ash, 2.4-8.9 ppm of Sodium (Na), 7-84.6 ppm of Potassium (K), 0.84-3.35 ppm of Phosphorus (P), 3-5 meq/L of Calcium (Ca) These results show that these species of mushroom are highly nutritive and can play a vital role in social upliftment and livelihood status of local people through creation of awareness among local people on the medicinal, nutritional, social and economic value of these wild species. The introduction of commercial varieties and wild spp to the production cycle will help the farmers to escape the consequence of seasonality of income, bring new uses of largely untapped agricultural waste and provide a model project for microfinance in Sudan.



## Poster Presentations

### I-P-1. Assessing biodiversity of wild mushrooms in Mizoram, Northeast, India

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Mizoram, one of the seven sister states of north east India, is a part of the Indo Burma biodiversity 'Hotspot'. The total forest cover of the state is 87.42% and holds third rank in India, with an annual rainfall ranging from 2160 mm to 3500 mm. Mizoram is divided into three agro climatic zones viz. tropical evergreen, sub-tropical semi-evergreen and montane. The study was conducted to collect the wild mushrooms from the three selected protected forest areas of Mizoram and their habit and habitat was studied. In total 182 wild mushrooms were collected and identified up to genus level. Tissue culture was attempted from all the collected fruiting bodies and forty two showed the growth. All isolates were screened for antifungal activity against three major fungal phytopathogens. Potential isolates were selected for molecular identification by amplification of internal transcribed spacer (ITS) region of ribosomal DNA. First time we have identified 13 mushrooms from Mizoram, among them *Trametes hirsutae* was the most pre- dominant. All the sequences have been deposited in the NCBI Gene Bank and accession numbers were obtained (KJ865831-KJ865843). The cultures have been deposited in the Gene Bank of DMR, Solan. We concluded that Mizoram has apparently rich in mushroom mycoflora, which is represented by species of *Trametes*, *Agaricus*, *Auricularia*, *Pleurotus*, *Coprinus*, *Ganoderma*, *Lentinus*, *Leucopaxillus* (edible), *Schizophyllum* (edible), *Calocybe*, *Marasmius* and one or two unique species of *Fomitopsis*, *Pholiota* and *Flammulina* (medicinal) etc. Hence, this region of India needs to be explored more extensively.

### I-P-2. Biodiversity of mushrooms of Sarpamari reserve forest of Dhubri district, Assam, India

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There is hardly any report of documentation of mushrooms from Sarpamari reserve forest. Hence, a survey was carried out (July 2013 to June 2014) to explore and inventories macrofungal diversity of the Sarpamari reserve forest of Dhubri District, Assam. The survey area is 168.80 ha with an average rainfall of about 2244 mm and humidity is 85%. Soil pH ranges from 3.0 to 5.70. Collected sporocarps of mushrooms were identified on the basis of morphological and anatomical characteristics. The genera like *Agaricus*, *Amanita*, *Auricularia*, *Clavaria*, *Cortinarius*, *Coprinus*, *Ganoderma*, *Lactarius*, *Lepiota*, *Lycoperdon*, *Shizophyllum*, *Polyporus*, *Marasmius*, *Mycena*, *Psilocybe*, *Pycnoporus*, *Russula*, and *Trametes* were identified. Out of 41 samples 32 samples were identified to species level. The preliminary study gives us a good picture of rich diversity of mushroom of Sarpamari reserve forest. It appears that lots of macro fungi are becoming extinct and facing threat of extinction because of habitat destruction and lack of awareness about the mushrooms among people. For conservation some awareness programmes need to be undertaken.

**I-P-3. Diversity of wild mushroom flora from Indian Thar desert****Praveen Gehlot\*, Ruchika Sharma and Kamna Sharma**

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The Indian Thar Desert (25°2' to 28°10' N and 69°3' to 74°0' E) forms the eastern extremity of the great arid and semi arid belt of the world. It is one of the smallest deserts of the world but exhibits a wide variety of habitats and unique biodiversity due to juxtaposition of Palaearctic, Oriental and Saharan elements. The climate of Thar Desert is characterized by low rainfall with erratic distribution, extremes of diurnal and annual temperature, low humidity, high wind velocity and frequent dust storms. In Thar Desert, mushrooms have an unestimated wealth of fungal diversity, which needs to be tapped properly as there may still be several undescribed species. Therefore, many periodic surveys were conducted for collection of wild mushroom flora from different sites and localities of Jodhpur, Bikaner, Barmer and Jaisalmer districts. During the course of investigation 48 species of 21 genera were collected from different habitat viz., grassland, pasture, roadsides, wooded area, sand dunes, over dead stumps and living trees. Collected mushroom were identified as *Agaricus alphitochrous* (Berk and Br.), *A. augustus* Fr., *A. bambusophilus* Heinem., *A. benzodorus* Heinem and Gooss, *A. bisporus* var. *hortensis* (J Lange) Pil., *A. purpurellus* (F.H. Moller) F. H Moller, *A. silvaticus* Schaeff ex Secr., *A. silvicola* (Vitt.) Peek, *A. trisulphuratus* Berk., *Clitocybe dealbata* Sowerb., *Coprinus extintorius* (Bull.) Fr., *C. lagopides* Karst., *C. sterquilinus* (Fr.) Fr., *Crepidotus herbarum* (Perk) Sacc., *C. quitensis* Pat., *Hemimycena pithya* (Fr.) Dorfelt, *Hygrophoropsis aurantica* (Wolf ex Fr.) Maire, *Lepiota americana* (Perk.) Sacc., *Leptonia sericella* (Fr.) Barbier, *Leucocoprinus cepaestipes* (Sow. Ex Fr.) Pat., *L. zeylanicus* (Berk.), *Macrolepiota exocortata* (Schaeff ex Fr.) Wasser, *M. rachodes* (Vittadini) Singer, *Marasmiellus* sp, *Marasmius confetus* Berk. and Br., *M. oreades* (Bolt ex Fr.) Fr, *Paneolus fimicola* (Pers. ex Fr.) Quel., *Pholiota squarrosa* (Pers exFr.) Kummer, *Pluteus subcervinus* (Berk. and Br.) acc., *Psathyrella magambica* Pegler, *P. pygmaea* (Bull. Ex Fr.) Singer, *P. spadicea* (Schaeff. ex Fr.) Singer, *P. tiarella* (Berk. and Br.) Sacc., *Stropharia semiglobata* Batsch, *Termitomyces eurrhizus* (Berk.) Heim, *T. heimii* Natrajan *T. microcarpus* (Berk. and Br.) Heim *T. tyleranus* Otieno, *Termitomyces* sp., *Tricholoma lobayense* Heim, *T. sulphureum* (Bull. ex Fr.), *Trogia in fundibuliformis* Berk and Br., *Volvariella bombycina* var. *flaviceps* (Murr.) Shaffer., *V. earlei* (Murr.) Shaffer, *V. hypopithys* (Fr.) Shaffer, *V. pusilla* (Pers. ex Fr.) Sing., *V. speciosa* var. *gloiocephala* (DC. Ex Fr) Sing., *V. speciosa* var. *speciosa* (Fr. Ex Fr.) Sing. Threats of mushroom in the Thar Desert are of much concern. Due to deforestation and ever increasing anthropogenic activities have been accompanied by changes in the traditional pattern of land use, resulting some mushroom species become endangered and hence, it is need of the hour to conserve them.

**I-P-4. Documentation of macrofungal flora of Western Kashmir Himalayas with special emphasis on diversity, distribution, their ethnomycological use and some recent accessions to the data base****Shauket Pala**

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The Kashmir valley located in the north extreme of the India lies between 33° 20' to 34° 54' N latitude and 73° 55' to 75° 35' E longitude forms an important region of north western Himalayas. The geographical area dominated by temperate climate with a huge forest cover of both coniferous and deciduous forest with a diverse tree species constituting them harbours a robust macrofungal wealth. However, the macrofungal diversity of Kashmir Himalayas particularly Western Kashmir Himalayas has not been documented completely as yet and is still in exploratory stage. In this backdrop, a systematic survey for exploration and inventorization of macrofungal species of Western Kashmir Himalaya was undertaken

during the year 2010-2013. During the study 75 species of mushrooms belonging to 50 genera and 32 families were collected. Conspectus of species belonging to different fungal groups revealed that *Coprinaceae*, *Agaricaceae*, *Amanitaceae* and *Russulaceae* were the dominant families, each represented by 8, 6, 6 and 7 species, respectively. During the study four species viz., *Amanita constricta*, Thiers and Ammirati, *Hygrocybe acutoconica* (Clem.) Singer, *Macrolepiota excoriata* (Schaeff.) Wasser and *Laccaria tortilis* (Bolton) Cooke are recorded for the first time from the state Jammu and Kashmir. Various features like habit and habitat, distribution, seasonal occurrence and usage of the collected species were also noted. During the study it was also found that 31 species of mushrooms belonging to different ecological and taxonomical groups were used for their nutritional and medicinal values. They were tested for their activities against a broad spectrum of diseases, ranging from simple skin diseases to present-day complex diseases such as diabetes and tumours. Factors causing their continuous declination are mentioned along with the possible *in-situ* and *ex-situ* conservation measures.

### **I-P-5. Ethnomycology in the “Tacaná volcano biosphere reserve”, Chiapas, Mexico**

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In order to contribute to the rescue of the fast disappearing knowledge of mushrooms and their use held by the Mam ethnic group living within the “Tacaná Volcano Biosphere Reserve”, in Chiapas, Mexico, the use and knowledge of mushrooms was investigated by applying open unstructured and spontaneous interviews, to elderly speakers of the Mam language who were presented fresh biological material. Of the 50 fungi shown, informants acknowledged 16. Some names of mushrooms in Mam have no meaning in Spanish or Mam although phonetically some names are very similar to Spanish words. Most mushrooms are used as food, important mushrooms include *Lycoperdon umbrinum*, which is both edible and medicinal, some fungi which are used as cattle feed and *Agaricus sylvaticus* known as Xch' kbi lak' in the Mam language. The Mam-speaking inhabitants have developed ways of preparation and treatment for mushroom consumption and can identify mushrooms depending on the shape, appearance (mature or immature) and size they present. It is stressed that the knowledge of mushrooms possessed by this ethnic group at the studied site, is limited but it is and on the threshold of disappearing.

### **I-P-6. Exploitation and utilization of mushrooms in North East India**

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Wild edible mushrooms like oyster (*Pleurotus* spp), split gill fungi (*Shizophyllum commune*), shiitake (*Lentinula edodes*) and wood ear (*Auricularia* spp) were collected and documented. More than 500 spp. of edible and poisonous mushrooms were collected and kept in the museum for ready reference to the scientific community and farmers of North Eastern Hill region. Ethnic knowledge was also explored during survey. Suitable substrates were evaluated for mass multiplication of spawn. It was observed that maize grain was one of the best substrates for multiplication of spawn. Low-cost model mushroom house was designed and constructed for trainings and demonstration to the farmers of NEH region. Paddy straw was the best substrate for mushroom bed preparation of oyster mushroom followed by maize stalk. Five model trainings were conducted for 450 farmers and five of them have undertaken oyster mushroom cultivation. Three self help groups have been created in three different villages and actively involved in disseminating the technology. The major threat to oyster mushroom cultivation was rat which damaged

25-30% beds followed by *Trichoderma* spp. weed mould (10% incidence), *Coprinus* (30% incidence), Beetle (25% infestation). Local bamboo trap was found to be the best to control the rats.

### **I-P-7. Exploitation of *Cordyceps sinensis* from Pithoragarh district of Uttarakhand and its impact on economy of Scheduled Tribes**

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Pithoragarh is a border district of Uttarakhand state with international boundary on the north with Tibet and on the east with Nepal. The district is gifted with a rich mushroom flora that includes one of the most valuable medicinal mushrooms *Cordyceps sinensis* which parasitizes the larvae of a moth of the genus *Hepialus* and survives in alpine grasslands of the high altitudes of Himalayas ranging from 3000 m - 5000 m. Tibetans know this fungus as Yartsa Gumbu, (summer grass –winter worm), and locally in Pithoragarh as 'Keera Ghas' (insect herb). Collection and trade of this caterpillar fungus is one of the most important sources of income for scheduled tribes of district Pithoragarh, Uttarakhand. It has tremendous impact on the economy of scheduled tribes and often derives over 75% of their annual cash income from its collection. A family of four to five collects about one to two kilogram *C. sinensis* and in two to three months period they earn over 5-10 lakhs or more which is enough to sustain them for rest of the year. Since, last few years *Cordyceps* has been traded very extensively in Dharchula and Munsyari blocks of Pithoragarh, Uttarakhand. The estimated volume of trade in Uttarakhand was 1500-2500 kg/year during last five years from 2009 to 2014. The fungus brought to the market by local gatherers particularly scheduled tribes of high altitudes and sold to agents or brokers in Munsyari and Dharchula which was further forwarded by them to traders coming from Delhi, Nepal, Bhutan and Tibet. Some brokers or traders buy the *Cordyceps* directly from the gatherers in the villages Chipla, Ralam, Laspa, Burfa, Karschila, Budhi Galja, Chalm, Boin, Bon, Dugtu, Panchachuli, Nampa and Api of high altitudes in Pithoragarh. In fact, there is no organized trade for the product so far. Therefore, price varies at the level of gatherers and brokers in different years from ₹ 1 lakh to ₹ 5 lakh and ₹ 2.5 lakh to ₹ 8.00 lakh per kilogram, respectively during the year 2009 to 2014.

### **I-P-8. Litter decomposing fungi in the forest of Southern Rajasthan, India**

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The aim of the present study was isolation and identification of fungi associated with decomposition of litter of Kamalighat, Anadara, Sitamata, Pipalkhut and Kumbalgarh forest of Southern Rajasthan. During rainy season of 2010-2013 surveys were conducted in pre-monsoon, monsoon and post monsoon seasons. Successional changes in the litter microflora were determined for the four main seasons of the year during July to October. The decomposition of the leaf litter continuously takes place throughout the year, however, the process intensified during the rainy season. The fresh litter is generally colonized by member of mitosporic fungi including genera *Alternaria*, *Cladosporium*, *Curvularia* and *Phoma*. The majority of basidiomycetes including ectomycorrhizal fungi appeared during August–September and this is the best period for the development of fungi and decomposition of the litter. Dematiaceous fungi mostly colonize litter in the later stage of decomposition. Fungi like *A. flavus*, *A. niger*, *A. fumigatus*, *Rhizopus stolonifer* and *Mucor* spp. were associated with the litter decomposition throughout the season. Some fungi including ectomycorrhiza forming fungi occur on the leaf litter in the rainy season (July, August and September) these are *Asteraeus hygrometricus*, *Pisolithus tinctorius*, *Boletus* and *Calvatia cythiiformis*. Macrofungi like *Podaxis pistrillaris*, *Phellorinia inquinans*, *Pleurotus* spp., *Clitocybe* spp., *Auricularia polytricha*, *A.*



*auricula*, *Polypores*, *Stropharia* spp., *Cyathus* spp., *Leucocoprinus* spp., *Boletus luridus*, *Bovista plumbea*, *Lepiota procera*, *Laetiporus* spp., *Volvariella bombycina*, *Psathyrella* spp., *Collybia* spp., *Agaricus augustus*, *A. arvensis*, *A. placomyces*, *Leucopaxillus giganteus*, *Calocera* spp., *Coltricia* spp., *Ganoderma applanatum*, *Lenzites* spp., *Mycena* spp., *Schizophyllum commune*, *Ganoderma tsugae*, *Tricholoma sulphureum*, *Lepista* spp., *Geaster* spp., *Fomes* spp., *Pycnoporus cinnabarinus*, *Gymnopilus* spp., *Entoloma* spp., *Hexagonia hirata*, *Pluteus*, *Hericius* spp., *Mutinus elegans*, *Inonotus* spp., *Calocybe*, *Cortinarius* spp., *Lycoperdon pyriforme*, *Scleroderma citrinum*, *Marasimus oreadus*, *Tremella mesenterica* were recorded during monsoon and post monsoon season.

## **I-P-9. Macrofungal assemblages in semi-evergreen forests of Western Ghats, India**

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Semi-evergreen forests of Western Ghats support rich and diverse macro-fruit bodies with a total of 124 macro fungal species (75 genera; 38 families) found fruiting on soil and wood. In total, 11 morpho-groups were identified. The macro-fungal assemblages were examined based on sporoma inventories over 5 years. The epigeous sporoma encountered were collected and analysed for their identity. Thirty six field expeditions were carried out from January 2007 to December 2011 in a 50 x 20 m transect in each surveyed area/year. Sampling of 10 plots revealed 22,334 sporomas of macrofungi. Of these, more than 20% of the sporoma (5,443 individuals) were found during 2007 and considered as highest during the study period. The time line for the fruiting was narrower than that for species on wood and soil, with peak fruiting occurring during June to August. The family Agaricaceae, Psathyrellaceae and Tricholomataceae dominated the macromycete communities during 2007 having the greatest number of species. The species belonged predominantly to the genera *Cyathus*, *Coprinellus* and *Xylaria*. The remainder appeared to emerge inconsistently over the survey. *Ascobolus stercorarius* (2007), *Pleurotus ostreatus* (2008), *Polyporus arcularius* (2009), *Calvatia craniiformis* (2008, 2010) and *Psathyrella candolleana* (2011) were most frequently recorded species. Rainfall and temperature were identified as key variables influencing species richness. Further, macro-fungal incidence was found to be significantly higher during 2007 and 2009, compared to other years of survey, indicating rainfall to be important for the development of fruitbodies. Family richness over rainfall was significantly correlated with two family variables, especially Inocybaceae and Lyophyllaceae. It is concluded that forest-tree composition may have fundamental effects on the community structure of macromycetes. The study provides benchmark knowledge on relationship between macrofungal community richness and amount of rainfall in semi-evergreen forests and briefly connected in the possibility of macrofungal families, genera and species. It will serve as a good basis in native forest types for further studies and in similar forests elsewhere in order to enrich the information on diversity of macro-mycobiota in India. This may also help identify possible novel species being extrapolated in the future for the betterment of mankind.

## **I-P-10. Morphological heterogeneity of the basidiocarps of cultivated *Ganoderma lucidum* strains**

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*Ganoderma lucidum* is a well-known and preferred cultivated medicinal mushroom all around the world. However, from the taxonomical point of view it is one of the most problematic species amongst polypores, that is why the morphologically similar *Ganoderma* species are often identified mistakenly as '*G. lucidum*'.

There are two main types of basidiocarps in the *Ganoderma* genus: (i) perennial, sessile with a non-laccate upper surface (Elfvigia group); (ii) annual and stipitate with laccate, shiny crust on the upper surface (*Ganoderma* group). *G. lucidum* belongs to the latter morphotype, which contains approximately 120 species. Recently, it has been proven, that the cultivated '*G. lucidum*' is not a single species, but represents *G. lingzhi* and/or *G. sichuanense* as well. In our study the aim was to observe the basidiocarp morphology of strains considered to be *G. lucidum*, cultivated under the same conditions. In our experiments two different substrates were used. Substrate 'A' consisted of 80% beech sawdust, 18% wheat bran, 2% gypsum and lime (3:2), while substrate 'B' contained 60% beech sawdust, 20% wood chips, 13% wheat bran, 5% bran, 2% gypsum and lime (3:2). Water content was set to 65-68% in both substrates. The mixtures were filled into 10 l PP bags with filters (Mycelia, Belgium). Following the sterilization on 128 °C for two hours, the bags were cooled down. Spawning with rye based spawn was carried out with six different cultivated strains (considered to be *G. lucidum*), spawn ratio: 5%. 10 bags of both substrate 'A' and 'B' were inoculated with each strain (total of 120 bags). After a 60 days spawn run and incubation period on 25 °C, the bags were placed on 10 °C in a growing room for 30 days. Ambient conditions during cropping: 17-20 °C and 90-95% relative humidity. From pinning stage a 12/12 day/night period were conducted at 700 lux, CO<sub>2</sub> was adjusted to 400-500 ppm. The bags on the shelves were opened either on their sides or on the top. Each strain had similar fruit bodies on both types of substrates, while between the strains significant differences were observed. These differences can be explained by plasticity of *G. lucidum* basidiocarp formation, but it can be presumed that in some cases a taxonomical error was made. Based on the observed basidiocarp heterogeneity and the preliminary studies it is likely that *G. lucidum* strains present in cultivation originate from different *Ganoderma* species.

## I-P-11. Mushroom diversity of Konkan region of Maharashtra, India

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The Konkan region is a narrow strip of 720 kms comprising four districts viz., Thane, Raigad, Ratnagiri and Sindhudurg of Maharashtra state. On the eastern side of the region lies the Sahyadri mountain range which is a part of Western Ghats- the biodiversity hot spot of the country. Sahyadri mountain range is bestowed with rich flora and fauna. The forays conducted in the diverse habitats of the Konkan region of Maharashtra for four consecutive monsoon seasons of 2007-2011, revealed the occurrence of 33 mushrooms. Among the collected mushrooms, 6 belonged to the family Agaricaceae, 5 to Lyophyllaceae, 3 to Pleurotaceae, 2 each to Hygrophoraceae, Marasmiaceae and Tricholomataceae, and 2 each to Phallaceae, Physalacriaceae, Xylariaceae, Tremellaceae, Sclerodermataceae, Dacrymycetaceae, Cantharellaceae, Entolomaceae, Plutaceae, Ganodermataceae, Bolbitiaceae, Psathyrellaceae, and some of mushrooms were unidentified. The morphological details of collected specimens were recorded following standard proforma. Microscopic features comprising basidia, cystidia and basidiospore morphology were recorded by taking the sections of the gills, in case of gilled mushrooms, while in case of other mushrooms only spore morphology was recorded. The lateritic soils of Konkan region are conducive for growth of termites. During the present investigation, five species of the genus *Termitomyces* were collected from all the four districts of the Konkan region. Out of these, three species, viz., *T. longiradicata*, *T. clypeatus* and *T. umkowaani* were collected from all the four districts, while *T. heimii* and *T. microcarpus* were observed only in Sindhudurg and Ratnagiri districts. In the genus *Agaricus*, three species e.g. *A. arvensis*, *A. placomyces* and *A. trisulphuratus* were recorded during the survey. Other members of the family Agaricaceae included *Macrolepiota procera*, *Leucocoprinus brinbaumii* and *Lepiota* sp. Three species of this genus viz., *Pleurotus ostreatus*, *P. pulmonarius* and one unidentified member was recorded from the region. Perennial trees such as on decaying plant litter of Ain (*Terminalia elliptica*) and Kinjal (*T.*

*paniculata*), *Hygrocybe miniata* was repeatedly recorded. The other member of this genus *H. conica* occurs in forests as well as in open fields. Members of the family Marasmiaceae which were collected from the region were *Marasmius haematocephalus* f. *haematocephalus* and *Marasmiellus ramealis*. *Tremella fuciformis* was the most fascinating mushroom among althea collected specimens. *Craterellus tubaeformis* which is known earlier from temperate regions found to occur in the warm and humid habitats of Konkan region as well. This mushroom and *Hygrocybe miniata* occurred in the same habitat. There is a vast scope for documentation of mushrooms from the region in future.

## **I-P-12. Mushroom flora of Jammu and Kashmir- an overview**

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Mushrooms produce large fleshy fruiting bodies especially during the monsoon season from branching mycelium that infiltrates the soil, leaf litter, wood of living and dead trees. Habitat wise they have commonly been referred to as humicolous (soil and humus loving) e.g. *Morchella* spp., *Volvariella* spp., *Lactarius* spp., *Russula* spp. etc, lignicolous (wood inhabiting) e.g. *Pleurotus* spp., *Agrocybe* spp., *Auricularia* spp., coprophilous (dung inhabiting) e.g. *Coprinus* spp., *Coprinellus* spp., *Coprinopsis* spp., *Panaeolus* spp., fungicolous (fungus inhabiting) e.g. *Asterophora* spp., bryophilous (living in association with bryophytes) e.g. *Omphalina* spp., *Galerina* spp., *Scutellinia* spp., parasitic (*Cordyceps* spp.) or may form ectomycorrhizal association with angiospermic and gymnospermic trees based on the substrate preferences. Owing to climatic variations, topography and rich floristic composition, the area harbours luxuriant mycota. In the present communication eighty five species of wild mushrooms belonging to Ascomycetes, Basidiomycetes and Gasteromycetes are described and illustrated. Brief macro and microscopic details, habitat description, edibility status and consumption modes have been incorporated in the communication.

## **I-P-13. Mushroom germplasm diversity in two forests in Ghana**

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Ghana is endowed with abundant genetic diversity of edible and medicinal mushrooms which are otherwise still unearthed and underutilised in Ghana. Surveys conducted over the years in some forests have shown several genera of edible and medicinal mushrooms. Statistics in Ghana have shown that the country's total forest cover which stood at 8.2 million hectares at the turn of the 20<sup>th</sup> century has decreased to about 1.6 million hectares, and it is estimated that in the next 23 years it will be totally lost if corrective measures are not put in place. As a result, both flora and fauna are threatened. Mushrooms and other macrofungi are therefore, no exception, and in recent years interest has grown in their nutritional, pharmacological, production of secondary metabolites and bioactive properties among others. The purpose of this study was to collect and examine the biodiversity of mushrooms in two Ghanaian forests, their similarities and differences in these ecological zones, with a view of conserving them and analysing them for secondary metabolites. Two main forests were studied in this survey: Atiwa Range Forest in the Eastern Region and Ayum Forest in the Brong Ahafo Region. These two forests are located in two ecological zones namely Semi-Deciduous rainforest and Forest Savanna transition, respectively. Identification of mushrooms was done following standard techniques and literature. A total of 40 macrofungi were collected: 17 from Atiwa Range Forest and 23 from Ayum Forest. Of these 40, the genus and species of 11 varieties from the Atiwa Forest and 13 varieties from the Ayum Forest were identified, while the identity of 16 varieties remains unknown. The edible mushrooms identified include specimens from *Auricularia*, *Volvariella*, *Pleurotus*,

*Termitomyces* and *Lentinus squarrosulus*. The medicinal mushrooms identified include *Pleurotus tuber-regium*, *Pycnosporus sanguineus* and *Daldinea concentrica*. Secondary metabolites such as ergothioneine, beta-glucans, ergosterol (pre cursor to vitamin D2) and vitamin D2 were analysed from mushrooms collected from different locations. These compounds have been suggested to be biologically active in humans. This study has profiled the biodiversity of mushrooms available in Ghanaian forests and the need to conserve them due to their potential health benefits.

#### **I-P-14. Species of genus *Agaricus* L.: Fr. - new to science**

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Genus *Agaricus* L.: Fr. is one of the major genera of family Agaricaceae. It is represented by 200 species the world over and is characterized by having carpophores with convex, smooth or scaly pileus, lamellae free, pinkish when young, chocolate brown in age. Stipe central, fleshy, annulate, annulus single ring like or double sheath like. Spore print greyish brown to dark brown. In the present studies taxonomy of the wild species of this genus is being presented with detailed description of macroscopic and microscopic characters and requisite illustrations 58 specimens belonging to 39 species were collected from various localities and sublocalities of Punjab state from 2008-2012. Of these 3 species viz. *Agaricus stellatus-cuticus* sp. nov., *A. punjabensis* sp. nov. and *A. patialensis* sp. nov. were observed to be new to science.

#### **I-P-15. Tropical semi-evergreen Sal (*Shorea robusta*) forests responding mushroom diversity in Chhattisgarh, Central-India**

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Chhattisgarh, the largest tropical forest state in the country, is more diverse in ecological systems and high endemism of tree species sustaining the richness of mushroom flora. In context to the present status of forest cover the state represents two types of forests (i) the primary forests with higher percentage of Sal tree providing ecological sustenance to wild life and the tribals (ii) the man-made secondary forests cutting down and reducing utility for wild life conservation. The purpose of present study is to relate mushrooms diversity in both the above forest types.

The study site Achanakmar-Amarkantak Biosphere Reserve (ABR), denotes an area with primary and secondary forests. During study 49 mushroom species belonging to 24 genera were collected from different sites of these forest types. In mixed old Sal forests a large number of ectomycorrhizal mushrooms have been found indicating a suitable environment for the growth and occurrence of variety of mushrooms.

The secondary forest cover showed certain limitations against the mushroom diversity. Because the leaf litter of other type of plants such as Teak is not being consumed by wild life and lying un-decomposed that does not provide humus suitable to mushroom growth. The preliminary information on mushroom diversity relating forest types and their environment will be discussed during the presentation.



## **I-P-16. Taxonomic and molecular studies of some resupinate Agaricomyceteous fungi from India**

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The resupinate Agaricomyceteous fungi belonging to class *Agaricomycetes* (subphylum-*Agaricomycotina*, phylum-*Basidiomycota*) are characterized by the gymnocarpic hymenium; hymenial surface varying from smooth, poroid (in case of poroid members), tuberculate, ridged, warted, toothed to merulioid; the color of the basidiocarp generally varying from different shades of white, gray or yellow to sometimes more bright shades of blue, red and brown; two to eight spored basidia and perforate to imperforate parentheses. Eleven species (*Antrodiella albocinnamomea*, *Chondrostereum purpureum*, *Hyphodontia niemelaei*, *Oxyporus corticola*, *Phlebia subserialis*, *Phlebiopsis gigantea*, *P. himalayensis*, *Schizopora paradoxa*, *S. radula*, *T. calothrix* and *T. effugiens*) have been taxonomically described. Of these, DNA based molecular studies of 7 taxa (except *S. paradoxa*, *S. radula*, *T. calothrix* and *T. effugiens*) were carried by targeting ITS and LSU regions by using different primers and confirmed the placement of these to their respective clades within class *Agaricomycetes*.

## **I-P-17. Agarics from South Kashmir: In putative ectomycorrhizal association with conifers**

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The present paper deals with the mycofloristic and putative ectomycorrhizal aspects of agarics of South Kashmir. Agarics are the gilled mushrooms belonging to subphylum *Agaricomycotina* under phylum *Basidiomycota* Moore. of subclass *Agaricomycetideae* under Class *Agaricomycetes* Doweld, growing in varid habitat. The region of South Kashmir is a part of hotspot mega centre of the Himalayan belt which has rich floristic diversity, represented by well demarcated vegetational zones, comprising of potential ectomycorrhizal hosts of agarics such as *Cedrus deodara*, *Pinus wallichiana*, *Abies pindrow* and *Picea smithiana* etc. The best seasons for the collection of the agarics being the spring, summer and autumn in the study area. In the present study the diversity of agarics along with their putative ectomycorrhizal associations with coniferous trees of the South Kashmir have been investigated. A number of fungal forays were undertaken to various localities, as a result of which a systematic account of the species of *Lepiota*, *Russula* and *Lactarius* along with the putative ectomycorrhizal association with *Pinus wallichiana* and *Cedrus deodara* is presecuted presently.

# **Session-II**

## **Genomics, Genetics and Breeding**



## Keynote Presentation

### II-K-1. Breeding edible mushrooms; progress from an applied to advance science

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Mushroom breeding has been, and partly still is, an applied science based on practices used for many decades. Among those are selection of fertile single spores, multi spore cultures or tissue cultures. In the past 10 years, however, research has enhanced our understanding of genomes of edible fungi and their life cycles. Mushroom breeding has advanced and has adapted breeding principles of plant breeders. Especially molecular techniques such as next generation sequencing have increased our knowledge of meiotic processes in fungi and enhanced the efficiency of breeding. This key lecture will highlight some of these new developments adapted from plant and animal breeding and give examples of how these are or can be used for mushroom breeding. Despite this progress, publications in peer reviewed papers on mushroom breeding are still very rare. The number of registered varieties for breeders right or plant patent are also low compared to crops comparable in size to edible mushrooms. The main reason is that investments in breeding programs is still low. This is mainly due to the insufficient protection of new varieties. Mushroom varieties can be copied and propagated easily as vegetative mycelium. For button mushrooms, fertile single spore cultures can be used to make small changes to existing varieties. These “look-alikes” can easily outcompete true bred lines and thus minimize return of investment. A European initiative will be presented that might be used as a template to come to a better of mushroom varieties. When effective, it might lead to a substantial investment in breeding research and breeding programs.

## Oral Presentations

### II-O-1. Spawn cryopreservation of *Agaricus bisporus* and *A. subrufescens* strains

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One of the main problems for conservation of germplasm of mushroom species is that the traditional method of sub culturing facilitates aging and contamination of strains. The conservation of strains at ultra-low temperature in liquid nitrogen (-196 °C) is a widely used method, however, this method become expensive in collections with large number of strains and requires highly specialized personnel. Cryopreservation of strains at - 80 °C has also been shown to be an efficient and low cost method. The aim of this study was to evaluate the viability of spawn of *A. bisporus* and *A. subrufescens* strains frozen at different temperatures. Two strains of *A. subrufescens* and one strain of *A. bisporus* were studied. The spawn was prepared in sorghum seeds and incubated for 3 weeks at 25 °C to allow the grains were completely covered by mycelium. Twenty five fully-incubated sorghum seeds were placed in polycarbonate vials containing 1.5 ml of sterile cryoprotectant solution (10% glycerol v/v) (G) or without glycerol (WG). The seeds in treatment G remained in contact with the cryoprotective solution for 1 h and then samples of both treatments were transferred directly into freezers at -20 or -80 °C. After 3, 6 and 12 months (3M, 6M, 12M) the samples were thawed in bath water (30 °C for 10 min) and the viability of the spawn seeds was evaluated placing seeds in Petri dishes containing potato dextrose agar (PDA) and incubated at 25 °C. The percentage of sample recovery was evaluated (50 spawn seeds by strain and treatment) through daily observations. Mycelial growth was also evaluated by placing a spawn seed in a Petri dish with PDA or PDA added with compost extract (C) and recording the diameter of the mycelium after 7 days of incubation in darkness at 25 °C, ten samples were prepared per treatment and strain. The results showed that in the samples frozen at -20 °C the recovery was minimal (2 to 4%) in only one strain. However, the samples frozen at -80 °C with or without cryoprotectant (G, WG) during the freezing times tested (3M, 6M, 12M) showed a recovery of 100% from 1 to 6 days after thawing. The recovered samples had a significantly higher growth in the medium C. In all cases the recovered samples produced normal basidiomata when cultivated on compost substrate prepared for commercial cultivation of *A. bisporus*. The proposed method may facilitate handling and reduce maintenance costs for *Agaricus* strains.

### II-O-2. Phylogenetic relationships of *Ganoderma* species based on mitochondrial and nuclear DNA sequences from Tamil Nadu, India

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Phylogenetic relationship of *Ganoderma* species confining to Tamil Nadu was assessed by three molecular markers viz., internal transcribed spacer region 1 and 2 (ITS),  $\beta$ -tubulin and ATP synthase subunit 6 (atp6). Forty-three isolates representing 11 species of *Ganoderma* were identified from ITS rDNA sequence data. The nucleotide variations found in the ITS region were suitable for discriminating the eleven species viz., *G. australe* (5 isolates), *G. cupreum* (1 isolate), *G. lucidum* (11 isolates), *G. resinaceum* (2 isolates), *G. tropicum* (2 isolates), *G. weberianum* (1 isolate), *Ganoderma* sp. 1 (1 isolate), *Ganoderma* sp. 2 (1 isolate), *Ganoderma* sp. 3 (7 isolates), *Ganoderma* sp. 4 (2 isolates) and *Ganoderma* sp. 5 (10 isolates). The terminal clade represent that species and/or species complex were consistent with geographical origin of the isolates and cultural characters. The  $\beta$ -tubulin gene phylogeny provided more robust

phylogenetic information for separating ten *Ganoderma* spp. (excluding *G. cupreum* and *Ganoderma* sp. 5) than the ITS region and found to be suitable in identifying the *Ganoderma* species and/or species complex. The *atp6* gene sequence data acquired highest phylogenetic informative character than nuclear DNA genes; however, due to the very low levels of nucleotide divergence, the gene is suitable at genus or lower level. *Ganoderma* spp. inferred from these three un-linked loci resulted in a robust phylogeny; the terminal clades were well resolved when compared to the individual genes. The tree topology of multigene analysis was in line with *atp6* because it might have contributed more phylogenetic informative characters than the nuclear DNA genes. In multi-gene analysis, the terminal clades showed similar pattern of geographical distribution between and/or within clades. This study implies that the higher variability in the nucleotide sequences of the nuclear DNA genes (ITS and  $\beta$ -tubulin) have the potential to be the robust markers for molecular identification than *atp6*. Chlamydospores in culture provided valuable information in identifying *Ganoderma* spp. The terminal clades of nuclear DNA genes represented the species or species complex and consistent with cultural characteristic, host relationship and biogeographical distribution rather than *atp6*.

### **II-O-3. DNA markers reveal genome-wide variations in the strains of button mushroom (*Agaricus bisporus*)**

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The wild strains of a cultivated species constitute an important breeding material for the genetic enhancement of commercial lines. Cultivated strains of button mushroom, *Agaricus bisporus* have narrow genetic base due to their origin from few high-yielding hybrids and single spore progenies through tissue culture propagation. The genetic identities of wild and cultivated strains of *A. bisporus* were established by DNA sequencing of internal transcribed spacer (ITS) regions of 5.8S rDNA. Molecular variation among the different strains was assessed using random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers. ITS1, 5.8S rDNA and ITS2 were 290, 154 and 208 bases, respectively in length in all the strains of *A. bisporus* studied. We report single nucleotide polymorphisms (SNPs) in ITS2 region, which distinguished different strains within the species. A total of 467 marker bands (92.3% were polymorphic) were generated from six AFLP primer-pairs which differentiated all the germplasm strains. White pileus cultivated strains were separated from the wild brown strains by a phylogenetic branch. The wild strains possessed a broad range of genetic variation (53.4%) and exhibited 53.7% genetic distance from the cultivated strains and hybrids, indicating a high level of DNA polymorphism in the germplasm. However, the cultivated strains (current and quondom varieties) showed less DNA polymorphism (16.8% genetic variation) as compared to the hybrid cultivars (26.8% variation). The cultivated strains showed a genetic distance of 33.6% from the hybrids and were clearly separated in the UPGMA dendrogram and PCO plot. The molecularly diverse germplasm strains were selected for genetic improvement. Breeding lines were developed through generation of single spore progenies (SSPs), protoplast regenerants (PRs) and EMS-induced mutants. These breeding materials were molecularly characterized using robust and polymorphic RAPD markers. DNA markers demonstrated their great value in the differentiation of pedigree related cultivated strains, SSPs, PRs, mutants and hybrids, and the

establishment of genetic relationships among germplasm strains of *A. bisporus*. These results have practical implications for the future breeding programmes of this commercially important button mushroom.

## **II-O-4. Genetic variability in strains of *Volvariella volvacea* collected from the state of Odisha, India**

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*Volvariella volvacea*, the straw mushroom is an edible mushroom of tropics and sub-tropics, valued for its flavor, texture and nutraceutical properties. However, it is among the least studied mushrooms in India with respect to genetic variability and strain improvement programme. The present study aimed at studying the genetic variability in wild *Volvariella* germplasm collected from different regions of the state of Odisha, India. *V. volvacea* fruit bodies with varied shape, size and colour were collected from nine different locations spread in eight districts of Odisha during rainy season of 2010. The mycelial cultures raised from fruit bodies collected from different regions were considered as separate strains, and were screened for their mycelial growth characteristics. Out of total ten strains, seven were recorded as fast growing (>90 mm radial growth on MEA in 6 days), while rest three were slow growing. Similar was the observation in downward mycelial growth on pounded paddy straw test tubes. The strains also exhibited variability in their colony colour as well as aerial mycelial growth and majority of the fast growing strains formed creamy white colonies. The strains also shown variations in their extracellular lignocellulolytic enzymes activity profiles. Fastest growing strain OSM-1 exhibited highest activity of exo-glucanase, low of endo-glucanase, and superior levels of  $\beta$ -glucosidase and xylanase. Laccase activity was comparatively low in slow growing strains compared to fast growing strains. In grow out trials, four strains including three slow growing (OSM-5, OSM-8 and OSM-10) and one fast growing (OSM-2) did not colonize the substrate. Highest fruit body yield was recorded in strain OSM-9 (23.60 kg/100 kg dry substrate), followed by strain OSM-3 (17.47 kg/100 kg dry substrate). Rest four strains gave negligible yield. The fruit bodies of strain OSM-9 were very light in weight (7.80 g) compared to strain OSM-3 (15.85 g). The repeat trial also proved non-fruiting attributes of these strains. The strains formed two separate groups with respect to length of their ITS regions and the two groups differ by 2 base pair length. The neighbor joining tree deduced from the ITS sequences also showed two groups, group-I comprised of all fast growing strains (OSM-01, OSM-02, OSM-03, OSM-04 and OSM-07), while group-II with three slow growing (OSM-5, OSM-8 and OSM-10) along with two fast growing strains (OSM-6 and OSM-9). In clustalW2 analysis, the strains of group-II exhibited deletions at two base pairs, one each in ITS-1 and ITS-2 regions. Strains of group-II also showed base substitution at three different places in ITS-1 region, where G was substituted with A at two places and T with C at one place. The present study paves the way for further breeding programmes in this mushroom.



## Poster Presentations

### II-P-1. Breeding for higher fruit body yield and quality in *Volvariella volvacea*

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*Volvariella volvacea*, the straw mushroom is in cultivation in different countries of the world but with very limited emphasis on its improvement programmes compared to that of *Agaricus bisporus*. The existing confusion on its primary homothalism or secondary homothalism way of breeding has further dented its breeding programmes. An impetus has been given on its breeding programme recently under a SERB, Govt. of India programme at DMR, Solan, India, where in approximately 420 single spore isolates (140 from each strain) isolated from three potential high yielding strains (DMRO-247, DMRO-484 and DMRO-187) of *V. volvacea* but with variable features, have been used for studying the variability in single spore isolates of this mushroom. The SSIs have been studied for variation in their mycelial growth characteristics on MEA petridishes and for downward mycelial growth on pounded paddy straw filled in wide mouth test tubes. In all 35 fast growing SSIs selected from three strains based upon their positive growth and fruiting related traits, were subjected to grow out trials using cotton ginning mill waste + paddy straw based composted substrate. In trial-I three SSIs, two of parent strain DMRO-247 and one of strain DMRO-484 gave higher fruit body yield compared to their parent strains. The second trial is in progress, where in a few SSIs have shown potential with respect to early first harvest (days post-spawning) and fruit body yield. A good numbers of non-fruiting types of SSIs have also been identified. Similarly, the slow growing (19) SSIs from these strains have been used for generating hybrids, followed by their screening for downward mycelial growth on pounded paddy straw filled in wide mouth test tubes. Out of 102 hybrids so generated, only eleven have shown faster mycelial growth compared to their parents based upon downward growth test criteria. Further studies pertaining to their lignocellulolytic enzymes activity profiles and grow out trials are in pipe line. The study will help in further understanding on breeding system in this mushroom and to have superior yielding strains for commercial use.

### II-P-2. Evaluation of phytochemical analysis and DNA fingerprinting by RAPD markers of some commercially cultivated edible mushrooms

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Mushrooms, belonging to the kingdom Fungi can be classified into four categories – edible mushrooms, medicinal mushrooms, poisonous mushrooms and other mushrooms whose properties are less well defined. Mushrooms are recognized as natural and healthy foods and credited to be the third largest macro-fungus cultivated for food and industrial purposes worldwide. Mushrooms contain high protein, vitamins, fibres and low calories; it also has many medicinal properties. In today's world with burgeoning population the cultivation of edible mushrooms holds great significance because of their nutritive and therapeutic properties. The advances in research on mushroom breeding and production are highly constrained compared to other crops; due to lack of previous knowledge in mushroom genetics. Present study focuses on establishing phylogenetic relationship among the eleven commercially cultivated edible mushrooms using RAPD markers. Eleven mushroom varieties namely *A. bisporus* (button), *A. bisporus* (portobello), *P. eryngii* (king oyster), *L. edodes* (shiitake), *H. tessellatus* (brown shimeji), *H. tessellatus* (white shimeji), *F. velutipes* (enoki), *P. ostreatus* (oyster), *P. djamor* (pink oyster), *C. indica* (milky), and *P. florida* (florida oyster) were used. In this study, mushrooms were screened for phytochemicals such as

cardiac glycosides, anthraquinones, terpenoids, proteins, flavonoids, saponins, tannins, lignins and phenol. All the samples showed positive result for terpenoids and proteins and showed negative result for anthraquinones, flavonoids, tannins, lignins and phenol. Most of the samples found positive for cardiac glycosides and saponins. Molecular markers serve as quick and reliable tool to establish the identities of mushrooms and are helpful in mushroom taxonomy. Among the various primers used, OPZ 10 gave the most distinguished and scorable band pattern. To estimate the similarity and genetic distance among different mushrooms cluster analysis based on frequency similarity was performed. The RAPD analysis in this study has proven to be useful in discrimination, characterization and differentiation of the fungal cultivars.

### **II-P-3. Genetic characterization of single spore isolates of *Agaricus bisporus* (Lange) Imbach**

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The yield and quality of the mushroom produced is determined by three factors: the genetic makeup of the mushroom strain, the environmental conditions in which the mushroom is grown and the quality of nutrition. In India, introduction of exotic strains of button mushroom and their selection on the basis of their evaluation is a continuing process. The present investigations were undertaken to develop improved strains of *Agaricus bisporus* through single spore isolates (SSIs) and genetic characterization of developed SSIs using SDS-PAGE protein profiling. On the basis of yield performance, four strains of *A. bisporus*, namely, S-11, MS-39, NCS-100 and NCS-101 were selected (designated as A, B, C and D, respectively) and used for the isolation of single spores. A total of 410 SSIs were developed out of which 255 SSIs performed below or at par to their respective parent while 155 SSIs out yielded the parent. Further, on the basis of higher yields, 5 SSIs of A (S-11), 2 SSIs of B (MS-39), 5 SSIs of C (NCS-100) and 6 SSIs of D (NCS-101) were selected and analysed by protein profiling. On the basis of protein profiles, the above 18 isolates were classified into seven groups. The total number of bands ranged from 5 to 13 and spread over three strains (A, B, C). One isolate was in 13 band group, while two isolates were in 5 band group, four in 6 band group, four in 7 band group, three in 8 band group, three in 9 band group and one in 10 band group. Within group, the isolates had very little difference in banding pattern both for position and intensities of bands, however, between group difference was observed. Irrespective of group, isolates varied more for high molecular weight protein band as compared to low molecular weight protein, which showed the variability among the isolates.

### **II-P-4. Molecular and morphological characterisation of mushrooms in Northeast India**

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Northeast region of India is blessed with many biodiversity hotspots. States like Arunachal Pradesh, Assam, Sikkim, Manipur, Nagaland, Tripura, Mizoram and Meghalaya are rich in mushroom biodiversity. Many times morphological criteria for identification of mushrooms often pose problem in accurate identification due to overlapping characters. Identification was attempted using molecular techniques involving amplification and sequencing of ITS 1-5.8s - ITS2 region of rDNA, etc. BLAST similarity searches were performed at NCBI website. The sequences have been deposited in Genbank. Phylogenetic analysis was also conducted for accurate placement of the species. Samples were *Polyporus* spp., *Termitomyces* spp., *Calvatia* sp. etc. Additional loci will be used for confirmation of the identity. Morphological characters along with molecular tools will help in reliable and accurate identification. This will definitely help in cataloguing mushroom diversity in northeast region.

## II-P-5. Sequence analysis of partial *tef1 $\alpha$* and *rpb2* genes of different *Pleurotus eryngii* isolates by means of PCR-RFLP

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The *P. eryngii* species complex includes several varieties and certain groups with ambiguous taxonomic position. At the moment the following taxa are recognized: var. *eryngii*; var. *ferulae*, *P. fuscus* var. *ferulae* var. *elaeoselini*; var. *nebrodensis*; var. *tingitanus*; var. *tuoliensis*; *P. hadamardii*; *P. fossulatus*. In the course of our studies partial sequence analysis of the *tef1 $\alpha$*  and *rpb2* genes was performed, in order to distinguish varieties and reveal variability between isolates. Specific regions of the *tef1 $\alpha$*  and *rpb2* genes were amplified by specific primer pairs (EF595F/ EF1160R; bRPB2-6.9F/ bRPB2-11R1) in PCR experiments. The fragments were cleaned, sequenced, aligned then BLAST searched against the NCBI Gene Bank nucleotide database. Some point mutations were detected in the sequences, which were used for selection of differentiating restriction enzymes for subsequent PCR-RFLP experiments. The *tef1 $\alpha$*  gene sequences showed 100% identity in each strain. In contrast to that, point mutations were detected in the 21, 372 and 957 positions of the *rpb2* sequences. Based on these nucleotide variations, *in silico* digestion was performed on the sequences and two restriction endonucleases, the BsmAI and TspDTI were selected for PCR-RFLP experiments. As a result of the digestions, the isolates could be grouped into two groups with both enzymes. At the same time, BLAST search with both amplified sequences did not give reliable information neither on varieties, nor on species level. The *rpb2* locus, with its higher level of polymorphism, is a potential candidate for differentiation between varieties or identification on varietals level. Based on the results we plan to investigate which loci and molecular methods may be suitable for differentiation of the isolates on varietals level. Today there are a lot of contradictions in the taxonomy of the *P. eryngii* species complex, but these discrepancies may be resolved by comprehensive molecular biology, speciation and coevolution research projects.

## II-P-6. A simple and efficient transformation method for the edible mushroom *Pleurotus eryngii*

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*Pleurotus eryngii*, an economically important edible fungus assigned to the Pleurotaceae, is a highly nutritious mushroom with important pharmaceutical and ecological attributes. However, few relevant molecular biological tools are available to meet the growing demand for the development of *P. eryngii* breeding and biotechnological applications. We now report the establishment of an efficient genetic transformation system to promote *P. eryngii* genetic research, mushroom breeding programmes, and the use of this mushroom as a potential expression system for the production of exogenous protein. Previously, *Agrobacterium tumefaciens*-mediated transformation (ATMT) has been successfully applied to *P. eryngii* and, in this present study, selected transformation parameters have been optimized. For the first time in a *P. eryngii* genetic transformation system, the Hsp70 promoter of *Cordyceps militaris* has been used to drive the expression of Enhanced Green Fluorescent Protein (EGFP), a nutrient-deficient medium has been employed to increase the receptiveness of the fungal mycelium to the transforming plasmid, and the glufosinate-ammonium (Bar) antibiotic resistance marker has been adopted for transformant selection.

All the putative transformants remained genetically stable even after five successive rounds of subculture, and produced normal fruiting bodies that were little different morphologically to non-transformed controls. Transformants were analyzed for the presence of the Bar gene by PCR and Southern blot, and EGFP expression was observed by fluorescence microscopy. Our data demonstrated that this improved ATMT system represents a simple and efficient tool for studying the molecular genetics of *P. eryngii*.

## **II-P-7. Development of browning resistant strains in white button mushroom (*Agaricus bisporus*)**

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Browning in white button mushroom after harvest is a major limiting factor determining the quality and marketability of mushrooms. In the present study, 361 hybrids using non-fertile isolates from 11 strains were developed and evaluated for their bruise resistance by applying double streak mechanical injury using a fork. In majority of the cases the browning started immediately after the injury whereas a few remained unchanged even after two hrs. Forty-one such browning tolerant hybrids were selected and evaluated for the yield. Five of these were evaluated on large scale in commercial units and two have been finally selected as these significantly out yielded the strains in use by the commercial units. The selected five strains along with three controls were also screened for total phenols, proteins and enzymes i.e. laccase, polyphenol oxidases, tyrosinase and peroxidase.

## **II-P-8. Evaluation of *Schizophyllum commune* strains for artificial domestication**

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Genus *Schizophyllum* is one of the most common mushroom widely spread in the Indian subcontinent ranging from temperate north evergreen forest of western Himalaya to north eastern Himalaya to tropical and sub tropical forest of central and south India. It is abundantly found in the states of Mizoram, Arunachal Pradesh and Meghalaya where it is collected during rainy season growing on logs of different types of tree and sold at premium price of ₹ 300-400/kg. The local people of Madagascar and Dutch East Indies also habitually chew carpophores of *Schizophyllum*. The Directorate of Mushroom Research Solan is maintaining a Mushroom Gene Bank of commercial as well as wild edible, non edible, medicinal, poisonous and wood rotting fungi for their beneficial commercial and industrial exploitation. The Gene Bank of DMR has more than 3000 culture accessions of *Agaricus* spp., *Pleurotus* spp., *Lentinula edodes*, *Volvariella* spp., *Auricularia* spp., *Ganoderma* spp., *Stropharia* spp. and *Flammulina* spp. from indigenous wild collections. Three strains of *Schizophyllum commune* were attempted for their artificial cultivation using wheat straw and saw dust. Spawn could be easily prepared on wheat grain and paddy grains. However, the spawn grain becomes very hard and difficult to separate the grains after mycelia colonisation. Wheat straw and saw dust were attempted for cultivation after soaking in water for 12 hours and then sterilized by autoclaving at 22 lb psi for 60 minutes. Mycelial growth was completed in 15 days on wheat straw while it took 20-24 days in saw dust. Strain DMRP-179 gave maximum biological efficiency of 55-70% on wheat straw. The fruit bodies were light brown, lobed and up to 3-4 cm in diameter. The low cost of cultivation of *Schizophyllum* can help the local people to get livelihood by cultivation of this mushroom. Several morphologically different collections of *Schizophyllum* have been made.

# **Session-III**

## **Bioinformatics and Nanotechnology**





## Keynote Presentation

### III-K-1. Mushroom mediated synthesis of inorganic nanomaterials: current status and future prospects

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Inorganic nanomaterials are conventionally synthesized under harsh environments like extremes of temperature, pressure and pH. These methods are eco-unfriendly, expensive, toxic, cumbersome, yield bigger particles which agglomerate due to not being capped by capping agents. In contrast, biological synthesis of inorganic nanomaterials occurs under ambient conditions viz. room temperature, atmospheric pressure, physiological pH and is reliable, non-toxic and cheap. Thus, the biosynthesis of nanoparticles is completely in harmony with the environment and should be further developed in order to obtain nanoparticles of variable sizes, shapes and chemical compositions which will find major applications in diagnostics, imaging, therapeutics, catalysis, electronics, etc.

India has a range of rich untapped areas which are home to a vast variety of rare endophytic fungi and mushrooms. These are an excellent source to work upon and should be explored for the bio-synthesis of a variety of bio-compatible nanomaterials, the fabrication of which is very difficult via chemical and physical methods. The bio-compatible nanomaterials will find use in a number of applications such as energy storage, agriculture, food and nutrition industry, smart delivery of herbicides and fungicides, etc.

Recently, we have started exploring the possibilities of nanoparticle synthesis employing various genera of mushrooms owing to the innumerable bioactive compounds with diverse biological activities present within them. A vast variety of proteins and polysaccharides found in the mushrooms have been utilized in the synthesis of both intracellular and extracellular nanoparticles. The compounds secreted by the mushrooms provide the nanoparticles so formed with high stability, extended shelf-life, water solubility and well dispersion properties. Also, we have employed different endophytic fungi for fabrication of nanoparticles of different sizes and shapes. Our experiments with mushrooms and endophytes so far seem to be very promising and open up a new area of green-chemical approach for synthesis of eco-friendly, non-toxic and stable nanomaterials.

In this talk, we describe our research into the biological synthesis of physically and chemically difficult to synthesize natural protein capped, biocompatible, fluorescent nanomaterials of different chemical compositions, sizes, shapes using endophytic fungi and mushrooms at room temperature. We have shown that endophytic fungi and mushrooms when challenged with aqueous metal ions are capable of reducing the ions both intra and extra-cellularly resulting in the formation of stable metal/quantum dots/oxides/biomineral/carbon nanoparticles. It has been confirmed by us very recently that the enzymes such as sulphite reductase, nitrate reductase and hydrolyzing proteins secreted by the fungus in response to the stress induced by the metal ions are responsible for the synthesis of nanoscaled particles.

We are further conducting research on above nanomaterials fabricated by us for safety/toxicity studies and hazard/risk/life-cycle assessment to check their impact on human health and environment. Once the safety studies are evaluated, we can further analyze our nanomaterials for applications in delivery systems, targeted delivery systems, imaging, diagnosis, therapeutics, energy conversion, energy storage, agriculture and environment.



## Oral Presentations

### III-O-1. The whole genome sequence of *Volvariella volvacea* will facilitate resolution of problems limiting its commercial exploitation

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*Volvariella volvacea* is widely grown in southeastern Asia as a high quality human food source, and is one of most important cultivated mushrooms worldwide. However, developments in *V. volvacea* cultivation have been limited due to a low biological efficiency (i.e. conversion of growth substrate to mushroom fruit bodies), sensitivity to low temperatures, and an unclear sexuality pattern that has restricted the breeding of improved strains. The Institute of Edible Fungi, Shanghai Academy of Agricultural Sciences, has recently completed the sequencing of the whole genome of *V. volvacea* strain V23-1, a single-spore isolate derived from *V. volvacea* strain V23. The sequence, which has been deposited in the NCBI database, is assembled into 62 scaffolds with a total genome size of 35.7 megabases (Mb), and contains 11,084 predicated gene models. Comparative analyses based on the whole genome sequence have been made with other basidiomycete mushrooms with regard to the mating type system, polysaccharide degrading enzymes and lignin oxidizing enzymes. Transcriptional analysis of the loss of hyphal viability following exposure to low temperature (4 °C) has also been undertaken. The *V. volvacea* genome contains numerous genes encoding enzymes involved in the degradation of cellulose, hemicellulose and pectin. Furthermore, the molecular structure of the mating type system is similar to that of the bipolar system of basidiomycetes, and indicates that *V. volvacea* has a secondary homothallic life cycle. The absence of genes encoding enzymes involved in initiating the biosynthesis of unsaturated fatty acids, trehalose and glycogen may be related in part to the sensitivity to low temperature exposure. Elucidation of the *V. volvacea* genome sequence will promote a deeper understanding at the molecular biological level of the mechanisms involved in substrate degradation and help to resolve problems limiting the industrial exploitation of this mushroom.

### III-O-2. Identification of the WRKY transcription factors in *Agaricus bisporus* (white button mushroom)

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With the changing variable environmental conditions, plants can reprogram their transcriptome through transcription factors. WRKY transcription factors are a class of sequence-specific DNA binding transcription factors found almost exclusively in plants and are key regulators, both positive and negative, of both biotic and abiotic stresses, seed development, seed dormancy and germination, development, plant hormone signaling, secondary metabolism and senescence. The WRKY protein family contains a highly conserved motif spanning about 60 amino acids and within this domain, there is an almost invariable heptapeptide signature WRKYGQK at the N-terminus and a novel zinc finger-like structure at the C-terminus. The WRKYGQK is the most dominant form of the signature followed by WRKYGKK and WRKYGEK, however, there are at least 35 variants of this motif present in plant and non-plant species. The WRKY domain can be characterized as WRRY, WSKY, WKRY, WVKY, or WKKY. WRKY proteins preferably bind to the consensus sequence TTGACC/T, the so-called W-box, which is usually enriched in the promoter region

of WRKY target genes such as stress responsive genes. So far, only two WRKY homologues have been identified from non-plant species, *Giardia lamblia* and *Dictyostelium discoideum*. Some WRKY proteins exist as chimeric proteins combining NBS-LRR (nucleotide binding site - leucine rich repeat) proteins and WRKY domains. During the study, WRKY domain was searched in silico in the genome sequence of *A. bisporus* and also the priming sites were determined. On the basis of in silico results, two WRKY and one Nucleotide Binding Site primers were tested for amplification of WRKY domains in white button mushroom. Surprisingly, *Agaricus* genome showed the presence of WRKY domain at multiple sites and also different type of WRKY domains could be identified in the genome. Also the Nucleotide Binding Site (associated with WRKY domain) primer for disease resistance gene amplified fragment in the mushroom showing the presence of disease resistance gene. This is a first report of presence of WRKY domain (specific to plants) in mushroom genome. Characterization of WRKY domain in mushrooms are in progress.

## Poster Presentations

### III-P-1. Transcriptome analysis of candidate genes and signaling pathways associated with light-induced brown film formation in *Lentinula edodes*

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High-throughput Illumina RNA-seq was used for deep sequencing analysis of the transcriptome of poly (A)+RNA from fungal mycelium grown under three different conditions: 30 days darkness (sample 118), 80 days darkness (313W), and 30 days darkness followed by 50 days in the light (313C), in order to gain insight into the molecular mechanisms underlying the process of light-induced brown film (BF) formation in the edible mushroom, *Lentinula edodes*. Of the three growth conditions, BF formation occurred in 313C samples only. Approximately 159.23 million reads were obtained, trimmed, and *de novo* assembled into 31,511 contigs with an average length of 1,746 bp and an N50 of 2,480 bp. Based on sequence orientations determined by a BLASTX search against the NR, Swiss-Prot, COG and KEGG databases, 24,246 (76.9%) contigs were assigned putative descriptions. Comparison of 313C/118 and 313C/313W expression profiles revealed 3,958 and 5,651 significantly differentially expressed contigs (DECs), respectively. Annotation using the COG database revealed that candidate genes for light-induced BF formation encoded proteins linked to light reception (e.g. WC-1, WC-2, phytochrome), light signal transduction pathways (e.g. two-component phosphorelay system, mitogen-activated protein kinase pathway), and pigment formation (e.g. polyketide synthase, O-methyltransferase, laccase, P450 monooxygenase, oxidoreductase). Several DECs were validated using quantitative real-time polymerase chain reaction. Our report is the first to identify genes associated with light-induced BF formation in *L. edodes* and represents a valuable resource for future genomic studies on this commercially important mushroom.

### III-P-2. *In silico* structure analysis of homeodomain proteins encoded *matA* locus in *Pleurotus ostreatus*

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The *matA* locus of Basidiomycetes plays the primary role in mating process and fertile dikaryon development. It encodes homeodomain transcription factors family (HD) that regulates expression of many genes involved in sexual development. The active transcription factor is a heterodimer that consists of two interacted homeodomain proteins (HD1 and HD2) from two different *matA* alleles originated from opposite mating partners. Therefore, heterodimerisation process depends on specific primary and secondary protein structure. In this study we searched for *HD* gene and protein sequence conservative regions among some *Pleurotus* species. Also some important differences between HD1 and HD2 protein sequences were found. Based on these differences we have predicted *in silico* secondary protein structure and tertiary structure for HD1 and HD2 protein families in *P. ostreatus*. Dimerization sites and DNA-binding domains of HD proteins were found. The structure of DNA-binding domain of HD1 and HD2 proteins was reconstructed. The heterodimer DNA-binding model was predicted by bioinformatics analysis *in silico*.

# **Session-IV**

## **Biology, Biochemistry, Physiology and Development**



## Keynote Presentations

### IV-K-1. Fungal unspecific peroxygenases : a new generation of oxygen-transferring biocatalysts

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The oxygenation of organic molecules is a challenging task in synthetic chemistry and therefore biocatalytic approaches using oxygen-transferring enzymes have come into the focus of chemists and biotechnologists. Fungal peroxygenases represent a unique enzyme type that selectively transfers oxygen from peroxides (R-OOH) to numerous substrates such as benzene derivatives, polycyclic aromatic hydrocarbons, *N*- and *S*-heterocycles, linear and cyclic alkanes, alkenes as well as to complex drug molecules and pesticides. Peroxygenases are heavily glycosylated heme-thiolate proteins that are actively secreted by fungi. Over 1,000 putative peroxygenase-like sequences, which form at least two distinct clusters, can be found in genetic data bases indicating the widespread occurrence of such enzymes in the whole fungal kingdom including true fungi and fungus-like heterokonts. Thus, peroxygenases represent, on the phylogenetic level, a fungi-specific superfamily of heme proteins. Their catalytic cycle combines the pathways of heme peroxidases and cytochrome P450 monooxygenases. Due to their high stability and the use of cheap peroxides as co-substrate, peroxygenases could become a powerful biocatalytic tool for applications in organic synthesis and other fields.

### IV-K-2. Genetic transformation of mushrooms and its utilization in basic and applied sciences

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Genetic transformation is a powerful tool for elucidating function of a gene of interest in any organisms and breeding of new species with desired phenotypes when it comes to agricultural crops. In the beginning, genetic transformation of mushrooms was successfully applied only to limited model species, such as *Schizophyllum commune* [1] and *Coprinopsis cinerea* [2] but, in these days, development of technical protocols which enable introduction and expression of genes have been spread to several mushrooms, including major cultivated edible mushrooms such as *Agaricus bisporus* [3], *Lentinula edodes* [4], *Flammulina velutipes* [5] and *Pleurotus ostreatus* [6]. To the best of my knowledge, no genetically modified mushrooms are on the market yet, transformation have been getting more and more popular and important for both of the basic and applied sciences in mushrooms, with the increase in the number of mushroom species of which genome sequence was analyzed.

For the basic science of mushroom-forming fungi, genetic transformation will lead us to understand fundamental molecular mechanism such as regulation of gene expression related to fruit body development or mating, substrate utilization, growth rate and disease control. And, in the long run, it may contribute to development of new commercial species with preferable properties, in respect with taste, flavor, yield and post harvest. Moreover, elucidation of the fundamental mechanism of vegetative growth and sexual development may lead to establishment of new and more effective cultivation protocol, especially for the

species which are difficult to be cultivated nowadays, including mycorrhizal fungi. Transformation technique were used to analyze molecular mechanisms for gene expression such as initiation and termination of transcription, maturation of mRNA, translational regulation, post-translational modification, and secretion pathway in *P. ostreatus*.

Besides food utilization, mushrooms have many potentials to be available in wide variety of applied fields: some wood rotting mushrooms can be used for pretreatment of woody biomass resources to be converted to various useful chemicals such as energy compounds or biodegradable materials; over-production of bioactive compounds both from homologous and heterologous origins; production of recombinant carbohydrate-modified proteins available for treatment of specific human diseases were tried in mushroom fungus; and so on.

In this keynote address, the author would like to introduce the past and current status of transformation in mushroom sciences and some of the on-going projects in his own lab.

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## Oral Presentations

### IV-O-1. A study on bioactive fluorescent compounds from selected mushrooms and its antimicrobial activity

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Mushrooms have long been appreciated for their flavor, texture, medicinal and nutraceutical attributes. Present report deals with fluorescent bioactive compounds from *Armillaria mellea* (MTCC 409) and *Omphalotus olearius* (MTCC 2790). The cultures were obtained from MTCC, Chandigarh. Time scale studies for the production, extraction and estimation of bioactive fluorescent compounds was made to evaluate their antibacterial activity. *A. mellea* (MTCC 409) and *O. olearius* (MTCC 2790) grown in potato dextrose broth recorded maximum mycelial dry weight of  $90 \pm 1.12$  g/50ml and  $0.57 \pm 0.025$  g/50ml, respectively on 28<sup>th</sup> day. *A. mellea* and *O. olearius* mycelium (1000 mg) recorded maximum crude fluorescent dyes in acetone (255 mg) and methanol (553mg), respectively. Acetone extracts of *A. mellea* and *O. olearius* recorded highest zone of inhibition against *E. coli* and *P. aeruginosa*, respectively.

### IV-O-2. Anti-fatigue effects of *Agaricus bisporus* extract in rats

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Mushroom antioxidants help in scavenging the free radicals formed during heavy exercise. Intake of yogurt helps to improve appetite and thereby the food consumption to yield more energy. So the addition of mushroom extract to curd may provides combinatorial effect. The present study was undertaken to investigate the anti-fatigue properties of *Agaricus bisporus* extract (ABE) and development of curd product using the same extract. Antioxidant properties of the extract was screened and the results showed increased free radical scavenging, reducing power and metal chelating property with increasing concentration of ABE. Total phenolic content was 7.5821 mg tannic acid equivalent/g extract (or) 27.8 mg quercetin/g of extract and total flavonoid content was 2.2385 mg quercetin/g of extract. HPLC profile of the polyphenols in the mushroom extracts revealed the presence of chlorogenic acid and p-coumaric acid. The anti-fatigue activity of ABE was measured using animal treadmill exercise. Endurance exercises reduced the levels of glycogen in control group. However, ABE supplementation enhanced liver and muscle glycogen levels ( $p < 0.05$ ). Lactic acid (LA) levels in muscle tissue were significantly increased in control exercise group when compared to sedentary group ( $p < 0.05$ ). The ABE supplemented group lowered LA levels in muscle tissue when compared to control rats ( $p < 0.01$ ) showing the efficient usage of glucose during exercise by the extract. In control exercised group of rats, the values significantly increased malondialdehyde (MDA) concentration in muscle and liver when compared with sedentary group. The ABE treatment decreased the MDA levels in muscle (37.7%) and liver (16.2%) to that of control group showing the *in-vivo* antioxidant property of the extract. Significant increase in ATP concentration was observed in ABE supplemented group compared to control group. Both the sedentary group (with or without extract) had similar concentration of ATP. A curd product enriched with ABE was formulated. In conclusion, the extract will have beneficial effect w.r.t its anti-fatigue property.

#### **IV-O-3. Comparative study on the production, purification and characterization of exopolysaccharides from *Pleurotus florida* and *Hypsizygus ulmarius* and their applications**

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Mushroom polysaccharides have attracted great deal of attention due to many healthy benefits such as immunomodulation, anticancer activity, prevention and treatment of cardiovascular diseases, antiviral and antimicrobial effects. The present work involved the production and purification of extracellular polysaccharides (EPS) from *Pleurotus florida* (PF) and *Hypsizygus ulmarius* (HU) and investigation of its effect in relation to antioxidant and anticancer activities. Several parameters such as pH, temperature and media for the growth of mycelial culture and for the production of EPS were optimized to be 4.5, 27 °C and GPKM media, respectively. Purification of exopolysaccharides were carried out by DEAE Sephacel (anion exchange chromatography) and high yield of exopolysaccharides were obtained as 0.726 mg/ml and 0.665 mg/ml for PF-EPS and HU-EPS, respectively. The samples were further subjected to characterization, antioxidant and anticancer studies. Characterization by NMR showed relative peaks corresponding to polysaccharides. The results of antioxidant assay for the two polysaccharide samples (PF-EPS and HU-EPS) by phosphomolybdenum method was found to be 20.57 and 21.93 µM AAE/g of tissue, respectively. Anticancer potential of purified polysaccharides were assessed by MTT assay on MCF breast cancer cell lines and two samples, PF-EPS and HU-EPS exhibited percentage of cell viability at 66.48% and 47.63%, respectively. On the whole comparatively, HU-EPS demonstrated encouraging results in terms of anticancer potential.

#### **IV-O-4. Laccase from selected mushrooms and its application in mycofilter for decolourization and removal of bacteria in textile dyeing effluents**

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*Pleurotus florida*, *Pleurotus sajor-caju* and *Trametes polyzona* were investigated for their ability to degrade textile dyes in the effluents. A cost effective enzyme mediated natural mycofiltration system was designed and developed incorporating spawns of *Pleurotus florida*, *Pleurotus sajor-caju* and *Trametes polyzona* along with different natural materials, and tested at laboratory level. The textile dyeing effluents were passed through this formulated natural mycofiltration system and periodical evaluation of decolourization and removals of pathogens were investigated. This mycofilter removed few types of bacteria available in the effluent and decolourized textile dyeing effluent.

#### **IV-O-5. Purification and characterization of an N-acetyl-D-glucosamine specific lectin from the Australian mushroom *Psathyrella asperospora***

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Australia has spectacular biodiversity including animal, plants and fungi. Australians have been using higher fungi traditionally as medicines and in religious practice for thousands of years. It has been estimated

that there is a large number of different mushroom species present in Australia that are poorly explored and catalogued. Importantly, very little is known about the extent and diversity of lectins from Australian mushroom species [1]. *Psathyrella asperospora* (Family: Psathyrellaceae) (synonym *Lacrymaria asperospora*) is an Australian indigenous mushroom from which we have isolated an N-acetyl-D-glucosamine (GlcNAc) specific lectin. De novo sequencing of *P. asperospora* lectin (PAL) using LC-MS/MS, identified 10 tryptic peptides that revealed substantial sequence similarity to the GlcNAc recognizing lectins from *Psathyrella velutina* lectin (PVL) and *Agrocybe aegerita* lectin (AAL-II) in both carbohydrate binding and calcium binding sites. Significantly, we also found that PAL has anti-proliferative effect on human colon cancer HT29 cells with an IC<sub>50</sub> of 0.48  $\mu$ M that represents one of the most potent mushroom lectin yet reported [2]. Further characterization of PAL's anti-proliferative activity using propidium iodide staining revealed that it induced cell cycle arrest at G2/M phase in a manner dependent on its ability to bind GlcNAc on the cell surface. Large scale purification of PAL has now been performed in order to fully characterize the carbohydrate binding specificity including its thermodynamic properties and structural determination using glycan array, isothermal calorimetry (ITC) and X-ray crystallography.

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#### **IV-O-6. Structural, antioxidant and functional properties of $\beta$ -glucan extracted from edible mushrooms *Agaricus bisporus*, *Pleurotus ostreatus* and *Coprinus atramentarius***

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$\beta$ -glucan fraction was extracted from three edible mushroom namely *Agaricus bisporus*, *Pleurotus ostreatus* and *Coprinus atramentarius*, using hot water extraction method and studies were carried out to investigate their structural, antioxidant and functional properties. Structural elucidation of the extracts was studied by FTIR (Fourier transform infrared spectroscopy) and NMR (Nuclear magnetic resonance) while antioxidant activities were determined using different assays viz., DPPH (2, 2-diphenyl-1-picryl-hydrazyl) radical scavenging, reducing power, metal chelating ability and ABTS (2, 2-Azino-bis, 3-ethylbenzothiazoline-6-sulfonoc acid). The FTIR and NMR studies elicited varied structural conformations of various  $\beta$ -glucans. The antioxidant activities varied significantly among all the sources of betaglucan, however the beta glucan from *C. atramentarius* showed highest values for DPPH (EC<sub>50</sub>=5.12 $\pm$ 0.205), reducing power (3.75 $\pm$ 0.195), metal chelating ability (2.89 $\pm$ 0.256) and ABTS (3.5 $\pm$ 0.503), whereas  $\beta$ -glucan from *Pleurotus* showed the strongest lipid peroxidation inhibition (EC<sub>50</sub> 4.15 $\pm$ 0.503) as compared to others. As far as the functional properties are concerned, *Coprinus*  $\beta$ -glucan also showed the highest swelling power, fat binding, foaming and emulsifying properties, however, the functional properties of  $\beta$ -glucan varied significantly depending upon its sources. It was concluded that the  $\beta$ -glucan from *C. atramentarius* showed better antioxidant and functional properties as compared to  $\beta$ -glucan from *Agaricus bisporus* and *Pleurotus ostreatus*.

#### **IV-O-7. Qualitative phytochemical screening, total phenolic content and antioxidant activity in methanolic extracts of *Morchella esculenta***

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Among wild edible species of mushrooms, morels rank first in choice and delicacy and have been one of the highly priced wild edible mushrooms in the world. The bioactive components present in *Morchella esculenta* are responsible for the nutraceutical potential of the mushroom. The present study was carried out to assess the phytochemical screening, total phenolics and antioxidant activities of methanolic extracts of *M. esculenta*. Qualitative phytochemical analysis showed the presence of tannins, saponins, flavonoids, terpenoids, phenolics, carbohydrates, as well as proteins and amino acids. The total phenolic content of methanolic extracts of morel was  $238.52 \pm 0.021$  (mg gallic acid equivalents per gram weight). The *in-vitro* antioxidant potential was analyzed by DPPH and Hydrogen peroxide method. The DPPH scavenging activity was  $85.2 \pm 0.371$  % and Peroxide was  $84.1 \pm 0.281$  % at 500 µg/ml concentration, comparable to that of ascorbic acid.

#### **IV-O-8. SEM study of exosporial ornamentation of basidiospores in genus *Russula*, *Lactarius* and *Lactifluus* from North-West Himalaya**

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Morphology of basidiospore surface in Basidiomycetes is used as an important taxonomic character. Different types of ornamentation exist in basidiospores of Russulaceous mushrooms. Every species has its unique ornamentation and is used as key characters to separate some closely aligned taxa in genus *Lactarius*, *Lactifluus* and *Russula*. To study the spore surface diversity Scanning Electron Microscopy (SEM) studies were conducted. For Scanning Electron Microscope examination, basidiospores were mounted on a double sided adhesive tape pasted on a metallic specimen-stub and after gold plating, the material was scanned at different magnification in high vacuum mode to observe pattern of spore ornamentation. SEM studies were carried out with JSM6610LV GEOL scanning electron microscope. During present investigation SEM studies have been conducted in 52 taxa of russulaceous mushrooms collected from North-West Himalaya spread over three investigated genera (*Lactarius*, *Lactifluus* and *Russula*). On the basis of SEM studies spores with eight varied ornamentation type on their surface were documented. These are: Tuberculate type (Warts completely isolated with no inter- connections); Catenulate type (Isolated warts with inter connections forming chain like rows or catenulations); Winged type A (Broad wings around the surface with isolated warts not forming reticulum (scattered or loose arrangement of wings) Winged type B (Winged all around the surface forming incomplete reticulum (compact arrangement of wings); Incomplete reticulate type (Wart to wart connections forming reticulum but isolated warts also exist in between); Complete reticulate type (No isolated warts present), Ridged type (When only 2-4 warts connected forming small to large ridges on the surface, no complete wing like structures formed. Isolated warts and catenulations) and Rugulose type (Very low warted spores).



#### IV-O-9. Lignocellulolytic enzymes of *Calocybe indica*

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*Calocybe indica* which is gaining popularity in the tropical parts of India and cultivated round the year while in subtropical parts it is preferred during summer seasons. The fruiting temperature requirement is from 28 °C to 35 °C. It is cultivated on wheat or paddy straw after chemical sterilization, hot water treatment or on pasteurized straw. The mycelial growth and fruiting takes place at higher temperature than the other cultivated mushroom like white button mushroom, shiitake mushroom or oyster mushroom. The lignocellulolytic enzymes responsible for growth on substrate and secretion of enzymes at higher temperatures of *C. indica* have not been thoroughly studied. So studies were undertaken to estimate influence of temperature on biomass production, changes in pH, total protein production, laccase activity, tyrosinase activity, aryl alcohol oxidase (AAO), manganese peroxidase (MnP), lignin peroxidase (LiP) and versatile peroxidase (VP) on wheat straw medium, Sabourauds medium and modified Czapek Dox medium (10 g/l glucose) after 6, 12, 18, 24 days. It was interesting to record that *C. indica* produced versatile peroxidase on all three culture medium and maximum quantity was recorded on wheat straw media after 12 days at 35 °C. Maximum protein was recorded on straw medium and least in Czapek Dox medium. Wheat straw medium produced maximum laccase after 24 days at 30 °C (187.38 µ/ml) followed by 35 °C and 25 °C. Maximum biomass was recorded on Sabourauds medium at 30 °C & 35 °C after 24 days followed by wheat straw and Czapek Dox medium. This is the first report of versatile peroxidase in *C. indica* in addition to *Pleurotus eryngii*, *P. pulmonarius*, *P. ostreatus*, *Bjerkandera adusta* and *Bjerkandera* sp. In all *Pleurotus* spp and *Bjerkandera* the production of VP is stimulated under peptone containing media. In the present case the wheat straw produced maximum VP followed by Sabourauds medium (peptone containing medium) and least in Czapek dox medium.

#### IV-O-10. Estimation of total phenol, flavonoid content and radical scavenging activity of a wild macrofungus, *Lenzites quercina*

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Extracts obtained from raw and fermented *Lenzites quercina* were assessed for total phenol, flavonoid content and antioxidant properties. Ethyl acetate extract from fermented *Lenzites quercina* (FEA) possessed higher phenolic content of 67.7±0.5 mg GAE/g while extract of ethyl acetate from raw *Lenzites quercina* (REA) have the highest flavonoid of 50.33±1.5 mg QE/g. Antioxidant properties measured by ferric reducing antioxidant power (FRAP) ranged from 9.67±0.6 to 62.33±3.2 mg AAE/g at concentration of 0.125 to 2.0 mg/ml. The scavenging properties of FEA were well pronounced against free radicals of nitric oxide, ferrous ion and exhibited better inhibition on lipid peroxidation at 93.93±0.9, 92.03±2.4 and 109.33±1.5, respectively. The study revealed that extract from fermented *Lenzites quercina* displayed better antioxidant property than unfermented *Lenzites quercina*. The phenol and flavonoid content found in *Lenzites quercina* suggest it has good antioxidant potential which could be extracted and used as alternative to synthetic antioxidants.

## Poster Presentations

### IV-P-1. Molecular and antibacterial profile of *Pleurotus sajor-caju*

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*Pleurotus* varieties cultivated in Mauritius include 3 strains: *Pleurotus sajor-caju* strain CC114, *Pleurotus sajor-caju* strain CC200 and *Pleurotus sajor-caju* strain CC201. In this study the chemical composition, antimicrobial properties and genetic variation of the three *Pleurotus* strains were explored. Chemical screening of crude extracts of the *Pleurotus* strains revealed the presence of terpenes, phenols, alkaloids, saponins and hydrolysable tannins. Flavonols were however identified only in *Pleurotus* strain CC200 extracts and leucoanthocyanins were detected only in the extracts of *Pleurotus* CC114 strain. Antimicrobial activity was tested against *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*. *Pleurotus sajor-caju* strain CC200 had more significant antimicrobial effect than *Pleurotus sajor-caju* strain CC114 and *Pleurotus sajor-caju* strain CC201 which both demonstrated nearly similar antimicrobial activity. Genomic DNA extraction was successfully carried out using the Phenol/Chloroform DNA extraction protocol and the DNA was purified using an RNase treatment. Genetic relatedness among the three strains of *Pleurotus sajor-caju* was assessed using the RAPD technique. Out of the 50 primers used, maximum polymorphism was observed using 8 Operon primers. Out of the 73 amplification products obtained with all three *Pleurotus* species, there was 28.8 % polymorphism which was observed. Maximum polymorphism was obtained following amplification using OPL 05. The fact that *Pleurotus sajor-caju* strain CC200 was least related to *Pleurotus sajor-caju* strain CC114 and *Pleurotus sajor-caju* strain CC201 could possibly explain the differences in the bioactivity of these mushrooms.

### IV-P-2. Analysis of N-glycan of the extracellular versatile peroxidase MnP2 secreted by *Pleurotus ostreatus*

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White-rot fungus *Pleurotus ostreatus* secretes an extracellular versatile peroxidase MnP2. To analyse structure and function relationship of the enzyme, we introduced amino acid substitution(s) using site-directed mutagenesis and overexpress the mutant enzymes in a homologous gene-expression system in *P. ostreatus*. Their catalytic properties for typical low-molecular-weight substrates such as Mn<sup>2+</sup>, H<sub>2</sub>O<sub>2</sub>, veratryl alcohol, as well as high-molecular-weight substrates, like RNaseA and Poly-R478, were investigated. An MnP2 variant R263N, which contains an Asn instead of Arg263, showed higher catalytic activity and wider pH stability compared to wild-type (wt) MnP2 enzyme. R263N showed elevated molecular mass in an SDS-PAGE analysis suggesting that it may possess an additional N-glycosylation at Asn263 located in a newly introduced NXS/T motif. It is possible that the newly introduced N-glycan may contribute to the functional improvement of the mutant enzyme. However, it remains unclear because there are only few studies on glycosylation of extracellular enzymes in basidiomycete fungi. To investigate structure of the carbohydrate moiety and its effect on the enzyme properties, we worked for the analysis of N-glycan composition in wt MnP2 and R263N enzymes. As the first step, we analyze carbohydrate modifications of wt MnP2 by MALDI-TOF-MS. In the amino-acid sequence of wt MnP2 protein, one consensus site for N-glycosylation (129-132 at amino acids) is predicted, and our previous study demonstrated that it has at least one N-glycosyl modification by the difference in SDS-PAGE analysis beforehand after digestion with



a N-glycosidase, glycopeptidase F. MnP2 enzyme expressed by a homologous expression system is purified and is subject to positive-ion MALDI-TOF-MS analysis after an in-gel digestion by trypsin. The MS spectra demonstrated that  $[M+H]^+$  3764.160 and 3926.031 were detected, suggested that a peptide fragment (amino-acids 128-152) generated by a trypsin digestion is conjugated with an N-glycan: Man5GlcNAc2 or Man6GlcNAc2: whereas predicted MS ( $[M+H]^+$  2546.026) for the intact peptide fragment did not appear. After treatment with glycopeptidase F, the peaks of  $[M+H]^+$  3764.160 and 3926.031 disappeared, whereas  $[M+H]^+$  2546.026 appear. These results strongly demonstrated that a high mannose type N-glycan, like Man5GlcNAc2 or Man6GlcNAc2, is attached at Asn129 of wt MnP2. Determination of N-glycan compositions in R263N and their effect for catalytic activity of the enzyme are under investigation.

### IV-P-3. Marine fish waste for better nutritional quality of mushroom (*Pleurotus florida*)

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Edible mushroom cultivation is a profitable cottage industry, in which oyster mushroom occupies a prominent place in India. A good substrate is a key factor that determines the profitability of the mushroom cultivation. The present study was aimed to analyze biochemical composition of mushroom *Pleurotus florida* and cultivation on different marine substrates along with paddy straw. The experimental work was designed with completely randomized design (CRD) with four treatments (375 g of paddy straw with 125 g of fish waste, shell waste, seaweed and seagrass amended separately) and a control (500 g of paddy straw) with three replications. Biochemical compositions of the cultured mushroom were analyzed by standard AOAC procedures. Results revealed that the rapid spawn running, primordial initiation and fruit body formations were observed in the fish waste substrate and it was slow in seaweed substrate. Total yield and biological efficiency were found maximum in fish waste substrate. The biochemical composition such as moisture, protein, carbohydrates, fiber, amino acids (arginine, methionine, aspartic acid, alanine, isoleucine, leucine, histidine and asparagine) and vitamins (ascorbic acid, riboflavin, niacin and cyanocobalamine) were found highest in the mushroom cultivated on fish waste as substrate and lowest in seaweed substrate. Results evidenced that fish waste amended with paddy straw was found to be best substrate for the cultivation of mushroom *P. florida*, which not only increased the mushroom yield but also enhanced the nutritional quality.

### IV-P-4. Comparative study of mineral composition of some wood rotting *Phellinus* mushrooms with wavelength–dispersive X-ray fluorescence spectrometry (WDXRF)

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Mushrooms are considered as nutritionally functional foods and important sources of physiologically beneficial medicines. Especially, *Phellinus* mushroom species (family: Hymenochaetaceae) are receiving special attention due to their potent pharmacological activities including immuno-stimulation, anti-tumor, anti-oxidant and anti-hepatotoxicity. Several mushrooms belonging to the genus *Phellinus* have been used as traditional medicines for the treatment of gastrointestinal cancer, cardiovascular disease, heart diseases, stomach ailments and diabetes. In recent years, the genus *Phellinus* is achieving much attention for its use in food and drug formulations. In the present investigation, the powdered basidiocarps of five different *Phellinus* species viz., *P. allardii*, *P. gilvus*, *P. fastuosus*, *P. extensus* and *P. conchatus* collected from different localities of district Dehradun (Uttarakhand, India) have been studied for their mineral constituents and various compositional properties. They showed variation in terms of their physical

characteristics (e.g. tapped density, bulk density, Hausner ratio, Carrs Index, oil absorption capacity, water absorption capacity, dispersibility, loss on drying and foreign matter) and proximate analysis (e.g. total ash value, acid insoluble ash, water soluble ash, alcohol soluble extractive, water soluble extractive, emulsion capacity and emulsion stability, total carbohydrates, total proteins and total phenols) and mineral composition. The mineral composition was determined by wavelength dispersive X-ray fluorescence (WDXRF) technique. The presence of various major and minor mineral nutrient elements have been detected. Pb and Hg were not detected as toxic elements in the present analysis technique. The present investigation revealed that in terms of both quality and quantity, the basidiocarps of these five *Phellinus* species are a good source of nutritional and mineral supplements to meet the dietary contents as well as to help the formulation of herbal drug preparations.

#### **IV-P-5. Estimation of cordycepin and determination of antioxidant properties in different species of *Cordyceps***

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*Cordyceps sinensis* that has emerged as an important medicinal mushroom globally is also gaining popularity in India now. This mushroom is known to occur naturally in India in few places like Chhipalakedar, Brahamkot, Ultapara, Ghwardhap, Chhipalakot and Najari areas in Dharchula region of Uttarakhand State. Recently its occurrence has also been reported from Arunchal Pradesh, Sikkim and locations along the Indo-Tibet and Indo- Nepal boarder namely Lashpa, Darti, Milam, Burfu, Mapa, Tola and Ralam in Johar Valley and Nagindhura, Golfa Bona and Chhipalakedar in basement of Panchachuli in Munsyari. *Cordyceps sinensis* grows naturally in the month of June and continues till late July.

Several *Cordyceps* species are regarded to have antitumour, antiaging, antidiabetic, immunomodulating, hypoglycemic, aphrodisiac and antimalarial activities (*Cordyceps sinensis* has been known and used for many centuries in Traditional Chinese Medicine TCM). *Cordyceps* is an important anti-aging medicine which inhibits the formation of active monoamine oxidase. It contains crude protein cordycepin an antibiotic (3'-deoxyadenosine) and d- mannitol. In China, this mushroom has been in traditional use for the treatment of chronic bronchitis, insomnis, anemia, night seats and cough. Major pharmacological actions by *Cordyceps* on hepatic, renal, cardiovascular and endocrine systems, anticancer, immunomodulation and hypoglycemic activity and effects on erythropoiesis and haematopoiesis. Antitumor polysaccharides such as galactomannan and  $\beta$ -(1-3)-D-glucan isolated from fruit body were shown to have hypoglycemic activity. Cultured hypha extract when orally administered produced anti-fatigue and improved motor functions. This mushroom is rich in polysaccharides having metastatic effect, proteins and nitrogenous compounds, sterols and 28 saturated and unsaturated fatty acids and their derivatives. Bio-medical application of antitumor polysaccharides galactomannan CI-P and CI-A and antitumor active  $\beta$ -(1-3)-D-glucan Co-1 have been isolated from different species of this mushroom. Current biomedical applications of *Cordyceps* spp. Include: anticancer, antitumor, immunopotential, vasoextension, antihypertension, arteriosclerosis, anti-allergy, anti-atopy, antibacterial, anti-hepatitis, hypoglycemic, asthma relaxation, fatigue recovery, brain illness prevention, robustness effect, stamina increasing, sedative, anticough, constipation and anti-baldness.

In present studies, detailed physiological studies, cordycepin content and anti-oxidant properties of two species of *Cordyceps* viz., *C. sinensis* and *C. bassiana* were carried out. Among the different media tested, maximum mycelia growth was recorded in Richards synthetic agar medium (71.2 mm) and maximum mycelia mass was recorded in Czapekdox broth (0.64 g) after 7 days of incubation. Among the different temperatures and pH regimes tested, best growth was recorded at 25 °C at 7 pH. Faster radial growth was recorded under dark conditions. Cordycepin content was determined by calorimetric method

using pure cordycepin (Sigma) at 280 nm. Cordycepin content was found to be higher at fruit body as compared to mycelium. In case of *C. sinensis*, it was 1.027 mg/g in mycelium and 2.048 mg/g at fruit body, respectively. In case of *C. bassiana*, it was 1.044 mg/g in mycelium and 11.78 mg/g in fruit body. The cordycepin content will be further verified by using HPLC. Studies on antioxidant activity were carried out in case of *C. sinensis*. Antioxidant activity was recorded to be 46.39% while reducing activity and scavenging effect on DPPH radical was 91.03 and 47.03%, respectively.

#### IV-P-6. Glutamate decarboxylase (GAD) of edible mushroom

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In recent years, there has been growing interest in GABA as a functional component of foods. GABA is widely distributed in nature, including mammals, plants and microorganisms. *In vivo*, GABA is reported to play several physiological roles such as neurotransmission, blood pressure regulation, acting as a diuretic and diabetes prevention. GABA is produced by decarboxylation of glutamic acid catalyzed by glutamate decarboxylase (GAD), which is a pyridoxal 5'-phosphate (PLP)-dependent enzyme. GAD is also observed in organisms ranging from microorganisms to mammals. Purification and characterization of GADs from the microorganisms *Escherichia coli*, *Neurospora crassa*, *Aspergillus oryzae* and *Lactobacillus* spp. have been reported. However, GAD purification and characterization from Basidiomycetes, including edible mushrooms, has not been reported, although a number of mushrooms contain glutamic acid and GABA in the fruiting body. If the relationship between the accumulation of GABA and GAD activity in mushrooms is clarified, it may be possible to improve the value of mushrooms as a commercial product by regulation of their GABA content and GAD activity. *Grifolia frondosa* GAD was purified 11.9-fold, with a yield of 1.24% by 4 steps of ammonium sulfate precipitation, hydrophobic chromatography (Butyl-Toyopearl 650C, twice), anion-exchange chromatography (DEAE-Toyopearl 650M) and gel filtration chromatography (Toyopearl HW65 and Sephacryl S-200 HR 16/60), and showed a single band on SDS-PAGE. The molecular mass of purified GAD was 42000 by SDS-PAGE and 97000 by HPLC-GPC. These results indicate that the enzyme probably consists of two identical subunits. Maximum activity was observed at 37 °C and pH 3.5. The enzyme was stable at 37 °C for 30 min and at values over pH 2-5.5. GAD of *G. frondosa* was specific for L-glutamate. The  $K_m$  and  $V_{max}$  of the enzyme were calculated to be 7.5 mM and 450  $\mu\text{mol min}^{-1}$ , respectively. The enzyme activity was strongly inhibited by  $\text{Hg}^{2+}$  and  $\text{Ag}^+$ . Although the N-terminal sequence of *G. frondosa* GAD was determined by a protein sequencer, no sequences showing significant homology were found. *Flammulina velutipes* GAD was slightly solubilized by Igepal CA-630, although strongly bound to cell walls. A nearly single protein band was observed in SDS-PAGE analysis after 9 treatments of solubilization and subsequent purification procedure with ammonium sulfate precipitation and ultrafiltration. Cell wall-binding enzyme experiments revealed GABA formation between pH 4 and 6, with the maximum GAD activity occurring at pH 6. However, GAD activity was lost after overnight dialysis against buffered at pH ranging from 6-10. The enzyme activity was optimum at 28 °C and stable below 50 °C. GAD of *F. velutipes* was specific for L-glutamate. The  $K_m$  and  $V_{max}$  of the enzyme were calculated to be 4.85 mM and 8.33  $\text{nmol min}^{-1}$  respectively. The enzyme activity was strongly inhibited by  $\text{Cu}^{2+}$ ,  $\text{Hg}^{2+}$  and  $\text{Ag}^+$ .

#### **IV-P-7. Growth of *Pleurotus ostreatus* on different concentrations of di (2-ethyl hexyl) phthalate in solid and in liquid media**

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Di (2-ethylhexyl) phthalate (DEHP) is one of the most widely used plasticizers, giving flexibility to the plastics. In addition, phthalates are important environmental contaminants and are difficult to degrade easily. Radial and specific growth rates, biomass, maximum biomass ( $X_{max}$ ), laccase and esterase activities, pH profiles and enzymatic kinetic parameters were evaluated in *Pleurotus ostreatus* grown in 0, 750, 1200 and 1500 mg of DEHP/l in solid and in liquid media. In liquid medium, the fermentation last 16 d and the highest  $X_{max}$  was observed in media containing 1500 and 1200 mg of DEHP/l. The esterase activity was much higher than the laccase activity at the beginning of the stationary phase (15 d) in medium containing 1000 mg of DEHP/l. In solid medium, the laccase activity was higher than the esterase activity in all the media containing DEHP. These results show that the production of esterase and laccase depends, at least in part, on the concentration of DEHP, culture system and time of fermentation. The rate of the enzymatic production was directly correlated to the amount of DEHP added to the media. Neither the culture system nor the culture medium showed carbon catabolite repression. These results suggest that the DEHP is used as carbon and energy sources by this fungus.

#### **IV-P-8. Laccase gene expression of *Pleurotus ostreatus* grown at different pH of the liquid culture medium**

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*Pleurotus ostreatus* is a white-rot fungus that produces laccase isoenzymes that have a potential use in bioremediation processes. It has been suggested that the number and type of laccase isoforms depends on the conditions of development of the fungus. In this study we evaluated the effect of initial pH of the culture medium on the expression of five laccase genes (Lacc1, Lacc4, Lacc6, Lacc9 and Lacc10) of *P.ostreatus* grown in submerged fermentation (SmF). *P. ostreatus* was developed in 125 ml flasks with 50 ml culture medium with glucose, yeast extract and mineral salts, the pH was adjusted to 3.5, 4.5, 6.5 and 8.5 with 0.1 M NaOH or HCl. Each flask was inoculated with 3 mycelium fragments of 4 mm of diameter obtained from the periphery of a colony grown on malt extract agar. The flasks were incubated at 25 °C for 23 days in orbital agitation at 120 rpm and sampled at 144, 168, 264, 312, 408, 504 and 528 h. Gene expression was observed by RT-PCR. *P. ostreatus* was developed at different pH.  $X_{max}$  (g/l) and ( $\mu h^{-1}$ ) values were 4.4, 5.2, 9.5, 7.9 and 0.006, 0.014, 0.018, 0.020, respectively at pH values of 3.5, 4.5, 6.5, 8.5. In PCR products of the five laccase genes expressed by *P. ostreatus* grown in SmF at different initial pH of the culture media, different level and intermittency of expression were observed. The initial pH of the culture mediums an important factor which regulates the expression of the laccase genes in addition to having an effect on the activity and number of isoenzymes produced. These results contribute with the understanding of the regulation of the expression of the laccase genes.



#### IV-P-9. Laccases and manganese peroxidases of *Pleurotus ostreatus* grown in solid-state fermentation

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White rot-fungi are capable of degrading some xenobiotics compounds and different kinds of environmental pollutant due to its ligninolytic enzymatic system. Ligninolytic enzymes have potential applications in a large number of fields, including the chemical, fuel, food, agricultural, paper, textile, cosmetic industrial sectors and more. Species of *Pleurotus* genus, including *Pleurotus eryngii*, *P. sapidus*, *P. pulmonarius* and *P. ostreatus* produce ligninolytic enzymes such as laccases, manganese peroxidases (MnP), veratryl alcohol oxidase (VAO) and versatile peroxidases (VP), in both, submerged (SF) or solid-state fermentation (SSF). In this work, laccase and MnP activities of *P. ostreatus* grown in SSF using polyurethane foam (PUF) as inert support were evaluated. The SSF was carried out in a flask of 250 ml containing 0.5 g of PUF of low density (PUF; 17 kg/m<sup>3</sup>) cubes (0.5×0.5×0.5 cm) as an inert support impregnated with 15 ml of sterile culture medium (pH 6.5) with glucose, yeast extract and mineral salts. Three mycelia plugs (4 mm diameter) taken from the periphery of colonies of *P. ostreatus* grown for 7 d at 25 °C in Petri dishes containing potato dextrose agar were used as inoculum for each flask. The cubes were washed twice with hot distilled water, oven-dried at 60 °C for 24 h, and then autoclaved at 120 °C for 15 min, before the culture. All inoculated flasks were incubated at 25 °C and samples were taken every 24 h after third day of growth. The enzymatic extract (EE) was obtained by soft pressing the PUF cubes and the broth was filtrated using filter paper (Whatman No. 4). Laccase activity was determined in each EE by changes in the absorbance at 468 nm using 2, 6-dimethoxyphenol (DMP) as substrate ( $A_{468} = 35645 \text{ M}^{-1} \text{cm}^{-1}$ ). MnP was determined by phenol red oxidation at 610 nm with extinction coefficient  $\epsilon_{610} = 4460 \text{ M}^{-1} \text{cm}^{-1}$ . The activity was expressed in U/L of EE. The maximum laccase activity was observed at 144-168 h of fermentation with a value of about 400 U/L, after that time, the laccase activity was constant of about 100 U/L. On the other hand, the MnP activity was approximately 3 U/L from the beginning of the fermentation until 456 h, peaking at 500 h with 5 U/L. The strain of *P. ostreatus* used in this study showed that it can produce ligninolytic enzymes by solid fermentation on an inert support, so it is suggested further studies to establish the optimal conditions for increased enzyme production.

#### IV-P-10. Monitoring lignocellulose degrading enzyme activities in a large scale oyster mushroom production facility

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Oyster mushroom (*Pleurotus ostreatus*) is a saprotrophic white rot fungi and it is the second most popular cultivated edible mushroom all over the world. In Europe it is grown on composted and pasteurized wheat straw (substrate), but it can be cultivated on a wide range of other lignocellulose due to its versatile enzymatic profile. The cultivation of oyster mushroom has two main parts. First, the oyster mushroom colonizes the substrate blocks in production house at 24-26 °C and relative humidity (RH) of 75-80% in dark. Second, during the fruiting body development the temperature is lowered to 12-14°C and RH is increased to 85-90% with high illumination. The aim of the present study was to monitor the lignocellulose

degrading enzyme activities during colonization and fruiting body development of oyster mushroom in a large scale production facility. During the whole oyster mushroom production period (10 weeks) every week three-five samples (colonized substrate, fruiting body) were collected from five different substrate blocks. Lignocellulose decomposing enzymes were extracted from the samples with phosphate buffer (pH 7, 50 mM), enzyme activities (endoglucanase, cellobiohydrolase, beta-glucosidase, endo-1, 4- $\beta$ -xylanase, 1,4- $\beta$ -xylosidase, laccase, manganese peroxidase and N-acetyl- $\beta$ -glucosaminidase) and reducing sugar content were determined. Lignin decomposition enzymes showed the highest activity during the first part of oyster mushroom colonization. Activity of laccase was the highest in the first week of production whereas the activity of manganese peroxidase reached its peak in the third week. Endoglucanase, beta-glucosidase, endo-1,4- $\beta$ -xylanase, N-acetyl- $\beta$ -glucosaminidase showed highest activity in the fourth week, which coincided with the development of fruiting bodies. Changes in cellobiohydrolase and 1,4- $\beta$ -xylosidase activity did not have any trend. The lignocellulose degrading enzyme activities of three blocks were similar during the first seven weeks. Nevertheless two blocks had a week delay in enzyme activity and fruiting body formation, as well.

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#### **IV-P-11. Mycelial growth of pink oyster (*Pleurotus djamor*) mushroom in different culture media & environmental factors**

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The study was undertaken to determine the optimum culture media and different other factors viz., optimum culture media composition, pH and temperature for a well known mushroom- pink oyster (*Pleurotus djamor*). Four different types of culture media as-potato dextrose agar (PDA), malt extract agar (MEA), potato yeast dextrose agar (PYDA) and yeast extract agar (YEA) was prepared as different media for pure culture growth. Calculation was conducted on average mycelium growth & duration of complete mycelium growth. The highest mycelia growth rate (0.24 cm) was observed for potato dextrose agar media and the lowest mycelia growth (0.11 cm) was observed in yeast media. The highest mycelia growth rate (0.41 cm) was observed at 25 °C and the lowest mycelia growth (0.20 cm) was observed at 30 °C temperature. Among the nutrient media compositions the fastest mycelium growth rate was observed in PDA media at ratio of 15:150 (Dextrose: Potato) and minimum mycelia growth rate was observed in PDA media at ratio of 25:250 (Dextrose: Potato). The highest mycelial growth rate (0.45 cm) was observed in the pH 6.5 and the lowest (0.11 cm) was observed in the pH 4.5 which was statistically similar with pH 9.0.

#### **IV-P-12. Physiological studies of the cultivable natural *Agaricus* flora in Kerala**

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Production of mushrooms, especially of the white button mushroom, in India has gone up in the last few years. The present production of white button mushroom is about 85% of the total production of mushrooms in the country. Even though Kerala with its congenial conditions is found to be the abode of a vast variety of mushrooms. However, only preliminary efforts have been made to record the highly priced mushroom of genus *Agaricus* of the state. The white button mushroom *Agaricus bisporus* cannot be grown in Kerala

due to its low temperature requirement. But there is immense scope for the exploitation of the natural flora. In this context a deliberate effort for the detailed study of natural agarics flora in the State from the different agroclimatic zones; isolation into pure cultures of collected promising species, studying the physiological aspects like role of carbon, nitrogen, temperature, light and pH in the growth of the fungus was made. Forty two species were collected from different agroclimatic zones of the state. Considering the morphological characters like size, shape, colour, smell, edibility and the abundance of occurrence *A. caroli*, *A. ochraceous*, *A. ochroflavus*, *A. stadii* and *A. squamuliferus* are recommended as potential species for cultivation under Kerala conditions. Growth of *Agaricus* species was highest on complete medium and the tropical species collected preferred a temperature range from 25-30 °C. *Agaricus* spp. were grown in different pH viz., 4,5,6,7 and 8, and observed that the mycelial growth increased up to pH 6 and then growth decreased. The results of the studies revealed that light did not play any significant role on the mycelial growth of the fungi. Among the different carbon sources viz., fructose, glucose, mannose, sucrose and xylose; *Agaricus* spp. preferred glucose, sucrose and fructose. Growth of *Agaricus* spp. on different inorganic nitrogen sources viz., ammonium nitrate, ammonium chloride and sodium nitrate, and organic nitrogen sources viz., peptone were studied. All species of *Agaricus* studied, showed maximum growth on media with peptone. From the study it can be concluded that there are many species of *Agaricus* that have the potential for cultivation under the normal climatic conditions prevailing in the state, but future studies are required for the screening of suitable species for Kerala and for the standardization of its cultivation techniques.

#### IV-P-13. Production of carboxymethyl cellulase from *Trichoderma* sp on solid state fermentation

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Cellulosic substrates are represented mainly by wood but also by recyclable waste products such as bran, wheat straw, peanut shell, saw dust and olive cake. Adding value to agricultural and industrial by-products by fermentation, such as cellulase production, is an attractive biotechnological option. Fungi are the primary source of the enzymes required to convert plant biomass into sugars, bio ethanol and other fermentation products and for improving the digestibility of feed. In this context, a local strain, *Trichoderma* sp, isolated from the Yakouren forest litter (Tizi-Ouzou-Algeria) was studied. The revelation of cellulases, conducted by the reactive iodo-iodized (iodine solution), showed a clear zone of cellulose hydrolysis of 6.5 cm diameter after 48 h at 30 °C. The cellulases biosynthesis from *Trichoderma* sp in wheat bran with solid state fermentation, showed CMCase activity of 1.64 U/ml and total proteins concentration of 2.39 mg/ml during 5 days of culture at 30 °C. The determination of optimal conditions of the enzymatic extract indicates a pH 5 and a temperature of 50 °C. Otherwise, the extract was characterized with an activity relatively stable between 30 °C and 50 °C during 10 min. However, storage at 4 °C for 2 weeks allows better activity.

#### IV-P-14. Role of enzymes in development of *Ganoderma lucidum*

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*Ganoderma lucidum*, specie belonging to the class basidiomycetes, family Polyporaceae of the order Aphyllophorales has been widely used as a source of potent nutraceutical products. Present study was planned to identify and characterize the role of extracellular (cellulases, xylanases and laccases) and



intracellular (peroxidases and esterases) enzymes in the developmental process of *G. lucidum*. Four strains of *G. lucidum* were grown in mushroom minimal medium (MMM) broth supplemented with wheat straw powder (@ 0.5/flask) showing a gradual increase in biomass to give 25.52 g to 31.72 g and on wheat straw supplemented with 5% wheat bran with maximum biological efficiency for GL-I strain (31.27%) followed by GL-II (26.76%). In all strains the enzyme activity was enhanced during spawn run on solid substrate in comparison to that grown in the broth. From the cellulose complex, endoglucanase specific activity ranged between 0.034 to 0.049  $\mu\text{g mg}^{-1} \text{min}^{-1}$  in broth with maximum for strain GL-IV (0.049  $\mu\text{g mg}^{-1} \text{min}^{-1}$ ), spawn run indicated endoglucanases in range of 0.033 to 0.048  $\mu\text{g mg}^{-1} \text{min}^{-1}$  with maximum in GL-I (0.048  $\mu\text{g mg}^{-1} \text{min}^{-1}$ ) followed by GL-II (0.045  $\mu\text{g mg}^{-1} \text{min}^{-1}$ ). The cellobiohydrolase activity in broth ranged from 0.014 to 0.016  $\mu\text{g mg}^{-1} \text{min}^{-1}$  while from spawn run it ranged between 0.011 to 0.043  $\mu\text{g mg}^{-1} \text{min}^{-1}$ . Similarly  $\beta$ -glucosidase in broth was in range of 0.008 to 0.089  $\mu\text{g mg}^{-1} \text{min}^{-1}$  while during spawn run it was 0.054 to 0.149  $\mu\text{g mg}^{-1} \text{min}^{-1}$ . Xylanase specific activity in broth culture was estimated in the range of 0.031 to 0.134  $\mu\text{g mg}^{-1} \text{min}^{-1}$  with maximum in GL-IV while during spawn run xylanase activity was between 0.128 to 0.405  $\mu\text{g mg}^{-1} \text{min}^{-1}$  again maximum with GL-IV. The laccase activity in broth ranged between 0.188 to 0.817  $\mu\text{g mg}^{-1} \text{min}^{-1}$  with maximum in GL-III while in spawn run it was maximum in GL-II (0.885 U  $\text{mg}^{-1}$ ), while it was not detectable in GL-IV. Peroxidases activity estimation from the broth culture and spawn run revealed that it was maximum in strain GL-II (0.064 and 0.096 U  $\text{mg}^{-1}$  respectively). A sharp decline in peroxidase activity was observed from the samples when peroxidase was estimated from fruit body. Esterase specific activity in broth was maximum for strain GL-IV (0.037  $\mu\text{g mg}^{-1} \text{min}^{-1}$ ) whereas it was maximum in GL-I (0.042  $\mu\text{g mg}^{-1} \text{min}^{-1}$ ) during spawn run. Unlike peroxidases, esterase activity increased at time of pin head formation but declined when esterase was estimated from fruit bodies. Thus, intracellular and extracellular enzymatic studies have indicated enhanced activity during spawn run on solid substrate in comparison to that grown in the broth. The esterase and peroxidase activity significantly increased during the pinning of the cultures thus, indicating a positive role of these enzymes in fructification process.

#### IV-P-15. Textile dye decolorization using laccase isolated from white rot Basidiomycete *Ganoderma lucidum*

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Oriental white rot Basidiomycete *Ganoderma lucidum* was collected from different agro-climatic zones of Haryana state in the year 2012-13 and cultured under *in vitro* conditions. Laccase production was carried out under submerged fermentation conditions in a 1 L fermenter from 9 different samples. The enzyme was isolated, precipitated, dialysed and partially purified using sephadex column. Specific activity of enzyme increased from 0.44 to 1.35 during subsequent steps of purification. The enzyme was then immobilised on polyvinyl alcohol membrane with 65.70% retention and showed higher  $V_{\text{max}}$  value (0.617 mM/min) with higher affinity for guaiacol (3.82 mM). The immobilized enzyme was used for decolourization of locally used textile dyes 'Reactive Blue 158' and 'Reactive Red 141'. The dyes were decolorized bio catalytically, where decolourization was correlated with laccase activity. The highest capacity for decolourization was showed by enzyme isolated and immobilized from samples of *G. lucidum* collected from *Delbergia sissoo*. The system seems to be suitable for use as bioreactor for effluent treatment from carpet industries as thermal stability of immobilised enzyme was also higher (35-75 °C) as compared to free enzyme (30-45 °C).

#### IV-P-16. Cultural practices and supplementation of cotton seed hulls for growing king oyster mushroom strains

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King oyster mushroom (*Pleurotus eryngii*) is one of the most popular and prized mushroom in the world due to its taste and flavour. The basidiocarps of *P. eryngii* are greyish colored large sized (6-15 cm) with enrolled cap margin. There is one another large sized popular *Pleurotus* species found in north western Indian Himalayas at an altitude of 3300 (m.a.s.l.) in the cold desert parts of Himachal Pradesh, Uttarakhand and Afghanistan which is *P. fossulatus*. The pileus of this species is creamish white, 6 to 20 cm in size with slightly eccentric stipe. The fruiting bodies are collected and sold in the Indian market as Kabul Dhingri or as king oyster. Due to its liking by the traders we have attempted to artificially cultivate on various substrates. Among different methods of substrate preparation autoclaving and steam pasteurization are the suitable methods. Chemical treatment with bavistin, hot water and pasteurized *Agaricus* compost did not support fruiting. Casing soil has no beneficial role for getting higher yield. However supplementation of cotton seed hulls has given more than 50% better yield than unsupplemented yield in seven different strains of *Pleurotus eryngii* and *P. fossulatus* while cotton linter waste was not a better supplement.

#### IV-P-17. Identification of ergosterol in mushrooms

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Mushroom has gained popularity as prized table delicacy with high nutritive and medicinal value. Ergosterol is a biological precursor. The contents of vitamin D2 and sterols in some wild and cultivated mushrooms were determined. Vitamin D2 was determined using an HPLC method, including saponification and semipreparative normal-phase HPLC purification. Currently HPLC methods are regularly practiced for the estimation/determination of ergosterol. Four mushrooms *Calocybe indica*, *Ganoderma lucidum*, *Pleurotus florida* and *Volvariella volvacea* were grown on two synthetic (complete yeast extract agar and Lambert's agar) media and three semi-synthetic (malt extract agar, rice bran decoction and wheat extract agar) media. The mycelial biomass of each mushroom was subjected to extraction of ergosterol and its identification using high performance liquid chromatography (HPLC). The ergosterol content ranged from 113 to 403 µg/g with lowest retention peak was observed in *Pleurotus florida* showing 113 µg ergosterol per gram whereas *Calocybe indica* showed 243 µg ergosterol per gram and *Volvariella volvacea* shows 159 µg ergosterol per gram and highest retention peak was observed in *Ganoderma lucidum* showing 403 µg ergosterol per gram of sample.

#### IV-P-18. Analysis of differentially expressed proteins in *Agaricus bisporus* fruit bodies at different post-harvest stages by using the technique of iTRAQ-MS/MS

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In order to identify the protein expression changes that occur during the post-harvest maturation of *Agaricus bisporus* fruit bodies, the technique of iTRAQ-MS/MS (i.e. isobaric tags for relative and absolute quantification

coupled two-dimensional liquid chromatography tandem mass spectrometry) was used to analyse the proteome of *A. bisporus* strain As-2796 fruit bodies at 0 h, 6 h, 12 h and 48 h after harvesting. As a result, a total of 1063 proteins and 5878 unique peptides, were identified. Among them, 1012 proteins provided quantitative information. Compared with the proteins at 0h post harvest stage, samples collected at 6h, 12h and 48h after harvesting produced 102, 106, and 160 different proteins, respectively, accounting for 10.1%, 10.5% and 15.8% of the proteins identified. The differentially expressed proteins among different post-harvest stages, as well as all of the identified proteins, were analysed via bioinformatics. Furthermore, the related genes of 8 proteins, either up or down expressed continuously at different post-harvest stages, were also analysed by real-time PCR, and 3 of them mismatched base pair. Cruciform DNA recognition protein, hydrophobin-B and protein transporter were confirmed to have a similar expression trend as the corresponding proteins.

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#### **IV-P-19. Biosorption of cadmium: a comparative assessment of fruit and spent substrate of oyster mushroom (*Pleurotus florida*) in the powder form**

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Heavy metals are a serious threat to the environment. These metals cannot be destroyed or degraded but have a tendency towards bioaccumulation and hence enter the food chains. A number of conventional techniques like adsorption, ion exchange, reverse osmosis and electro-dialysis have been developed for their removal which have their disadvantages. Hence, biosorption, which involves heavy metal uptake by live or dead microorganisms, has emerged as an effective technology. The most important benefits of biosorption technique over usual treatment methods include its low cost, high efficiency, minimization of sludge, re-use of biosorbent and metal recovery. The macrofungi (mushrooms) can be used as biosorbents for adsorbing heavy metals from industrial effluents. *Pleurotus* species (oyster mushroom) is a type of gilled mushrooms which grows normally on wood. The mushroom has the ability to sequester heavy metals in dead as well as live states. The spent mushroom substrate (SMS), the post-harvest waste product, can also be utilized for the biosorption of heavy metals. The present work represents the assessment of biosorption potential for cadmium from the aqueous solution onto the biomass (powder form) made from the first flush fruiting bodies and SMS of *Pleurotus florida*. Sorption experiments have been conducted to determine the values of the variables that provide the greatest biosorption efficiency.

# **Session-V**

## **Waste Conversion & Utilization, Substrates, Casing and Crop Management**



## Keynote Presentations

### V-K-1. Biochemical features influencing mushroom-substrate compatibility

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The capacity of mushroom fungi to utilise the wide range of lignocellulosic substrates adopted for cultivation depends in large part on the production of hydrolytic and oxidative enzymes (cellulases, hemicellulases and ligninases) that degrade the major component macromolecules (cellulose, hemicellulose, lignin). The proportion of these macromolecules in different lignocellulosic materials, and the ability of a given mushroom species to degrade them, can vary significantly. In particular, although the cellulose and hemicellulose components are readily solubilised by the fungal hyphae to provide nutrients for growth, considerable variation exists in the ability of a given species to degrade the more complex and recalcitrant lignin element. This, in turn, is dependent on the different combinations of ligninases (e.g. lignin peroxidases, manganese peroxidases, laccases) produced by the fungus. Thus, while *Volvariella volvacea* is a prolific producer of key cellulolytic enzymes (endoglucanases, cellobiohydrolases,  $\beta$ -glucosidases), among the recognized ligninolytic enzymes it appears able to synthesize only laccases. This is reflected in the ability of the straw mushroom to grow well on cotton wastes consisting largely of cellulose but not on more “woody” materials such as sawdust. Conversely, mushroom species able to synthesize ligninolytic peroxidases (*Lentinula edodes*, *Pleurotus* spp.) can grow on a wider spectrum of lignocellulosic wastes. Also linked to mushroom-substrate compatibility is the enzymic capacity of the former to detoxify harmful compounds (e.g. phenolics) often present in the latter. Using specific examples, this presentation will detail key biochemical features involved in lignocellulose degradation and in the neutralisation of toxic constituents, and will correlate these features to mushroom-substrate compatibility.

### V-K-2. Production of mushroom spawn in a clean room- environment: problems and challenges

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The aim of spawn producers is to deliver axenic mycelium with a constant quality, which is a prerequisite for a good mushroom yield. Very often, mycelium producers have a poor knowledge as how to estimate the microbiological quality of their working environment. This leads to the building and organization of production labs with an insufficient level of security. A lot of problems in mycelium production can be brought back to an incorrect product flow, as well as to wrong rules of conduct. The aim of this talk is to analyse a number of problems, while offering a remedy for the latter, so as to prevent financial disasters.

### V-K-3. Biotechnological potential of ten *Pleurotus djamor* strains

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Ten strains of *Pleurotus djamor* were characterised and studied for their mycelial growth, sporophore production, laccase (on ABTS), Mn-peroxidase (on dimetoxiphenol), aryl alcohol oxidase (on veratryl alcohol)

and phenol oxidase activities (on catechol). Also the antioxidant capacity was determined. The radial extension rate varied between 1.12 mm/d (*P. djamor* ECS-0150) and 3.9 mm/d (*P. djamor* ECS-0143). Biological efficiency, production rate, performance in two harvests varied between 36.4 and 71.4%, 1.14 and 2.1% and 0.033 and 0.069, respectively. All the strains tested have at a certain degree, laccase, Mn-Peroxidase and phenol oxidase, except for aryl alcohol oxidase. The strain ECS-0123 showed important ligninolytic activity, good antioxidant capacity, good growth and an average capacity to produce sporocarps (mushrooms).

#### **V-K-4. *Lentinula edodes* cultivation technique and models in China**

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*Lentinula edodes* (Xianggu) is a major cultivated mushroom in China, with the widest production area, highest total output and best economic returns. The mushroom was originally cultivated in China around 1100 AD using the 'hatchet-notching' method. Later, in the 1960s, the cultivation technique involved inoculating pure culture spawn into natural wood logs. During the following decade, the Institute of Edible Fungi, Shanghai Academy of Agricultural Science, developed the sawdust-based cultivation method whereby it became possible to cultivate the mushroom under natural environmental conditions using a small-scale, family-oriented production model. After popularization of this technique, *L. edodes* cultivation areas expanded rapidly to all provinces in China southwards from the Yangtze River Basin. Since 1989, China has been the world's largest producer and exporter of *L. edodes*, and total output in 2012 reached 6.35 million tons.

However, with China's fast economic growth in recent years, problems have arisen with the family-oriented *L. edodes* cultivation model. It is a heavily labour-intensive production process with a low level of mechanization, year-round production cannot be sustained due to the seasonal production cycle, product quality is often unstable, and there is a limited ability on the part of growers to resist fluctuations in market prices. Currently, an intensive cultivation model has been adopted in China's main *L. edodes* production areas which, compared with the traditional model, ensures the safety and security of the raw materials used for cultivation, and lends itself to increased mechanization. Standardized spawn production, inoculation procedures and management practices have helped to ensure higher yields, and integrated *L. edodes* product collection and distribution has served to reduce the risks associated with market price fluctuations. However, the intensive *L. edodes* cultivation model requires more careful supervision and stronger technical support, especially in the control of spawn quality. Furthermore, the intensive *L. edodes* cultivation model is still limited to seasonal production. Therefore, in order to ensure an all-year-round supply of fresh *L. edodes*, an industrialized cultivation model has emerged, and the development of a technological process with Chinese characteristics is in progress.



## Oral Presentations

### V-O-1. Biological efficiency of wild type and commercial *Agaricus bisporus* strains

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In the cultivation of the button mushroom (*Agaricus bisporus*), the cost of substrate production represents a large part of the total production cost. The quality of the substrate (compost) determines the yield of mushrooms, their quality and sensitivity to pathogens. In Europe, the button mushrooms use only 25% of the organic matter in the compost in two flushes. Increasing the efficiency with which the substrate is converted into mushrooms will therefore have a considerable impact on the economics of production. It will also impact the environmental burden of the crop; a higher biological efficiency would result in less transport of compost and also in a smaller quantity of spent compost. Currently, mushroom strains are only able to use a relatively small portion of the organic matter present in compost. Cellulose and hemicellulose represent the major source of carbon and constitute only 32% of the dry matter in the compost. Basically, compost consists of a large part of an inert carrier material which provides a number of important physical characteristics such as water-holding capacity and structure allowing gaseous exchange. Development of an improved substrate/compost for cultivation of *Agaricus bisporus* has proven to be quite difficult. On the other hand, most white button mushroom strains used worldwide today are closely related to the first hybrid strains developed in the Netherlands over 30 years ago. Wild strains may provide the possibilities to improve the biological efficiency with which compost can be converted into mushrooms. The department of Plant Breeding at Wageningen UR has a large collection of mushroom strains, many of which are derived from the ARP collections. After analysis of the genetic relationships between strains in our collection, a selection was made representing the major genetic groups within the collection. These selected strains were studied for the biological efficiency (conversion of compost into mushrooms in two flushes)). Our main results are: The collection of mushroom strains at Wageningen UR (comprising mainly strains from ARP collection and old commercial strains) represent a large genetic variation and the genetic relations among the strains have been well studied. The strains tested show a large variation in biological efficiency and the present commercial strains are among the ones with the highest biological efficiency. There was a clear correlation between the dry weight of mushrooms produced and the drop in pH in the compost. Loss in dry matter in compost as a result of mushroom production can be attributed for 95% to loss in hemicellulose and cellulose. Total amounts of lignin and undefined organic matter do not decrease much during the cultivation of mushrooms. There is a clear correlation between mushroom production and consumption of hemicellulose from the substrate. This correlation is less clear for cellulose.

### V-O-2. Vegetative growth of different strains of *Pleurotus* and *Lentinula* species on cassava (*Manihot esculenta*) and yam (*Dioscorea rotundata*) wastes in Ghana

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Mushrooms are fast becoming important components of diets worldwide and it is necessary to find out appropriate substrates on which they can be grown. Eight strains of mushrooms: *Pleurotus ostreatus* strain MES11797, 03416, 03772, 03364, 03216 and *Lentinula edodes* strain MES 02008, 02052, 12060

were cultivated on substrate formulated from cassava and yam wastes such as Cassava peels, Cassava sticks and Yam peels. Sawdust of *Triplochiton scleroxylon* which has been the traditional substrate for the cultivation of *Pleurotus* spp. in Ghana was used as the control substrate. Cassava peels agar supported the best growth for four *Pleurotus* spp. (MES 11797, MES 03772, MES 03216 and MES 03416) and one *Lentinula* strain (MES 12060). Yam peels however support the best growth for *Pleurotus* strain MES 03364. Cassava sticks supported the best growth for *Lentinula* strain MES 02008. Sawdust supported the best growth for *Lentinula* strain MES 02052.

### **V-O-3. Cultivation of oyster mushrooms on cassava waste**

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Cassava is a major food crop for approximately 700 million people, especially in African countries. A large quantity of waste is produced after processing of cassava mainly consisting of tuber peels. Although previous research has shown that these peels can be an ingredient as substrate to cultivate mushrooms, yields were usually inferior compared to traditional substrates such as saw dust. In a project funded by the European Union Commission Community (<http://www.fp7-gratitude.eu/>), trials were done for the production of oyster mushroom using fermented peels and stems from cassava crop produced in Ghana. Four strains representing two species (*Pleurotus ostreatus* and *P. pulmonarius*) were grown on substrates made from cassava waste. Peels and cassava stems were tested in different ratio's and supplemented with different amounts of rice or wheat bran. All substrates were colonized quickly and time to pinning varied between 18 and 24 days. The *P. pulmonarius* strains gave three flushes within 47 days (starting from inoculation) and the *P. ostreatus* strains needed 57-63 days completing 3 flushes. Biological efficiencies after 3 flushes varied between 38% and 102%. The effect of bran supplementation on yields depended on the concentration (0, 1, 3 and 5% w/w), type of bran (rice or wheat) and strain used. The trials have shown that cassava waste (stems and peels) can be used well for the production of oyster mushroom and that substrate containing up to 75% cassava peels have productions well comparable to yields obtained on the traditional saw dust substrates.

### **V-O-4. Exploitation of thermophilic fungi in compost production for white button mushroom (*Agaricus bisporus*) cultivation- A review**

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Compost production is the most important and integral part of button mushroom cultivation and its quality parameters mainly determine success or failure of a crop. It is a product of fermentation brought about by the activities of thermophilic organisms and among them fungi especially, *Scytalidium thermophilum*, *Humicola insolens* and *Humicola grisea* play a decisive role in bringing about the selectivity and productivity of the compost. In a pursuit to improve the present day composting procedures and also to prepare compost in most environment friendly manner in shortest possible time, series of experiments were conducted at Directorate of Mushroom Research, Solan, India. Role of thermophilic fungi and especially that of *Humicola insolens* was thoroughly investigated. Based on many physiological, enzymatic and under in vitro studies, I-1 and X-21 strains of *H. insolens* and *S. thermophilum*, respectively were short listed as potent strains for their further exploitation in compost production. Artificial inoculation of these fungi on day zero under long method of composting (LMC) not only brought the composting period to 20 days but *S. thermophilum* significantly increased the button mushroom yield compared to check. Inoculation of these fungi under short method of composting was not successful in improving its productivity; however,

composting period (phase-II) could be brought from seven to five days. These two fungi and their combinations were further utilized in improving the environment friendly indoor composting (Anglo-Dutch method). High conversion of compounding mixture to compost was found in inoculated treatments with increased yield over control. Total composting operation lasted for 13 days. Highest degradation in cellulose, hemicellulose and carbon was observed in inoculated pile, which contributed in good spawn run and highest fruit body yield. Another study was conducted on INRA method (hot process) using above fungi as inoculants. Highest count of inoculants along with other thermophilic fungi was observed in consortium treatment, which led to less emission of ammonia and more conversion of compounding mixture to compost in shortest possible time. This resulted in excellent spawn run and highest fruit body yield. In control pile least count of *S. thermophilum* and *H. insolens* was observed resulting in increased concentration of ammonia, which led to high incidence of *Coprinus* sp. and delayed spawn run. Inoculation of substrate in both kinds of composts was successful and compost prepared in 13 days time gave more compost output and satisfactory yields.

#### **V-O-5. Metagenomic profile of the bacterial community changes that occur in the casing layer during cropping of the white button mushroom, *Agaricus bisporus***

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Decreases in yield occur after each subsequent flush, or break, of the commercially produced white button mushroom, *Agaricus bisporus*. The exact cause behind the reduced yield is unknown, though there are several theories. One theory is that chemical by-products (eg. salt crystals) form on the mycelium thereby reducing nutrient uptake and subsequent fruiting. A second theory is that the nutrition of the substrate is reduced throughout the cropping process thereby limiting yield potential and subsequently reducing the yield of each break. Another explanation for the reduced yield may be related to the diverse microbial communities present in either the casing layer or the compost substrate itself. It is believed that certain microbial communities are needed and are responsible for promoting primordial formation and maturity. This study was an investigation into the differences that occurred in the major bacterial groups present in the casing layer during cropping. The casing layer consisted of a sphagnum peat moss buffered with calcium carbonate that was added to the compost after a 17 day spawn run. Samples of the casing layer from *A. bisporus* crop were collected on days 6, 13, 22, and 29 (days post casing) during the production period. DNA was extracted from the replicate casing samples and bacterial DNA was amplified using PCR and then isolated from each sample for metagenomic analysis. Statistically significant changes in populations of Actinobacteria, Firmicutes, Bacteroidetes, and Proteobacteria were observed, with minor changes also seen in Chloroflexi, Gammatimonadetes, and Planctomycetes. A better understanding of the microbial communities in the compost and casing will hopefully allow us to increase the bioefficiency during production as well as possibly help us to better understand the microbial community-pathogen relationships as they relate to disease development.

#### **V-O-6. Performance of straw mushroom (*Volvariella volvacea*) raised as an intercrop in coconut plantations of coastal Odisha**

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Paddy straw mushroom (*Volvariella volvacea*) is an edible mushroom of the tropics and sub-tropics. In India, Odisha is the leading state in term of straw mushroom production (8129 tons/annum) sharing

80% of the total production of the country (10,000 tons/annum). *V. volvacea* is the ruling species of the state because of its soft and fleshy texture and excellent flavour. The hot and humid coastal agro-ecological situation with abundance of manpower and agricultural waste (paddy straw) has made it most suitable for cultivation of straw mushroom. It is often cultivated outdoor as an intercrop in the coconut plantations in the coastal belt of Odisha comprising the districts of Ganjam, Khurda and Puri contributing to 74% of total straw mushroom production of the state. The favourable climate of the coastal areas experienced from the month of February to November is fully exploited by the growers to raise straw mushroom crop for 10 months a year. However, conventional method of farming in tree shade leads to unstable and low yields. Therefore, an attempt was made to ascertain the seasonal productivity of straw mushroom under natural climatic conditions and to correlate the yields with the prevalent weather conditions. Straw mushroom beds of dimension 1.5'x1.5'x1.5' were raised at monthly intervals round the year. Each treatment (month) was replicated 12 times in Randomized Block Design in the coconut plantation of the Central Research Station, Orissa University of Agriculture and Technology, Bhubaneswar. Beds were spawned in three layers @3% of the dry weight of the substrate with incorporation of 3% wheat bran as the organic supplement. Beds were maintained with due aftercare. Two flushes were harvested from the beds at two and three weeks of spawning. Observations were recorded on yield and yield attributing parameters all through the crop period. Analysis of data indicated that there was significant variation among the treatments in respect of all the parameters recorded. The significantly highest yield of 1771.16 g/bed was obtained from the crop raised in the month of July with a biological efficiency of 25.30%. Superiority of the treatment was observed in terms of number (84.16) and average weight of sporophores (21.50 g) with modest crop duration of 14.66 days. Appreciable yield levels obtained during the period from the month of June to September (rainy season) were in the range of 1263-1771.16 g/bed in comparison to the summer crop (724-1097.50 g/bed). However, the yields obtained from the month of December and January were substantially low (165.33 and 136.66 g/bed, respectively). Therefore, it appeared that the winter months are not favourable for raising straw mushroom beds in outdoor condition in the coastal belts of Odisha. Multiple regression analysis showed that the correlation between both the independent variables (mean day temperature and relative humidity) and the biological efficiency realized was significant both at 5% and 1% levels. The data recorded from the investigation indicated that raising of straw mushroom in outdoor situation could be profitable from the month of February to November with acceptable yield levels (10.34-25.30% biological efficiency). However, crop grown during the months of December and January are less productive and therefore, un-remunerative.

#### **V-O-7. The research, development and application of the fermentation accelerator, a new material for edible mushroom cultivation on unsterilized substrate**

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The fermentation accelerator was a product resulted from the research on the differences in biological characteristics of various fungi. The purpose of the research was to develop a substance that can accelerate fermentation of the substrate, thus realize sterilization at a lower temperature and in a shorter time period, as well as reduce consumption of energy, cost and labor. We found that by using it in the traditional sterilization method, the fermentation accelerator could help ammonia production and help beneficial micro-organisms to accelerate the death of contaminants at a temperature between 70 and 80 °C in 5-7 hours, realizing the purpose of sterilization. The production of 10,000 bags of *Lentinula edodes* (shiitake) or *Auricularia auricular-judae* (black wood ear) could save 720 kg of coal and 2 labor forces and increase the yield by over 10%. Our conclusion was that edible mushrooms could not only be cultivated on sterilized substrate, but also on unsterilized substrate with the help of the fermentation accelerator, which could



bring about considerable economic and ecological benefits. This achievement passed the expert appraisal of scientific and technological administration sector and over 290 million bags were supplied and used in 26 provinces (regions, municipalities) nationwide.

#### **V-O-8. Effect of *Pleurotus ostreatus* colonized substrate on broiler chicken growth**

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Alternative compounds have been used in ethnoveterinary to enhance growth performance and/or prevent common bacterial infections to poultry. However, few of them have used mycelial-colonized substrate to partially replace standard diet in broiler chickens. The objective of this study was to evaluate broiler chicken production with partial replacement of standard diet by *P. ostreatus*-colonized substrate. 150 one-day-old male Cobb chicks were given standard diet partially replaced by 0, 5, 10, 100 or 200 g kg<sup>-1</sup> of *P. ostreatus*-colonized substrate and randomly distributed into five treatments. Each treatment had 3 replicates, with 10 birds per replicate, totalizing 30 birds. The replacement of the standard diet by 100 and 200 g kg<sup>-1</sup> (P100 and P200) of colonized substrate for 21-day-old chickens presented up to 35% and 40% higher feed intake ( $p \leq 0.05$ ), respectively, than the control. For body mass of P100 and P200, it was 50% and 58% higher ( $p \leq 0.05$ ), respectively, than control for 21-day-olds. The use of *P. ostreatus*-colonized substrate in chicken feeding is an alternative to improve broiler chicken production.

#### **V-O-9. Drip irrigation, a new way of watering *Agaricus bisporus* crop: increased production and lower carbon footprint**

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Drip irrigation was invented in Israel, more than 50 years ago. Today, it is the most efficient way for irrigation of field crops and orchards worldwide. Nevertheless, there was no attempt to adjust this way for water supply, to mushroom cultivation, probably due to technical constraints. The development of new low flow, pressure adjusted, leak less, drippers enabled the needed accurate water spread and opened the way to their use in mushroom cultivation. Drip irrigation overcomes the watering obstacles for mushroom cultivation. The experiments with the new watering system were carried out in a commercial mushroom farm, in rooms of 400 m<sup>2</sup> shelves area. The control was watering with a conventional spraying watering machine. Drip irrigation enabled the keeping of optimal water contents in compost and casing during entire crop cycle. The value of mushroom yields (quantity and quality), was found to be higher with drip irrigation, mainly at the third picking flush. Decreased casing soil thickness by 35%, in the drip irrigation plots, did not harm mushroom's yield or quality, due to the ability of drip irrigation to add water to the casing soil, during the entire cropping cycle. The casing and compost moisture decrease, was minimized using drip irrigation, leading to the ability to grow the mushrooms during three flushes. Decreasing relative humidity (RH), in the cultivation room, after spraying, is avoided with drip irrigation, decreasing the energy needs for room's drying. Decreased bacterial blotch incidence, due to dryer mushrooms using drip irrigation, enhanced the mushrooms quality. Higher mushroom quality, using drip instead of spray irrigation, mainly at the second and third flushes, increased the entire crop value. Special machinery was developed for the placement of the drip lines into the casing, on the mushroom cultivation beds. This machinery was fixed on Phase 3 compost filling machine. With the use of this machinery, introduction of drip irrigation to mushroom cultivation rooms became almost fully automatic, and the use of manpower was reduced, to

the level of use in a conventional watered room. Drip pipelines can serve as tools for the delivery of food supplements and pest control agents too. Algorithm for control system, based on data collected from the room, was developed enabling automatic irrigation control. The reduction in energy needs and in thickness of casing reduces the carbon “footprint” of mushroom cultivation. The mentioned data collected until now, reveals the commercial advantage of drip irrigation, over spray watering for mushrooms cultivation.

#### **V-O-10. Bioethanol: hydrothermal pretreatment of the spent mushroom substrate to increase the efficiency of enzymatic hydrolysis**

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The uncertainty of the availability of fossil fuels such as oil and natural gas demands search for a new and more sustainable energy source that produces lesser harm to the environment. Thus, researchers have tried to obtain an economically viable alternative using lignocellulosic biomass and wastes applied in bio-refinery. So it is necessary to have research for microbial enzymes that can help viability of employing lignocellulosic biomass and its potential biotechnological applications. This study aimed to evaluate the activities of holocellulases of filamentous fungi isolated from decaying trunk of Brazilian savannah, when grown in culture media with different lignocellulosic biomass as carbon sources. One hundred thirteen (113) filamentous fungi were isolated from decomposing wood. All were screened using a test IAE index (enzyme activity), where 5 fungi were chosen for submerged cultivation, containing sugarcane bagasse, elephant grass or okara as carbon sources. Three other filamentous fungi, pigment producers, belonging to the collection of Embrapa Agroenergia (AR) were also used. The crude enzymes extracts of these fungi were found to produce holocelulases. Activities of holocelulases (xylanases and CMCases) exhibited best results with crude extracts of fungi MD-22 and AR-133, with culture medium containing elephant grass as a carbon source. The optimum temperature for CMCases and xylanase activities of these crude extracts was 50°C and pH 4.5. These enzymatic activities showed higher thermo stability at 45°C. The efficiency of the biological pre-treatment was also evaluated using white-rot fungi, *Pleurotus ostreatus* and *Ganoderma lucidum* under solid state fermentation with sugarcane bagasse, elephant grass or combinations thereof (spent mushroom substrate – SMS). The efficiency was determined by chemical analysis, hydrolases activity and oxy-reductases. After the biological pretreatment the biomass was subjected to hydrothermal treatment. *P. ostreatus* exhibited highest level of biomass delignification. Enzymatic hydrolysis of pre-treated elephant grass was performed with implementing trade Cellic CTec3 enzyme and enzyme extracts of fungi (MD-22 and AR-133). Significant release of glucose outside the crude extract of MD-22 from the culture medium was obtained with elephant grass. These results had been compared with the hydrolysis of biomass by commercial enzyme Cellic CTec3 (Novozymes).

## Poster Presentations

### V-P-1. A comparative study of growth behaviour, quality attributes and yield potential of *Pleurotus* spp. on oil palm factory wastes

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Three *Pleurotus* species (*P. sajor-caju*, *P. florida* and *P. djamor*) were cultivated on oil palm factory wastes viz., bunch refuse and mesocarp waste, which constitutes about 50% of the Fresh Fruit Bunches (FFB), and are available in bulk quantities and sterilized form. Present study indicates the bioconversion of these wastes by solid state fermentation, paddy straw as control and chooses the higher yielding varieties of oyster mushroom in commercial scale. The experiment was conducted in three seasons (June-Sept, Oct-Jan, and Feb –May) with six replications by poly bag method, showed significant variation in growth behaviours and quality attributes during June-September 2013-2014. Biological efficiency (BE) was high in *P. florida* (68.70%) in mesocarp waste and bunch refuse (63.47%). Maximum number of sporocarps produced by *P. florida* (121.83) in bunch refuses with high individual weight (5.61g.) in mesocarp waste followed by *P. sajor-caju* (116.5) with individual sporocarps weight (4.91g.) In mesocarp waste and bunch refuse, respectively. The spawn run period and first appearance of pinhead was in *P. djamor*, 7.83 and 10.83 days, respectively in bunch refuse and *P. sajor-caju* taken more days (19 and 23). Mature fruit bodies was harvested in the range of 5 -7 days in the first flush and 8-12 days in the second flush from *P. djamor* in bunch refuse and 9-13 and 8 -10 in mesocarp waste followed by *P. florida* (9-14 and 8-11 from bunch refuse and 7-14, 9-12 from mesocarp wastes in first and second flush, respectively). The stipe length, pileus diameter ratio was high in *P. sajor-caju* (2.85:8.8) in bunch refuse and *P. florida* (2.23:8.02) in mesocarp waste. The crude protein content was high in *P. sajor-caju* (17.66%) in bunch refuse compared to paddy straw (13%). The present study revealed that *P. florida* gave the best in biological efficiency.

### V-P-2. An innovative method of preparation of healthy grain spawn

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Mushroom spawn is prepared on cereal grain media by inoculating them with pure cultures of selected mushroom species under sterile conditions, yet the problems of contamination of spawn is a major bottleneck in the growth and spread of mushroom farming in developing countries. Species of *Trichoderma*, *Aspergillus*, *Penicillium*, *Rhizopus*, etc. besides wet spot causing bacteria are known to be major contaminants of mushroom spawn in eastern India, including Jharkhand affecting commonly grown mushrooms viz., *Pleurotus* spp., *Hypsizygus ulmarius* and *Volvariella volvacea*, which need urgent action for their management. In light of reports of appearance of fungicide-resistant strains of *Trichoderma*, therefore, a Neem- based herbal formulation, namely Mahaneem containing 0.15% Azadirachtin was tried as a prophylactic pre-treatment of the wheat grains used for the spawn medium together with some empirical changes in the current method of preparation of the grain spawn medium. The modified method tried successfully for raising healthy, contamination-free and productive Master and Planting spawns of oyster and paddy straw mushrooms has been discussed.



### **V-P-3. Assessment of low density polyethylene (LDPE) biodegradation potential of an edible mushroom, *Pleurotus florida***

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Increasing amounts of synthetic polymers produced places pressure on capacities available for plastic waste disposal which results in increasing interest in polymer biodegradation. This study aims in investigating the low density polyethylene (LDPE) biodegradation potential of *Pleurotus florida* under various *in vitro* conditions. The mushroom was grown on potato dextrose agar (PDA) medium and mineral salt agar medium (MSM) and their mycelia growth on both the medium was recorded at periodic intervals. The mushroom mycelia was inoculated in the liquid media supplemented with the pre-weighed low density polyethylene sheet as the only carbon source. The culture flasks were maintained at optimum temperature and the LDPE sheet after 30 days incubation with mushroom was analysed for various changes through Fourier Transform Infra Red spectroscopy and Scanning electron microscope imaging. The FTIR results of control and treated LDPE sheets showed different peaks based on the chemical groups present which indicated the oxidative degradation of the polymer. The LDPE sheets were weighed after a month of incubation and the weight loss percentage were calculated. The scanning electron microscope analysis of the LDPE sheet revealed the regions of fungal mass colonization and degradation patterns. Analysis of extracellular enzymes liberated in the liquid medium supported the biodegradation potential of *P. florida*.

### **V-P-4. Bioremediation of heavy metals through cultivated mushroom *Pleurotus florida***

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During the last two decades, attention has been paid on the management of environment pollution caused by hazardous materials such as heavy metals with the help of living organisms. The present study was designed to assess the potential of cultivated mushroom, *Pleurotus florida* to act as bioremediation agent against 10 different heavy metals (Cu, Zn, Pb, As, Cd, Cr, Al, Mg, Mn and Co). For this effect of various concentrations (10, 25, 50 & 100 ppm) of heavy metals on mycelial growth (*in vitro*) and fruit bodies production (*in vivo*) of oyster mushroom (*P. florida*) was evaluated by involving various parameters like weight of mycelial growth (mg.), number of days taken for mycelial colonization of substrate, first harvest and last harvest, total mushroom yield, total number of mushroom fruit bodies, average weight of fruit bodies, average size of fruit bodies and biological efficiency. The accumulated concentration of heavy metals by mushroom mycelia and fruit bodies were recorded with the help of Atomic Absorption Spectrophotometer (AAS). *P. florida* can tolerate heavy metals toxicity up to certain extent.  $\text{CrSO}_4$ ,  $\text{AlSO}_4$ ,  $\text{MgSO}_4$  and  $\text{MnSO}_4$  didn't have any adverse effect on mycelial growth in all concentrations whereas, higher concentrations (25 ppm and above) of the salts of  $\text{CuSO}_4$ ,  $\text{AsSO}_4$ ,  $\text{CdSO}_4$  and  $\text{CoSO}_4$  were found toxic for the mycelial growth. Moreover,  $\text{PbSO}_4$  and  $\text{ZnSO}_4$  were found average in its effect on mycelial growth. Mushroom yield was highest in presence of Copper, Zinc, Manganese and Magnesium at 10 and 25 ppm and Cobalt at 10 ppm concentration of heavy metal salts in the substrate. Mushroom yield was lowest in presence of heavy metal salts of Lead, Cadmium and Aluminium at 100 ppm concentration in the substrate. The average yield of fruit bodies for a heavy metal in all four concentrations viz., 10, 25, 50 and 100 ppm was recorded in following order from higher to lower i.e.  $\text{Mg} > \text{Zn} > \text{Cu} > \text{Mn} > \text{Co} > \text{Cr} > \text{As} > \text{Cd} > \text{Pb} > \text{Al}$ ; i.e. 523.24, 468.24, 467.82, 454.47, 419.25, 328.72, 288.07, 285.49, 268.34 and 256.05 g, respectively. All treatments of *P. florida* from 10-100 ppm concentration of heavy metals in substrate were able to form fruit bodies. Heavy metal accumulation in mushroom mycelia varied significantly with the type of metal and total concentration of metals in the substrate. The most abundant metal recorded in mycelia of *P.*

*florida* was Magnesium followed by Manganese, Zinc and Cadmium, respectively. The least abundant metals found in mycelia of *P. florida* were Lead, Arsenic and Cobalt, followed by Chromium. Moreover,  $\text{MgSO}_4$  was found highest in fruit bodies of *P. florida* in the range of 188.12-278.36 ppm.  $\text{ZnSO}_4$  and  $\text{CuSO}_4$  were found next highest in the range of 15.87-52.49 ppm and 7.84-38.87 ppm, respectively.  $\text{CrSO}_4$  and  $\text{MnSO}_4$  were accumulated at average level with 4.81-9.12 ppm and 11.18-16.41 ppm, respectively. Other heavy metals found in minimum amount were  $\text{PbSO}_4$  (0.76-5.75 ppm),  $\text{AsSO}_4$  (0.32-1.83 ppm),  $\text{CdSO}_4$  (2.28-6.83 ppm),  $\text{AlSO}_4$  (2.13-5.46 ppm) and  $\text{CoSO}_4$  (1.12-2.01 ppm). Also, it could be concluded from results that, as the concentration of heavy metals in substrate increases, the accumulation of heavy metals in fruit bodies of cultivated mushroom *P. florida* increases and vice versa. It shows that *P. florida* has potential to bio-accumulate tested heavy metals.

#### **V-P-5. Chemical attributes of local flora in Spiti Valley of Himachal Pradesh tested as substrate for cultivation of oyster mushroom**

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An experiment was conducted at Regional Horticultural Research Sub- Station, Tabo, Distt. Lahaul & Spiti for the production of *Pleurotus sajor-caju* on different locally available flora as substrates under cold desert conditions of Himachal Pradesh. The different flora tested included dried and chopped leaves of *Populus ciliata* (poplar), *Lactuca sericola* (vern. nechaapa); whole plant parts of *Iris* sp. (vern. thehma) and one local grass called Congsasa Grass as well as dried straw of *Hordeum* sp. (vern. Gandhum or Fungma) and *Triticum* spp. (wheat). Different chemical properties of these substrates were analysed at Deptt of Soil Science and Water Management, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan. Chemical properties analysed included pH (1:5), electrical conductivity, and content of organic carbon, nitrogen, phosphorus, potassium, copper, iron, manganese and zinc on dry weight basis of substrates. The data were also recorded in terms of number of days for spawn run, pin head formation, days for first flush, number of fruit bodies and yield. Biological Efficiency (BE) was also calculated as per cent of fresh mushroom yield to dry weight of substrate at spawning. The pH was recorded maximum (6.77) in wheat straw and minimum (4.82) in nechappa while, maximum electrical conductivity (7.16 mmho) was recorded in wheat straw and minimum (2.52) in thehma. Organic carbon content ranged between 32.69% in wheat straw to 48.33 % in thehma while, NPK contents varied between 1.03% in fungma to 2.88% in thehma, 0.15% in fungma to 0.38% in nechappa and 0.50% in poplar to 3.16% in wheat straw, respectively. Copper content was minimum (0.5 ppm) in fungma and maximum (3.16 ppm) in wheat straw while, iron content was maximum (2267 ppm) in congsasa grass and minimum (873 ppm) in thehma. Manganese content was maximum (140.7 ppm) in poplar and minimum (49.4 ppm) in wheat straw while, zinc content ranged between 44.0 ppm in fungma to 130.4 ppm in popular. The time taken for spawn run on different substrates ranged between 21 and 33 d while, it was 26 to 38 d after spawning for first flush. On an average wheat straw supported the production of maximum number of sporocarps (82.61), followed by gandhum and poplar species. The maximum yield (1.256 kg) was recorded with wheat straw (standard check) followed statistically by gandhum (0.911 kg) and poplar (0.732 kg). The biological efficiency of oyster mushroom on different substrates varied from 32 to 77% being highest in *P. sajor-caju* grown on wheat straw and lowest in *P. sajor-caju* grown on nechappa.

## V-P-6. Collection, isolation, identification and cultivation of *Pleurotus pulmonarius* in India

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*Pleurotus* mushroom generally referred as oyster mushroom all over the world is known as Dhingri in India. Sufficient information is available on the sexual cycle of this mushroom and for improvement of this mushroom genetic variability is available. Potential strains of this mushroom suitable to different agro-climatic zones are available and new species are being collected from wild for domestication/ inclusion in the improvement programmes. In an effort to enrich the collection and domestication of oyster mushroom species, surveys were conducted especially in the upper reaches of HP for collection of different macro-fungi during the month of June-July (2011). Mature fruit bodies growing on willow tree stump (10-30 °C; < 45% RH) were collected. The geographical location of the place was 32.58 °N 77.03 °E located at an altitude of 3,080 meters from mean sea level. There is little or no rain fall. The climatic conditions are extremely cold. Fruiting bodies were sterilized for 1 minute with mercuric chloride (0.1%) under aseptic conditions. Small tissue of the fruit body was taken from the junction of stipe and pileus. and placed in petri plates containing PDA. Genomic DNA from the isolates were extracted using approximately 100 mg mycelial cultures raised in malt extract broth (Malt extract 10 g l<sup>-1</sup>; Glucose l<sup>-1</sup>) for 10 days at 25±1 °C as stationary culture. Universal primers ITS-1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS-4 (5'-TCC TCC GCT TAT TGA TAT GC-3') were used for PCR-amplification of the internal transcribed spacer (ITS) region of ribosomal DNA, including the 5.8S rRNA gene, ITS-1, and ITS-2 regions. Nucleotide sequence comparisons were performed by the basic local alignment search tool (BLAST) network services against the National Centre for Biotechnology Information (NCBI) database. The mushroom species were designated to the sequenced cultures and analyzed based on similarity with the best-aligned sequence of BLAST search. 5.8S rRNA gene sequence alignments were performed using ClustalX 1.83 software. Comparison of sequences confirmed the identity as *Pleurotus pulmonarius*. This mushroom was cultivated on mixed saw dust supplemented with 4% wheat bran. Spawn run was completed in 25-30 days at 25-28 °C. Pinning started after 10 days of opening the bags. First flush was harvested after 15 days when blocks were placed at a temperature range of 10-25 °C.

## V-P-7. Comparative cultivation experiments of *Pleurotus eryngii* isolates

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The King oyster mushroom (*Pleurotus eryngii*) is a well-known species and has may be one of the best taste within the *Pleurotus* genus. There are several cultivation technologies and substrates described in the literature, but the improvement of those are always necessary. In these experiments a comparative cultivation of *P. eryngii* isolates of mostly Hungarian origin was performed. Sixteen *P. eryngii* isolates were cultivated on lignocellulose substrate containing 65% beech sawdust, 17% bran, 9% beech woodchips, 3.5% gypsum and 5.5% soybean supplement (Promycel 480). Water content of the substrate was adjusted to 60%. The mixture was filled into plastic bags, sterilized and spawned. The blocks were cased by peat-based commercial *Agaricus* casing soil. Yield, number of fruiting bodies, period of flushes, average weight of fruiting bodies, biological efficiency (BE %) and productivity (P %) for each strains was recorded. The amount of yield was calculated for 100 kg of substrate. A photo documentation and description of the strains were also prepared. The highest yield was produced by the Ple-4V (41.5 kg/100 kg) and Ple-5V (39.5 kg/100 kg) strains, whereas the lowest yield was found in case of the PEL (9 kg/100 kg) and PEG (11 kg/100 kg) strains. Average yield of the strains was 27–53 kg calculated for 100 kg substrate. The number

of fruiting bodies was 1488, average weight of fruiting bodies (concerning the species) was 19.95 g. Very high biological efficiency was produced by the Ple4V (156.2%) and Ple5V (140%) strains. The lowest efficiency was found at the PEL (28.5%) and PEG (37.8%) strains. Our results showed that the average yield was over 25 kg/100 kg spawned substrate, which means that this species might be a successful rival of the oyster mushroom (*P. ostreatus*) hybrids that are very popular amongst the growers nowadays. In the future it is necessary to select the best strains for cultivation, start cross breeding experiments, determine the optimal substrate composition adapted to the local lignocellulose sources and define the most suitable environmental conditions.

#### **V-P-8. Comparative evaluation of casing mixtures for yield potential of button mushroom (*Agaricus bisporus*)**

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In order to study the effect of different casing mixtures on the growth and yield of common button mushroom (*Agaricus bisporus*) an experiment was carried out with seven casing mixture formulations. Maximum sporophore (12.33) were obtained by casing mixture of CCP + VC + FYM + SD + Sand and minimum sporophore (5.67) on casing mixture CCP + FYM. The highest yield of first flush was obtained from the casing mixture of CCP + VC + FYM + SD + sand (270.33 g) followed by CCP + FYM + SD, VC + FYM, VC + SD + FYM + Sand and CCP + VC + Sand (266.67, 216.67, 213.33 and 196.67), respectively. Casing mixture CCP + VC + FYM + SD + Sand recorded the highest yield (320 g) whereas CCP + FYM showed lowest (250 g) yield in the harvesting of second flush. The total highest yield (1112.26 g) was obtained from casing mixture, CCP + VC + FYM + SD + Sand and lowest yield (736.67 g) from CCP + FYM. Casing mixture of CCP + FYM + SD recorded second highest yield (1033.67 g). Finally all the casing materials were evaluated for their effect on growth parameters and yield of *Agaricus bisporus*. Among the different casing mixtures tested CCP + VC + FYM + SD + Sand and CCP + FYM + SD were found to be better in yield when compared to other casing mixtures. These studies will help to mushroom growers for selecting the most suitable casing materials for better growth behaviour and optimum yield potential of common button mushroom (*Agaricus bisporus*) grown in our country.

#### **V-P-9. Evaluation of various substrates and supplements for enhancing yield of *Pleurotus eous* (Berk.) Sacc**

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Oyster mushroom (*Pleurotus* spp.) cultivation has increased tremendously throughout the world during the last few decades. Oyster mushroom accounted for 14.2% of the total world production of edible mushroom in 1997. Oyster mushroom cultivation can play an important role in managing organic wastes whose disposal has become a problem. Among the tested grains, minimum (7.33 days) period for substrate colonization by *P. eous* was recorded in sorghum grains with whitish pink compact mycelium growth. All grains were completely colonized, tightly held with each other followed by paddy grain (8.66 days). The slowest and poor mycelium run was observed in soybean (15.33 days) and grains were not fully covered. The effect of different supplementation on straw substrate play important role in boosting the yield potential of pink oyster mushroom. Supplementation with pigeon pea bran gave highest yield (640.66g) with BE (96.05 %), followed by wheat bran (588.00g, BE 88.15%), chickpea bran (564.65g, BE 84.65 %), soybean



flour (552.76g, BE 82.87 %), lathyrus bran (538.33g, BE 80.70 %), while minimum yield was observed in rice bran (520.66g, BE 78.05 %) followed by control (un supplemented) (534.00g, BE 80.05 %).

#### **V-P-10. Comparative evaluation of growth behaviour and yield potential of five strains of milky mushroom (*Calocybe indica*)**

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Milky mushroom is the third most largely growing mushroom in India and has the good demand in the market as well as in world trade. An experiment was carried out with five strains of *Calocybe indica* viz., CI-4, CI-13, CI-14, CI-15 and CI-18 for growing behaviour and yield potential. Minimum spawn run period (15-66 days) was observed in CI-14 while CI-15 took maximum (18 days) time for complete spawn run. Pinheads appeared early (28 days) in CI-14 while strain CI-15 took maximum time (34 days). The harvesting of 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> flushes were completed early (35, 47 and 58.33 days, respectively) in strain CI-14 followed by CI-4, CI-18, CI-13 and CI-15. Strain CI-14 gave the highest number of fruit bodies (25). Strain CI-14 gave the highest yield while CI-13 showed lowest yield. Highest average yield of 1<sup>st</sup> and 2<sup>nd</sup> flush was also in CI-14 (441.67 g and 285 g) strain but the yield of 3<sup>rd</sup> flush was higher in strain CI-13 (75.33 g). Overall total yield was better in CI-14 (811.67 g).

#### **V-P-11. Comparative performance of five strains of oyster mushroom (*Pleurotus* spp) on paddy straw and rubber saw dust and extent of their adoption and nutritional acceptability**

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The genus *Pleurotus* includes several edible species that are known for their exceptional nutritional and medicinal properties and relatively low cost method of cultivation. These are efficient lignin degraders and can grow well on different types of ligno cellulosic materials. The present investigation is on the comparative performance of five strains of oyster mushroom viz.; three strains of *Pleurotus florida* designated as F-1 (Vellayani Strain), F-2 (Changanassery Strain) and F-3 (Attingal Strain) and two strains of *Pleurotus eous* designated as E-1 (Vellayani Strain) and E-2 (Ponmudi Strain) on paddy straw and rubber saw dust. The extent of adoption of strains by mushroom growers as well as overall nutritional acceptability was also evaluated. Polybag method of cultivation was followed. One kg each of substrates, thirty days old spawns and 60 x 30 cm polypropylene covers were used for bed preparation. Three strains of *P. florida* showed marked difference in colour and shape. The sporophores of F-1 strain are bigger sized, more weight per mushroom, and creamy white coloured, while F-2 sporophores are ashy white coloured and smaller sized than F-1 strain. F-1 strains appear in two forms -one completely spread out form and another blooming and curved upwards. The fruit bodies of F-3 strain are smaller than F-2 and ashy white coloured. Among the three strains, F-1 recorded maximum production on both substrates followed by F-3 and F-2 strains. Time taken for mushroom formation was recorded and rubber saw dust took more time than paddy straw and no significant difference was observed among the strains with respect to duration of spawn run or mushroom formation. The total weight from three harvests was the highest in the case of F-1 strain on rubber sawdust followed by F-3 and F-2. Out of the two substrates tried, rubber sawdust was found to give maximum yield in all the strains. The sporophores of pink oyster, *P. eous* (E-1 Strain) are light pink coloured, slightly harder at maturity and smaller sized compared to E-2 strain, which are dark pink coloured, soft and bigger sized. Both the strains produced fruit bodies in 14 to 15 days on paddy straw and

no significant difference was observed between the strains with respect to the time taken for fruit body formation or spawn run days. Between the two substrates, rubber sawdust took more time for fruit body formation (19 days) than paddy straw, but the total weight as well as weight of individual mushroom was the highest for two strains in rubber sawdust followed by paddy straw. Maximum yield was recorded by E-2 strain. These five strains are being grown on entrepreneurship basis by mushroom growers of Kerala and survey on the adoption of these strains by selected growers in seven districts of Kerala indicated that 94.29% of white oyster mushroom growers preferred F-1 strain and 92.87% pink oyster growers preferred E-2 strain. Organoleptic trials on the nutritional parameters indicated the maximum overall acceptability score for F-1 followed by F-2 and in pink oyster, E-2 recorded the maximum score.

#### **V-P-12. Cultivation of four summer species of *Pleurotus* on different wild grasses in Arunachal Pradesh**

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*Pleurotus* spp. are edible basidiomycetes cultivated worldwide on various ligno-cellulosic agro-forest wastes for their rich nutrition content and culinary properties. Four summer species of *Pleurotus*, namely *P. sajor-caju*, *P. sapidus*, *P. citrinopileatus* and *P. flabellatus* were studied for their ability to utilize three different wild grasses as substrates namely *Saccharum spontaneum*, *Neyraudia reynaudiana* and *Imperata cylindrica*. Substrates were prepared for inoculation with mushroom mycelia by chemical sterilization method using formalin (500 ppm) and carbendazim (75 ppm). The spawn of all the species of *Pleurotus* were maintained on wheat grains and were inoculated to freshly prepared substrates @ 3% (w/w). The number of days for mycelial run, pinhead formation, first flush yield, average weight of fruit body, weight of quality fruit body and biological efficiency was recorded. Mycelial run was completed earliest on *S. spontaneum* followed by *N. reynaudiana*. In all the three wild grasses, the substrate colonization was fastest by *P. citrinopileatus* and *P. flabellatus* whereas, *P. sapidus* took more time. Pinhead formation was observed earliest on *S. spontaneum* and *N. reynaudiana* by *P. sajor-caju* and *P. citrinopileatus*, while it was the most delayed in *I. cylindrica*. The highest biological efficiency was observed for *P. citrinopileatus* on all the substrates tested followed by *P. sajor-caju* and *P. sapidus*. *P. flabellatus* gave the least BE. The first flush yield was highest on *S. spontaneum* followed by *N. reynaudiana* and the least on *I. cylindrica*. Highest first flush yield was produced by *P. citrinopileatus* followed by *P. sajor-caju* and *P. sapidus*. *P. flabellatus* gave the lowest yield. The average weight of fruit body was highest on *S. spontaneum* and *N. reynaudiana* and least on *I. cylindrica*. *P. citrinopileatus* and *P. sajor-caju* produced bigger fruit bodies on *S. spontaneum*, whereas *P. sapidus* and *P. flabellatus* on *N. reynaudiana*. The smallest fruit body size was observed on *I. cylindrica* by all the four spp. of *Pleurotus*. Weight of quality fruit bodies produced was more on *S. spontaneum* followed by *N. reynaudiana* and it was the lowest on *I. cylindrica* for all the four species. Comparatively, *I. cylindrica* proved to be a much inferior substrate

#### **V-P-13. Cultivation of oyster mushroom (*Pleurotus sajor-caju*) in North Chota Nagpur Region of Jharkhand by using locally available substrates**

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North Chota Nagpur region of Jharkhand, India falls under rain fed area with high rainfall up to 1100-1350 mm. Mushroom is becoming popular in Jharkhand. The mushrooms are nutritionally endowed fungi (mostly Basidiomycetes) that grown naturally on trunks, leave and roots of trees as well as decaying woody materials. In Jharkhand wild edible mushroom are available in rainy season in market so people are well



adapted to mushrooms but commercially oyster mushroom is becoming popular for livelihood security and substitute of malnutrition problem. Malnutrition is a serious problem of most of the population in this tribal state. Oyster mushroom cultivation has a very good potential in this zone as livelihood security, which can be a major sources of required nutrients for the growth and even required low cost designed mushroom house. A on campus trial was conducted by Holy Cross Krishi Vigyan Kendra, Hazaribag, Jharkhand for exploring the possibility of mushroom cultivation on locally available substrate of different crop residues. Under this experiment eight different locally available substrate viz., dry pod and stem of mustard, dry leaf and suckers of banana, paddy straw, husk of maize cob, dry branches and leaves of pigeon pea, dry leaves and stem of groundnut were evaluated as comparative with wheat straw as standard check for the production of *Pleurotus sajor-caju* during the year of 2012-2013. The data were recorded in term of time taken for mycelium growth, yield of mushroom, total harvesting days. The every bundle has 5 kg weight after packing. The groundnut substrate was found to be the best treatment in term of number of days taken (21 days) and yield (2.016 kg/bundle), cost of cultivation was ₹ 22, and C:B ratio was 1:6.5. The highest yield of mushroom were recorded on banana substrate, in terms of yield (2.105 kg/bundle) but, it took 55 days of cropping cycle, cost of cultivation was ₹ 28 per bundle and C:B Ratio was 1:58. However, other treatment like mustard substrate produce (1.725 kg/bundle) and cropping period in terms of day was 37 days, cost of cultivation is ₹ 28, and C:B ratio was 1:3.6 were also good. Another experiment on paddy substrate took 38 days of cropping cycle and yields was recorded (1.009 kg/bundle) and cost of cultivation was ₹ 28 and C:B ratio was 1:2.29. As far as cultivation days were the concerned maximum in banana substrate was 55 days and minimum in groundnut substrate used bundle was 21 days. The cost of cultivation was lowest in groundnut substrate used bundle ₹ 28 and yields were also higher 2.105 kg/bundle. The standard check of wheat treatment yield were 1.9 kg/bundle and took 28 days cropping cycle and cost of cultivation was ₹ 28 and C:B ratio was 1:3.27. Holy Cross Krishi Vigyan Kendra, Hazaribag is disseminating the technology for popularizing the mushroom cultivation on banana and groundnut substrate.

#### V-P-14. Cultivation of *Tuber aestivum* in northern Israel

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The location and weather of Israel were thought to be unsuitable for the cultivation of black truffles. Nevertheless parts of northern Israel, namely "The Upper Galilee" is mountainous with heights of 700-900 meters, above sea level. At this region weather and soil conditions are suitable for growing crops like apples and cherries. Therefore it was decided to make an attempt to introduce truffles cultivation to this region too. In order to achieve this goal, *Tuber melanosporum* fruit bodies were imported from Italy and used to inoculate seedlings of local oaks and introduced oaks and hazelnuts.

Few years later, once truffles appeared, it was demonstrated that although the trees were thought to be inoculated by *T. melanosporum* the collected truffles were of *Tuber aestivum* [1]. Truffles were collected and yields were calculated separately for each tree. Highest truffle yields were collected around the local (endemic) oaks. The Upper Galilee region, unlike the known truffles cultivation regions in Europe, lack summer rains. Therefore lack in soil humidity during the summer, was thought to be, the main limitation for fruit bodies development. Recent preliminary irrigation experiments have proved this hypothesis. The irrigation treatments brought to longer fruit body appearance in season. Truffles yields were also higher in the irrigated plots as compared to the un-irrigated ones. The collected truffles were sent to known chefs for actual organoleptic studies. They were found to be of the expected truffles quality. These results are an encouraging sign that truffles cultivation could be possible in this region.

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### V-P-15. Cultivation of *Volvariella volvacea* using spent mushroom substrates

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Spent growth substrates remaining after the cultivation of *Pleurotus eryngii* (SPES), *Hypsizygus marmoreus* (SHMS) and *Agaricus bisporus* (SABC) were used to cultivate the edible straw mushroom *Volvariella volvacea*. Several formulations were used in the cultivation experiments: 30% SPES + 67% cotton waste (CW), 60% SPES + 37% CW, 97% SPES, 30% SHMS + 67% CW, 60% SHMS + 37% CW, 97% SHMS, 30% SABC + 67% CW, 60% SABC + 37% CW and 97% SABC. Cotton waste (97%) served as the control. The duration of the spawn running period, the times elapsing prior to the formation of pin-heads and the maturation of fruiting bodies, and mushroom yields were then compared. Our data revealed no significant differences in mushroom mycelial growth rates on the different formulations. However, pin-head formation occurred earlier on growth substrates consisting of 60% SPES + 37% CW and 60% SABC + 37% CW. Highest biological efficiencies were recorded on the formulations comprised of 60% SABC + 37% CW, and 60% SPES + 37% CW.

### V-P-16. Developing spore deficient strain of *Pleurotus flabellatus*

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Oyster mushroom is the 3<sup>rd</sup> largest cultivated mushroom in the world and preferred in tropical and sub tropical parts of the world due to faster substrate colonization and early fruiting. The fruit bodies of *Pleurotus* spp. (oyster mushroom) is gymnocarpous and produce billions of spores during cultivation. The spore formation and spore dispersal starts in the very young fruiting bodies (1-2 days old) and continue up to the harvesting. Sometimes these spores are visible in the packing material as greyish white slimy powder. These spores cause allergies to workers who are frequently exposed in the cropping rooms during cultural operations like harvesting, water spraying or cleaning. The problem of spore allergies is more prominent during winter season when the outside temperature is low and very less air circulation is being provided in the cropping rooms to maintain higher temperature. Due to spore allergies several entrepreneurs have stopped cultivation of oyster mushroom. Not only the spores cause allergies but sometime in a growing unit producing two different types of mushrooms the spores of oyster mushroom may germinate on the casing soil or substrate and one can found two different mushroom growing in the same bag or tray. Sporeless or spore-deficient strains of oyster mushroom is important to propagate this mushroom on large scale in tropical and subtropical parts of world. There are several reports of developing spore-deficient or sporeless strains developed by mating of spores of mutant sporeless parent with single spore of sporulating parent. In our breeding experiment we have observed that some of the single spores of *Pleurotus flabellatus* on mating within species produced spore deficient strains. One of the promising strain PI-50 (H-16) so developed was evaluated for fruiting and gave 60% biological efficiency on pasteurized wheat straw. The colour of the fruit bodies was slightly brown with drooping type morphology and infundiulform pileus centre. We have examined the fruiting bodies after taking anatomical sections of pileus and it was interesting to note that the hymenium produced large number of cystidia while basidia were very difficult to find and mostly basidioles were present. The strain was able to tolerate higher temperature. DNA profiling of the newly developed strains using ITS sequencing was done for its genetic cataloguing.

## V-P-17. Diversity of culturable thermophilic fungi and actinomycetes in mushroom compost

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Thermophilic fungi and actinomycetes are the components of microflora of composts, which play an important role in decomposition of organic matter [1, 2]. Thermophilic fungi were isolated from mushroom compost by the dilution plate method. Fifteen species of thermophilic fungi were identified based on morphological characteristics and Internal Transcribed Spacer (ITS1, 5.8S rDNA, ITS2) sequences. Among them, *Chaetomium thermophilum*, *Malbranchea cinnamomea*, *Myceliophthora thermophila*, *Myriococcum thermophilum*, *Rhizomucor pusillus*, *Scytalidium thermophilum*, *Thermoascus aurantiacus* and *Thermomyces lanuginosus* were frequently obtained. The most dominant species was *S. thermophilum*. For actinomycetes, we focused on searching the rare actinomycetes. Eighteen species of thermophilic actinomycetes were isolated from the compost and identified based on the phenotypic and phylogenetic data. Among them, strain AG2-7T represents a novel species of a new genus in the family Pseudonocardiaceae, and *Thermotunica guangxiensis* gen. nov., sp. nov. have been described earlier [3]. Several novel species in genera of *Actinomadura*, *Laceyella*, *Nonomuraea*, *Pseudonocardia*, *Sphaerispora* and *Thermomonospora* will be described elsewhere.

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## V-P-18. Effect of different substrates on yield and biochemical composition of *Pleurotus* spp.

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Studies on cultivation of *Pleurotus sapidus* and *P. florida* on different lignocellulosic agrowastes revealed that lowest cropping period (74.67 and 70.67 days) was observed in *P. sapidus* and *P. florida*, respectively with paddy straw + wheat bran +  $\text{CaCO}_3$  while maximum cropping period of 90.33 and 86.33 days, respectively in *P. sapidus* and *P. florida* was in sugarcane baggase +  $\text{CaCO}_3$ . The effect of substrates on biological efficiency showed paddy straw + wheat bran +  $\text{CaCO}_3$  giving the highest biological efficiency of 83.33 and 80.43% in *P. sapidus* and *P. florida*, respectively while, lowest biological efficiency of 52.97 and 44.77% recorded in sugarcane bagasse +  $\text{CaCO}_3$ . The proximate analysis of fruit bodies harvested from different substrates revealed that substrates had significant effect on biochemical composition of sporophores. The moisture percentage varied between 87.11 to 90.11% and 87.07 to 90.98%, carbohydrates from 49.53 to 54.40% and 49.40 to 53.73% in *P. sapidus* and *P. florida* respectively. The highest protein, crude fibre, ash content of 26.58 and 25.30, 7.74 and 7.15, 6.62 and 6.99% respectively was observed in fruit bodies of *P. sapidus* and *P. florida* respectively harvested from paddy straw + wheat bran +  $\text{CaCO}_3$ . Highest fat content of 2.99 and 2.96 percent was found in fruit bodies of *P. sapidus* and *P. florida* harvested from wheat straw + wheat bran +  $\text{CaCO}_3$ . Test substrates showed variation in the cellulose, hemicelluloses and lignin content before *P. sapidus* and *P. florida* cultivation and after the mushroom crop harvest. The highest cellulose (66.72 and 65.61%), hemicellulose (25.00 and 25.33%)

and lignin (20.00 and 20.02%) degradation was observed with paddy straw while lowest cellulose (12.92 and 11.27%), hemicellulose (7.53 and 6.40%) and lignin (1.88 and 2.07%) degradation in saw dust bags.

### **V-P-19. Evaluation of *Agaricus bisporus* Lange (Sing.) strains in the plains of Punjab**

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*Agaricus bisporus* (Lange) Sing. strains were screened for their yield potential with the objective of selecting strains capable of growing best in Punjab conditions. Twenty seven strains of *Agaricus bisporus* (Lange) Sing. (AVT 01- AVT 06, AVT 07-AVT 11, U3 and SSI 01/12- SSI 15/12) were screened for their yield potential and quality parameters (color, texture, stipe length and diameter) with the objective of selecting strains for post harvest processing such as canning and pickling. The mushroom strains were cultivated on short method compost using wheat straw as substrate. Out of white strains, SSI 04/12 (18.22 kg/100 kg compost), SSI 08/12 (17.94 kg/100 kg compost) and AVT- 02 (17.48 kg/100 kg compost) gave highest yield followed by AVT-06 (14.02 kg/100 kg compost). From brown strains, AVT 11 gave highest yield of 10.78 kg/100 kg compost followed by AVT 08 giving 8.34 kg/100 kg compost. The color index indicated maximum L Value for six strains SSI 07/12, SSI 09/12, SSI 12/12, AVT 02, AVT 04 and AVT 06 with lowest values for brown strains ranging from 30.00 to 31.15. Texture analysis indicated the hardness highest for strains SSI 08/12 followed by six other strains SSI 02/12, SSI 06/12, SSI 12/12 and U3. When compared with white strains, brown strains showed less hardness.

### **V-P-20. Evaluation of different strains of oyster mushroom for their cultural, morphological and yield attributes**

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Oyster mushroom is one of the important edible mushrooms grown in India as well as in world having high nutritive value and manifold uses but due to non-availability of suitable strains, this mushroom contributes only 14.2 per cent in the total world mushroom production. Different strains of oyster mushroom (PL-1, PL-3, PL-4 and PL-5) were evaluated for their cultural, morphological and yield attributes. It was found that strain PL-3 grew best (maximum mycelia growth) at temperature 30 °C and at pH-6 however, higher temperature (30 °C) and higher pH level (pH 8) suited for PL-1. The low temperature (20 °C) and higher pH-8 required for strain PL-5 strain. Among different media tested, maximum radial mycelia growth was observed on oat meal agar (OMA) medium in PL-3 and PL-1 strain. In morphological parameter, the stipe length was highest in strain PL-3, whereas shortest stipe was in PL-5 strain. The pileus diameter was highest in strain PL-1 whereas minimum in PL-5 strain. On the basis of yield performance, strain PL-4 was found the best giving highest yield i.e. 395.42 g/2kg wet substrate with biological efficiency 65.90% on wheat straw + waste paper (1:1). It was observed that the supplementation of waste paper (50%) to the wheat straw is suitable for strains PL-1, PL-3 and PL-4. However, supplementation of palm leaves (50%) to wheat straw suited the best only for PL-5 with 53.94% biological efficiency.



## **V-P-21. Evaluation of substrates and supplements for enhancing the productivity of paddy straw mushroom (*Volvariella volvacea*)**

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Paddy straw mushroom (*Volvariella volvacea*) commonly known as the straw mushroom or the Chinese mushroom, is popular for its taste and excellent flavour, short cropping cycle, ability to grow on various crop residues and at wide range of temperature and humidity. However, it has low yield and poor shelf life. Keeping in view an experiment was conducted during two cropping period (July and August) in 2013, to find out the best substrate and supplement for enhancing the productivity of this mushroom. Out of four locally available substrates, cotton waste substrate gave significantly high yield (734.8 g and 796.2 g/ 5.0 kg dry substrate) and biological efficiency (14.7 & 15.92%), followed by pea straw (12.96% and 14.16%), paddy straw (9.32% and 12.68%) and wheat straw (8.54% and 11.32%), respectively, in two cropping period. Cotton waste substrate also gave highest number of fruiting bodies (62.4 in first and 57 in second time), followed by pea straw, wheat straw and paddy straw substrates. Five supplements, namely, rice bran, wheat bran, chick pea grain powder, pea grain powder and pigeon pea grain powder were evaluated on paddy straw substrate. Out of these, rice bran gave highest yield (821.0 g/ 5.0 kg dry substrate), followed by wheat bran (791.3 g), chick pea grain powder (743.0 g), pigeon pea grain powder (704.3 g) and pea grain powder (683.0 g). Yield obtained from each supplement differ significantly from one another, and the yield enhancement and biological efficiency were significantly higher over the control.

## **V-P-22. Optimizing mushroom spawn production in Uganda**

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Most farmers in Uganda own less than an acre of land, which is typically used to grow crops such as bananas, pineapples and cassava. Mushroom cultivation requires less space, takes place near the home and has the potential to improve the lives of thousands of small land holders, especially women. However, for various reasons, farmer groups in Uganda are presently unable to meet the demand for high quality, fresh and dried mushrooms. This project, initiated in March 2014 and funded through the Agri TT (Agricultural Technology Transfer) programme, is directed at addressing current deficiencies in, and impediments to, the development of the Ugandan mushroom industry. The project introduced several key innovations that have made China the world's pre-eminent mushroom producer. These include: the provision of uninterrupted supplies of robust, high quality, genetically-stable spawn (through the Mushroom Training and Resource Centre [MTRC] and registered spawn producers) capable of generating high yields of quality mushrooms and using a wide range of cheap, readily-available cultivation substrates under the diverse climatic conditions prevailing in different regions of Uganda. It also includes establishing a germplasm bank to protect and conserve indigenous mushroom resources and a comprehensive breeding programme for enhanced strain performance. The other issues are introduction of a Field Technical Service coupled to educational and training elements to ensure a sustained supply of 'home-grown' mushrooms and engagement with the Ugandan government to establish a National Mushroom Policy and Strategy.

### **V-P-23. Performance evaluation of *Volvariella volvacea* (Bull. ex Fr.) Sing., strain PS1 for outdoor cultivation in maize fields**

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A rapidly growing *V. volvacea* strain, PS1 was identified to produce more chlamydospores in culture. This strain showed molecular variation by exhibiting a unique protein band at 100 kDa. Protein band of 68 kDa was very conspicuous in PS1 strain and the DNA of this isolate was also amplified at 660 bp. Sugar alcohols (sorbitol and mannitol) greatly encouraged both biomass production and chlamydospores formation. Glycine was the best organic nitrogen source which helped to increase biomass production (3.14 mg) and chlamydospores density. *V. volvacea* strain PS1 greatly narrowed down the C:L and C:N ratio in paddy straw within 15 days. In an outdoor system of cultivation as an inter crop in maize fields, the fungus colonized paddy straw substrate. The average yield of PS1 strain was around 2.44 to 3.50 kg of mushrooms at egg stage from each bed containing 25 kg of paddy straw (dry weight), placed in the inter row space of maize field after 20-25 days of sowing (9.76 to 14.00% bio-efficiency) over a period of 15 days. Further inoculation of *T. viride* to the spent mushroom substrate was quite promising for the *ex-situ* multiplication of the biocontrol fungus ( $129.96 \times 10^6$  cfu/g of spent mushroom substrate within 7 days). Application of this paddy straw spent mushroom substrate fortified with *T. viride* has helped to reduce both pre emergence (38.5 per cent) and post emergence (39.3 per cent) damping-off of tomato seedlings, in addition to increasing the seedling vigour.

### **V-P-24. Quality improvement of casing material and yield in milky mushroom (*Calocybe indica*) by using biofertilizers and different substrates**

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The study was undertaken to assess the effect of nitrogen fixing and phosphate solubilizing biofertilizers and different substrates for improvement of casing quality and yield in milky mushroom (*Calocybe indica*). The experiment was conducted in completely randomized design with eight treatments of biofertilizers viz., *Azotobacter* and PSB i.e. Phosphate solubilizing bacteria (*Bacillus megaterium* + *Pseudomonas striata*) and their combinations. The quality parameters of casing material for milky mushroom viz., mycelial growth, microbial count, C:N ratio and bulk density were analysed in the laboratory and are interpreted. The results revealed that inoculation of bacterial inoculants either in alone or in different combinations resulted an increase in mycelial growth of *Calocybe indica* compared to uninoculated control in in vitro. Highest fresh weight (20.52 g) as well as dry weight (0.65 g) of mycelium was obtained in consortium of *Azotobacter* + PSB (*B. megaterium* + *P. striata*) followed by treatment of PSB resulting in 20.08 g and 0.62 g fresh weight and dry weight of mycelium respectively. The total nitrogen content of casing material increased significantly from 0.11 to 0.55 percent due to biofertilizers treatment with soil + FYM + coir pith. However, organic carbon decreased from 1.46 to 0.55 percent in same treatment. Further, the C: N ratio also decreased from 13.0 to 7.9 due to *Azotobacter* inoculation in casing soil upto harvesting of crop. The microbial count of *Azotobacter* and PSB was also found higher in the same treatment and recorded  $21 \times 10^5$  and  $23 \times 10^5$ , respectively at casing, whereas it decreased upto harvest of mushrooms with greater magnitude. The yield of milky mushroom increased from 12.89% to 79.81% due to inoculation of biofertilizers. In another experiment, the wheat and soybean straws and their mixture were evaluated for optimization of yield of milky mushroom. The data revealed that the stipe length, pileus diameter and average fruit weight were high in mixture of wheat and soybean straw (1:1). The highest yield/kg substrate and biological



efficiency were recorded in mixture of straw, followed by soybean and wheat straw alone and these ranged from 382 to 714 g/kg substrate and 38.2 to 71.4% respectively. The results in general indicated that N<sub>2</sub> fixing and phosphate solubilizing bacteria could increase the quality of casing material. Further, the mixture of wheat and soybean straw (1:1) as a substrate performed better than individual substrates, reflected in higher yields of milky mushroom.

## **V-P-25. Spent mushroom substrate as mulch for yield enhancement and management of rhizome rot complex disease of ginger**

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Spent mushroom substrate (SMS) is the composted organic material released after the harvest of a mushroom crop. After mushroom cultivation, a considerable amount of spent substrate remains as residual material. It creates various environmental problems and nuisance if not handled properly. Recent findings illustrate that this substrate can be used for disease management. So far no attempt has been made on the use of SMS from oyster mushroom, against rhizome rot complex disease of ginger. Under these circumstances, a study was conducted at Vellanikkara, Thrissur (Kerala) to assess the effect of SMS from *Pleurotus florida* and *P. sajor-caju* on growth parameters, yield and rhizome rot complex disease caused by *Pythium aphanidermatum* and *Ralstonia solanacearum* in ginger. The substrates used for oyster mushroom cultivation were agricultural waste materials like paddy straw, saw dust and neopeat. Quantitative estimation of bacteria, fungi and actinomycetes from different SMS was carried out by serial dilution method. For that samples were collected at the time of bed preparation, spawn running period (15<sup>th</sup> day), during harvest (30<sup>th</sup> day) and after harvest (50<sup>th</sup> day). The antagonistic effect of isolated organisms against *R. solanacearum* and *P. aphanidermatum* was tested under laboratory conditions using dual culture method. SMS was rich in microflora with antagonistic effect against pathogenic microorganisms. Evaluation of SMS against rhizome rot complex disease of ginger was conducted under pot culture condition. Challenge inoculation of the pathogens, viz., *P. aphanidermatum* and *R. solanacearum* was done at 45 days after planting. SMS was used as mulch at the time of planting, 60 DAP and 120 DAP. Observations on per cent disease incidence, germination percentage, plant biometric characters and yield at different intervals were recorded. The treatment with paddy straw SMS of *P. sajor-caju* as mulch was significantly superior to all other treatments and the incidence of disease was not observed in this treatment, whereas control plants recorded 100 per cent disease incidence. The observations on number of tillers, number of leaves per tiller, height of tillers and rhizome yield were also recorded highest in the treatment with paddy straw SMS of *P. sajor-caju*. Other treatments with SMS as mulch also showed less disease incidence, better growth parameters and yield compared to control. Hence from the present study it was concluded that SMS from *Pleurotus* spp. is rich in antagonistic microorganisms and it can be used for disease management. Among different SMS, paddy straw SMS of *P. sajor-caju* as mulch is most effective for the management of rhizome rot complex disease as well as plant growth promotion in ginger. *P. sajor-caju* is having better substrate degradation capacity and that facilitates better colonization by plant growth promoting and disease controlling microorganisms.

## **V-P-26. Suitability of shiitake mushroom (*Lentinula edodes* (Berk.) Pegler) for cultivation under Kerala conditions**

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Shiitake mushroom is the one of the most important culinary and medicinal mushroom in the world which ranks number two in terms of total mushroom production. Traditionally, shiitake has been cultivated on

oak logs but recently there is a trend to cultivate it in on sterilized or pasteurized substrates in order to increase yield and reduce the time of its culture cycle. The great interest in shiitake's commercialization is due to its unique flavour, nutritive value and its medicinal properties. Mushroom cultivation has received little attention in most developing countries where millions of tons of lignocellulose rich wastes are unused. The present study will help to add one more edible medicinal mushroom to the edible mushroom flora of Kerala where agricultural byproducts like sawdust, paddystraw and wood shavings of hardwood trees are available in plenty. This work aimed to evaluate six strains viz. Le 1, Le 2, Le 3, Le 4, Le 5 and Le 6 of *Lentinula edodes* for its cultivation on some lignocellulose rich by-products and their effect in yield when supplemented with rice bran and wheat bran. Mycelial growth and biomass production among the six strains were compared using seven culture media viz., malt extract peptone dextrose agar, malt extract agar, potato dextrose agar, Czapek Dox agar, carrot agar, yeast extract agar and oat meal agar. They were conditioned in an incubator at a controlled temperature of 25 °C. Various grains viz., paddy, wheat, maize, bajra, ragi and sawdust were used as substrates for spawn production. For the substrate supplementation studies, the treatments used were Treatment 1: 80% sawdust and 20% wheat bran, Treatment 2: 80% sawdust and 10% wheat bran, Treatment 3: 80% sawdust and 20% rice bran, Treatment 4: 80% sawdust and 10% rice bran, Treatment 5: paddy straw and 20% wheat bran, Treatment 6: paddy straw and 20% rice bran, Treatment 7: paddy straw alone, Treatment 8: 80% wood shavings of hardwood tree pincoda, 20% rice and wheat bran. The moisture of each substrate mixture were adjusted to 60% by proper mixing and filled in polypropylene bags @500 gm substrate per bag and sterilized in autoclave for 2 hours at 121 °C and 15 lbs pressure. After cooling the substrate was inoculated @300 gm spawn per bag under hygienic condition and kept for incubation. The conditions provided for mycelial run was 25 °C at 80% relative humidity. Bags were allowed to turn brown and opening of bags were done after complete browning and bump formation had taken place in bags. A cold water treatment was given by dipping them in chilled water (4-5 °C) and kept for incubation at 18-20 °C and 85-95% relative humidity. Among the physiological studies, malt extract peptone dextrose agar gave best results in mycelial and biomass production of *Lentinula edodes*. Yield of shiitake was recorded higher with rice spawn. The colonization of different substrates was achieved at durations varying from 60-90 days after inoculation. First harvest was obtained 100 days after inoculation. Maximum number of fruiting bodies developed on teakwood sawdust followed by wood shavings of pincoda. Teakwood sawdust produced maximum biological efficiency in Le 1 (70%) and Le 2 (58%) strain. Twenty percent supplementation of wheat bran was the best among the supplements used. Highest weight of fruiting bodies was observed in Le 1 strain (75 g). In the paddy straw substrate the mycelium colonized the substrate after a much longer time but produced no primordia even under favourable conditions. Le 6 was the least productive with a maximum biological efficiency of 28%. Yield continued upto four to five harvests in two weeks interval for a period of three months.

#### **V-P-27. Use of local glutinous rice (Buhbai) for the mushroom spawn production in Mizoram, North-East, India**

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Glutinous rice (*Oryza sativa* var. *glutinosa*) is a type of rice grown mainly in Northeast part of India, which has opaque grains, very low amylose content, and is especially sticky when cooked. Henceforth, attempts were made to use this locally available rice variety for the production of mushroom spawn by using two species of *Pleurotus* and one species each of *Lentinula* and *Schizophyllum* obtained from Directorate of Mushroom Research (DMR), Solan. Five different treatments (T1 to T5) were tried with gradient in soaking time from 30 minutes to 120 minutes and a last treatment was soaking with boiling for 10 minutes. In treatment T3 soaking the rice for 60 minutes was found to be more promising because the mycelium was grown fully in 9 days and mycelia colonization of 1kg saw dust substrate bag was completed in 12 days.

While spawn preparation for *Pleurotus djamor* took 25 days. There was very less mycelia growth for *Pleurotus sajor caju* and *Lentinula edodes* on the rice grains. We concluded that the rice grain soaking for 60 minutes will be sufficient and energy saving method for the spawn production of *Schizophyllum*. Small scale production is underway for the *Schizophyllum commune*.

## **V-P-28. Utilization of biodiesel industry wastes as substrate for mushroom production and animal feed**

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*Jatropha curcas* is a promising oil seed for high quality bio-diesel production. It presents key features such as: (i) high oil yield per grain, (ii) non-edible oil, (iii) and adaptation to different climates. The residue generated after mechanical oil extraction is called *Jatropha curcas* cake (JCC). The cake is constituted of lignocellulosic fibres, proteins, lipids, soluble carbohydrates and minerals (nitrogen, phosphorus and potassium). These characteristics demonstrate the possibility of the use of this waste as animal feed supplement, organic fertilizer or raw material for production of second generation ethanol (cellulosic). This co-product of bio-diesel production can be used in the production chain of animal feed. However, it is necessary to remove or process the toxic compounds and anti-nutritional factors that are present in the plant biomass. The phorbol esters are the main components of this toxic by-product. The biological effects of these esters in animals depend on the molecular structures that can induce acute inflammatory responses and tumour formation. The macromycetes (mushrooms), mainly basidiomycetes are known for their ability to excrete enzymes such as laccase, manganese peroxidase and lignin peroxidase, capable of degrading lignin structures. Also, they can work as bioremediating agents against pollutants derived from industrial processes such as textile dyes. This work aimed to select basidiomycetes able to grow on and detoxify the JCC generated by the oil extraction process. For the initial screening, we developed a specific culture medium containing agar + JCC in Petri dishes. Fungal growth was measured at 24 hours intervals and those who showed superior mycelial growth (cm/day) were selected for cultivation under solid-state fermentation (SSF) condition. The culturing of selected basidiomycetes was performed by using two treatments: i) 100 g of substrate JCC, and ii) 90 g of cake with supplementation of 10 g of *Jatropha curcas* fruit shell. The mycelia were inoculated at 28 °C with 60% humidity for 30 days. After the biological treatment, the methanolic extraction of the cake/substrate was performed to determinate the remaining phorbol esters levels using UHPLC-DAD. The fungus EF-87 exhibited the best efficiency in the reduction of phorbol ester of the cake, reaching 94% reduction of this compound. Further study by involving nine formulations, using 80% of JCC and 20% supplementation with high lignocellulosic agro-industrial waste, to assess the best treatment for conversion of biomass into fruiting body of the selected fungus is under investigation. Thus, the prospect of this work is to promote detoxification of JCC using biological treatment (mushrooms), enabling the use of this biomass as a substrate for fungi cultivation and its subsequent use for animal feed.

### **V-P-29. Yield performance and element profiling of different strains of *Lentinula edodes* (Berk.) Pegler**

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Shiitake (*Lentinula edodes*) has been reported to boost the immune system, lower cholesterol, function as an anticoagulant and may have use in treatment of some cancers as it contains lentinan, a polysaccharide with strong anti-cancer properties. The fungus is saprophytic and grows on dead material. Five strains (OE-16, OE-22, OE-28, OE-38 and OE-388) of shiitake were grown at Mushroom Research and Training Centre (MRTC), Pantnagar, in the two consecutive years (2011-12 & 2012-13) using wheat straw as substrate, supplemented with wheat bran @10% on the dry weight basis. Data were recorded for spawn run, number of sporophore, yield and weight per fruiting body. Among five strains OE-388 took minimum period (62 and 58 days) for spawn run and produce significantly higher yield (2468.33 and 2070.50 g/08 kg wet wheat straw) and maximum number of sporophores (80.16 and 69.50) in both the years, respectively. Whereas strain OE-28 showed poor result in terms of spawn run and produce minimum no of sporophores. The fruit bodies of these mushrooms were analyzed for Mg, Fe, Mn, Zn, Cu, As and Cr by using Atomic absorption spectroscopy. Amongst five strains, OE-388 exhibited high value of magnesium (109.51 mg/100 g dry wt.), iron (3.83 mg/100 g dry wt.), zinc (7.71 mg/100g dry wt.), manganese (4.59 mg/100 g dry wt.) and copper (1.5 mg/100 g dry wt.) followed by OE-28. However, toxic elements were not detected in any strains.

### **V-P-30. Yield potential of *Pleurotus (sajor-caju) pulmonarius* WC-537(Jacq.Fr) on soybean straw with combined effect of various cellulosic wastes**

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Different substrates were evaluated for cultivation of exotic strain named *Pleurotus (sajor-caju) pulmonarius* WC-537. The mushrooms have long ago drawn attention of human beings as a food and now-a-days it is a leading food component. Among all the three flushes of *Pleurotus sajor-caju pulmonarius* WC-537, the treatment T1 (soybean 100%) produced highest yield of (312.2 g). Nevertheless, treatment T5 (Rice husk 100%) gave very poor yield of (199.6 g) with biological efficiency of 19.96%. The soybean straw proved one of the best growing substrate among others for getting high yield of oyster mushroom and has been recommended for farmer's community to fulfill the gap of malnutrition as it is a cheap source of protein.



### **V-P-31. Use of *Trichoderma* enriched spent mushroom compost (TESMC) for enhancing yield and quality of Kinnow (*Citrus reticulata*)**

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The left over compost after harvesting full or remunerative crop of button mushroom is called as spent mushroom compost (SMC). SMC is good quality organic manure, rich in major and micro-nutrients required for plant growth. Several modes have been documented to recycle the SMC for various purposes i.e. use as organic manure in field and horticultural crops, reclamation of soil and bioremediation of contaminated soil and water. An investigation was carried out at Integrated Farming Systems unit of Project Directorate for Farming Systems Research, Modipuram, Meerut, India, during 2013-14 to evaluate the alternative use of SMC for mass multiplication of *Trichoderma harzianum* and its further use in the form of *Trichoderma* enriched BMSC (TEBMSC) for enhancing the growth and yield of potential fruit crop, Kinnow. The powdered formulation of *T. harzianum* was mixed in button mushroom spent compost to obtain an initial inoculum (0 day) of  $10^4$  cfu/g of the substrate. Colony forming units (cfu) of *T. harzianum* were estimated in the laboratory following serial dilution technique. One month old TEBMSC was applied in root zone of Kinnow @ 25 kg/plant in the month of April (flowering/fruiting stage). Un-inoculated plants were treated as control. The cfu of *T. harzianum*, which was in the range of  $10^4$ /g substrate at the beginning (0 day), reached  $8.72 \times 10^6$ /g substrate at the end of 4<sup>th</sup> week. It indicates better growth and sporulation of *T. harzianum* in the SMC. In Kinnow, the average leaf area in treated plants was 24.40 cm<sup>2</sup> against 14.23 cm<sup>2</sup> in control. The SPAD and NDVI values were also significantly higher in treated plants. The number of fruits/plant (395), average fruit circumference (22.87 cm) and fruit weight (148.57 g) were significantly higher in treated plants as compared to control. There was over 3 folds higher fruit dropping in control plants during May-June and September-October when Kinnow fruits are generally vulnerable for dropping. The increasing leaf area, general greenness and canopy cover; increased fruit numbers, circumference and weight and decreased fruit dropping in TEBMSC treated Kinnow plants clearly exhibited the alternative use of SMC for multiplication of the fungal bio-agent and its potential use in increasing the growth and yield of fruit crops like Kinnow.

### **V-P-32. Technological interventions for saving labour and energy in oyster mushroom cultivation**

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*Pleurotus* species commonly called as 'oyster mushroom' has two major components namely spawn production and crop production. Both these components are indoor activities which require labour, energy and water as the major input resources for successful cultivation. Every operation in oyster mushroom cultivation is manual and requires 3-4 labourers for a small mushroom farm. Energy is mainly utilized during substrate pasteurization, grain boiling and sterilization during spawn (mushroom seed) production and environmental maintenance in spawn running and cropping rooms. Hence, the necessity of the problem was felt to develop and integrate labour and energy efficient technologies. A series of indigenous machinery for mushroom spawn production and cultivation have been designed and developed at IIHR, Bangaluru. The machinery are grain cleaner, grain boiler, boiled grain and chalk powder mixer, bag filler, spawn inoculator, paddy straw soaking, pasteurizing and moisture removing machine. This machinery are useful to reduce the electrical power consumption up to 60% and labour requirement about 50% besides increasing

the labour efficiency and production capacity in comparison to the conventional method. Presently the energy requirement in the process of oyster mushroom spawn production is being met by utilizing electrical, gas, wood and by burning other agricultural wastes. These conventional techniques are expensive, cumbersome and create environmental pollution. The energy requirement of an oyster mushroom farm producing approximately 50 kg of fresh mushrooms/day would be approximately 55kW (electrical), 60 kW (gas) and 99 kW (wood/other agricultural wastes). Hence, system was developed to integrate solar energy in the production process. Water heating was integrated with the evacuated tube solar hot water input system and Scheffler reflectors type solar steam generation system. The commercial vertical autoclave was redesigned and modified to make it suitable for solar energy integration. The mechanized spawn production resulted in doubling spawn production within the same time and 50% labour in comparison to manual method. The solar energy intervention resulted in saving electrical energy up to 45 to 100%. The redesigned vertical autoclave has 30% more available volume with the same energy input in comparison to conventional model.

### **V-P-33. Cultivation of edible mushroom, *Coprinus comatus* (O.F. Müll.) Pers on substrates with addition of anaerobically digested food waste**

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*Coprinus comatus*, known as shaggy mane, is the valuable culinary ingredient, nutritious and healthy. As a member of Agaricales family, *C. comatus* is frequently seen on the lawns, along gravel roads and in waste areas all over the world, although in some areas limited by seasonal occurrence. Conventional cultivation of *C. comatus* is performed as bags or bed cultures, and the substrate used is composted wheat straw, cotton waste, corncobs, rice straw, with chicken manure supplemented by solid sisal waste as the main source of nitrogen triggering the composting process. Recent interest in improving production technology focused on waste utilization, such as pulp and paper waste. Anaerobically digested organic waste is a rich source of nitrogen, with high pH (8.2) which is favourable for mushroom substrate preparation. Renewable energy production in recent times became more popular; therefore, digestate is easily accessible agricultural waste material. The main goal of our investigation was to combine waste from renewable anaerobic biogas production with recyclable waste office paper as a basal component in mushroom substrate for *C. comatus*. Yield, biological efficiency (BE), carpophores morphological features and chemical composition of *C. comatus* were compared. Mushrooms were cultivated on substrates with increasing amounts of dewatered digestate, ranging from 0 to 40% (DW/DW), with intervals of 10%. For the control, commercial compost was used. Dewatered digestate was added at the start of phase I composting. The addition of anaerobically digested organic waste positively affected yield and biological efficiency of investigated species in comparison to control compost. In addition, dry matter content of mushrooms harvested from substrates made with digestate ranged from 6.6 to 15.3%.



### V-P-34. Effect of spent mushroom substrate and vermicompost on cultivation and fungal infections in Lettuce

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Mushroom production is increasing each year and it is leading to increasing amount of post-production wastes generation with high level of organic contents. Spent mushroom substrate (SMS) is considered as a rich source of nitrogen and can be successfully used as organic supplement in vegetables cultivation. Whereas vermicompost, a product of organic substances processing using earthworms, used as substrates supplement increases biological activity and water holding properties. Organic matter enrichment is a viable solution to provide macro elements and improve physical properties of substrate. In the present study, the suitability of vermicompost (VC) and SMS as cultivation substrates supplement, replacing the peat in growing media, and its influence on lettuce cultivation and fungal infections of plants was investigated. Three mixtures comprised of garden compost (GC), vermicompost (VC) and spent mushroom substrate of *Agaricus subrufescens* (SMS) were used for lettuce cultivation. The proportion of SMS was 5, 10 and 15%, while that of GC it was 75, 80 and 85%. Vermicompost was kept @10% in each mixture. Mixture of GC (90%) and VC (10%) was used as a control. Addition of SMS to the cultivation substrates affected the yield of lettuce. All substrates supplemented with SMS gave higher yield of lettuce than the control substrate. Scientific work financed from the funds for science (2012-2015) granted to an international co-funded project No 179/CIPUE/2012.

### V-P-35. Potential use of different lignocellulosic by-products for the cultivation of *Lentinula edodes*: effect on yield and total phenolic content

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*Lentinula edodes* (shiitake) is generally cultivated on hardwood logs, mainly oak trees. However, this cultivation system represents a limiting factor and potential danger to the environment due to the slow growth rate and overuse of the oak, jeopardizing the population of this important forest element. Furthermore, it has been observed that the composition of the substrate influences the chemical content of the harvested carpophores. Therefore, this study evaluated the productivity of four shiitake strains on different lignocellulosic by-products with the objective of correlating the composition of the substrate with the yield of carpophores and their polyphenol content. The IE-40, IE-105, IE-245 and IE-256 strains were evaluated in this study. The fungus was produced using vineyard pruning (VP), sorghum stubble (SS), sugar cane bagasse (CB) and oak shavings (OS) (as control). The productivity of the strains was evaluated based on biological efficiency (BE), production rate (PR) and yield (Y). In order to determine the chemical composition of the substrates and the effect on shiitake growth, the content of NDF, hemicellulose, cellulose and lignin were initially determined and subsequently at 13, 26, and 69 days after inoculation. Total phenolic compounds were quantified by spectrophotometry. For the production of the carpophores, the substrates were maintained at an average moisture of 70% and were supplemented with wheat bran, Ca (OH)<sub>2</sub> and CaSO<sub>4</sub>. In polypropylene bags (19.5 x 48 cm) 1.2 kg (wet weight) of substrate was added and sterilized for 1.5 h at 121 °C. Then spawn previously prepared from each strain was added at 5% (w/w). The largest BE was obtained for IE-256 SS (145.11%), IE-245 SS (142.61%) and IE-105 VP (110.23%). The highest PR was observed for IE-245 SS (1.69%), IE-256 SS (1.57%) and IE-105 SS (1.34%), while the highest Y was

recorded for IE-256 SS (41.96%), IE-245 SS (41.23%) and IE-105 SS (35.77%). The fiber results demonstrated that carpophores were significantly affected by growth period, strain variety, substrate type and the selected combinations of strains and substrates. In general, it was observed that VP and SS had the highest percentage of biodegradation, where the highest hemicellulose and cellulose degradation occurred with IE-256 strain and the highest lignin degradation with IE-245 strain. The analysis of variance indicated that substrate ( $p<0.0001$ ), strain ( $p<0.0001$ ), and interaction substrate-strain ( $p<0.0001$ ) had a significant effect on the content of phenolic compounds in the carpophores. The lowest and highest values were obtained with IE-256 strain on OS (1.5983 mg EAG\*g<sup>-1</sup>) and SS (2.7197 mg EAG\*g<sup>-1</sup>), respectively, which verifies that the interaction of substrate-strain was highly significant. SS was the best substrate for producing carpophores, while the IE-245 strain presented the greatest values for BE, PR and Y. Thus, the carpophores of the IE-256 strain cultivated in SS could potentially present greater antioxidant activity due to highest polyphenols content.



**Session-VI**  
**Myco-molecules, Medicinal,  
Nutritional and Nutraceutical  
Properties**



## Keynote Presentations

### VI-K-1. Potential of mushroom bioactive molecules to develop healthcare biotech products

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Since ancient times mushrooms were widely appreciated all over the world for their nutritional value and medicinal properties. They have low fat, high protein and vitamins contents. Mushrooms contain several minerals and trace elements, as well as substantial amount of dietary fibers. Modern scientific data has documented that they are also producers of bioactive molecules. Basidiomycetes mushrooms (phylum Basidiomycota) including agaric and bracket fungi are natural source of bioactive molecules and valuable enzymes with different therapeutic effects. Therefore, they are considered as perspective organisms to develop different healthcare biotech-product.

The main groups of bioactive molecules produced by different mushrooms are polysaccharides, terpenoids, phenolics, lectins, etc. Research on medicinal mushrooms has markedly increased due to therapeutic potential of these compounds. More than 126 therapeutic effects (immune-modulating, antimicrobial, antiviral, antioxidant, hypocholesterolemic, etc.) of bioactive molecules with mushroom origin were described.

Nowadays, interest to biotechnological cultivation of Basidiomycetes mushrooms is related with the growing demand of different mushroom-based biotech-products in pharmaceutical, food and cosmetic industries. Two approaches - fruiting bodies production and mycelia cultivation are currently used in biotechnological cultivation of mushrooms. Fast mycelial growth and easy reproduction in culture conditions is assisting biotechnological cultivation of medicinal mushrooms. The submerged cultivation of mycelia has significant industrial potential and it is the best technique to obtain biomass and desired bioactive molecules for further development of consistent and safe healthcare mushroom-based biotech-products.

Presently, several pharmaceuticals (krestin, lentinane, coriolan, schyzophyllan, etc.) formulated from medicinal mushrooms are available in the world market. The majority of mushroom products possesses beneficial health effects owing to the synergistic action of present bioactive molecules and can be used on a regular basis without harm. Nutritive, anti-inflammatory, regenerative and antioxidant properties of several mushrooms (*Lentinula edodes*, *Ganoderma lucidum*, *Fomes officinalis*, *Tremella* species) makes their usage perspective in manufacturing of cosmetic products. A new area of application of mushroom biotech-products is formulation of balanced food for pets.

Establishment and maintenance of culture collections play important role in studies of biodiversity, genetic resources and biotechnological potential of Basidiomycetes mushrooms.

### VI-K-2. Advances in cultivation of medicinal fungi biomass and pharmaceutical compounds in bioreactors

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Medicinal mushrooms *Ganoderma lucidum* (W.Curt.:Fr.) Lloyd, *Grifola frondosa*, *Trametes versicolor* and *Hericium erinaceus* were cultivated by a submerged liquid substrate cultivation as well as in solid



state cultivation in various types of bioreactors. Laboratory and pilot bubble columns, fix bed, stirred tank and horizontal stirred tank reactors were used. Extracellular and intracellular polysaccharides were obtained by extraction, ethanol precipitation, and purification by ion-exchange, gel and affinity chromatography. The results showed that polysaccharides from *G. lucidum* induced moderate to high amounts of innate inflammatory cytokines. Fungal intracellular polysaccharides were stronger innate inflammatory cytokines inducers while extracellular polysaccharides demonstrated higher capacity to modulate cytokine responses of IONO+PMA activated lymphocytes. The results indicate that *G. lucidum* polysaccharides enhance Th1 response with high levels of IFN- $\gamma$  and IL-2, and display low to no impact on IL-4 production. Similar pattern was observed at regulatory cytokine IL-10. All the fractions tested enhanced IL-17 production at different levels. After obtaining different fractions of two polysaccharide groups, polysaccharide-protein complexes (PPK) and polysaccharides with removed proteins (P), the cytokine responses of peripheral blood mononuclear cells from leukocyte concentrates (PBMCs) were studied *in vitro*. The cells were activated by various polysaccharide fractions and hence the ability to induce inflammation in a cell culture was determined by the measurement of TNF- $\alpha$ , IFN- $\gamma$  and IL-12. The ability of different polysaccharide fractions to modulate cytokine responses of lymphocytes, activated with polyclonal activators ionomycin (IONO) and forbol-12-myristate-13-acetate (PMA) was studied. The concentration of IL-2, IL-4, IFN- $\gamma$ , IL-10 and IL-17 in the cell cultures was determined. Polysaccharides stimulated cytokine responses of polyclonal activated lymphocytes and polarized into Th1 response. The strength of responses was dependent on the type, purity and concentration of polysaccharide fractions. Intracellular polysaccharides had a greater impact on the inflammatory immune response, however, exopolysaccharides were stronger stimulators of the immune response of T lymphocytes. Present research has also revealed the effects of polysaccharides on synthesis and secretion of IL-17, what represents a new, not yet published original contribution to the knowledge of controlling effector functions of lymphocytes.

## Oral Presentations

### VI-O-1. Ameliorative potential of polysaccharides from *Calocybe indica* fruiting bodies on oxidative stress in STZ induced diabetic rats

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The present study was undertaken to evaluate the possible protective effects of *Calocybe indica* fruiting body polysaccharides (CIFBP) against oxidative stress in streptozotocin (STZ) induced diabetic rats. Diabetes was induced in overnight fasted adult Wistar strain albino female rats weighing 150-180 g by a single intraperitoneal injection of freshly prepared streptozotocin (60 mg/kg body weight) in 0.1 M citrate buffer (pH 4.5) and NAD (110 mg/kg body weight). Three days after STZ induction, diabetic rats received CIFBP orally at the doses of 200 and 400 mg/kg daily for 30 days. The effects of CIFBP on glucose, glycosylated haemoglobin (HBA1C), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), glutathione reductase (GR), reduced glutathione (GSH), vitamin C, thiobarbituric acid reactive substances (TBARS) and hydroperoxide in serum, liver, kidney and pancreas were studied. The levels of glucose, HBA1C, TBARS and hydroperoxide were increased significantly whereas the activities of enzymic and levels of non-enzymic antioxidants were decreased in STZ induced diabetic rats. Administration of CIFBP to diabetic rats showed a decrease in glucose, HBA1C, TBARS and hydroperoxide. In addition, the activities of enzymic and levels of non-enzymic antioxidants were increased in CIFBP treated diabetic rats. The above findings were supported by histological observations of the liver, kidney and pancreas. The antioxidant effect of CIFBP was compared with glibenclamide, a well-known and anti-hyperglycemic drug. The present study indicates that the CIFBP possesses a significant favourable effect on anti-oxidant defense system in addition to its anti-diabetic effect.

### VI-O-2. Antifungal activity of *Fomitopsis pinicola* collections against potentially pathogenic for humans and animals filamentous fungi

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It is reported that polypore mushrooms (Basidiomycota, Polyporales) from different genera (*Ganoderma*, *Coriolus*, *Fomes*, *Fomitopsis*, etc) are producers of biologically active metabolites, such as polysaccharides, triterpens, phenolics, flavonoids and other compounds with immune-modulating, hypoglycemic, antibacterial, antifungal, antioxidant and other therapeutic effects. Medicinal properties of polypore mushrooms have been used in traditional medicine of many countries. Modern scientific data has documented that polypores represent an unlimited source of bioactive metabolites and are an example of molecular diversity with recognized potential in drug discovery and development. In nowadays biotech-products, such as dietary supplements and functional food additives with different formulations (tablets, powders, teas, etc.) obtained from biotechnologically produced fruiting bodies and cultivated mycelium of polypores are available in the world market. In the current study antifungal activity of 7 collections of polypore species *Fomitopsis pinicola* with different geographical origin (France and Russia) is reported. Experiments were carried out by two approaches: in dual culture by growing *F. pinicola* collections with potentially pathogenic for humans and animals test filamentous fungi (*Chrysosporium keratinophilum*,

*Penicillium griseofulvum* and *Penicillium* sp.) in Petri dishes using PDA (potato-dextrose agar) medium and by added cultural liquid samples of *F. pinicola* collections obtained after 2 weeks submerged growth of mycelia in malt-extract medium into PDA (100 ml PDA was added by 75 ml cultural liquid). The obtained results showed that in dual culture experiment collections of *F. pinicola* in 66.7% of antagonistic interactions overgrew on colonies of filamentous fungi. In 33.3% of antagonistic interactions mutual inhibition of contacted colonies was described. No overgrowth by test filamentous fungi on colonies of *F. pinicola* was observed. The samples of cultural liquid of *F. pinicola* collections significantly suppressed growth rate and sporulation of filamentous fungi, particularly tested *Penicillium* species on agar media. In our experimental conditions fungistatic effect was observed toward colony of *C. keratinophilum* by cultural liquid of strain Ha-2 of *F. pinicola*. Thus, mycelium of *F. pinicola* is producer of extracellular antifungal metabolites and possesses antifungal activity against potentially pathogenic for humans and animals filamentous fungi. Further studies of antifungal activity of mycelial extract of *F. pinicola* will assist to develop antimycotic biotech products from this mushroom.

### **VI-O-3. Antioxidant properties of methanolic and aqueous extract from *Lentinula edodes* cap and stipe**

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Mushrooms have been part of the human diet for thousands of years. Edible mushrooms are traditionally used in many countries as food and medicine. Recently, they have been become an attractive functional food mainly because of their chemical composition and this can be explained by the antioxidant capacity of mushrooms to scavenge free radicals which are responsible for oxidative damage of lipids, proteins and nucleic acids. Mushrooms accumulate a variety of secondary metabolites including phenolic compounds, terpenes and steroids. The antioxidant metabolites present in mushrooms is of great interest as protective agents to help the human body in reducing oxidative damage without any interference. Although research has focused mainly on the therapeutic effects and cultivation methods of mushrooms, little information is available about their biological properties. In addition there are limited data in the literature concerning the antioxidant properties of the mushrooms from India. Hence, the main objective of the present study was to determine the anti-oxidative properties of methanolic and water extract from *Lentinula edodes* cap and stipe. Freshly harvested mushroom fruiting bodies were shade dried and grinded in a super mill grinder. Mushroom powder (2 g) was extracted with methanol and water separately, using semiautomatic soxhlet apparatus. The methanolic and aqueous extracts from cap and stipe of *Lentinula edodes* were evaporated at 40 °C to dryness, re-dissolved in methanol and stored at 4 °C to measure antioxidant activities and related parameters. Estimation of different antioxidant activities and bioactive compounds was carried out in triplicate. The findings indicated that different mushroom extracts contained 2.40-5.60 mg GAE of phenolics/g extract, 1.23-3.26 mg catechins equi. of tannins, and 2.3-11.9 mg ascorbic acid per gram of extract. The assayed mushrooms contained 4.40–89.58% DPPH scavenging activity, 9.32-99.61% ABTS scavenging activity, FRAP value of 23.17-332.77 µM FeSO<sub>4</sub> equivalent, total antioxidant activity of 67.81-215.49 µM trolox equivalent, reducing power of 0.16-0.31 and 7.61-88.86% of metal chelating activity. Two way clusters based on ward method showed that aqueous extract from cap and stipe contained higher antioxidant potential in comparison to methanolic extract. In present investigation, it was found that all the extract present antioxidant potential, especially high for water extract from cap of *L. edodes*. The variety of compounds and the antioxidant potential revealed by *L. edodes* represent an important contribution of their possible beneficial effect for human health.

**VI-O-4. Antiproliferative activity of bioactive compounds from mushrooms of Indian isolates****Venkatesan Kaviyarasan\* and Ramalingam Shenbhagaraman***Centre for Advanced studies in Botany, University of Madras, Chennai, Tamil Nadu, India**Department of NanoTechnology, Anna University, Chennai, Tamil Nadu, India**manikavi53@gmail.com*

Antiviral and antiproliferative therapeutics compounds of allopathic origin in general are known to have more non-target effects. Hence the world is searching for the bioactive compounds from natural sources, which can act as medicine and nutrition without many side effects. Mushrooms have been valued as nutritional and medicinal resources. Bioactive compounds from mushrooms are the best known as potent substances with anti-proliferative and immunomodulating properties. Although the process of isolation, structural characterization and antitumor activity of mushroom bioactive compounds have been extensively investigated in the past three decades, the relationship between the antitumor activity and the chemical composition as well as the high order structure of their active components is still not well established. In our study we have isolated bioactive compounds from indigenous mushroom isolates such as *Lentinus tuberregium*, *Neolentinus kauffmanii* and *Trametes hirsuta*. The isolated bioactive compounds were terpenoid, steroid and polysaccharide in nature. The studies on these bioactive compounds showed that these substances are active against many cancer cell lines such as breast, liver, lung, ovarian and prostate cancer through their antiproliferative therapeutic properties.

**VI-O-5. *Calocybe indica* polysaccharides alleviates cognitive impairment, mitochondrial dysfunction and oxidative stress induced by D-galactose in mice****Sudha Govindan<sup>1\*</sup>, Pramila Jaba<sup>2</sup>, Elizabeth Johnson Elcy Rani<sup>2</sup>, Jayasakthi Shanmugam<sup>2</sup> and Sadish Kumar Palur Manoharan<sup>1</sup>**<sup>1</sup>*Department of Biochemistry, Periyar University, Salem, Tamil Nadu, India*<sup>2</sup>*Department of Biochemistry, Kongunadu Arts and Science College, Coimbatore, Tamil Nadu, India**sudhabiochempu@gmail.com*

Mushrooms have been reported to have a therapeutic potential against several age related processes. The aim of this study was to investigate the protective effect of *Calocybe indica* crude polysaccharides (CICP) against D-galactose induced cognitive dysfunction, oxidative damage and mitochondrial dysfunction in mice. Mice were subcutaneously injected with D-galactose (150 mg/kg per day) for 6 weeks and were administered CICP simultaneously. Aged mice receiving vitamin E (100 mg/kg) served as positive control. Chronic administration of D-galactose significantly impaired cognitive performance oxidative defense and mitochondrial enzymes activities as compared to control group. The results showed that CICP (200 and 400 mg/kg) treatment significantly improved the learning and memory ability in Morris water maze test. Biochemical examination revealed that CICP significantly increased the decreased activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST), mitochondrial enzymes-NADH dehydrogenase, malate dehydrogenase (MDH), isocitrate dehydrogenase (ICDH), Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup> ATPase activities, elevated the lowered total anti-oxidation capability (TAOC), glutathione (GSH), vitamin C and decreased the raised acetylcholinesterase (AChE) activities, malondialdehyde (MDA), hydroperoxide (HPO), protein carbonyls (PCO), advanced oxidation protein products (AOPP) levels in brain of aging mice induced by D-gal in a dose-dependent manner. In conclusion, present study highlights the potential role of CICP against D-galactose induced cognitive impairment, biochemical and mitochondrial dysfunction in mice.

## **VI-O-6. Cardioprotective and antioxidant activities of polysaccharide from *Auricularia polytricha***

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The present study evaluated the protective potential of polysaccharide extract of *Auricularia polytricha* (APP) against isoproterenol (ISO) induced myocardial infarction in rats. The rats were pre-treated orally with (APP) extract at two different doses (250, 500 mg/kg) daily for a period of 21 days. After the treatment period, ISO (85 mg/kg) was subcutaneously injected to rats at an interval of 24 h for 2 days. Rats receiving a-tocopherol (100 mg/kg; p.o.) served as positive control. Isoproterenol caused a significant increase in the activity of cardiac marker enzymes like CK, CK-MB, LDH, AST and ALT. Oxidative stress produced by isoproterenol was significantly lowered by the administration of APP which was evident from increased activities of antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase) and reduced concentration of lipid peroxidation products (TBARS and conjugated dienes). The levels of vitamin C, protein sulfhydryl groups and reduced glutathione (GSH) were also high in APP pretreated rats. The histopathological studies also showed that APP treatment significantly minimized the damage induced by isoproterenol. Results of our in vitro findings also confirmed that APP exhibits significant reducing power, lipid peroxidation inhibition and radical scavenging activity against DPPH, ABTS radicals. These findings provided evidence that APP was found to be protecting the myocardium against ischemic insult and the protective effect could attribute to its antioxidative and antihyperlipidemic activities. Thus, APP provided cardioprotection against isoproterenol induced myocardial infarction in rats.

## **VI-O-7. Comparison of the anti-tumor activities of polysaccharides from 7 macrofungi against lewis lung-cancer**

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The number of macrofungi is estimated at 15,000 species in the world. Macrofungi comprise vast and yet largely untapped source for powerful new pharmaceutical products, in particular, and most importantly for modern medicine. The anti-tumor activity differences of 7 selected macrofungi against Lewis lung cancer were studied in this paper to supply scientific basis for future development and utilization of macrofungi. Experiments were carried out by extracting polysaccharides from the macrofungi with hot water, applying to mice via gastric perfusion, taking mice applied with cyclophosphamide (CTX) and normal saline as contrast, and detecting tumor suppressing rates, thymus indices, and spleen indices. The experimental results show that: CTX has highest tumor suppressing rate against Lewis lung cancer but low thymus index ( $0.49 \pm 0.14$ ) and spleen index ( $2.11 \pm 0.34$ ); all the polysaccharides extracted from the tested macrofungi have certain suppressing effect on solid tumor of Lewis lung cancer, and have tumor suppressing rate of 11-86.8%. The polysaccharide from *Coriolus versicolor* has best tumor suppressing effect and has tumor suppressing rate up to 74.3-86.8%. The polysaccharides from *Coprinus comatus* and *Schizophyllum commune* have worst tumor suppressing effect and tumor suppressing rates lower than 27% and 21%, respectively. The experiments also indicate that the thymus indices of the polysaccharides from the macrofungi except *Cordyceps sobolifera* are higher than that of CTX group; and the polysaccharides from the macrofungi have higher spleen indices than that of CTX group. The polysaccharides except PAP and LSP have spleen indices higher than that of normal saline group, and the difference is significant. The 7 macrofungi polysaccharides have good effect in suppressing tumor,



and recovering and protecting thymic and splenic functions, and have the tumor growth suppressing mechanism probably realized through regulating immunologic function.

#### **VI-O-8. Effect of culinary medicinal mushrooms, *Pleurotus ostreatus* and *P. cystidiosus* on fasting and postprandial glycaemia in healthy volunteers**

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*Pleurotus ostreatus* (Jacq.: Fr.) P. Kumm. and *P. cystidiosus* O.K. Miller (*Pleurotaceae*, higher Basidiomycetes) are culinary-medicinal mushrooms grown worldwide. The *P. ostreatus* and *P. cystidiosus* commonly known as American oyster and abalone, respectively were shown to possess antioxidant, antitumor, antinociceptive, antifungal, hypocholesterolaemic and hepatoprotective activity. We have reported the promising oral hypoglycaemic potential of *P. ostreatus* and *P. cystidiosus* in both normal and alloxan-induced diabetic rats as well as the anti-inflammatory activity of *P. ostreatus* in our previous studies. According to our previous findings, the *P. ostreatus* and *P. cystidiosus* did not exert any toxic effects after long term administration to rats. This study evaluates the effect of *P. ostreatus* and *P. cystidiosus* on the fasting and postprandial serum glucose levels in healthy volunteers. Safety of *P. ostreatus* and *P. cystidiosus* after long term consumption by healthy volunteers was also investigated. Fasting serum glucose levels of healthy volunteers (n=22/group) were measured and distilled water was administered as the control. Postprandial serum glucose levels were measured after 2 hours of administration of 75 g of glucose. Two groups of subjects (test 1 & test 2) received suspensions of freeze dried and powdered *P.ostreatus* or *P.cystidiosus* at a dose of 50 mg/kg for two weeks, respectively. At the end of two weeks, fasting serum glucose levels were determined. The same procedure was repeated with administration of suspensions of freeze dried and powdered *P. ostreatus* or *P. cystidiosus* and postprandial serum glucose levels were measured. The healthy volunteers were monitored for one month for any adverse effects and at the end of the one month period, serum levels of ALT, AST, ALP, Gamma GT and creatinine were determined. Creatinine clearance was also estimated. The results were analyzed for statistical significance using Student's t test. Statistical analysis was done using SPSS 17. *p* values <0.05 were considered as significant. There was a significant fasting and postprandial serum glucose reduction in *P.ostreatus* or *P.cystidiosus* groups when compared with respective control groups (*p*<0.05). The percentage reduction in the fasting serum glucose levels for *P. ostreatus* and *P. cystidiosus* groups were 6.1 % and 6.4 % respectively and the postprandial serum glucose reductions were 16.4 % and 12.1 %. There were no significant differences in serum levels of liver enzymes, creatinine as well as estimated creatinine clearance before and after one month from the treatment. This indicated that the consumption of *P. ostreatus* and *P. cystidiosus* over a period of time did not cause any significant hepato cellular damage and detrimental effects in the renal system of the healthy volunteers. In conclusion, significant decrease in both fasting and postprandial serum glucose levels in healthy volunteers after administration of *P. ostreatus* and *P. cystidiosus* suggest that long-term consumption of *P. ostreatus* and *P. cystidiosus* may be beneficial to mankind. Moreover, as it is considered that tight glycaemic control is essential in lowering the risk of developing complications of diabetes, *P. ostreatus* and *P. cystidiosus* may possess immense value as a functional food for improving glucose control. Hence, this study confirms the suitability of *P. ostreatus* and *P. cystidiosus* as a functional food for diabetics.



### **VI-O-9. *Ganoderma lucidum* (Curtis:Fr.) P.Karst: a unique natural product useful as adjunct in the treatment of cardiovascular diseases**

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Despite the substantial improvements in the treatment of cardiovascular diseases (CVD) such as atherosclerosis, ischemic heart disease, heart failure (HF), stroke, and hypertension. CVD still remains one of the major causes of human mortality. Advances in the mitochondrial research during the last decade focused on the preservation of its function in the myocardium, which is vital for the cellular energy production. Mitochondrial dysfunctions increase the risk for a large number of human diseases, including CVDs. Since, slowly dividing/post-mitotic cardiac myocytes are highly dependent on energy produced from oxidative phosphorylation, the cardiac myocardium will be affected especially when the proportion of the damaged mitochondria become considerably high. Chronic increases in oxygen free radical production can lead to a catastrophic cycle of mtDNA damage, mtDNA copy number as well as decline in the activity of complexes I, III and IV of electron transport chain (ETC) as well as cellular injury status. Recent investigations in our laboratory have demonstrated that *Ganoderma lucidum* was able to alleviate the declined complex I-IV activities of ETC in the isoproterenol-induced myocardial infarction in rat. Furthermore, the cardioprotective effect of *G. lucidum* is supported by its protective effect against the doxorubicin induced myocardial damage in rat. In both models, the protective effect was found to be due to the significant reactive oxygen species scavenging, increasing the antioxidant enzymes and Krebs cycle dehydrogenases activities in the cardiomyocytes. Several phytochemicals mainly terpenoids, adenosine and guanosine were experimentally proved to be beneficial in CVDs. Fruiting body of *G. lucidum* is reported as a treasure chest of bioactive compounds. The results of the studies indicate that *G. lucidum* possessed significant cardioprotective effects. The distinctive cardioprotective activity of *G. lucidum* suggests that it qualifies to be a unique adjunct in the conventional standard therapy for treating heart diseases.

### **VI-O-10. Hypolipidemic and antioxidant effects of polysaccharides from *Tremella fuciformis* in hyperlipidemic rats induced by high-fat diet**

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A high-fat diet (HFD) has been reported to adversely affect health. The prevalence of dyslipidemia resulting from excess energy intake and physical inactivity is increasing worldwide. Abnormal lipid metabolism is a main cause of dyslipidemia, which is a major risk factor for cardiovascular disease, obesity and overall mortality. It has been reported that high levels of fat increase fat-mediated oxidative stress and decrease antioxidative enzyme activity. The current study evaluated the *in vitro* antioxidant activity and hypolipidemic effect of *T. fuciformis* polysaccharides (TFP) in the hyperlipidemia rats induced by a high-fat diet. *In vitro* antioxidant activity of the polysaccharide extracts was assessed by using the DMPD, ABTS, OH radical scavenging assay, Ferric Reducing Antioxidant Power assay (FRAP), ferrous ion chelation and lipid peroxidation inhibition assay. Crude polysaccharide extract of *T. fuciformis* (250 and 500 mg/kg, p.o.) was administered to the high fat-diet induced hyperlipidemic rats for 30 days to evaluate its antihyperlipidemic activity. Atorvastatin (10 mg/kg; p.o.) was used as a standard drug. At doses of 250 and 500 mg/kg, oral administration of TFP to hyperlipidemia rats was highly effective in decreasing the levels of serum total cholesterol (TC), triacylglycerols (TG), low-density lipoprotein cholesterol (LDL-C), phospholipids and increasing the levels of serum high-density lipoprotein-cholesterol (HDL-C). TFP significantly suppressed lipidperoxidation by decreasing malondaldehyde and increasing antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase and non-enzymic

antioxidants glutathione and vitamin C in serum and liver. Besides, histological morphology examination showed that TFP prevented the damage of liver tissues induced by high-fat diet. The results demonstrated that the polysaccharide extracts possess radical scavenging and antioxidant activity. Thus, the findings indicate that TFP might provide protection against cardiovascular diseases.

#### **VI-O-11. Prebiotic activity of polysaccharides extracted from some *Pleurotus* sp. from Konkan region of Maharashtra, India**

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*Pleurotus* is an edible mushroom commonly found in the Western Ghats of Maharashtra, India. We get around 16 species of *Pleurotus* in the Konkan region of Maharashtra, of which *P. citrinopileatus* Singer, *P. cytidiosus* Miller, *P. djamor* (Fr.) Boedeijn, Kummer, *P. eous* (Berk) Sacc. *P. floridanus* Singer, *P. ostreatus* (Jacq.) P. Kummer, *P. sajor-caju* (Fr.) Singer are very common. These mushrooms are easily cultivated at low cost hence the polysaccharides obtained from fruit body of some of these mushrooms were evaluated for their prebiotic activity. Polysaccharides were extracted from fruit bodies by hot water and alkali extraction. Some of the crude polysaccharides of *Pleurotus* species exhibited potential prebiotic activity when tested against four probiotic strains of *Lactobacillus* viz., *L. lactis* (ATCC No. 8000), *L. acidophilus* (ATCC No. 4963), *L. plantarum* (ATCC No. 8014) and *L. bulgaricus* (ATCC No. 8001) as a carbon source. These polysaccharides exhibited significant increase in the growth of *Lactobacillus* as compared to 1% (w/v) Lactulose (Duphalac, Abbott) used as a positive control.

#### **VI-O-12. Prospective aspects of myco-chrome as promising future textiles**

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Colour plays a vital role in life of each and every one through industries like textiles, paint, food, clothing, art and cosmetics. The traditional natural dyes from the plants were quickly replaced by synthetic dyes even since the discovery of synthetic organic dye, mauveine by William Henry Perkin in 1856. Thousands of synthetic dyes have been prepared with several advantages like low cost, vast range of new colours and ability to impart better properties upon the dyed materials. Considerable research work is being undertaken around the world on the application of natural dyes. The industries are continuously looking for cheaper, more environment friendly routes to existing dyes in order to minimize the damage to the environment. Pigments from microbes especially fungi are considered as a good alternative to hazardous synthetic dyes. In this paper experience of working with different dyes and pigments from *Polyporus* sp. and their application as mushroom dyes for textile industries is presented. The work was initiated from isolation, followed by production of dyes on mycelial cultures, simple and cost effective cultivation with successful fruiting body production and extraction of dyes and pigments, and application of the mushroom dyes with cotton and silk yarns and fabrics. Pilot scale cultivation was successfully carried out and dyeing experiments were carried out at an industry, where the pigments and dyes were tested for their suitability as mushroom dyes.

## VI-O-13. Status of edible and medicinal mushroom research in Sri Lanka

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Sri Lanka is an island country situated 32 km away from southern most part of India and covers an area of 65,610 square kilometres. Despite its relatively small land area, the island is blessed with rich biodiversity similar to that of Western Ghats of India. This paper discusses the present status of edible and medicinal mushroom research in Sri Lanka and suggests future needs for a sustainable expansion of mushroom sector. The cultivable mushrooms were introduced to Sri Lanka in 1985 under the auspices of the World Bank funded UNDP program and *Pleurotus ostreatus* (oyster mushrooms) is acclaimed as the first domesticated species. Although *Pleurotus* mushrooms are the preferred species in terms of production and consumption, straw mushrooms, milky mushrooms and button mushrooms are also popular but cultivated on a lesser enormity. In view of the scarcity of mostly used softwood sawdust, used for *Pleurotus* cultivation, several cost effective substrate mixtures have been formulated as plausible alternatives. This has enabled a two fold increase in yield. Bioassays revealed antioxidant activity and cytotoxicity of *P. cystidiosus* against Hep-2 carcinoma cells and also its pain alleviating potential. In straw mushroom (*Volvariella volvacea*) research, indoor cultivation using amended straw substrate was found superior to the conventional outdoor method. Further research reported that ergosterol peroxide, a compound isolated from *V. volvacea* had significant antibacterial activity against *Escherichia coli*. Research on medicinal mushrooms is still at its preliminary level. A protocol has been developed for the bag cultivation of *Ganoderma lucidum* using sawdust based substrates. Although button mushroom (*Agaricus bisporus*) cultivation is relatively new and confined to areas at higher altitude above 300 meters, the growers have found a niche export market. *Cyrtospora bifacies* and *Gyrophanena* species have been identified as major pests of oyster mushrooms and integrated measures have been adopted to effectively control them through non chemical strategies paying major emphasis on pest exclusion. Four species of fungi namely *Aspergillus fumigatus*, *Chetomium thermophile*, *Mucor pusillus* and *Trichoderma harzianum* were identified from straw and oyster compost substrates with reported anti bacterial activity against three human pathogenic bacteria, at relatively low concentrations (10-40 µg). Multifaceted utility of spent mushroom substrate was enumerated firstly as a mushroom substrate component and secondly as bio-fertilizer and bio-pesticide in leafy vegetable cultivation. Studies also revealed dienecompounds isolated from *P. cystidiosus* could effectively control *Colletotrichum gleosporioides* at 40 percent level. Several value added mushroom products have been developed to prolong the shelf life of mushrooms. As a future strategy, comprehensive ethno mycological studies are needed to explore the indigenous mushrooms in Sri Lanka and to investigate their nutritional and therapeutic properties. Precise identification tools need to be formulated since there have been few studies on taxonomy and phylogeny of mushrooms in recent times. Introduction of exotic mushrooms and the application of conventional and molecular tools for crop improvement are also envisaged in the expansion of the mushroom industry.

## VI-O-14. Histomorphometric and biomechanical analyses of the axial and peripheral skeleton in rats treated with mushroom extract

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Effect of mushroom extracts on morphological and biomechanical parameters of vertebrae, diaphysis and metaphysis of femurs and articular cartilage in ovariectomized rats investigated. Four- month-old female Sprague Dawley rats were ovariectomized (OVX) or sham operated and treated for seven weeks.

Animals were operated under general anesthesia using an IP injection of ketamine (0.8 ml/kg) and diazepam (0.6 ml/kg). A small incision was made on the lower ventral side of the animal through which ovaries were clamped and excised. The surgical incision was closed with three sutures and disinfected. SHAM animals were only opened and ovaries were touched by a sterile needle. After ovariectomy animals were randomly assigned into 4 experimental groups according to BMD values measured on the DXA device: (1) Negative control – SHAM (n=6); (2) Positive control - OVX + vehicle (n=8); (3) Group B - OVX + extract B (n=8); (4) Group C- OVX + extract C (n=8). Two blended mushroom extracts (B and C) were dissolved in H<sub>2</sub>O and administered in a dose of 1.5 g/kg of animal weight. Therapy was administered using an oral gavage six days per week. 2 ml of vehicle or extract suspension was administered while SHAM rats were left untreated. After seven weeks of therapy hind limb with the preservation of the knee was collected for the ex vivo analyses. Tissues were fixed in freshly prepared 4% buffered paraformaldehyde for 48 h and afterwards stored in 70% ethanol. Biomechanical testing was performed by using the three point bending test for the cortical bone and the indentation test for the trabecular bone. Micro CT analysis was performed using a Sky Scan 1076  $\mu$ CT device. The cortical and trabecular bone were analyzed at the site of distal femur and lumbar vertebrae. The measured parameters included thickness and structure of joint cartilage and underlying epiphyseal bone above the epiphyseal growth plate. Percentage of the trabecular bone in the epiphyseal region of distal femurs and vertebrae was quantified and data regarding trabecular number, thicknesses and separation were provided. SHAM animals had significantly higher bone volume than ovariectomized rats. Mushroom extract B increased the bone volume and strength of vertebrae, while at the distal femur trabecular number and volume was increased in the epiphyseal area above the articular cartilage of the knee joint which supports the normal function of the joint surface cartilage. Collectively, in OVX rats, mushroom B extract suppressed the axial bone loss, juxta-articular osteoporosis and prevented subchondral bone loss.

## VI-O-15. Mushrooms in longevity and health care: contribution of Taurine

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Longevity is not an isolation identity; in fact it is a dependent consequence of sound health, which in term is composite in nature; build up and maintain through strengthen immune system and host defence management. The best example of longevity is Okinawa inland of Japan, regarded as Island of longevity with highest life span, due to disease fighting menu of islanders. Since ancient time, their menu is compose of aquatic sources; fish, red alga (lever for making sushi) and other marine products along with variety of mushrooms. While there was no definite answer how it help to keep fit and stay longer, but modern analytical tool conforms the presence of several active known health ingredients; but surprise presence of a common molecules a sulphur containing amino acid Taurine in all these nutrients sources; fish/liver mushrooms, indicating a unified command of action mechanism. A large section of world community bearing oriental nations, till recently has not accepted mushrooms on menu plate as it belongs to such part of plant kingdom which gives bitter impression and this label has hampered its acceptability for long. Mushrooms has wide range of therapeutic properties; anti-oxidants, anti-hypertension, cholesterol lowering, liver protection, anti inflammatory, anti diabetic, anti virus, anti-microbial and many more but the happiest addition to this long list is, its immunological and anti cancer properties. Mushroom taurine interaction is a significance finding, with high content of Taurine in *Ganodrema lucidum* which ranges; 37-83  $\mu$ mole/100g (Fresh wet), another mushroom, *Lentinus velutips* contains highest level of taurine; 83.2  $\mu$ mole/100g. *Agaricus bisporus* and *Lentinus edodes* also contains relatively higher level; 49-65  $\mu$ moles/100g, where as oyster and *Pleurotus* containing 8.9 and 3.7. Besides all above, shiitake also contains high amount of taurine and king oyster contains 48 mg/100g. Several extracts using various solvent systems provides taurine contents of *G. lucidum* as high as 140 mg/100g to 157mg. Besides this many other mushrooms also contains good amount of taurine. *G. lucidum* which in china known as Lingzhi but is



Japan it is Reishi or Manner take. This mushroom is regarded as double agent with spiritual potency and essence of immortality with answer to success, well being, divine power and longevity. Its application in health benefits has a long list; to name few; diabetes, modulation of immune system, hepato-protection, neuro and cardio protection to prevention of modern diseases like cancer, AIDS. In similar way Taurine; which is chemically, 2-amino ethane Sulfonic acid is an endogenous natural substance constituting 0.1% adult body weight; exhibit parallel biological/pharmacological properties with participation in host defence, is also recognised as anti-oxidant, and has been patented for several disease/symptoms /conditions, from epilepsy to Congestive Heart Failure (CHF), bone formation to inhibition of bone, hearing and hair loss, diabetes to cancer and many more. It is logical to think why a significant amount of Taurine is found in mushrooms? It seems that Mushrooms therapeutic potential is basically taurine centric and perhaps many of its pharmacological properties may be channelized through taurine action mechanism; linking mushroom disease prevention/control/disease Free State initiative through taurine; providing an inner circuit of mushroom -taurine -good health- longevity as a part of longevity cycle.

## **VI-O-16. Mushroom science in Cuba: towards new opportunities for developing functional foods/nutraceuticals**

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Health and sustainability have definitively become strategic issues for today's agriculture. Mushroom science in Cuba allows the valorization of agricultural by-products, such as coffee pulp, shells, husks - among many others- into functional foods/nutraceuticals for human consumption to address objectives of sustainability and biotechnological development. Much research work done in Cuban eastern region has been performed in the genus *Pleurotus*, one of the most popular basidiomycetes edible mushrooms whose cultivation has increased greatly throughout the world during the last few decades. *Pleurotus* species, like many edible and medicinal mushrooms, are a good source of immunomodulators and substances considered as "host defense potentiators" (HDPs) as judged by their immuno-stimulating properties. In this context, dietetic supplements with a high therapeutic potential acting on the immune system and formulated from refined or partially refined mushroom extracts, or from dried mycelia/fruited bodies biomass are referred as "mushroom immunocuticals". The present study examined the synergy exerted by the vast structural diversity of biomolecules found in *Pleurotus* crude extracts, powders and other preparations on immune responses on both immuno competent and immunodeficient (irradiated, cyclophosphamide-treated and malnourished) BALB/c mice. *Pleurotus* derived-products could potentiate the host defence mechanisms *in vivo* and should be promising for further pharmacological studies. The effects on cell immunity are especially valuable in the prophylaxis of tumours, immune-deficiencies and as co-adjuvant in chemotherapy. The results also demonstrate that not only mushrooms but also their mycelia maybe a good candidate for nutraceuticals production. Through this immunological "window" we are assisting to a revolution in mushroom science characterized by the diversity of natural compounds found in mushroom (both chemical and biological diversity) and on the other hand by the possibilities given by the abundance of specific molecular targets. In sum, mushroom science in Cuba opens new opportunities for developing functional foods/nutraceuticals. An extended knowledge of the immuno-enhancing activity of *Pleurotus* nutraceuticals would be useful in understanding their potential applications for immunonutrition and immunotherapy.

## Poster Presentations

### VI-P-1. Anti-anxiety activity of various extracts of *Ganoderma brownii*

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The genus *Ganoderma* consists of cosmopolitan polypore mushrooms, many of which can cause different types of rots in plants. Many species of this genus have been used in traditional medicines for centuries, particularly in Asian countries, to treat central nervous system disorders such as insomnia, nervousness, stress, anxiety, depression etc. Despite their long traditional use, systematic studies regarding anti-anxiety activity have not been carried out on these mushrooms. Thus, the present study has been undertaken to evaluate the anxiolytic potential of *G. brownii* collected from Uttarakhand. In the present study, various extracts of basidiocarps of this mushroom were prepared in petroleum ether, chloroform, methanol and distilled water by successive soxhlet extraction. All the extracts were tested for anti-anxiety activity using elevated plus maze (EPM) model in Swiss albino mice. The methanol extract of *G. brownii* at a dose of 200 mg/kg, p.o. showed a significant increase in the average time spent in the open arms of the EPM when compared to the control. However, this activity is less when compared to the standard drug (diazepam, 2mg/kg). Hence, it can be concluded that methanol extract demonstrates mild anti-anxiety activity.

### VI-P-2. Antifungal/antagonistic activity of different *Ganoderma* collections against plant pathogenic fungi and their antagonists

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Research on biology of Basidiomycetes mushrooms has markedly increased due to their ability to synthesize bioactive metabolites and potential for the production of different biotech-products used in medicine, pharmaceutical, food and agriculture industries. Nowadays, there are no effective mushroom-based biological control products against plant pathogenic fungi. Meanwhile, study of antifungal activity of Basidiomycetes mushroom and their antagonistic effect against plant pathogenic fungi could be of practical interest to develop new bio control agents applicable in agriculture. Antifungal/antagonistic activity (AFA/AA) of 22 strains of 4 *Ganoderma* collections (*G. adspersum*, *G. applanatum*, *G. lucidum*, *G. resinaceum*) with different geographical origin (Armenia, China, France, Iran, Italy) were studied against species of phytopathogenic fungi (*Bipolaris sorokiniana*, *Fusarium culmorum*, *Fusarium oxysporum*, *Pestalotiopsis funerea*, *Rhizoctonia cerealis*) and their antagonists (*Trichoderma asperellum*, *Trichoderma harzianum*, *Trichoderma pseudokoningii*, *Trichoderma viride*) in dual cultures experiment in vitro. All *Ganoderma* collections, particularly *G. adspersum*, *G. resinaceum* and *G. applanatum* possess significant AFA/AA against test phytopathogenic fungi. High antagonistic activity against *Ganoderma* species, except *G. resinaceum* was detected by phytopathogen *F. culmorum* and *Trichoderma* species. No significant difference in growth rate indicators of mushrooms and test filamentous fungi compared with control data was revealed. Thus, tested *Ganoderma* collections are potential producers of antifungal metabolites which significantly suppressed the growth of test phytopathogenic fungi. Further studies will assist to develop novel bio-control agents from wood inhabiting *Ganoderma* mushrooms against certain plant pathogens.



### VI-P-3. Antihyperlipidemic effects of *Hypsizygus ulmarius* mycelial polysaccharides in high-fat diet fed rats

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The present work was undertaken to investigate the effects of polysaccharide extract from *Hypsizygus ulmarius* in experimentally induced hyperlipidemia in rats. Crude mycelia polysaccharide extract of *H. ulmarius* (HUIPS) (200 and 400 mg/kg, p.o.) was administered to the high fat-diet induced hyperlipidemic rats for 30 days to evaluate its antihyperlipidemic activity. Atorvastatin (10mg/kg; p.o.) was used as a standard drug. Female Albino rats were divided into five groups each comprising six rats. Initially all the animals were given the normal diet for 1 week. Group I served as normal control and fed with normal diet throughout the course of study. Animals of Group II to V were fed with high-fat diet for 30 days. Subsequently, the high-fat diet fed animals were replaced by normal diet. Group I received normal diet only. The groups II and III received HUIPS in divided doses of 200 and 400 mg/kg; p.o. and Group V, served as positive control was treated with Atorvastatin suspension prepared with Tween 80 (10 mg/kg; p.o.) in addition for next 30 days. Treatment of hyperlipidemic rats with HUIPS led not only to significant decreases in ALT, AST, LDH, lipid levels of total cholesterol, total triglyceride, phospholipids and LDL-C but also resulted in an increase in HDL-C, consequently the atherogenic index and a increase in HDL-C in serum, but also a significant decreases in total lipids, total cholesterol, triacylglycerol and phospholipids in the liver, heart and adipose tissue. The polysaccharide extract showed a significant ameliorative effect on the elevated atherogenic index as well as LDL/HDL-C ratio. Histopathological observations indicated that PEPE could effectively prevent excessive lipid formation in liver, heart and adipose tissue. The study suggested that HUIPS had significant health benefits and could be explored as a potentially promising food additive for the prevention of hyperlipidemic diseases.

### VI-P-4. Antimicrobial activity of macrofungi collected from the dry zone of Sri Lanka

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A number of novel and important bioactive compounds such as Ganomycines (antibacterial), Schizophyllan (Antitumor) and Lentinan (antiviral) have been isolated from macrofungi during the last few decades. However, a large majority of macrofungi found in Sri Lankan forests is not yet abundantly investigated for their antimicrobial potentials. This study was carried out to investigate the antimicrobial activity of macro fungi collected from the dry zone of Sri Lanka. Fruit bodies of macrofungi were collected from the forest reserves in Dambulla, Sigiriya, Moneragala, Minneriya and Kawudulla areas. Metabolites of fruit bodies were extracted serially with CH<sub>3</sub>OH, CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub> (1:1, V/V) and CH<sub>2</sub>Cl<sub>2</sub> using 10 g of air dried and powdered macro fungi samples. Three extracts were combined and filtered using Whatman no. -1 filter paper. Subsequently it was evaporated to dryness under vacuum in a rotary evaporator to obtain the total crude extract of each macro fungus. Crude extracts were re-dissolved in CH<sub>3</sub>OH to prepare stock solutions. Antibacterial activity of macro fungi extracts was tested against gram positive *Staphylococcus aureus*, *Bacillus cereus*, *B. subtilis* and gram negative *Pseudomonas aeruginosa* and *Escherichia coli* bacterial strains. Antifungal activity was tested against a *Candida* sp. and three plant pathogenic fungi *Rigidoporus microporus*, *Colletotrichum* sp, and *Curvularia lunata*. Disc diffusion method was used to test the antimicrobial activity of crude extracts at 1mg/disc concentration against bacteria and *Candida* sp. Antifungal activity of crude extracts against plant pathogenic fungi were tested at 400 ppm by the poisoned food technique. Gram positive bacterial strains *S. aureus*, *B. cereus* and fungal

strain *R. microporus* were inhibited by most active macro fungi extracts while gram negative bacteria and *C. lunata* were shown resistant to most of the macro fungi extracts. Therefore, these susceptible microorganisms may be used as indicator organisms in preliminary screening of macrofungi extracts for bioactivity. Of the tested 90 samples 8 macro fungi extracts namely *Anthracoephyllum lateritium*, *Schizophyllum commune*, BBL1 (yet unidentified species), *Hymenochaete* sp., SGR4 (Polyporales), *Ceriporia purpurea*, *Coriopsis byrsina* and *Coriopsis caperata* showed strong antibacterial properties against gram positive bacteria. *Daldinia concentrica* and *Amauroderma rude* were active against gram negative bacteria while MK-17 (yet unidentified species) extract was active against *Candida* sp. six macrofungi extracts *Inonotus* sp., *Coriopsis caperata*, *Flavodon flavus*, *Polyporus varius*, *Trichaptum* sp. and *Trametes elegans* showed inhibition of growth or static growth conditions against the plant pathogenic fungi. Genus *Coriopsis* exhibited activity against both bacteria and fungi indicating the presence of broad spectrum of antimicrobial compounds. Accordingly these macro fungi species may be used as potential sources to isolate important antimicrobial compounds.

#### **VI-P-5. Antimicrobial potential of wild *Ganoderma* spp. against drug resistant *Candida* strains**

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Extracts of five wild *Ganoderma* fruiting bodies collected and their cultured mycelium were screened against azole resistant *Candida* strains using agar well diffusion method to identify a new natural antibiotic from natural resources against multidrug resistant *Candida* strains. All species of *Ganoderma* and cultured mycelial extracts were found effective against tested pathogens. The antimicrobial results indicated that the ethanolic extracts of wild fruiting body as well as cultured mycelium inhibited all the tested pathogens. The Minimal Inhibitory Concentration (MIC) of the ethanolic extracts of the fruiting body extract was  $3.05 \times 10^{-5}$  while MIC of cultured mycelial extract was  $2.10 \times 10^{-5}$  against tested pathogens. The extracts inhibited potency against heavy doses of inoculum at MIC. The extracts were found thermostable up to 80 °C and its antifungal activity was not affected. The ethanolic extract of cultured mycelium of *Ganoderma* spp showed significantly more inhibitory activity compared to the fruiting body extracts. The study indicated that these extracts are potential medicinal resources that can be used as a natural antifungal agents. Since the mycelium can be produced throughout the year in the lab, so it has no production limitations and the yield of desired bioactive metabolites can be increased by optimizing the environmental conditions.

#### **VI-P-6. Anti-oxidant and anti-herpetic activities of *Ganoderma lucidum* indigenous isolates**

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*Ganoderma lucidum* is an economically important medicinal fungus utilized for its nutraceutical properties and is major source for many bioactivities. *G. lucidum* mushroom does not have cyto toxicity and has been demonstrated to be safe as food supplement due to its' long history of oral administration not associated with toxicity. *G. lucidum* is very rich in antioxidants and other properties like anti-viral and anti-cancer potentials. Natural and synthetic antioxidants have been widely reported. However, synthetic antioxidants have adverse effects like toxicity and carcinogenicity. The present study focused on the antioxidant potential and anti-herpetic activities of fruit body and mycelia of *G. lucidum*. The highest free radical scavenging activity, 84.3% was recorded in isolates of GI- 3 on 9th day and 94% in fruiting body of GL 01. The anti-herpetic activity for water extract of fruiting body of *G. lucidum* against the HSV1 was evaluated and no cytotoxicity was observed even in 2000 µg/ml. Hence a detailed study on significance of *G. lucidum* will unfold ways to new findings in field of pharmaceuticals.

## **VI-P-7. *Antrodia cinnamomea* mycelium from submerged fermentation reduces thioacetamide-induced liver fibrosis in rats**

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*Antrodia cinnamomea*, a fungus unique to Taiwan, is considered the ideal liver remedy. This study aimed to investigate the protective effects of *A. cinnamomea* mycelium from submerged fermentation against thioacetamide (TAA)-induced liver fibrosis in rats. Animals were allocated into four groups of which one group received saline and served as normal control. Three groups of male rats were treated with TAA administrated intraperitoneally at doses of 100-200 mg/kg three times per week for 8 weeks and simultaneously were given a daily oral dose of 0.5% carboxyl methyl cellulose and *A. cinnamomea* mycelium (131, 393 mg/kg). Experimental results showed that *A. cinnamomea* mycelium significantly reduced the TAA-induced serum levels of ALT and AST at week 1, 3 6 and 8. In addition, *A. cinnamomea* mycelium increased the TAA-reduced albumin levels and lowered the TAA-induced  $\gamma$ -GT activities. The spleen weight and liver collagen content that were induced by TAA and the liver protein content that were decreased by TAA were brought back to normal ranges by *A. cinnamomea* mycelium. Meanwhile, the rise of glutathione content and glutathione peroxidase activity and the fall of lipid peroxidation in the TAA receiving groups also were significantly reversed by *A. cinnamomea* mycelium administration. In the liver pathological examination, *A. cinnamomea* mycelium can inhibit liver nodules and bile duct fibrosis production. In conclusion, *A. cinnamomea* mycelium can reduce liver fibrosis in rats induced by TAA.

## **VI-P-8. *Calocybe indica* mycelial polysaccharides prevents mitochondrial damage in isoproterenol-induced cardiotoxicity in Wistar rats**

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Altered mitochondrial function and free radical-mediated tissue damage have been suggested as important pathological events in isoproterenol (ISO) induced cardiotoxicity. This study was undertaken to know the preventive effect of *Calocybe indica* mycelial polysaccharides on mitochondrial damage in ISO induced cardiotoxicity in male Wistar rats. The rats were pretreated orally with (CIMP) extract at two different doses (200, 400 mg/kg) daily for a period of 21 days. After the treatment period, ISO (85 mg/kg) was subcutaneously injected to rats at an interval of 24 h for 2 days. Rats receiving  $\alpha$ -tocopherol (100 mg/kg) served as positive control. ISO induced rats showed significant increase in mitochondrial lipid peroxidation products (thiobarbituric acid reactive substances and lipidhydroperoxides) and significant decrease in mitochondrial antioxidants (superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, glutathione reductase and reduced glutathione, vitamin C). Also, significantly decreased activities of tricarboxylic acid cycle enzymes such as isocitrate, succinate, malate and  $\alpha$ -ketoglutarate dehydrogenases and respiratory chain marker enzymes such as NADH-dehydrogenase and cytochrome-c-oxidase were observed in mitochondrial heart of myocardial infarcted rats. Prior treatment with CIMP significantly prevented these alterations and restored normal mitochondrial function. *In vitro* studies on the effect of CIMP on scavenging 1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2, 2'-azinobis-(3-ethylbenzothiazoline-6- sulfonic acid) (ABTS+), superoxide anion ( $O_2^-$ ), and hydroxyl ( $OH$ ) radicals, reducing power and lipid peroxidation inhibition confirmed the free radical scavenging and antioxidant activity of CIMP. Thus, the observed effects are due to the free radical scavenging and antioxidant potential of CIMP. Thus, this study confirmed the preventive effect of CIMP on isoproterenol induced mitochondrial damage in experimentally induced myocardial infarction in Wistar rats.

## VI-P-9. Extraction of bioactive compounds in mushrooms by supercritical fluid extraction technology

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Mushrooms are a well known functional food and the natural bioactive compounds present in them lead to great pharmacological and health benefits. The inherent difficulties in screening and production of these compounds have led to the development of advanced technologies. Various novel techniques like ultrasound and microwave-assisted extraction, supercritical fluid extraction, and accelerated solvent extraction have been developed for the extraction of nutraceuticals in order to shorten the extraction time, decrease the solvent consumption, increase the extraction yield, and enhance the quality of extracts. The traditional methods used earlier to obtain extracts have the main disadvantage of using toxic solvents (e.g. methanol and acetone). In the present study, supercritical CO<sub>2</sub> extraction of one of the medicinal mushrooms i.e *L. edodes* was investigated. Fatty acid esters, fatty acids, triterpenes, diterpene alcohols and phytols were identified as the major chemical groups in the *L. edodes* extract. The paper provides theoretical background on supercritical fluid extraction technology as one of the important techniques for the production of specific compounds from mushrooms.

## VI-P-10. Hypocholesterolemic effect of blue oyster mushroom in male albino rats

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Effect of *Hypsizygus ulmarius* at the rate of 2.5 and 5.0% dosage on cholesterol accumulation in blood and liver of weaning male white Albino Wistar rats was assessed. The serum total cholesterol level of rats fed with 5 % of mushroom recorded 3.52, 18.62 and 18.68% decrease when compared to control over a period of 30, 60 and 90 days. The reduction of cholesterol was due to the decreased cholesterol content in low- density lipoproteins (LDL). There was no significant difference found in serum high-density lipoproteins (HDL) concentration. It is suggested that prolonged exposure to *H. ulmarius* administrations reduced lipid and lipoprotein concentrations significantly in rats.

## VI-P-11. Identification of polysaccharide fractions of *Pleurotus ostreatus* with antioxidant and antimicrobial properties

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Mushrooms are nutritionally rich food and a source of physiologically beneficial and nontoxic medicines. Biologically active substances originating from Basidiomycetes have beneficial effect on human health. Among these compounds, polysaccharides (especially  $\beta$ -glucans) play an important role due to their anticancer and immunomodulating activities. In present study, polysaccharide fractions were obtained from edible mushroom *Pleurotus ostreatus*. Three polysaccharide fractions were obtained with ethanol precipitation from cold water, hot water and hot aqueous NaOH extracts. The fractions were subjected to Fourier Transform Infra-red Spectroscopy (FTIR) for structural analysis. Spectra of three fractions showed several intense overlapped IR bands in the region of 950-1200 cm<sup>-1</sup> (mainly CC and CO stretching vibrations in pyranoid rings) indicating the presence of polysaccharides as the major component. All fractions also



showed peaks near 1078 cm<sup>-1</sup> indicating presence of  $\beta$  (1  $\rightarrow$  3) glucans. Hot water fraction showed peak at 1374 cm<sup>-1</sup> that further confirmed presence of  $\beta$  glucans. Cold and hot water fractions showed peak for stretching vibrations of C-H bond (2921 cm<sup>-1</sup>). Cold water fraction also showed peak for hydroxyl group stretching vibration. The polysaccharide fractions were tested for their antibacterial potential using well diffusion technique. All fractions showed moderate activity against *Yersinia enterocolitica*, while cold water fraction also indicated antagonistic activity against *Salmonella typhi*. The antioxidant activity of *P. ostreatus* polysaccharide fractions was determined via the 2, 2-diphenylpicrylhydrazyl (DPPH) radical neutralization assay. Relative to DPPH, the NaOH soluble fraction showed maximum antioxidant activity (0.21) followed by cold (0.02) and hot water fractions (0.01), respectively.

## **VI-P-12. Importance of understanding pelletization in *Termitomyces* Heim species for potential applications to produce edible nutritious mycoprotein**

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The mutualistic paleotropical termite exosymbiont fungal genus *Termitomyces* having 54 distinct species is represented by 85 taxa (www.indexfungorum.org). Although all species are harvested from the wild and reported to be edible, popular and gourmet quality nutritious mushrooms, due to their complex mutualistic nature it has evaded attempts of domestication and commercial production. These species indicate a lot of promise for nutritional SCP/Mycoprotein production. The lipid content of these mushrooms is reported [1] to vary from 2.5–5.4 g/100 g dry weight (with high proportions of polyunsaturated fatty acids (45.1–65.1% of total fatty acid methyl esters) with crude fibre content showing remarkable proportions (17.5–24.7 g/100 g dry weight) and protein content between 15.1 and 19.1 g/100 g dry weight. Good cultural growth of this species on solid and in liquid media indicates its' potential to produce edible mycoprotein. Fungi can be grown in submerged cultures in several different morphological forms: suspended mycelia, clumps, or pellets [2]. Pelletization or the cubic form of microbial growth associated with reduction of medium viscosity has been reported in many edible mushrooms. For any large scale industrial process to produce edible mycoprotein to succeed, standardization of pelletization process would be necessary by identifying a suitable, morphologically stable culture [3]. In this direction the work presented in this paper was aimed at morphological characterization of pellets using nine pure *Termitomyces* cultures namely- *T. albuminosus*, *T. auranticus*, *T. bulborhizus*, *T. clypeatus*, *T. globulus*, *T. heimii*, *T. medius*, *T. petaloides*, *T. striatus* in shaken submerged conditions. Pelletization was carried out in 100 ml of 5 gm/l strength of carbon source -sucrose in Czapek dox solution with pH 5.5 in 250 ml conical flasks kept on rotary shaker with 150 rpm in light for upto 20 days. Except *T. bulborhizus*, *T. heimii*, *T. medius* and *T. petaloides* rest of the species produced good pelletization by displaying micro (less than one mm) and macro pellets (more than 10 mm). Spiky, loose and dispersed mycelial morphology was found to be common in most of the *Termitomyces* species. *T. clypeatus* emerged as the most promising, morphologically stable culture producing white to cream, spherical with spiky surface pellets (1.0-1.5 cm), few fluffy irregular pellets and mycelia aggregates (0.2-1.0 cm), indicating further potential for mass production.

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### VI-P-13. *In vitro* and *in vivo* antidiabetic activity of *Calocybe indica* P & C

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*In vitro* and *in vivo* antidiabetic activity of milky mushroom (*Calocybe indica*) exhibited significant results for its  $\alpha$ -amylase ( $89.49 \pm 3.54$  % at 1.0 mg/ml) and  $\beta$ -glucosidase inhibitory activity ( $67.30 \pm 2.93$  % at 1.0 mg/ml) in a dose-dependent manner. The methanolic extract showed significant activity ( $p < 0.05$ ) at the tested dose level (200 mg/kg b.w) which was comparable to glibenclamide, a standard antidiabetic drug. Presence of phytochemicals namely phenols, flavonoids, saponins and tannins may be responsible for such antidiabetic activity. These results reveal that *C. indica* can be used as a potential antidiabetic agent.

### VI-P-14. Investigation of the impact of edible medicinal mushroom species on probiotic bacteria

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At present we have very less information about the impact of edible mushrooms on those probiotic bacteria that live in the human intestines and have beneficial effects on the human health. We investigated the effects of extracts of five mushroom species, namely, *Agaricus bisporus*, *A. subrufescens*, *Pleurotus ostreatus*, *Ganoderma lucidum* and *Trametes versicolor*, on probiotic bacterial growth. The cultivated fruiting bodies of each species were dried. The grinded powders were then used for water, alkali, acidic and ethanol extractions. These extracts were used to prepare bacteria specific carbohydrate-free liquid broth medium and inoculated with the following probiotic bacteria, namely, *Lactobacillus acidophilus*, *Lb. casei*, *Lb. paracasei*, *Lb. rhamnosus* and LGG. Two fecal species, *Enterococcus faecium* and *Escherichia coli* were also included in the present investigation. Colony forming units were counted after 24 and 48 hour of incubation. Prebiotic (technical grade and pure inulin) was used as positive control while distilled water as negative control. We found that the water extract had the strongest positive effect on the proliferation of probiotic bacteria, followed by the acidic, alkalic extracts and the solid fraction of water extracts. *E. coli* cell numbers did not show significant differences in the various treatments. Effect of the *A. bisporus*, *A. subrufescens* and *P. ostreatus* extracts were found to be the strongest on bacterial proliferation. These results showed that certain mushroom species could have prebiotic effects. The study also demonstrated that mushrooms may have positive impact on the human health, in addition to their beneficial nourishing physiological effects.

### VI-P-15. Qualitative and quantitative evaluation of different strains of *Ganoderma lucidum* for the production of lignin degrading enzymes

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*Ganoderma lucidum*, a white rot fungus well known for its medicinal properties, produces various extra cellular enzymes such as laccases, lignin peroxidase and manganese peroxidase which are mainly responsible for the mineralization of lignin and lignocellulosic compounds in nature. Hence, this fungus can be used to degrade various dyes and xenobiotics compounds. Present study was conducted to



screen four strains of *G. lucidum* (GL-1, GL-2, GL-3 and GL-4) for the potential production of these three lignin modifying enzymes by using ABTS and guaiacol as indicators on rice bran agar (RBA) plates and rice bran broth (RBB) media. Different concentrations of guaiacol (0.75 ml/l, 1.50 ml/l, 2.25 ml/l and 3.00 ml/l) and ABTS (1 ml/l, 2 ml/l, 3 ml/l and 4 ml/l of 1% ABTS in dH<sub>2</sub>O) were used to study their effect on the production of lignin modifying enzymes. In qualitative study on solid medium supplemented with guaiacol, the growth of all four stains was retarded as compared to control plate (without guaiacol) and appearance of brownish red zone around the mycelial bit indicated the secretion of lignin modifying enzymes. All four strains were able to grow on RBA with 0.75 ml/l guaiacol with potency index (PI) ranging from 0.67 to 1.27. There was no growth on RBA plates supplemented with 1.50 ml/l, 2.25 ml/l and 3.00 ml/l concentrations of guaiacol, although the brownish red colour zone was observed around each bit. The intensity of brownish red zone was very high in GL-2 strain, followed by GL-1, GL-3 and GL-4 strains. In ABTS supplemented plates, all the four strains grew well and produced blackish red zones. PI ranged from 0.00 to 1.20 over the period of 7 days for all four strains. The quantitative analysis was done in RBB supplemented with varying concentrations of guaiacol and ABTS for 8 days at 30 °C. In control, maximum production of lignin modifying enzymes was observed in GL-2 strain, followed by GL-1, GL-3 and GL-4. The production of laccase enzyme was found to be 44, 38, 24 and 20 U/ml in GL-2, GL-1, GL-3 and GL-4, respectively at fifth day of incubation. The amounts of MnP enzyme were 85, 65, 35 and 35 U/ml in GL-2, GL-1, GL-3 and GL-4 respectively at sixth day of incubation. The LiP enzyme was present as 120, 97.5, 72.5 and 67.5 U/ml in GL-2, GL-1, GL-3 and GL-4 respectively at sixth day of incubation. It was found that Guaiacol suppresses the production of the lignin degrading enzymes studied. In case of ABTS supplemented broth, the secretion of lignin modifying enzymes was enhanced as compared to control. Supplementation of 2 ml/l of 1% ABTS gave maximum stimulation to enzyme production. GL-2 strain produced 76 U/ml, 160 U/ml and 75 U/ml of laccase, LiP and MnP enzymes, respectively on the sixth day of incubation in 2 ml/l of 1% ABTS supplemented RBB. The results indicated the potential use of these strains for degradation of lignocellulosics and xenobiotic compounds.

## **VI-P-16. Screening of high yielding *Ganoderma lucidum* strains based on mycelial growth rates and substrate degrading enzymes**

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Twenty-seven strains of *Ganoderma lucidum* were verified as distinct strains through antagonistic tests conducted on potato dextrose agar. Mycelial growth rates, and laccase and CMCase activities, were then measured and correlations between these parameters and fruit body yields were evaluated using Pearson correlation analysis. This revealed strong correlations between mycelial growth rates, enzyme activities and fruit body yields. In the case of mycelial growth rate and CMCase activity, these two parameters exhibited a positive linear correlation with mushroom yields, whereas a negative linear correlation was observed between yields and laccase activity. Analysis of these indices facilitates preliminary forecasts of yield levels without cultivation tests, and is a simple and economic model for selecting suitable *G. lucidum* parental strains for hybridization breeding.

### **VI-P-17. Selenium induced anti-oxidant activity and generation of selenoergothioneine in *Agaricus bisporus* cultivated on selenium hyper accumulated agri-residues**

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Mushrooms have been reported to be good sources of Selenium (Se), but vary significantly depending on the type of Se supplementation and the compost (substrate) used for their growth. Most of the studies have been focused on Se fortification in mushrooms through exogenous supplementation dominantly as selenite. Agricultural residues naturally enriched with Se can effectively be used for generating Se-enriched mushrooms. The prime objective of the study was to study the accumulation of Selenium in mushrooms cultivated on selenium rich agricultural residues collected from seleniferous sites across north India. Modulations in antioxidant properties of button mushroom, *Agaricus bisporus*, cultivated on selenium-rich wheat straw from the seleniferous belt of Punjab (India) was examined in comparison to the mushrooms cultivated on normal straw. Selenium concentration in Se-fortified mushrooms ( $122 \pm 1.8 \mu\text{g g}^{-1}$ ) was significantly higher than control ( $14 \pm 0.7 \mu\text{g g}^{-1}$ ) as analyzed by fluorimeter. The antioxidant activity, as determined by various assays such as free radical scavenging, metal chelating activity, reduced lipid peroxidation, as well as inhibition of cancer cell proliferation was higher in experimental mushrooms as compared to control. Further analysis of Se rich extracts, using FTIR and MS indicated possible presence of selenoergothioneine-like moiety as an expression of Se uptake by mushrooms. The present study demonstrates the use of Se hyper accumulated agricultural residues as substrates for producing Se-rich mushrooms, which can be potential sources of Se supplementation/nutraceutical applications.

### **VI-P-18. Some interventions in the cultivation technology of important medicinal mushrooms**

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Of the 14,000 species of mushroom in the world, around 700 have been known for medicinal properties. Much work on medicinal mushrooms has been done in countries like China, Korea, Taiwan, Japan etc. Cultivation of medicinal mushrooms in India is still in preliminary stages though cultivation packages has been developed for *Lentinula edodes*, *Ganoderma lucidum*, *Flammulina velutipes* and *Auricularia* spp. In the present studies, some refinements in the existing cultivation technology of important medicinal mushrooms were made. Among different substrates tested for *G. lucidum* and *L. edodes*, maximum yields were recorded on the mixture of saw dust + rice bran + lantana wood chips. *F. velutipes* was successfully cultivated on a mixture of wheat straw + rice bran (3:1 w/w) in polypropylene bags. Safe chemicals like sodium hypochloride @3% and calcium oxycloide @4% were found best for sterilization of substrates. Containers like plastic baskets, bamboo baskets and polypropylene bags were successfully used for the cultivation of *Pleurotus ostreatus*. Vertical and slanting wall stacking methods gave about 20% higher yield as compared to traditional shelf methods. Environmental conditions were also standardized for their cultivation.

## VI-P-19. Role of mushroom in selenium supplementation

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Selenium (Se) is an essential trace element that plays various crucial roles in mammalian biology. The food we take is the only source of selenium, and it is absorbed from the soil in the form of sodium selenite, sodium selenate, selenomethionine, selenocysteine, etc. Due to low therapeutic index, toxicity of Se increases abruptly with a minute change in concentration. And thus, excess of selenium in the soil leads to toxicity in dependent population. Bioremediation through microbial transformations of toxic Se species ( $\text{Se}^{+4}$ ,  $\text{Se}^{+6}$ ) into nontoxic forms ( $\text{Se}^0$ ) is being considered as an effective remedial tool. An alternative approach of utilizing selenium from selenium contaminated sites and its direct application in Se supplementation in Se deficient population would be progressive. Mushroom production is among the large scale commercial applications of microbial technology. Here we suggest, fortification of selenium in mushroom by growing them on Se rich soil can be used as a selenium supplement.

## VI-P-20. Anti inflammatory properties of *Cordyceps militaris* hexane extract and its fractions

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Microglial cells are the resident macrophages of the brain and spinal cord, and thus act as the first and main line of active defence in the central nervous system against assaults including inflammation. For decades, inflammation has been one of the most potent but unknown causes for many diseases ranging from atherosclerosis to arthritis rheumatism and even cancer and Alzheimer's diseases. In the search for safe anti-inflammatory agents, mushrooms are being actively sought. In this study the anti-inflammatory effects of hexane extract and its subfractions of *Cordyceps militaris* (L.) Link was investigated. The anti-inflammatory effects were determined by nitric oxide (NO) production assay using Griess reagent. The reduction in NO production by hexane fraction was 7.03% and 85.15% at 10  $\mu\text{g/ml}$  and 100  $\mu\text{g/ml}$  concentrations respectively compared to 1  $\mu\text{g/ml}$  of *Escherichia coli* (O55:B5) lipopolysaccharide (LPS) activated cells without treatment (negative control). The extract was fractioned into four subfractions and tested for the effect on nitric oxide production in BV2 microglial cells at treatment dose of 0.1  $\mu\text{g/ml}$  to 10  $\mu\text{g/ml}$  as higher concentrations showed reduction in cell viability. The anti-inflammatory activity was highest when cells were treated with fraction  $\text{CH}_2$  and  $\text{CH}_3$ . Fraction  $\text{CH}_2$  treated cells showed about three fold reduction at 10  $\mu\text{g/ml}$  compared to 1  $\mu\text{g/ml}$  with values 70.26% and 26.62% compared to negative control. Cells treated with fraction  $\text{CH}_3$ , however, showed a gradual nitric oxide reduction ranging from 14% to 94% with the increase of extract concentration (0.1  $\mu\text{g/ml}$ -100  $\mu\text{g/ml}$ ). The bioactive components in the fractions were identified with GC-MS. The sub fraction  $\text{CH}_3$  contained linoleic acid (44.50%), as the major compound and (22E)-ergosta-5, 7, 9 (11), 22-tetraen-3.beta.-ol (1.64%) minor compound. The  $\text{CH}_2$  fraction contains linoleic acid (32.25%) and ethyl linoleate (20.60%) as major compounds and traces of (22E)-ergosta-5,7,9 (11), 22-tetraen-3.beta.-ol (3.75%), ergosta-5,7,22-trien-3-ol (1.84%), ergosta-4,6,8(14),22-tetraen-3-one (3.08%). Fatty acids and sterols from *C. militaris* are also potential anti-inflammatory agents besides cordycepin. Further studies are being conducted to obtain the pure compound and test for its anti inflammatory activity.

# **Session-VII**

## **Mycorrhizal, Entomopathic and Other Novel Mushrooms**



## Keynote Presentation

### VII-K-1. Mycorrhizal, entomopathic and novel mushrooms

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Mushrooms have always been a source of good health and are being used since time immemorial as medicines. They are pro-biotic i.e. they help our body strengthen itself and fight off illness by maintaining physiological homeostasis. Mycorrhizal, entomopathic and novel mushrooms are gaining popularity since last few decades worldwide. Mycorrhizal mushrooms comprise a specific group of edible fungal species that form symbiotic associations with their host plants and includes *Tricholoma matsutake*, *Tuber melanosporum*, *Tuber magnatum*, *Lactarius deliciosus*, *Lyophyllum shimeji*, *Tuber borchii*, *Tuber formosanum*, *Rhizopogon rubescens*, *Terfezia laveryi*, *Boletus edulis*, *Cantharellus cibarius*, *Lactarius hatsutake*, *Lactarius akahatsu* and *Morchella* spp. However, currently only *Tuber melanosporum* and *Tuber uncinatum* have been cultivated commercially, although some success has been achieved with *Lactarius deliciosus*, *Lyophyllum shimeji*, *Tuber borchii*, *Tuber formosanum*, *Rhizopogon rubescens*, *Morchella esculenta* and *Terfezia laveryi*. Entomopathic fungi usually attach to the external body surface of insects in the form of microscopic spores. The most common and important entomopathic fungus is *Cordyceps* (*Ophiocordyceps*). *Cordyceps sinensis* has long been used in folk medicine and is known to have remarkable medicinal properties. It has been determined that there is perhaps a greater biodiversity of compounds within different strains of this single species. Due to the great difference in the concentration of native compounds, a wide range of quality is found in *Cordyceps* cultivated from different strains and utilizing different culture methodology. Due to its peculiar characteristics, habitat, morphology and being a store house of medicinal properties, it is a highly prized mushroom. *Ganoderma lucidum* has gained wide popularity in recent years as a dietary supplement, not only in China and Japan, but also in North America and other parts of the world. The reason it attracts international attention as a valuable Chinese herb is due to the wide variety of its biological activities such as antitumor, immunomodulatory, cardiovascular, respiratory, antihepatotoxic and antinociceptive effects. The diversity in the biological actions may be attributed to the fact that it is composed of different chemical entities including triterpenoids, polysaccharides, alkaloids, amino acids, peptides, inorganic elements, steroids, fatty and organic acids. *G. lucidum* products with different triterpenes and polysaccharides or combinations of these two groups are most likely to result in different pharmacological activities. However, there is lot more to be explored of this wonderful gift of nature and requires the attention of scientific community to exploit this mushroom to the benefit of mankind.



## Oral Presentations

### VII-O-1. Role of copper on growth and development of *Pleurotus nebrodensis*

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*Pleurotus nebrodensis* is an important rare edible fungus with independent intellectual property rights in China, its cultivation has been commercialized and large scale culture was firstly realized in 1990s in Beijing. It is a widely popular mushroom in the market, and has current yield over 200,000 ton per annum in China. This paper focused on influence of manganese on growth and development of *P. nebrodensis* and on activities of extracellular laccase and manganese peroxidase. Change of flow rate of H<sup>+</sup> and Ca<sup>2+</sup> on mycelia surface under the action of copper is determined, to make physiological effect of copper clear. It was shown by research result that, copper supplemented substrate can obviously promote growth rate (4.35 mm/d) of *P. nebrodensis* mycelia at a content of 50 mg/kg, significantly different with that of the control group. At copper content of 50mg/kg, growth cycle and biological efficiency of *P. nebrodensis* were 166.22 days and 50.66%, prolonged by 16 d and improved by 4.61%, respectively. In growth period of *P. nebrodensis* mycelia, laccase had high activity, and even higher (significantly different with that of the control group) when the copper content in the substrate was 25-50 mg/kg, but had activity decreased when the copper content was over 50 mg/kg. In growth period of *P. nebrodensis* fruiting body, manganese peroxidase had activity higher than that in growth period of mycelia, and had the highest activity at copper content of 50 mg/kg, indicating certain correlation with yield. The content of copper in the substrate can change flow direction of extracellular H<sup>+</sup> and Ca<sup>2+</sup> on mycelia surface. All these results should provide scientific information for culture techniques and theory of *P. nebrodensis*.

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### VII-O-2. Orange dye from *Pycnoporus* sp. for textile industries

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Colour plays a vital role in life of each and every one through industries like textiles, paint, food, clothing, art and cosmetics. The traditional natural dyes from plants were quickly replaced by synthetic dyes ever since the discovery of synthetic organic dye, mauveine by William Henry Perkin in 1856. Thousands of synthetic dyes have been prepared with several advantages like low cost, vast range of new colours and ability to impart better properties upon the dyed materials. Pigments from microbes especially fungi are considered as a good alternative to hazardous synthetic dyes. The *Pycnoporus* sp. is a source of orange dye that has application as mushroom dye for textile industries. The work was initiated with isolation, followed by production of dyes from mycelial culture, simple and cost effective cultivation with successful fruiting body production, and extraction of dyes. It was followed by application of the mushroom dyes with cotton and silk yarns and fabrics. Pilot scale cultivation was successfully carried out and dyeing experiments were carried out at an industry for testing its suitability as natural dye.

### VII-O-3. The prospect of rot fungi in controlling of *Trichoderma* spp. in mushroom cultivation

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*Trichoderma* spp. are often encountered in mushroom cultivation. They could contaminate substrates at the time of spawning or after. The heavy infections could reduce the yield considerably. The JPA isolate is a *Lentinus* sp. (rot fungus) that was found in oil palm plantations. Based on the initial detection by paper disc method, the methanolic extract of JPA mycelia cultivated in 1.5% of malt extract could inhibit the growth of *Bacillus subtilis*. Based on the bioautography test, the crude extract gave the inhibition zone as well with the  $R_f$  in a range of 0.7-0.8. The antagonistic test of JPA isolate with *Trichoderma* sp. S2-2 was conducted in the bag logs containing 1 kg of oil palm empty fruit bunches (OPEFB) supplemented with 15% bran, 1.5% lime and 1.5% gypsum. Both isolates were inoculated at both sides of the bag logs simultaneously. Based on this test, JPA isolate could inhibit the growth of *Trichoderma* sp. S2-2 and covered the colony of *Trichoderma* sp. S2-2 and the OPEFB substrates after 8 weeks of incubation. Furthermore, the methanolic extract of the OPEFB substrates inoculated only with JPA isolate were tested against *Trichoderma* sp. S2-2 by paper disc method. The results showed that the extracts could inhibit the growth of *Trichoderma* sp. S2-2 with formation of very thin *Trichoderma* colony zones or clear zones around the paper discs containing the extracts. Based on ITS1 and ITS4 sequences data, this isolate was *Lentinus* sp. but the data did not match closely with any species registered in Gene Bank.

### VII-O-4. Toxicological studies on *Inocybe virosa*

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Western Ghats of the Indian sub-continent harbours great fungal diversity with a wealth of mushroom flora, a few genera of which have been found to be poisonous. A new endemic species, *Inocybe virosa*, reported in the Kerala state, is an addition to the group of poisonous mushrooms and is the focus of the present study. HPLC technique was employed for the qualitative analysis and the quantitative estimation of muscarine in *Inocybe virosa*. The HPLC data revealed a concentration of 0.3 mg per unit gram of the hydro-ethanol extract. Further, the toxicological evaluation was carried out *in vitro* by subjecting the mushroom extract to different digestive enzymes and pH variations, simulating the *in vivo* digestive conditions. The toxin was present in the digested fraction and was identified using the chromatographic technique. The digestate obtained on *in vitro* digestion was studied for its cytotoxicity on intestinal Caco-2 cell line and its *in vivo* toxic potential was verified in mice. On oral administration, characteristic symptoms of toxicity such as perspiration, lacrimation and salivation were observed. As commonly found in the species of Inocybaceae, the basidiomata of this new endemic *Inocybe virosa* too contains muscarine in a concentration which causes undesirable effects and hence, is not recommended for consumption.

## VII-O-5. Identification of *Tolypocladium paradoxum* and *Ophiocordyceps yakusimensis* from China

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Two kinds of *Cordyceps* parasited on cicada were collected from Zhejiang province in eastern of China and they were studied by morphological and molecular biology. Morphological characters demonstrate that the two kinds of *Cordyceps* were similar to *Elaphocordyceps paradoxa* and *Ophiocordyceps yakusimensis*, respectively. Phylogenetic analyses of DNA sequences from 18S-28S rDNA Internal Transcribed Spacer ITS4/ITS5 sequence and/or nuclear ribosomal large subunit (26S rDNA) D1/D2 domain sequences analyses were most similar to *Elaphocordyceps paradoxa* and *Ophiocordyceps yakusimensis*, respectively. Therefore, morphological and molecular biology studies demonstrated the two kinds of *Cordyceps* were *Elaphocordyceps paradoxa* and *Ophiocordyceps yakusimensis*.

## VII-O-6. Taxonomy, sociobiology, nutritional and nutraceutical potential of termitophilous and lepiotoid mushrooms from North West India

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The present paper deals with biology and the evaluation of nutritional and nutraceutical potential of wild edible lepiotoid and termitophilous mushrooms from North West India. The studies reveal that presence of appreciable amount of most of the essential nutrients. Out of the total wild mushrooms analysed, *Termitomyces medius* possessed maximum (46.2%) amount of protein followed by *T. badius* (44%) whereas *T. striatus* possessed the least amount (12.95%) on dry weight basis. Maximum percentage of fat was in *Macrolepiota procera* (3.4%) followed by *Termitomyces mammiformis* (3.3%). All other mushrooms had considerably low percentage of crude fat per 100 g of dry sample. The crude fibre content was highest in *T. mammiformis* (8% of dry weight) while *T. badius* and *Macrolepiota rhacodes* possessed minimum percentage of crude fibre (2.5%). The carbohydrate content was highest in *Termitomyces striatus* was evaluated to contain (60.27%) followed by *T. mammiformis* (47.65%) while lowest carbohydrate content was in *T. microcarpus* (33.5%) and *T. medius* (33.3%). The overall energy value was maximum in *M. rhacodes* (364.7 kJ) as compared to the species of *Termitomyces* and *Macrolepiota*, evaluated. Amongst the minerals, Fe content was maximum in *T. mammiformis* (673 mg/100 g dry weight) followed by *T. radicans* (482 mg/100 g) and minimum in *T. striatus* (82 mg/100 g). In addition *Termitomyces* species are quite rich in Mg. *T. medius* (330 mg/100 g) had maximum amount of Mg followed by *T. heimii* (287 mg) and minimum in *T. microcarpus* (6 mg/100 g). Mn (13mg/100 g) and Ca (204 mg/100g) content was highest in *T. medius* and minimum amount of Manganese (1 mg/100 g) was recorded in *M. dolichaula* and *T. microcarpus* (6 mg), respectively. Cu was maximum in *T. striatus* (11 mg/100g) followed by *T. radicans* (9 mg/100 g) and *T. badius* (7 mg/100 g). As compared minimum quantity of this element was in *T. mammiformis* (4 mg). Zn was maximum (94.3 mg/100 g) in *M. rhacodes* followed by *T. microcarpus* (79.5 mg/100 g), whereas minimum quantity of Zn was recorded in *T. radicans* (35.9 mg/100 g). The nutraceutically important nutrient Se was maximum in *T. microcarpus* (123.2 mg/100 g) followed by *T. heimii* (113.10 mg/100 g) whereas minimum amount of this element was recorded in *T. striatus* (46.5 mg/100 g). The dry mushroom samples were also evaluated for the presence of heavy metals. Traces of some heavy metals viz., Hg, As and Cd were detected in *T. radicans*, *T. medius*, *T. badius*, *T. microcarpus* and *M. rhacodes* while Cr, Ag, and Pb were found to be absent in these mushrooms.

Phenolic content in *T. microcarpus* (25.85 mg) was maximum, followed by *T. mammiformis* (22.5 mg) and minimum in *Macrolepiota dolichaula* (5.9 mg). Flavonoids were found in small amount ranging between 1.36 mg/g in *T. microcarpus* and 2.02 mg/g in *M. rhacodes*. The  $\beta$ -carotene content was found in very low amount in different species ranging from 1.1  $\mu$ g/g in *T. heimii* to 1.5  $\mu$ g/g in *T. badius*. Lycopene was found to be least in these species ranging from 1.03  $\mu$ g/g in *T. heimii* to 1.27  $\mu$ g/g in *T. striatus*. Alkaloids were found in very small concentration ranging between 0.046 mg/g in *T. radicans* and *T. heimii* and 0.103 mg/g in *M. dolichaula*. Substantially low amount of phenolic content, carotenoids, alkaloids and flavonoids detected in these mushrooms account for their antioxidant property. Maximum content of vitamin A (retinol) in the analysed mushrooms was observed in *Lepiota humei* (0.17 mg/100 g) followed by *T. heimii* (0.12 mg/100 g) and minimum in *M. dolichaula* (0.075 mg/100 g). As compared thiamine (Vitamin B1) content ranged from 0.75 mg/100 g in *M. rhacodes* to 0.21 mg/100g in *T. heimii* and the riboflavin (Vitamin B2) content was maximum in *T. heimii* (0.25 mg/100g) and minimum in *M. rhacodes* (0.13 mg/100 g). Vitamin C (Ascorbic acid) was maximum in *T. reticulatus* (1.45 mg/100 g) and minimum in *Lepiota humei* (0.18 mg/100 g). The nutritional and nutraceutical constituents in these mushrooms are comparable to commonly cultivated mushrooms. Their culinary credentials also compares well with the commonly consumed vegetables. These mushrooms need to be explored further with a view to conserve them and domesticate them so that these can be fruitfully used for human welfare.

## VII-O-7. Biodiversity exploration of milky mushroom (*Calocybe indica* P&C)-concept to commercialization

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Indian mushroom industry is witnessing a fabulous change in recent years with respect to the types and strains cultivated. *Calocybe indica* P&C popularly known as milky mushroom is relatively new to world mushroom lovers. This species is highly suitable for commercial exploration in the warm humid tropical zones around the world. Limited attempts were made in cultivation of this species until 1990s. During 1992, an isolate of *Calocybe indica* was collected near a coconut tree (*Cocos nucifera*) at Coimbatore, Tamil Nadu, India. This isolate was found to out yield hither to known cultivated mushrooms around the globe (with an average bio-efficiency of 142 per cent in paddy straw). Further, this tropical edible mushroom variety possessed incomparable shelf life. During 1994-1998 the technology was severally field tested under University Adoptive Research Trials (ART) and Multi Location Trials (MLT) involving mushroom farmers in the entire state. Finally, the technology for commercial cultivation and the new variety APK2 has been introduced for the first time in the world from Tamil Nadu Agricultural University, Coimbatore, India during 1998. From the concept to commercialization, the journey was very tough and challenging. Systematic studies were undertaken during 1992-94 to standardize the physical, physiological, biochemical and cultural requirements for the commercial cultivation of milky mushroom. Continuing with the efforts through a research project funded by ICAR to develop milky mushroom hybrids (2003-2006); and also through ICAR - All India Coordinated Research Project on Mushroom (2000-2014), we have made sustained efforts to collect more than 25 wild isolates from different locations in Tamil Nadu. Many of these isolates were found to have association with the finer roots of *Cocos nucifera*, *Bororus flabellifer*, *Peltaphorum ferrugenum* and *Tamarindus indicus*. Sometimes they have also been found exclusively humicolous. Cultures of at least five isolates have been deposited in the National Repository (NCBI Gen accession No. AY636067) at the Directorate of Mushroom Research (DMR), ICAR, Solan, Himachal Pradesh, India. At present, the annual milky mushroom production in Tamil Nadu state alone is approximately 530 t worth of ₹ 8.0 crore. More than 120 milky mushroom growers are distributed throughout the state. Horizontal spread of the technology is done through ICAR-AICRP, KVKs and State extension functionaries.



## VII-O-8. Chinese caterpillar mushroom *Ophiocordyceps sinensis* industry in China- collection sites, processing plants and medicine preparation, an overview for the outside world

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Deng Chong Xia Cao, winter worm summer grass, the Chinese caterpillar mushroom *Cordyceps sinensis* is gaining importance as a potent source of medicine for critical human disease control in the world. Locally this mushroom is known as 'Yarsha Gumba', Yarsha Gamboo or 'Kira Ghas' since it parasitizes the Lepidopteran insect larvae of the *Hepialus armoricanus* under the soil. The first ever report of this fungus dates back to 18th century when Torrubia, a Franciscan friar in Cuba described it as the tree growing out of the bellies of wasps. That is the reason why genus *Cordyceps* is sometimes referred to as the Torrubia in honour of its inventor. The combination of an insect larvae or rather the mummy of a insect larvae converted into fungus mycelium along with the fungus stalk is known as 'Chinese plant worm' in the ancient literature, and now named as *Cordyceps sinensis*. This mushroom is reported to be used for cure of wide spectrum of human diseases, especially terminal diseases where there is no known cure available in other forms of medicine world over. There are two sources of *Cordyceps sinensis* medicine available in the world market, one is the medicine made from the fruit body collected from nature, and the second is the *in vitro* pure culture mycelium of the fungus grown on substrates like rice grain / wheat grain / liquid medium. While Chinese medicine makers use rice as a substrate, the American medicine makers use wheat grain as a substrate. This paper will give details about the preparation of medicine from the fruit body harvested from nature. The collection is done from the high mountains at elevations above 14000 feet above sea level, especially in heavy snow bound areas of higher hills in Tibet plateau extending into Himalayan mountains touching India, Nepal and Bhutan. The mushroom is collected in summer months of May -June when snow melts in higher mountains. The collection is done by the local people around the collection sites of the particular region who go for mushroom collection forays into higher hills and return to base camp after collection. The mushroom is then brought for marketing to the auction house (Mandi in Indian language) for this commodity, located at Xining, Qinghai Province, China. This is the only organized auction house for selling this mushroom in the world and mushrooms from other countries like Nepal, Bhutan and India find their way to this auction house in Xining for sale at attractive prices. This trade is completely in the hands of the minority community of China and auction house is predominantly crowded by these Chinese traders with their produce in polythene /cloth bags, selling this mushroom in the well organized auction house. The mushrooms are sold at very high prices, and price is determined by the quality of the fruit body, especially its size, colour and thickness. The prices in the US market have reportedly gone as high as US\$ 70000 per kg. These mushrooms are collected from most difficult areas in the mountains, and its occurrence in nature is rare and this medicinal mushroom is sold like gold and silver in the market. This mushroom is processed in the pharmaceutical factories located around auction house in Xining area in Qinghai Province, China. These mushrooms on arrival in the factory are first graded according to the size, thickness and colour (quality), and different grades separated. The mushroom product is marketed as mushroom powder, capsules, wine, ointment, cream and in many other forms.

### VII-O-9. Molecular characterization and *in vitro* evaluation of indigenous *Suillus* isolates for the production of mycorrhizal blue pine (*Pinus wallichiana*) seedlings

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Fresh basidiocarps of *Suillus* spp. were collected from conifer forests of the North-Western Himalayan region of India during monsoon season. Basidiocarps were identified using morpho-anatomical characteristics. Eight pure cultures were obtained from the basidiocarps of a range of *Suillus* spp. *Suillus* isolates obtained were identified and characterized molecularly by direct sequencing of internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) genes. Variation within the ITS region of the rDNA genes of *Suillus* was examined by restriction fragment length polymorphism (RFLP). Inter-specific variations in the length and number of restriction sites of the amplified ITS region was observed. Restriction enzyme digests of the ITS–rDNA products for eight *Suillus* isolates separated the isolates into five different groups. When comparing ITS sequences and RFLP patterns, the *Suillus* spp. could be reliably distinguished into five different species, namely *S. sibiricus*, *S. granulatus*, *S. triacicularis*, *S. himalayensis* and *S. indicus*. Among these five species, *S. triacicularis*, *S. himalayensis* and *S. indicus* were found to be new to the science and the phylogenetic analysis clearly distinguished these species as distinct species from other closely related species of *Suillus*. In addition, some physiological attributes of all the *Suillus* isolates, such as radial growth, biomass yield and mycorrhizal capacities were evaluated to select efficient native fungal inocula for the production of mycorrhizal blue pine (*Pinus wallichiana*) seedlings in nursery. Inter-specific and intra-specific variations were observed in radial growth, biomass yield and mycorrhizal capacities of different *Suillus* isolates. Furthermore, the effects of fungal isolates on growth, biomass yield and nutrient contents of *P. wallichiana* seedlings were assessed after four months of the mycorrhizal inoculation. All the *Suillus* isolates enhanced the growth, biomass yield and nutrient contents of *P. wallichiana* seedlings as compared to control treatment, but at different rates. *S. sibiricus* isolate SNW06 showed the highest improvement in plant growth, biomass and concentration of most nutrients, whereas *S. himalayensis* isolate SNW03 was found to be least effective. On the basis of physiological analysis, mycorrhizal colonization and growth response of *P. wallichiana* seedlings, *S. sibiricus* isolate SNW06 was found to be the most effective *Suillus* isolate for mycorrhizal inoculation of *P. wallichiana* seedlings in nurseries and experimental plantations, followed by *S. indicus* isolate SNW02.

### VII-O-10. Applicability of mushroom *Agaricus blazei* in diabetic BALB/c mice

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Some of the filamentous fungi and mushrooms that belong to the class Basidiomycetes are toxic and/or hallucinogens, however, they have other nutritional and pharmacological properties. They generally have high amounts of protein and essential amino acids, minerals, vitamins and fibre, the major one being  $\beta$ -glucans. Studies confirm the use of the mushroom *Agaricus blazei* for the prevention of cholesterol, atherosclerosis, hepatitis, cancer, diabetes and ulcers. The focus of this academic work is to treat diabetes mellitus (DM), a disorder in the metabolism of carbohydrates and lipids which implies poor manufacture and use the hormone insulin by the body leading to hyperglycemia and numerous secondary discomforts such as kidney and heart failure. The overall objective of the project was to verify the effect of consumption of *Agaricus blazei* in BALB/c mice carrying the DM metabolic disease and identify favorable relations that led to significant improvements in the clinical state of diabetic animals. To achieve this goal 45-days old BALB/c mice from the Central Animal Laboratory of the University of São Paulo at Ribeirão Preto (USP - RB) were used, weighing between 30 and 40g, divided into six distinct groups. The first was the negative



control (G1) consisting of healthy animals. The second was the positive control group (G2) with untreated diabetic animals. The third group (G3) was insulin-treated diabetic animals. The fourth (G4) consisted of diabetic animals treated with the mushroom *A. blazei* flour diluted in water with a concentration of 3g/kg of the animal, while the fifth group (G5) consisted of diabetic animals treated with the *A. blazei* flour 3g/ kg diluted in gelatine. The sixth group (G6) was treated with the hormone insulin and a diet with mushroom in gelatine. The animals were evaluated by digital glucometer at intervals of two days with the glucose test method, the samples were collected by pique tail of each animal. Euthanasia was caused by overdose of ketamine and xylazine. The total leukocyte count in a Neubauer chamber and differential counts were performed by analysis of thin blood smears stained with Panoptic method. The results showed an improvement in both the physical condition, and the metabolic condition of the animals, as well as a significant decrease in the glycemic profile of the treated groups.

## Poster Presentations

### VII-P-1. Crop cycle time, yield and protein content of *Lentinus squarrosulus* produced on some organic wastes

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Agricultural production and agro-food industry furnish large volumes of solid wastes, which when unutilized could lead to environmental pollution. An attempt was made to utilize wastes from oil palm and timber industries for the production of *Lentinus squarrosulus*, a Nigerian edible mushroom. Mahogany sawdust, *Gmelina* sawdust, oil palm fruit fibre and oil palm empty fruit bunch significantly influenced crop cycle time, yield and protein content of the mushroom. The shortest crop cycle time achieved (46 days) was with *Gmelina* sawdust, while oil palm fruit fibre produced mushrooms with the highest protein content (27.42%). Although there were no significant differences ( $p > 0.05$ ) in yield of mushrooms produced with oil palm fruit fibre and mahogany sawdust, oil palm fruit fibre gave the highest and most consistent yields. Considering the desirable characteristics of yield and protein content, oil palm fruit fibre proved the best waste for commercial production of *L. squarrosulus*.

### VII-P-2. Comparative study on bioactive constituents and biological activities of indigenous cultivated strains of *Ganoderma lucidum*

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Various conditions for successful cultivation of twenty indigenous strains of *G. lucidum* were optimized by studying the mycelial growth at different temperatures, pH and media. The quality differences of these cultivated strains of *G. lucidum* from different origins were investigated by comparing total polysaccharide, total triterpenoid and Ganoderic acid A (GAA) contents. All the strains were also evaluated in vitro for cytotoxic and antioxidant properties. The cytotoxic effect was determined against HepG2 cancer cells using MTT assay while the antioxidant activity was assessed using the DPPH radical scavenging assay. The total triterpene content was determined by spectrophotometric method based on colouring reaction of vanillin-perchloric acid and glacial acetic acid reacting with triterpenes. The total triterpene content varied among the strains and was in the range of 5.902 mg/g to 16.810 mg/g of dry powder. Total polysaccharide content was determined by anthrone-sulfuric acid method and was in the range of 0.609 mg/g to 4.174 mg/g dry powder. GAA accounts for more than half of the total triterpene content of *G. lucidum* and so the determination of GAA content can be used as one of the scientific basis for judging quality of *G. lucidum*. A simple, accurate and reliable RP-HPLC method using an isocratic mobile phase of acetonitrile and 0.1% aqueous acetic acid (80:20) was used to quantify GAA. The established method was validated in terms of linearity, sensitivity, precision, accuracy, stability and the content of GAA varied between the strains. The cytotoxic assay of the above strains against HepG2 cancer cell lines showed their cytotoxicity with the IC<sub>50</sub> values in the range of 43.39 µg/ml to 471.18 µg/ml. All the studied strains had profound antioxidant activity when tested using the DPPH radical with IC<sub>50</sub> values ranging from 34.674 µg/ml to 181.337 µg/ml. Furthermore, hierarchical cluster analysis (HCA) was also applied to evaluate the variation of chemical components. Results from all the above studies provided a basis to serve as a measure for quality control to validate and for selecting best strains for cultivation.

### VII-P-3. *In vitro* antioxidant properties of cultivated edible mushroom: *Agrocybe aegerita*

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The antioxidant activity of cultivated edible mushroom *Agrocybe aegerita* was analysed by testing its methanolic extracts with that of cultivated mushrooms *Lentinus edodes* and *Calocybe* species. Results showed predominant activity with respect to all the *in vitro* assays with different chemical systems including reducing power, DPPH free radical scavenging, ferric reducing antioxidant power (FRAP), superoxide scavenging, peroxide scavenging, ferrous ion chelating, total phenolic & flavonoid content. Scavenging effects of the mushroom extracts on 2, 2-diphenyl-1-picrylhydrazyl radicals were moderate to high (66.23-92.27%) at 1.5 mg/ml. Chelating effects on ferrous ions were moderate to excellent (78.41-96.65%) at 20 mg/ml. At 12 mg/ml, the reducing powers were excellent (84.04-92.82%). FRAP results were moderate in the range (18.60-72.35%) at 12 mg/ml. The ability to scavenge super oxide (% SOD scavenging) was moderate to excellent (26.21-92.32 %) at 20 mg/ml. The total phenols in the extracts ranged from 0.64-0.26 at 20 mg/ml. The total flavonoid content in the extracts ranged from 0.307-0.055 at 10 mg/ml. The results indicated that the antioxidant capacity of *A. aegerita* was much higher compared to other studied mushrooms.

### VII-P-4. *In vitro* optimization studies on mycelial cultivation of *Cordyceps tuberculata*

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Cultural studies like evaluation of solid medium, liquid medium, temperature and pH were conducted on *Cordyceps tuberculata* collected from Coleopteran insect. Seven solid and liquid media were tested for mycelial growth. Amongst seven solid media [yeast extract agar (YEA), malt extract agar (MEA), Dimmic medium (DM), yeast potato dextrose (YPD), potato dextrose agar (PDA), wheat grain agar and Czapek Dox agar (CDA)], maximum mycelial growth was observed in MEA (4.24±0.1 cm) and least in DM (3.00±0.0 cm). Amongst seven liquid media, [yeast glucose medium (YGM), potato dextrose broth (PDB), Glucose Asparagine Medium (GAM), Asthana & Hawker Medium (AHM), Dimmick Medium (DM), Peptone Water (PW) and Glucose Peptone Medium (GPM)], maximum mycelial dry weight was obtained in GAM (4.00±0.0 g/l) and least in PDB (1.00 ± 0.1 g/l). Maximum growth was observed at 24 °C (4.21 ± 0.012 g/l) at 6.5 pH.

### VII-P-5. A low cost technology for the cultivation of *Calocybe gambosa* (Fr.) Donk-a promising milky mushroom

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Milky Mushroom *Calocybe* spp. is an attractive mushroom with large, milky white sporophores, belonging to the family Tricholomataceae of the order Agaricales. The natural habitat of Kerala is blessed with different strains of milky mushrooms. A survey conducted during South-West monsoon season (2009-2010) in Koliyoor area of Thiruvananthapuram district resulted in the collection of *Calocybe gambosa* (Fr.) Donk, which was isolated into pure culture. Morphological characters were studied in detail. The sporophores

have bigger-sized pileus and club-shaped, stout and elongated stipe. Cultivation technology was standardized by polybag method on paddy straw and 1:1:1 sand-soil-vermicompost mixture as casing media. Compared to the commonly cultivated milky mushroom, *C. indica*, the sporophores of *C. gambosa* were more robust. The biological efficiency, size and weight of individual sporophores were more in *C. gambosa*. Average fruit body weight ranged from 250–620 g. Different substrates viz., paddy straw, sugarcane bagasse, banana waste, rubber sawdust and neopit were tried for its cultivation and maximum yield was recorded in paddy straw followed by rubber sawdust. Farm trial conducted in ten locations of six districts of Kerala-Thiruvananthapuram, Kollam, Pathanamthitta, Alappuzha, Kottayam and Ernakulam indicated the highest biological efficiency of 137.14 percent compared to 90.06 percent for *C. indica*. First flush appeared early (32 days) in *C. gambosa*, while it took 39.5 days in *C. indica*. Comparison of sensory evaluation of these two species based on colour, appearance, flavour, texture, taste and overall acceptability revealed 96.08 percent appreciation for *C. gambosa*, compared to 80.67 for *C. indica*. A low-cost substrate was tried for its cultivation in open conditions and greenhouses. A benefit-cost ratio of about 3:1 was achieved owing to low-cost substrates.

#### **VII-P-6. Proximate nutritional composition of wild edible mushroom *Astraeus hygrometricus* (pers.) Morgan**

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*Astraeus hygrometricus*, commonly known as Rugra in Jharkhand, is found to grow on sandy or lateritic soil in the forests of Jharkhand, West Bengal, Odisha, and Madhya Pradesh. Fruiting frequently occurs in rainy season between May-August. The fungus depends on trees for its growth and cannot be cultivated artificially. It occurs in association with sal (*Shorea robusta*) trees. Local people use it for short period for edible purposes. However, its nutritional benefits have not been fully explored. It then either rots or shrivels up limiting thereby its marketability. To determine the nutritive value of *A. hygrometricus*, the proximate nutritional composition of its fruiting bodies for content of moisture, crude protein, crude fat, total carbohydrate and minerals (Ca, Fe, Mg, Mn, and Se), employing the AOAC methods, was done. The fruiting bodies were found to contain moisture, 78.66%; protein, 21.56%; crude fat, 3.30%, total carbohydrate, 30.96%, and minerals (Ca, 171 mg/100g; Fe, 16.48 mg/100g; Mg, 53.51 mg/100g; Mn, 2.66 mg/100g, Se, 2.38 mg/100g). Gross energy was determined to be 240 kcal/100 g. Further comprehensive nutritional composition of the mushroom for validating its nutritional claims and adding value to increasing its utilization and marketability still needs to be established.

#### **VII-P-7. Taxonomic studies, pharmacognostic and phytochemical evaluation of *Trametes leonina* and *T. menziesii***

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Genus *Trametes* Fr. (Family Polyporaceae, Order Polyporales, Class Agaricomycetes, Phylum Basidiomycota) is characterized by sessile, pileate, dimidiate to fan shaped, single/imbricate basidiocarps; hispid to glabrous, often zonate abhymenial surface; whitish to creamish to pale grey hymenial surface; trimitic hyphal system; clamped generative hyphae; tortuous, solid binding hyphae; straight, thick-walled to solid skeletal hyphae; absence of cystidia and ellipsoid to allantoid, smooth, thin-walled, in amyloid basidiospores. Species of this genus are receiving attention because of their immunomodulatory, anti-

cancer, anti-tumor, hepatoprotective and lipid lowering properties. This paper provides information on the taxonomic details (macroscopic and microscopic), pharmacognostic parameters and phytochemical screening of two tropical species (*T. leonina* and *T. menziesii*). The material of these has been collected from various localities of district Haridwar (Uttarakhand) and has been deposited at the Herbarium of Botany Department, Punjabi University, Patiala, India. Both *T. leonina* and *T. menziesii* are being reported for the first time from the state of Uttarakhand. Pharmacognostic parameters (tapped density, bulk density, Hausner ratio, Carrs Index, oil absorption capacity, water absorption capacity, dispersibility, lesson drying, foreign matter, total ash value, acid insoluble ash, water soluble ash, alcohol soluble extractive, water soluble extractive, emulsion capacity and emulsion stability) of *T. leonina* and *T. menziesii* have been standardized and phytochemical screening of their aqueous extracts revealed that these can be a source of carbohydrates, phenols and tannins.

## VII-P-8. Taxonomy, physiology, cultivation, nutritional and nutraceutical studies on *Lentinus sajor-caju* (Fr.) Fr.

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Edible mushrooms are rich sources of nutritional and nutraceutical components. One such edible mushroom is *Lentinus sajor-caju* (Fr.) Fr. it is a white rot mushroom which degrades both cellulose and lignin biomolecules. The fruit body of *L. sajor-caju* was collected from the stem of *Bauhinia variegata* from Sirmour (Himachal Pradesh) in North-West India. Basidiocarps of this mushroom are large sized, variously coloured (whitish/ pale ochraceous/ yellowish brown) and characterized by infundibuliform pileus having in rolled margin, crowded linear gills and annulated stipe. It was taxonomically investigated, identified and brought into pure culture on Potato Dextrose Agar medium which was maintained through periodic sub-culturing. Cultural studies were carried out by selecting 14 different solid media and 14 different liquid media, 7 different temperatures (17 °C, 21 °C, 25 °C, 29 °C, 33 °C, 37 °C, 41 °C); varied pH levels (3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0); darkness and light conditions. Malt yeast extract agar, yeast glucose, 33 °C temperature, pH 4.0 and darkness were observed to support the maximum vegetative growth of the mushroom when incubated for 11 days. Preliminary studies were undertaken for its domestication. Spawn was prepared on wheat grains and cultivation was attempted on 3 lignocellulosic substrates i.e. sawdust, mixture of wheat straw and paddy straw (1:1) and mixture of wheat straw, paddy straw, sawdust and wooden flakes (1:1:1:1). Mixture of wheat straw, paddy straw, sawdust and wooden flakes (1:1:1:1) yielded maximum number of carpophores and gave 18.93% biological efficiency. The nutritional and nutraceutical attributes were evaluated from the fruit bodies of this mushroom collected from wild. *L. sajor-caju* contain 85.82% carbohydrates, 1.05% protein, 0.80% fat, 1.91% ash content, 3.99% fibres and rest was moisture content. Other constituents evaluated include xylose (0.011%), glucose (0.2%), sucrose (0.585%); ascorbic acid (0.42 mg/100g);  $\beta$ -carotene (0.22  $\mu$ g/100g); lycopene (0.086  $\mu$ g/100g); phenolic compound (8.83  $\mu$ g/100g) and alkaloids (0.80%). Amongst the fatty acids Palmitic Acid (41.29%) was found in maximum quantity. SFA (53.89%) were in maximum amount in comparative to MUFA (16.27%) and PUFA (1.31%). Amino acids and mineral profile was also studied using standard biochemical techniques. Fifteen amino acids were estimated quantitatively. Amino acid analysis showed that it contains both essential (Lysine, Phenylalanine, Leucine, Histidine, Isoleucine, Valine, Methionine, Glutamic Acid, Threonine) as well as non-essential amino acids (Alanine, Cysteine, Tyrosine, Glycine, Aspartic Acid, Serine). Mineral estimation study revealed the presence of C, O as main frame work elements; K, P, S, Ca, Mg, Na as macro elements; Cl, Si, Fe, Zn, Cu, Mn, Ni as micro elements and Ru, Sr and As heavy elements.



## VII-P-9. Testing of entomopathogenic fungi in biological control against pine weevil

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Coniferous forests in Slovakia, as well as in other European countries in recent decades are attacked by various harmful factors. The most important abiotic factor is the wind, which can in a short time destroy large complexes of spruce stands. After processing such calamities frequently create huge areas for afforestation, making very good conditions for pine weevil presence. It is a beetle causing a lot of damage by eating bark on the trunks of young seedlings, thereby causing a weakening or dieback of them. Actual research is focused on study of reactions of the pine weevil adult (*Hylobius abietis* L.) (Coleoptera: Curculionidae) to infection caused by various entomopathogenic fungi species (*Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria fumosorosea*). *Beauveria bassiana* is one of the longest known insect pathogens. The infection of insect occurs via conidia of fungus, which germinate on the surface of the cuticle, which after a short period of time grow on the surface vertically, penetrating the chitin exoskeleton. Actual research was based on infection of its adults with spores of various fungal species. 'Dry' and 'wet' conidia of entomopathogenic fungi were applied at concentrations of 105-108 conidia/ml. An important part of the research was to observe the intensity of infected beetle's feeding process. The main result of the research is the evaluation of the entomopathogenic fungi impact on mortality of pine weevil. The most significant effect in reducing of food intake has fungus *B. bassiana*, *M. anisopliae* had no effect on food intake, and beetles infected with fungus *I. fumosorosea* had even increased food intake. Mortality of pine weevil has increased by fungus *B. bassiana*, and good efficacy in this regard has also fungus *M. anisopliae*. *I. fumosorosea* had almost no effect on their mortality rate.

## VII-P-10. Use of Phase II mushroom compost in *Agaricus subrufescens* production

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In the last years, the cultivation of the medicinal mushroom *Agaricus subrufescens* Peck has evoked great interest worldwide, becoming increasingly popular and facilitating its expansion through many countries, mainly as a result of being a high value product in the international markets. During the crop development, techniques and methods previously established for *A. bisporus* production has been adapted, trying to take advantage of existing buildings and technologies. In this sense, the goal of this work outlines the assessment of *A. subrufescens* cultivation from substrates specifically prepared for the production of *A. bisporus*. A balanced factorial plan 3x2 with six replicates was used as experimental design. Factor 1, with three levels, corresponded to three phase II commercial composts (obtained from three different plants of composting) initially prepared for the production of *A. bisporus*. Factor 2, with two levels, corresponded to the loading density of compost (60 to 70 kg m<sup>-2</sup>). The strain used was ABL99/30 (Modulo de Cogumelos, FCA-UNESP, Brazil). Mushroom growing cycle was carried out according to the rapid induction model: incubation at 28 °C, induction up to 20 °C and cultivation at 26 °C and [CO<sub>2</sub>] 700 ppm. A peat based casing was used. The main production parameters (earliness, number of mushrooms and yield per unit area, biological efficiency, unitary weight and dry matter content) were assessed and the proximate analysis of harvested mushrooms was performed. There were no significant differences among treatments for any of the considered parameters. Biological efficiency values were between 46.10 and



58.52 kg dt<sup>-1</sup>, resulting in general higher than those registered in the references consulted. The fact that significant differences in behaviour between different composts used have not been observed, along with the good agronomic performance of them, allows to confirm its suitability for the production of *A. subrufescens* in the conditions used. As regards the loading density, although neither significant difference were registered, it is remarkable that a higher yield was obtained when 70 kg m<sup>-2</sup> was used instead of 60 kg m<sup>-2</sup> (10.96 against 10.19 kg m<sup>-2</sup>), although the biological efficiency registered (49.81 kg dt<sup>-1</sup>) was lower than that obtained with the lowest density (54.19 kg dt<sup>-1</sup>). This range between 60 and 70 kg m<sup>-2</sup> could be applied to commercial scale. The proximate analysis of the harvested carpophores did not provide significant differences between treatments or factors. Harvested mushrooms showed, on average, the following content on dry matter: protein 291.1 g kg<sup>-1</sup>; crude fat 20.4 g kg<sup>-1</sup>; total carbohydrates 615.6 g kg<sup>-1</sup>; available carbohydrates 540.9 g kg<sup>-1</sup>; crude fibre 74.7 g kg<sup>-1</sup>; ash 73.0 g kg<sup>-1</sup>; energy value 353 kcal per 100g. With regard to the content of other cultivated species of edible fungi, *A. subrufescens* is remarkable in general for its low content of moisture, crude fat, crude fibre and ash, and high content in protein and total carbohydrates.

## VII-P-11. Mycelial characteristics of several *Psathyrella* collections

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Based on Index Fungorum presently the genus *Psathyrella* together with 24 other genera (*Coprinellus*, *Coprinopsis*, *Drosophila*, *Lacrimaria*, *Macrometrula*, *Parasola*, etc.) from former family Coprinaceae Sing. is included in the family Psathyrellaceae, phylum Basidiomycota. Molecular data shows that genus *Psathyrella* is polyphyletic and species of this genus are phylogenetically closer to species from new clades *Coprinellus* and *Coprinopsis*. Currently *Coprinellus* and *Psathyrella* are discussed as sister clades. Taxonomically significant biological (morphological, ecological, physiological, genetic, etc.) characteristics of mycelia of Basidiomycetes mushrooms together with characteristics of fruiting bodies play important role in their phylogenetic reconstruction. From this point of view study of biological characteristics of mycelia of 12 *Psathyrella* collections and evaluation of their taxonomic significance is topical. Cultural observations were carried out on malt-extract and potato dextrose agar media (MEA, PDA). All studied collections formed white cottony colonies with well developed aerial mycelium on both media. The colonies were fast growing (up to 15.4 mm/d) on MEA, however, on PDA they formed denser colonies growing with decreased growth rate (up to 11.0 mm/d). Hyphae are often with round shape clamp cells similar to hyphae of *Coprinopsis* species. Not well developed irregular hyphal loops previously described in *Coprinopsis* species were also observed in the tested *Psathyrella* collections. In old cultures rare chlamydospores and Hormographiella type arthro spores described previously in other Psathyrellaceae species were observed. Cylindrical crystals were abundantly present in the medium. Study of interspecific antagonism between *Psathyrella* collections and 7 coprinoid species (*Coprinus comatus*-3 strains, *Coprinellus disseminatus*, *C. radians*, *C. flocculosus* and *Coprinopsis atramentaria*, *C. strossmayeri*, *C. cinerea*-1 strain of each species) from 3 clades *Coprinus*, *Coprinellus* and *Coprinopsis* in dual culture revealed high interspecific antagonism between *Psathyrella* collections and *C. comatus* (family Agaricaceae). Mycelial growth rate indicators in *C. comatus* strains were decreased significantly (60.3%) while in *Psathyrella* collections they changed insignificantly (9.5%). Interspecific antagonism between *Psathyrella*, *Coprinellus* and *Coprinopsis* collections (family Psathyrellaceae) was weaker and not clade specific which shows their phylogenetical relatedness. Thus, mycelial characteristics and inter specific antagonism are taxonomically significant and their further studies will assist in phylogenetic reconstruction of Psathyrellaceae species/clades.

# **Session-VIII**

## **Pests and Diseases**



## Oral Presentations

### VIII-O-1. *Cladobotryum mycophilum* - causal agent of cobweb disease on commercial *Agaricus bisporus* and *Pleurotus eryngii* crops in Spain

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Severe outbreaks of cobweb were observed in commercial white button mushroom (*Agaricus bisporus*) and king oyster mushroom (*Pleurotus eryngii*) crops in Castilla-La Mancha (Spain) between 2008-2011. Based on Koch's postulates, morphological characteristics and DNA sequence data identity of the pathogen was established as *Cladobotryum mycophilum*. Two pathogenicity trials were performed in *A. bisporus* growing rooms, using in each assay 24 blocks containing pasteurized *A. bisporus* substrate. Nine days after casing, conidial suspension of one isolate of *C. mycophilum* was sprayed onto the surface of the casing layer of twelve blocks at a rate of 10<sup>6</sup> conidia m<sup>2</sup>. Twelve blocks were sprayed with sterile distilled water as control. The first cobweb symptoms developed after 25 days of incubation, between the 2nd and 3rd flush (trial A) and after 11 days, between the 1st and 2nd flush (trial B) after inoculation. *C. mycophilum* was consistently re-isolated from eight inoculated blocks (67%) in trial A and eleven inoculated blocks (92%) in trial B. The total area of the crop affected by cobweb was 30% in the inoculated blocks (trial A) and 45% (trial B). The non-inoculated blocks remained healthy. Compared with the uninoculated controls, decrease in the yield of 10.7% was observed in trial A and 9.1% in trial B. Pathogenicity tests were also performed using 46 blocks containing sterilized, spawned and incubated *P. eryngii* substrate. The blocks were placed in a mushroom growing room and cased with a mineral casing layer. Five days after casing, a conidial suspension of one isolate of *C. mycophilum* was sprayed onto the surface of the casing layer of 24 blocks at a rate of 10<sup>6</sup> conidia m<sup>-2</sup>. Twenty-two blocks were sprayed with sterile distilled water as a control. The first cobweb symptoms developed 23 days after inoculation and *C. mycophilum* was consistently re-isolated from nine (37.5%) of the inoculated blocks. Un-inoculated blocks remained healthy. In a second test, conidial suspensions (3.4 x 10<sup>5</sup> ml<sup>-1</sup>) of three isolates of *C. mycophilum* were inoculated (50 µl) onto 20 *A. bisporus* and 20 *P. eryngii* fruit bodies. Sterilized distilled water was used as a control. All fruit bodies were then incubated at 22 °C in a moist chamber. Assays were conducted twice and the results were recorded after 7 days. *C. mycophilum* grew in 100 % of inoculated *A. bisporus* carpophores while between 80 and 85% of *P. eryngii* ones, while the control fruit bodies remained symptomless. The identification and characterization of *C. mycophilum* causing cobweb disease in *A. bisporus* and *P. eryngii* in Spanish mushroom crops is expected to provide useful information for controlling this economically important disease.

### VIII-O-2. Effect of essential oils and *Bacillus subtilis* on mycopathogens of *Agaricus bisporus*

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The control of mycopathogens in mushroom cultivation is based on the usage of very limited active substances, cultural protection practices and sanitary precautions. The lack of permitted/ recommended

chemicals is a problem for mushroom farmers and fungicide residuals are serious issues in mushroom cultivation as well. Integrated disease management is used in mushroom cultivation, but there is a need for novel selective fungicides and cheap, reliable disinfectants. The main target of the present work is to find natural products – either for prevention and/or for treatment – which can be tested in cultivation against the major *Agaricus bisporus* fungal diseases. Essential oils of aromatic plants, *Bacillus subtilis* and active substance prochloraz-Mn (Sporgon 50WP) were tested in vitro. Tested essential oils: *Cinnamomum zeylandicum*, *Citrus aurantium*, *Matricaria chamomilla*, *Mentha spicata*, *Pelargonium graveolens*, *Salvia officinalis*, *Thymus vulgaris*. The examined pathogens: *Clabobotryum dendroides*, *Mycogone perniciosa*, *Lecanicillium fungicola* and *Trichoderma aggressivum* f. *aggressivum*. The effect of essential oils on the growth of a cultivated hybrid variety of *A. bisporus* was also tested. The efficiency of the chemicals was tested with four methods: 1. Hole-test: PDA media were slanted into petri-dishes, then 3 holes were cut in the media. Two holes were filled with agents to be tested and one with sterilized DW for control. The inhibition zone of developed colonies was monitored. In Contact test the chemicals were mixed into the liquid PDA media after cooling, then slanted. The fungi were placed in the centre of the petri-dishes, then the colonized area were measured and compared to non-treated media. In volatile fraction test PDA media were slanted into petri-dishes, then inoculated with fungus mycelia and turned down on the lid a sterilized paper was placed and sprayed with essential oils and other chemicals. Each above mentioned treatment had four replicate and the petri-dishes were incubated at 25 °C for 5 days. In the latter two test fungal colony diameters were measured. Test on mushroom fruit bodies, the effect of the tested material was also investigated on fruit bodies. The essential oils in different concentrations (cc., 10x, 100x and 1000x dilution) were pipetted onto mushrooms, then lesions were monitored. Among the tested oils *Cinnamomum*, *Mentha* and *Pelargonium* proved to be the most effective, as they completely inhibited the growth of all four mycopathogens. It can be concluded that Citrus and Tyhmus were not effective in this case. *Bacillus subtilis* was effective against all tested pathogens in contact test. Further research is needed to determine correct application and concentrations, because almost all tested oils inhibited the growth of the cultivated mushroom hyphae as well. From that point of view, only *B. subtilis* approved prospective in cultivation of mushroom.

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### **VIII-O-3. Integrated approaches for the management of *Mycogone perniciosa* causing wet bubble disease**

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Wet bubble in white button mushroom (*Agaricus bisporus*) incited by *Mycogone perniciosa* Magn has been reported as one of the serious diseases from almost all the major mushroom growing countries of the world. Bubble or mole, first described from Paris in 1888, has been reported to assume serious proportions in other major mushroom growing countries of the world., In India, this disease was reported for the first time in 1978 from some mushroom farms in Jammu and Kashmir. Later, this disease has been reported from the states of Himachal Pradesh, Haryana and Maharashtra. As the pathogen inflicts serious damage to the crop, various attempts have been made to manage the disease through various means. Interaction between *A. bisporus* and *M. perniciosa* studies conducted in dual, half plate and paired cultures. The average growth of *A. bisporus* and *M. perniciosa* in either dual culture was 16.13 and 28.86 mm, respectively. Growth of *A. bisporus* remain unaffected (16.02 mm) and *Mycogone* enhanced to 36.91mm (21.80% increase) when both grown in dual culture. Pre spawning of casing soil 5-20 days prior to pasteurization resulted in reduced incidence of wet bubble disease. Thermal death point of *Mycogone* was observed to be at 44-45 °C. Moisture contents of casing soil less than 60% at the time of pasteurization

favoured the survival of *M. perniciosus*. *Mycogone* failed to survive in casing soil having moisture contents of 60% or above at 60 °C or above temperature. Two bacterial isolates B-9 (*Bacillus*) and B-18 (*Alcaligenes*) proved to be very promising bio control agents for the management of wet bubble disease both under laboratory and mushroom house conditions. Out of five fungicides and two other chemicals tried, carbendazim proved most effective in managing wet bubble disease among all the fungicides/ chemical tested.

#### VIII-O-4. Investigation on mushroom diseases in China

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Systematic investigation on the edible mushroom diseases was carried out in China since 2009. Fourteen competitive diseases, 11 mycelial mycoparasites, 20 fruit body diseases and 5 physiological diseases were found successively. Occurrence of dry bubble disease (*Verticillium fungicola*) and wet bubble disease (*Mycogone perniciosus*) were common in *Agaricus bisporus*, which were caused by Ascomycetes and its anamorph. Fruit body diseases in *Pleurotus* mushrooms occurred seriously including *P. eryngii* and *P. ostreatus* was caused in *Pseudomonas tolaasii*. The bagged sawdust cultivation method has been popular in China and as such it was more likely to result in substrates infection by fungi. Mycelia of edible mushroom were often decomposed under these circumstances. The slippery scar disease in *Auricularia polytricha* caused by *Scytalidium lignicola* and mycelia apoptosis in bagged sawdust of *Lentinus edodes* by *Trichoderma* spp. occurred seriously and often result in enormous losses. The former is due to improper sterilization of bag breakage, while the latter was due to mycelial growth at high temperature that caused decline in mycelia resistance to the *Trichoderma* spp. The spawn decline seems to be caused by mycovirus, but its pathogen remains to be uncertain which need further studies. Moreover, poor ventilation lead to fruit body malformation, and the other physiological disorders were common.

#### VIII-O-5. Impact of leached spent mushroom compost extract on yield, quality and disease suppression of *Agaricus bisporus*

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The leachate of spent mushroom compost (spent mushroom compost tea) was pasteurized and utilized for irrigating the casing soil in *Agaricus bisporus* cultivation. The concept behind this was to suppress dry bubble disease and its impact on mushroom yield and quality. The result of chemical analysis of pasteurized leached compost extract (PLCE) showed that there were more nitrate, less potassium and salinity in PLCE as compared to pasteurized spent mushroom compost. The result also showed that there was no significant difference in growth of vegetative mycelium but a positive effect was estimated in yield, quality and disease suppression in when casing soil was irrigated with pasteurized leached compost extract.



## Poster Presentations

### VIII-P-1. *Agaricus bisporus* and *Mycogone perniciosus*: *in vitro* hyphal interaction and enzymatic studies

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Host-parasite interactions of *Agaricus bisporus* (button mushroom) and *Mycogone perniciosus*, a causal agent of wet bubble disease were studied using paired cultures and the infection was studied using phase contrast and scanning electron microscopy (SEM). Three strains (U3, P1 and S11) of *A. bisporus* were used to study their interaction with *M. perniciosus*. Total hyphal damage was observed at the point of interaction using scanning electron microscopy, but no apparent alterations could be detected using phase contrast microscopy. *In vitro*, different cell wall degrading enzymes were produced by *M. perniciosus* when it was grown along with different strains of *A. bisporus* in potato dextrose broth. The hydrolytic enzymes were induced by the presence of *A. bisporus* mushroom extract, quantification of enzymes revealed that the mycopathogen is capable of producing considerable levels of enzymes like chitinase (23.4 U/ml), protease (25 U/hr), amylase (7.89 U/ml) and cellulase (42.18 U/ml). The presence of enzymes like amylase, protease, glucanase and chitinase was further confirmed by SDS- PAGE using crude extract of interaction broth. Since, enzyme production in pathogenic fungi is specific and forms an important criterion for successful development of disease, these results suggest that the primary action of mycopathogen in establishment of infection was to attack the host cell wall.

### VIII-P-2. Chemical and biological control of diptera in Spanish mushroom crops

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The phorid *Megaselia halterata* and the sciarid *Lycoriella auripila* are common pests in Spanish mushroom farms. *M. halterata* has been seen to be the dominant species (ratio 4:1), with the highest number of phorids in spring and autumn. In Spanish farms, sciarids have been best controlled with diflubenzuron and ciromazina, but phorids have only been controlled with diazinon. The evaluation of active substances set in motion by the EU has considerably reduced the number of insecticides authorized, with diazinon one of them. The main objective of this study was to look for alternatives to control mushroom flies, especially phorids. Several assays were carried out using entomopathogenic nematodes, *Steinernema feltiae* and *S. carpocapsae*, and the insecticides diflubenzuron and triflumuron. The effectiveness against flies and effect on yield were evaluated. Effectiveness trials were set up in an experimental mushroom growing room. At the end of the spawn running, some trays were exposed to infestation in a commercial mushroom crop, where they were left for 48 hours. Afterwards, the trays were placed in the experimental room again, where they were covered with a structure made of antitrips mesh. A yellow sticky trap was placed inside each of them to capture the flies. On days 16-17, after casing, treatments were applied. The experiments finished after the emergence of the first generation of adults. The captured flies were counted and identified and the percentage reduction in adult emergence was calculated. Phytotoxic trials were set up in the same way as described before, but there was no infestation. The effect of treatments on mushroom productivity was evaluated from the yield of three flushes, the biological efficiency of the crop and the earliness of the harvest. A randomised complete block design with four treatments and ten replicates was used in the nematode trials: C, uninfected control; CI, infested control; Sf, fly infested trays and subsequent application of 106 IJ m<sup>-2</sup> of *Steinernema feltiae*; Sf+Sc, infested trays and application of 0.5 106 IJ m<sup>-2</sup> S.

feltiae + 0.5 106 IJ m<sup>-2</sup> S. carpocapsae. Phytotoxic trial treatments were C, Sf, Sf + Sc. No decrease in the population of *M. halterata* was detected with either nematode application used, while the number of *L. auripila* fell with both treatments, particularly with the Sf treatment. The production parameters were not affected by any treatment. A randomised complete block design with four treatments and six replicates was used in the insecticide trials: C1, infested control; D, infested trays and diflubenzuron (1 g m<sup>-2</sup> a.i.); A1, infested trays and triflumuron (0.5 g m<sup>-2</sup> a.i.); A2, infested trays and triflumuron (1 g m<sup>-2</sup> a.i.). The phytotoxic trial treatments were: C, D, A1 and A2. Diflubenzuron and triflumuron were highly effective against sciarids, regardless of the application dose. However, the application of these insecticides had no effect on *M. halterata* populations. The production parameters were not affected by any treatment. Further, efforts to develop methods to control mushroom phorids are necessary.

### VIII-P-3. *In vitro* lethal capability of ten strains of edible mushrooms against *Haemonchus contortus* (nematode) infective larvae

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*Haemonchus contortus* is considered to be the most pathogenic haematophagous gastro-intestinal parasitic nematode in small ruminants. The most common control method for this and other nematodes is the continuous administration of chemical anthelmintic (AH) drugs in the host animals; however, the indiscriminate use of these products has triggered some problems including anthelmintic resistance. New alternatives of control are necessary to reduce the use of AH drugs. This study was conducted to evaluate the *in vitro* nematicidal activity of mycelia from ten strains of edible mushrooms against *Haemonchus contortus* infective larvae. This study was carried out using water agar plates. Eleven groups of plates were established: One control (without mycelium) and ten groups each containing mycelia of each selected fungi. Five hundred *H. contortus* L<sub>3</sub> were released on each plate (n=10) and incubated at 18-25°C, for 5 days. The highest lethal effects (82-99%) were recorded with *P. ostreatus* ECS-1123 and ECS-0152, *P. eryngii* ECS-1292, *P. cornucopiae* ECS-1328 and ECS-1330 and *L. edodes* ECS-0401.

### VIII-P-4. Molecular characterization of *Cladobotryum* species associated with cobweb disease of mushrooms

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*Cladobotryum* species associated with cobweb disease of edible mushrooms were isolated from fruit bodies of *Agaricus bisporus*, *Calocybe indica*, *Pleurotus sajor-caju*, *P. sapidus*, *P. florida* and *P. ostreatus*. The nucleotide sequence comparisons of 5.8S rRNA identified 15 *Cladobotryum* isolates into three taxa, *Cladobotryum dendroides*, *C. mycophilum* and *C. asterophorum*. The RAPD primers exhibited both inter and intra-specific variations among the test isolates and separated them into seven distinct phylogenetic sub-clades. In the light of molecular identification the cultures of *C. dendroides* were redesignated as *C. mycophilum* and *C. asterophorum*. The present studies indicates that at least three species are associated with cobweb disease of different cultivated mushrooms in India and *C. mycophilum* is potential cause of cobweb disease in *Agaricus bisporus* and not *C. dendroides* as described earlier. *C. mycophilum* has wide host range and it can also infect milky mushroom. *C. asterophorum* was found associated with different species of oyster mushrooms and suggests wide geographical distribution and is a potential threat to the *Pleurotus* cultivation.

### VIII-P-5. Morphological characteristics of wet bubble disease (*Mycogone pernicioso*) isolated from button mushroom (*Agaricus bisporus*) and assessment of factors affecting disease development and spread

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Wet bubble disease (*Mycogone pernicioso*) is a devastating disease in the crop production of mushrooms. In India, it has been reported to cause serious crop losses. It is also common contaminant, occurring in mushroom houses in the Kashmir valley. The aim of the present study was to investigate physiological characteristics of *M. pernicioso* isolated from button mushroom (*Agaricus bisporus*). The assessment of the factors affecting the development and spread of Wet bubble disease was also done. The isolated fungus produced copious flocculent mycelia and the colour of colonies changed from white to pale brown and finally dark brown after 12-14 days of incubation at  $24 \pm 1$  °C. The maximal radial growth (90 mm) was recorded on compost agar medium after 15 days, followed by potato dextrose agar (75.5 mm) and malt extract agar (72.0 mm). Microscopic examination of the pathogen revealed that the mycoparasite was both inter and intra-cellular. The conidiophores were erect, long and verticillately branched. Conidia were oval, single, 2-celled and thin walled. The pathogen was successfully re-isolated when admixed with the compost at 6<sup>th</sup>, 7<sup>th</sup> or 8<sup>th</sup> turnings i.e. on the 22<sup>nd</sup>, 25<sup>th</sup> and 29<sup>th</sup> day of composting with a pile temperature of only (0-30 °C). The pathogen was also present in viable form in all the test samples of casing mixture in varied populations. The pathogen was continuously present in samples from spent compost of diseased trays. It was also observed that only the garden soil and the spent compost carried the wet bubble pathogen varying in populations each year. The peat soil, virgin soil, farm yard manure and sand did not yield any *M. pernicioso* propagules during either year.

### VIII-P-6. Mycophagous nematodes : a threat to button mushroom cultivation in Haryana

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Haryana is one of the leading states in seasonal mushroom cultivation. Farmers are facing serious problems due to nematodes. The button mushroom compost samples from different locations of Haryana collected/received in the Mushroom Technology Laboratory (MTL), Department of Plant Pathology, CCSHAU, Hisar during 2012-2014 were analysed for the presence of nematodes. The survey and analysis revealed that the predominant nematodes in the samples were *Aphelenchoides* spp., *Aphelenchus* spp., and *Ditylenchus* sp. Almost 80% of the samples were having these myceliophagous nematodes. A few of the samples contained *Seinura* spp. and *Fictor* spp. the predatory nematodes. It was also observed that the samples which had predatory nematodes were lacking or having very less number of mycophagous nematodes. The other nematodes present in the samples were *Rhabditids*, *Cephalobids* and *Dorylaimids*. The losses observed in the mushroom houses were 20% to complete crop failure. In Haryana, since the button mushroom is cultivated under seasonal conditions using compost prepared by long method and many times casing mixture was not properly sterilized. This has lead to the build up of pathogenic nematodes and due to this the growers are shifting the mushroom houses/beds every year from their existing places to new areas. The negligible presence of mycophagous nematodes in the samples having predatory nematodes may present an avenue for the biocontrol of these pathogenic nematodes.

### VIII-P-7. Prevalence of competitor moulds and diseases in straw mushroom (*Volvariella volvacea*) beds and their management

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*Volvariella volvacea*, commonly known as paddy straw mushroom is the most popular and palatable mushroom in south-east Asia. It ranks sixth among the edible mushrooms accounting for 3% of the global production. Odisha is the leading state in terms of straw mushroom production in India. Over 8,000 tons of straw mushroom is produced every year generating a turnover of approximately 80 crores of rupees. However, a number of competitor moulds infest the beds at different stages of crop growth and bring down the mushroom productivity substantially. *Coprinus* spp., *Aspergillus* spp. and *Rhizopus* sp. are frequently encountered in mushroom beds both in summer and kharif seasons which compete for space and nutrients in substrate inhibiting growth of *V. volvacea*. Besides, bacterial button rot disease is an emerging problem in the hot and humid coastal belt. Considering the gravity of the situation a survey was conducted during the summer season of 2013 in the coastal agro-ecological situation of the state to identify the competitor moulds and diseases encountered in the straw mushroom beds. Besides, the performance of physio-chemical and biological methods was evaluated for their management. Survey was conducted in the leading mushroom growing districts of Khurda and Puri at 10 locations both in outdoor and indoor situations. A minimum number of 100 beds per location were observed for the contaminants and diseases and per cent incidence was recorded. A trial was designed with 10 treatments each with six replications including the untreated control in randomized block design to evaluate various physico-chemical and biological means for management of contaminants and diseases. Straw mushroom beds of size 1.5'x1.5'x1.5' were raised. Beds were spawned @3% of the dry weight of substrate in three layers at 1:1:2 proportions. Beds were supplemented with wheat bran @200 g each. Appropriate aftercare of beds were taken till fruiting. Observations on yield and yield attributing parameters were recorded from two flushes at two and three weeks of spawning. Data revealed as many as eight competitor moulds contaminated the straw mushroom beds during the fruiting stage. *Coprinus* spp. was predominant of all in both outdoor and indoor farming situations. However, outdoor farming recorded more bed contamination (46.8%) than the indoor one (27%). Bacterial button rot disease was recorded to the tune of 13 to 9% in indoor and outdoor situations, respectively. The data further revealed that the pre-soaking of the substrate with 2% calcium carbonate solution for six hours was significantly superior among the treatments in giving a yield of 1016.67 g/bed with a corresponding biological efficiency of 14.52%. Application of 2% neem leaf extract was next in order in respect of yield (946.67 g/bed) and biological efficiency (13.52%). Benomyl (0.15%) with streptocycline (0.01%) application yielded 943.33 g/bed which were at par with the treatment that received neem leaf extract. Further, the intensity of *Coprinus* spp. was found to be low in the above mentioned treatments as compared to the untreated control. The improvement of pH of the substrate through calcium carbonate supplementation could be the sole factor in suppression of *Coprinus* and consequently yield improvement. Hence, this seems to be a viable proposition for small growers in the hot and humid coastal belt of Odisha.

### VIII-P-8. Studies on abiotic disorders of button mushroom

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In addition to biotic agents, which adversely affect mushroom production, there are a large number of abiotic agents which create unfavourable environment for the proper growth of mushrooms resulting in both quantitative and qualitative loss. These abiotic agents include low or high moisture in the substrate, pH, temperature, CO<sub>2</sub> concentration in the room, air velocity, fumes of hydrocarbon and other related products and variability in relative humidity. Many of these agents make the substrate non-selective for



mushroom mycelium and encourage other moulds and pests while some interfere with the normal mushroom growth. Management of environment is of great significance in mushroom cultivation and any deviation from the optimum requirements may lead to various kinds of abnormalities. Since a major proportion of button mushrooms is being produced under natural climatic conditions in India, studies were carried out to simulate the symptoms of abiotic disorders. Various abiotic factors viz., effect of diesel and kerosene oil fumes, smoke, excessive use of pesticides, excessive aeration/ no aeration, high temperature, low RH were evaluated and very specific and clear symptoms of abiotic disorder like stroma, rose comb, scales, onion shaped, long stipe, mass pinning and brown discoloration were recorded. Fumes of kerosene oil caused barrel shaped mushrooms and scales. Various abnormalities simulated so resulted in 11.56-94.75% loss of button mushroom yield.

## VIII-P-9. Microbial contaminants in oyster mushroom (*Pleurotus ostreatus*) cultivation, their management and role of meteorological factors

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Oyster mushroom (*Pleurotus* spp.) is an efficient lignin degrading mushroom that can grow easily on different types of ligno-cellulosic materials including agricultural and forest waste. Despite of its successful cultivation, the main obstacle for increased production is frequent contamination of the mushroom beds with micro flora. An attempt was made to study the role of environmental factors on the development of competitor mould fungi and their management with the help of phyto-extracts during 2010 and 2011. Survey revealed the occurrence of seven contaminants in mushroom beds and out of which *Trichoderma harzianum*, *Penicillium notatum*, *Sclerotium rolfsii*, *Aspergillus niger* and *Coprinus* spp. were the most dominant fungal contaminants and were high during June and July (28.4 & 35.8%) causing maximum loss in mushroom yield. The incidence of *T. harzianum*, *P. notatum*, *S. rolfsii*, *Aspergillus niger* and *Coprinus* spp. was less during the month of January, October, November and December in both seasons (2.80 to 5.60%) and good harvest of mushroom (81.5% to 105% biological efficiency) can easily be obtained. Minimum Incidence (2.80%) was noticed during the month of January. A range of average maximum temperature (24.63-33.18 °C), minimum temperature (9.40-25.51 °C) and average relative humidity (68.90 -85.27%) was found most appropriate for the cultivation of oyster mushroom in this region. High R<sup>2</sup> values of (+1.00) and (+0.916) indicated 100% contribution of all meteorological factors on the incidence of contaminants and about 91.6% contribution of meteorological factors and microbial contaminants towards the yield of *Pleurotus oestratus*. Eight different botanicals were tested along with the most popular chemical treatment (carbendazim 75 ppm + formalin 500 ppm) against the competitor mould fungi *in vitro* and *in vivo*. Chemical treatment (bavistin 75 ppm + formalin 500 ppm) was found to be most effective in reducing the mycelial growth of five contaminants (65.4 to 86.6%) *in vitro* and checked incidence of competitor moulds 81.36% *in vivo*, which increased the yield up to the tune of 35.20% (106% BE). Among the botanicals, *A. indica* (neem) showed its supremacy among all the botanicals tested and gave minimum effect on the growth of mushroom mycelium (4%) and exhibited maximum inhibitory effect (54.1 to 71.6 %) against *Aspergillus* spp., *Trichoderma* spp., *Coprinus* spp., and *Penicillium* spp. but was less effective against *Sclerotium rolfsii* *in vitro* followed by extracts of *Pongamia pinnata* (6.7%) and (42.4 to 61.3%) inhibition, respectively against the mycelium of *P. ostreatus* and mould fungi. A range of 35.3 to 62.4% reduction in the incidence of inky caps (*Coprinus* sp.) and 26.3 to 68.4% reduction in green moulds (*Trichoderma* spp) were recorded with different phyto-extracts. All the botanicals except *Acacia nilotica* reduced the incidence of competitor moulds (18.18 to 70.91%) in mushroom beds which resulted in an increase of mushroom yield up to the tune of 21.3%. The information will provide an idea about the appropriate cultivation period of oyster mushroom in the lateritic belt of West Bengal as well as provide an alternative method of surface sterilization of substrates which can minimize the use of fungicides in mushroom cultivation.

## VIII-P-10. Exploration of antifungal bioactive compounds of *Pisolithus tinctorius* (Pers.) coker against soil borne plant pathogens

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*Pisolithus tinctorius* (Pers.) Coker is an ectomycorrhizal fungus that forms abundant wealth of biomass in forested ecosystem, with hidden treasure of bioactive compounds and secondary metabolites having multifaceted use in health and agro chemical industries. The potential of this fungus is yet to be explored in India. During the present investigation, basic studies were undertaken to identify an effective isolate of *Pisolithus tinctorius* for the biomass production, extraction and testing of its bioactive compounds and secondary metabolites against soil borne plant pathogens viz., *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Synder and Hansen; *Macrophomina phaseolina* (Goid); *Rhizoctonia solani* (Kuhn) and *Sclerotium rolfsii* (Sacc). An isolate of *P. tinctorius* MTP1 was isolated from Eucalyptus plantations from Jakanarai Forest Range, Meetupalayam, and Tamil Nadu during November 2013. Appearance of sporocarps coincided with North-East monsoon. Modified Melin Norkrans (MMN) growth medium was found to be ideal for the mass production of the fungus and an average 6.80 g of biomass per 100 ml of MMN broth adjusted to pH 5.5 was obtained in 30 d at 30 °C. Complete darkness during incubation favoured the biomass production. The stationary growth phase of *P. tinctorius* isolate MTP1 for the extraction of secondary metabolites was found to be beyond 45 d. The cell free culture filtrate (CFC) at 1500 ppm showed the maximum mycelial inhibition of *F.o.f.sp. lycopersici* (52.00 percent); *R. solani* (51.92 percent) and *M. phaseolina* (48.23 percent). The CFC mixture did not inhibit the growth of *S. rolfsii*. Comparative bio-efficacy studies of CFC and fungicides viz., carbendazim, tebuconazole and chlorothalonil used at 0.1 percent indicated that, carbendazim and tebuconazole had completely inhibited growth of *F.o.f.sp. lycopersici*, *R. solani*, *M. phaseolina* and *S. rolfsii*. However, *P. tinctorius* CFC also inhibited the pathogens, except *S. rolfsii* considerably (around 50 percent) as compared to control. Ethyl acetate fraction of whole fruiting body (inclusive of basidiospores) extract yielded 0.2 percent bioactive composite while, that of CFC condensate yielded 0.0057 percent recovery of biomolecules respectively. In well diffusion technique, the biomolecules composite of CFC (secondary metabolites) at a concentration of 150 µl exhibited the maximum inhibition of *F.o.f.sp. lycopersici* colony (920mm). GC-MS analysis of biomolecules composite of ethyl acetate fraction of CFC and whole fruiting bodies indicated the presence of active compounds. Prominent among the biomolecules were benzene-ethanol; 2-Allyl-5-t-butylhydroquinone; 1,2-benzenedicarboxylic acid, diethyl ester; 1,4-diaza-2,5-dioxoisobutylbicyclo [4.3.0] nonane; Benz [j] aceanthrylene, 1,2,6,7,8,9,10,12 b-octahydro-3-methyl; 3-benzyl-1,4-diaza-2,5,dioxo-bi - cyclo[4.3.0] nonane; Phthalic acid nonyl 4-octyl ester. Similarly, the biomolecules composite of sporocarps contained several organic compounds including hexadecanoic acid; 2-penta-deuterio-isopropenyl-3-hepta-deuterio-isopropyl naphthalene; tetrakis-dimethyl-silyl-carbodiimide), Di-(2-ethylhexyl) phthalate and Hesperetin (1-[2,4,6-tris (trimethylsiloxy) phenyl] 3- [3-methoxy-4-(trimethylsiloxy) phenyl] - 2- propen - 1-one). Likewise, the diethyl ether fraction of mycelial mat contained cyclohexane, 1,4-dimethyl-2-octadecyl-, methyl -2-(3',3' - dimethyl-1' - butyn-1' - yl)-1-cyclohexenecarboxylate and 7,9- di-tert-butyl-1-oxaspiro (4,5) deca-6,9 - diene-2,8-dione.



**VIII-P-11. Efficacy of bioactive molecules of *Ophiocordyceps sinensis* [Berk] Sacc. against *Fusarium oxysporum* f.sp.cubense [E.F. Smith], the Panama wilt pathogen of banana, and its predisposing nematode, *Meloidogyne incognita* (Kofoed & White) Chit.**

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*Ophiocordyceps sinensis* [Berk] Sacc. the “Chinese caterpillar fungus” is one of the most valued mushroom fungus by pharmaceutical industry. In nature, the fungus parasitizes the larvae of ghost moths (*Hepialus armoricanus*). The close anamorphic state of the fungus, *Paecilomyces lilacinus* is known to have nematophagous activities. Studies were conducted to evaluate the efficacy of bioactive molecules of *Ophiocordyceps sinensis* [Berk] Sacc. against *Fusarium oxysporum* f.sp.cubense [E.F. Smith], the Panama wilt pathogen of banana, and its predisposing nematode, *Meloidogyne incognita* (Kofoed & White) Chit. Dual culture assay indicated 46.67 percent inhibition of *Fusarium oxysporum* f.sp.cubense by *Ophiocordyceps sinensis*. Secondary metabolite composite obtained on 15 days old culture, at 500 ppm showed significant level of inhibition of the pathogen 49.63 percent as compared to control in a well diffusion assay. Cell free culture filtrate collected on 15<sup>th</sup> day exhibited 77 percent inhibition of egg and 41 percent death of juveniles of root knot nematode. Bioactive molecules from *Ophiocordyceps sinensis* were extracted in different solvents viz., methanol, ethanol, hexane, butanol, ethyl acetate, petroleum ether, acetone, acetonitrile, chloroform and aqueous phase. Ethyl acetate fraction of the culture filtrate condensate showed the maximum mycelial inhibition of the pathogen (up to 51.48 percent). GC-MS analysis of biomolecules composite of ethyl acetate fraction of cell free culture filtrate (CFC) indicated the presence of ten different organic compounds viz., 1H-Imidazole-4-carbonitrile, 3,5-Dimethyl-1,2,4-thiadiazole, ergotamine, Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl), 2-tert-Butyl-4-isopropyl-5-methyl phenol, Phthalic acid, hept-4-yl isobutyl ester, N-Methyl-2-Propyl-5-Butylpiperidine, Cycloheptadecanone, 1-Propyl-1-[(tert butyldimethylsilyl) oxy] perfluoroheptene. Ergotamine has been reported to have several pharmacological applications.

# **Session-IX**

## **Value Addition and Mushroom Products**



## Oral Presentations

### IX-O-1. Development and evaluation of mushroom assorted food products

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Mushroom, a macro fungus with a distinctive fruiting body either epigeous or hypogeous, rich in many bioactive components are considered as a good source of proteins, vitamins, fats, carbohydrates, amino acids and minerals. These are highly perishable commodity with shelf life of 3-5 days at refrigerated ( $\pm 2$  °C) and 1-2 days at ambient (tem.  $25 \pm 2$  °C, RH 70%) conditions. The short shelf life of mushroom is an impediment to the distribution and marketing of the fresh produce hence novel techniques such as value addition and drying enhanced shelf-life of mushrooms. Dried Mushrooms were converted in to powder further utilized for the preparation of value added products such as composite flour, instant products, indigenous food products, extruded and baked products. In composite flour, the wheat flour was fortified with mushroom powder at different concentration from 10 to 50 percent and on the basis of quality parameter 10 percent mushroom fortification was found best with 35 percent increase in protein content. In the extruded products macaroni and noodles were prepared from fresh as well as powdered mushroom. Generally macaroni and noodles are prepared from durum wheat which is deficient in essential amino acid however mushroom is good source of essential amino acid. On the basis of quality parameters 20 percent fresh mushroom and 10 percent mushroom powder fortification was found best in case of macaroni and noodles. Instant products like soup mix and tikki mix were also developed with 20 percent and 30 percent mushroom powder fortification with energy value of  $383.33 \pm 0.21$  kcal/100g and  $308.04 \pm 0.30$  kcal/100 g respectively. In baked products buns, bread and biscuits were developed. On basis of quality parameter 15 percent mushroom powder fortified biscuits secured highest score for colour, crispiness, overall acceptability and for physico-chemical characteristics where as in bread and buns 10 percent mushroom powder fortification was adjudged the best. Nuggets and *papad* are indigenous food products prepared in every Indian household. Both these products contains the good source of digestive fibre and also act as appetizer, inspite of these, the product is deficit in quality protein so mushroom is better option for fortification of these indigenous products. Using response surface methodology (RSM), 20 percent mushroom powder in nuggets and 10 percent mushroom powder fortification in *papad* were recommended for these indigenous products. These mushroom fortified products were found economically market viable.

### IX-O-2. Effect of cooking on antioxidant activity and phenolic content of various species of edible mushrooms of India

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Mushrooms are known for medicinal value and various health benefits are associated with dietary intake of mushrooms. Some of the mushrooms have anti-carcinogenic properties which are attributed to its antioxidant potential due to various bio-molecular components. Cooking of mushrooms has an effect on the antioxidant potential to various extents. In the present study, effect of various cooking methods on antioxidant potential by DPPH inhibition, thiobarbituric acid (TBA) reactive compounds and total phenols on common edible mushrooms of India viz., *Agaricus bisporus*, *Calocybe indica*, *Volvariella volacea*, *Lentinula edodes* and *Pluerotus ostreatus* was done. It was found that antioxidant potential as DPPH inhibition in fresh mushrooms was found to be in the decreasing order as *A. bisporus*, *V. volacea*, *C. indica*, *L. edodes* and *P. ostreatus*. TBA reactives were found to be in the decreasing order as *A. bisporus*, *V. volacea*, *C. indica*, *P. ostreatus* and *L. edodes*. Total phenols as estimated by Folin ciocalteu assay was found to be in decreasing order as *P. ostreatus*, *C. indica*, *V. volacea*, *A. bisporus* and *L. edodes*. These mushrooms were also analysed after cooking by various methods as microwaving for 2 min,

boiling in water for 5 min and sautéing in sunflower oil for 2 min. The prepared samples were analyzed for antioxidant potential, TBA reactives and total phenols. Trials were done in triplicates and results were compared statistically using t test, it was found that antioxidant potential as DPPH inhibition in case of all the mushrooms decreased in microwave treatment as well as in boiling which can be attributed to leaching of biomolecules whereas increased slightly in sautéing of mushrooms that can be attributed to concentration of biomolecules due to frying. Similar results were observed in case of total phenols with high content measured in sautéed mushrooms because of higher rate of conversion to quinones during sautéing. TBA reactives decreased in all the mushrooms by all forms of cooking that indicates less of carbonyl compounds which are measured by TBA assay.

### **IX-O-3. Relating material properties of mushroom (*Agaricus bisporus*) to the loss of water holding capacity upon its thermal treatment**

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Canning is one of the most common and old methods of mushroom preservation. Processed mushrooms lose their water holding capacity (WHC) as a result of the thermal treatment in canning process. Several studies have been carried out in the past regarding the reduction in WHC upon the heat treatment and protein denaturation is identified as the main reason for this. In this study, we measured WHC change in mushroom upon thermal treatment in relation to protein denaturation and cell wall integrity loss. Freshly harvested mushrooms were vacuum impregnated in the isotonic solution which consisted of natural sugars present in mushrooms, namely mannitol and trehalose, and its natural salt potassium phosphate. The solution was maintained at a pH value of 6.3 which is native pH of mushroom. Protein denaturation in the hydrated mushrooms was carried out with the thermal treatment. Cell wall integrity was destroyed with the milling. The protein denaturation and cell wall integrity losses were measured with the dynamic scanning calorimetry and the electrolytic conductivity measurement, respectively. The WHC was measured with the centrifugation technique and was expressed as the polymer fraction in the sample after centrifugation. The experimental results showed a significant loss in WHC in protein denatured sample. A bigger loss in WHC was found in the samples where cell wall integrity was destroyed.

### **IX-O-4. Improvement in nutritional and therapeutic properties of daily meal items through addition of oyster mushroom**

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Mushroom is the choicest food of nutritionists because of its hypolipidemic, hypocholesterolemic, hypoglycaemic and antitumor properties. Moreover it has antibiotic factors like antibacterial, antiviral, antiprotozoal and antifungal properties. Keeping in view unique chemical composition of mushroom, it was thought to incorporate in daily diet for benefit of normal as well as diseased individuals. About twenty four food items were selected from daily meals. Fresh as well as dry mushrooms were incorporated in suitable food item at 25 percent to 50 percent and 10 percent level, respectively. Ingredient, their proportion and procedure of each control and experimental food items was standardized and subjected to study the acceptance on a nine point Hedonic Scale by a panel of ten judges on a three consecutive days. Nutritive value was calculated and therapeutic use was found out. The study concluded that mushroom is a suitable food for incorporation in breakfast, lunch, dinner and snacks food items. It can be use in sweet as well as salty and spicy food. All the mushroom added foods were acceptable. Additions of mushroom improve the nutritional quality and therapeutic properties as well. Mushroom added daily meal food items are recommended for growing children, obese as well as underweight adults, diabetic, heart patients and in most of the ailments. Human and animal feeding experiments need to be carried out to study therapeutics effects of mushroom.



## Poster Presentations

### IX-P-1. Biopotentialities of Actinomycetes metabolites in enhancing shelf life of button mushrooms (*Agaricus bisporus*):- some promising results from benchtop trials

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Despite rising production, consumption and global demand for edible mushrooms popular gourmet species such as *Agaricus bisporus* (button mushrooms) are perishable and tend to lose their fleshy white appearance post harvest due to action of enzymes such as tyrosinase (EC 1.14.18.1), thus, creating a problem for marketing and consumer acceptance. Extension of the quality and shelf-life of this perishable commodity is therefore, a scientific, technical and economical challenge. Although, there are several approaches for extending the shelf-life of mushrooms, an alternative safe, cheaper and biological and eco-friendly approach is of utmost importance. Systematic bioprospecting of interesting prokaryotes such as Actinomycetes yield useful chemical leads. Testing promising metabolites from Actinomycetes thought to be excellent tyrosinase inhibitors is one unexplored possibility. The aim of the work presented in this study was to explore on a benchtop trial scale, the effect of unidentified but interesting metabolites produced by chemically creative strain of Actinomycetes, SBSK- 430 in solubilized form in cell free culture filtrate (CFCF) on the quality of button mushrooms (*Agaricus bisporus*) as determined from color, weight loss and physical attributes as indicators of quality. Four different treatments based on standardized nebulizer microspray technique were used under controlled laboratory environment for targeting fresh handpicked whole mushrooms (*A. bisporus*) (30 in one set) which included one set – the test sample sprayed with CFCF, a positive control treated with sterile distilled water; negative control with sterile membrane filtered media and the untreated control without any treatment. The reduction in loss of biomass, change in pileal diameter and other physical attributes such as aroma, texture and extent of discoloration or browning were analyzed during storage at 25 °C for a period of 10 days. The extent of browning of mushrooms was impedimental with time, with the test sample as compared to the controls. In contrast, the loss in biomass of mushrooms treated with test sample ( $22.4\% \pm 0.8$ ) was found to be equivalent to the positive control ( $22.4\% \pm 1.9$ ). In addition, there was a higher biomass loss in negative control ( $30.3\% \pm 11.0$ ) and untreated mushrooms ( $30.5\% \pm 9.6$ ). The aroma and texture of the pileus was unaffected with the treatment. It is claimed that this trial benchtop study indicates the biopotentialities of the Actinomycetes metabolites as useful candidates for enhancing the quality and shelf life of *Agaricus bisporus*. Further research to pinpoint the candidate biomolecules is in progress.

### IX-P-2. Effect of packaging material and temperature on the firmness of the minimally processed button mushrooms (*Agaricus bisporus*)

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Mushrooms (*Agaricus bisporus*) are one of the most perishable horticultural produce with high nutritional value and short shelf life; usually 1-3 days at ambient temperature. The production and consumption of freshly cultivated mushrooms have undergone an important rise during last decade. The market acceptance of mushroom is mainly affected by its color and firmness. In the current study, experiments were carried out to evaluate the effect of different storage conditions on firmness, weight loss and chemical composition of mushrooms. To investigate the influence of storage conditions and properties, mushrooms were stored under different temperatures (i) 13 °C (ii) 18 °C (iii) 24 °C (iv)  $4 \pm 1$  °C (refrigeration temperature) (v) -18°C

(deep freezer) and packaging material (i) polythene bag (a) 100 gauge (b) 200 gauge (c) 300 gauge (with and without macro-perforations). The results showed the samples stored at refrigeration temperature had a longer shelf life in comparison to other temperatures. The mushrooms stored at deep freezer (-18 °C) showed a different trend in comparison to the samples stored at room temperature and refrigeration temperature. The results indicated there was a significant difference in the weight loss, firmness and chemical composition of the mushrooms packed under different conditions.

### **IX-P-3. Effect of post harvest treatment to extend the shelf life of white button mushroom (*Agaricus bisporus*)**

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Production and consumption of mushrooms has been tremendously increased in India due to increased awareness of commercial and nutritional significance of this commodity. *Agaricus bisporus* (white button) are appreciated not only for texture and flavour but also for its nutritional characteristics. White button mushrooms is rich in quality protein and low in fat content. Mushrooms are mainly marketed in fresh form due to their highly perishable nature. To extend the shelf life the freshly harvested mushrooms were washed with different solutions of CaCl<sub>2</sub>, citric acid, KMS and NaCl and their combinations for 2 min and analysed for best washing treatment. The treatment with 0.5% KMS + 0.5% NaCl + 0.5% CaCl<sub>2</sub> showed minimum change in degree of white colour with maximum total soluble solids. The moisture content differed non-significantly different from each other and ranged from 93.56% to 88.76%. The washing of mushroom with 0.5% KMS + 0.5% NaCl + 0.5% CaCl<sub>2</sub> has not shown any effect on the essential amino acids profiling. This was the best washing treatment and when packed in polypropylene pouches the mushrooms maintained quality for 3 days under ambient conditions and 5 days in refrigerated condition.

### **IX-P-4. Exploiting natural wealth to sustain the much fancied *Calocybe* in Kerala, India**

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Kerala is one of the small states of India. The climate of Kerala is hot and of humid nature. During summer there is maximum temperature and rainfall hence, a typical tropical monsoon climate. It is essential to find newer wild strains of mushrooms that can be domesticated to meet the growing demand for food. Selection of new improved strains of wide edible mushrooms is one of the best ways to improve quality and yield without increasing cost of production. Milky mushroom (*Calocybe indica*) is a tropical mushroom that can be cultivated throughout the year. This highly esculent and large mushroom occurs in abundance during and after the monsoon showers. Survey conducted revealed that the growth occurrence and distribution of different species of wild milky mushroom depended on the rainfall which maintains the adequate moisture content in the soil. Most collections were made from the basins of coconut palms and humus rich soil. This particular species is seen in abundance even after the monsoon showers when there is abundant sunshine. Large sized milky mushrooms have also been obtained in large numbers on lawns during the pre monsoon season. The frequency of occurrence of the same species continuously from different localities of the state irrespective of the soil type indicated their cosmopolitan nature of occurrence. *Calocybe* ranging from small size to huge ones have been collected from various locations throughout the state. The samples collected were tissue isolated and brought in to pure culture. Morphological characterization of collected wild milky mushrooms were compared for pileus diameter, stipe length and thickness, pileus, cuticle, color, shape and position of stipe. Five different isolates obtained from diversified habitat were selected for the study and they were designated as AK, BK, JK, RK, SK.

Wide screening of *Calocybe* from diversified habitat for yield assessment was done by observing the growth and yield in beds. Biological efficiency ranging from 80 to 90 percent were obtained for the different isolates. Selection of different casing materials like coir pith compost vermi compost and sand soil cow dung mixture etc, to suit the physiological requirement after screening the efficient strains for commercial production was done. Once planted out of soil with the right characteristics and due care the wild strains started producing the fruit bodies in less than 25 days in vermi compost cased beds and maximum 40 days were taken for initiation of fruiting bodies in beds cased with coir pith compost. Short thick stipe with dense tissue and pure white fruiting body was obtained in majority of the isolates under study. The fruiting bodies of one strain (RK) was firm pure white, smooth and with hairs. The fruiting body of another wild strain (AK) had long thin stipe small cap with fibrous tissue and cream colored gills. As expected the wild strains of *Calocybe* though they exhibited more than 90 percent relatedness to reigning strain APK2 in terms of yield, they did not have the resinous after taste of *Calocybe* at all. In fact the taste of the local wild strains was pleasant and more relishing to the consumers. Our motto was improvement of quality through research development, innovation and domestication. Will a comparison between the several wild edible *Calocybe* and cultivated species be effective? Highlights of this comparison will be presented focusing in particular on the prospects of cultivation of this tropical milky mushroom in the south.

### **IX-P-5. Concentration of multiple pathogens in different food matrices**

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Conventional pathogen detection methods, such as microbiological and biochemical identification are time-consuming and laborious, while immunological or nucleic acid-based techniques require extensive sample preparation and are not amenable to miniaturization for on-site detection. Biosensors have shown tremendous promise to overcome these limitations and are being aggressively studied to provide rapid, reliable and sensitive detection platforms for such applications. Recent advances in molecular cloning and recombinant DNA techniques have revolutionized the detection of pathogens in foods. In this study the development of a PCR-based technique for the rapid identification of the food-borne pathogens *Salmonella* and *Escherichia coli* was undertaken. The possibilities of combining different rapid methods, including improved technologies for separation and concentration of specific bacteria, and for DNA extraction and purification, will facilitate the direct detection of pathogens in food. The goal is to avoid the enrichment, providing rapid alternatives to conventional quantitative culture methods. Further improvements, especially in genetic methods, can be expected, including the use of DNA microarray technology. In this approach, the bacteria are attached to magnetic beads by immunological reaction with specific antibody against *Salmonella*. Further amplification of the genetic material with a double-tagging set of primer is then performed and the amplicon captured with streptavidin-magnetic beads.

### **IX-P-6. Mushroom fortification – enhancing biscuit nutritious**

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Mushroom is a unique member of third food Kingdom which is without leaves, without buds & without flowers yet forms fruits. This fruit is a marvelous combination of food, tonic and medicine and solves the quest of human being to live long and live healthy. The virtues of mushroom are milestone in this regard. Even the prehistoric men acknowledged this and regarded mushroom as “bread from heaven.” Human body needs energy for its organs to function which goes on uninterrupted. For their smooth functioning both energy and nutrition are essential. Mushroom though energy poor, is nutrition rich food. It supports

the biological functions and makes them healthier and fitter. As far as mushroom as industry is concerned it can be categorized as edible, medicinal and wild mushroom. All these three categories are however, complimentary to each other. Mushroom industry like another industry ought to undergo constant change and innovation. This is important because mushroom is a perishable edible substance. In our country with a changing lifestyle and increasing health awareness, mushroom is coming up as one of the most favoured food item of every section of society. To further enhance its acceptability we have to present mushroom as more tongue friendly and in ready to eat form and in a form which is imperishable for quite long period. Biscuit can be one such form which is relished and easily affordable by one and all, young and old. It accompanies morning breakfast and evening tea of person of every economic strata – affluent and poor and is available everywhere cosmopolitans and villages. Mushroom though perishable food item, is versatile and this quality of mushroom can be utilized to overcome its short coming. It is compatible with very many edibles in its various forms, fresh, dry, powder and other processed forms. It adds to the value of the mother ingredient in terms of both nutrition and cost. People very much like something to munch, which are light, tasty and nutritious. Mushroom biscuit will fulfill all these criteria. It will provide all the benefits of mushroom in a sustainable way. Biscuit production is a small cottage industry requiring small industrial unit, not a big investment and labor intense. It's technology is well-known. Considering its market potentiality it will be very much sought after edible product, sold by every section of business community vender to big confectioneries. It is thus clear that mushroom biscuit offers a bright future to mushroom producer mushroom lover and mushroom sellers.

#### **IX-P-7. Mushroom recipes tianguis markets and state of Hidalgo, Mexico**

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Mexican cuisine is known for its great variety of dishes, reflecting the biodiversity of our country, in which organisms interact with cultural expressions and traditions of each geographic region, which gives to each a hallmark. The state of Hidalgo ranks third nationally with more than 260 species of wild edible mushrooms (WEM) and the tradition continues in the consumer markets and swap meets some municipalities: Acaxochitlán, Huasca, Huejutla, Mineral del Monte Mineral del Chico, Molango, Omitlán, Pachuca, and Zacualtipán Tlanchinol mainly. Going to these sites, buying and selling is an enjoyable experience as they become excellent “information centers” of knowledge, which are provided by “hongueros”, the people who are responsible for their collection and marketing. Species, prices and sales units vary according to the region of the state be purchased heaps, sardine, quadron, bucket, piece or kilo and prices range according to the species, the highest are for the most defendants and/or hard to find. Variety of dishes prepared with 24 WEM and 3 cultires, acquired in these municipalities are presented in this cookbook. Each recipe is explained step by step and is accompanied by a picture, the names of the people who prepared and traditional and scientific name of the species. Finally some tabs are displayed with information of each of the fungal species: scientific name, traditional name, brief description of morphological, vegetation type, fenology and market where they are available. The recipes are the result of creativity and collaborative effort of the people in the communities, teachers, students, friends and family who have participated in various culinary samples that have occurred over more than ten years in the UAEH. In this type of event is intended that people come to taste the different dishes and put aside their fear and distrust. This cookbook offers the opportunity to spread the culinary tradition of Hidalgo and promote consumption of these organisms, which are natural food with a good flavor, nutritious and healthy and add to the great global trend for Food in the XXI century.

### IX-P-8. Some products made from fourteen species of wild and cultivated mushrooms

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Edible and medicinal mushrooms have great potential as functional foods that have different properties than other food products. They are a source of protein, vitamins (A, B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, B<sub>12</sub>, C, D<sub>2</sub>, niacin, pro-vitamin D<sub>2</sub>), minerals (Fe, K, P, Cu, Se, Ca, Mg, Mn and Zn), dietary fibre, are low in fat and have digestible carbohydrates. Likewise, these exhibit anticancer, antibiotic, antithrombotic, anti-diabetic properties, act as antioxidants and reduce cholesterol level and hypertension. The preventive and therapeutic effects of its components and modes of action have been documented across different biological systems. At present, the development of new products based on mushrooms in Mexico is emerging as a new alternative. In the Laboratory of Ethno botany UA EH, 23 products have been developed based on mushrooms. These are; jams, facial cream, ointment, stuffed cheese, traditional drink, seasoning, choco-amaranth, sausage, terrine, crowbars, bread puff, pastry, stuffed mushrooms, coffee, tea, tortillas, fortifying drink, pickled mushrooms, ice cream, yogurt, salad dressings, mayonnaise, shampoo and soap. All with 14 species viz, four macroscopic commercially grown *Agaricus bisporus*, *Pleurotus ostreatus*, *Lentinula edodes* and *Pleurotus djamor*, Microscopic: *Saccharomyces cereviceae*; four medicinal wild and cultivated: *Ganoderma curtisii*, *G. applanatum*, *G. brownii* and *Pycnoporus sanguineus*; five edible wild: *Ramaria botrytis*, *Boletus edulis*, *Ustilago maydis*, *Lactarius indigo* and *Lepista nuda*.

### IX-P-9. Studies on nutritional and medicinal characteristics of some edible mushrooms

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Mushrooms are extensively used in recent days because of their nutritional and medicinal traits. This study focused on examining nutritional and medicinal characteristics of hot water, sodium hydroxide, aqueous ethanolic extracts of *Agaricus bisporus*, *Pleurotus florida*, *P. eous* and *Hypsizygus ulmarius*. The nutritional characteristics were studied by estimating total carbohydrate, total crude protein and total reducing sugars for hot water extracts of mushrooms. Total carbohydrate content revealed the variations in the mushrooms, the highest carbohydrate content was observed in *A. bisporus* (4.5 g/100 g dry wt) and protein and reducing sugars content observed in *H. ulmarius* of 2.5 g/100 g and 59 mg/100 g, respectively. This study also investigated the DPPH free radical scavenging activity of the hot water, sodium hydroxide and aqueous ethanolic extracts of mushrooms. The highest antioxidant activity of mushroom extracts on oxidation was in *P. florida* followed by *A. bisporus*, *H. ulmarius* and *P. eous* for hot water extract. Similarly, for sodium hydroxide and aqueous ethanolic extracts, the orders were *A. bisporus* > *H. ulmarius* > *P. eous* > *P. florida* and *P. eous* > *H. ulmarius* > *A. bisporus* > *P. florida*, respectively. Among all the extracts of mushroom aqueous ethanolic extract of *P. eous* showed the highest value of 82%. *A. bisporus* and *H. ulmarius* showed effective antibacterial action against *Pseudomonas aeruginosa* with inhibition zone diameter of 9 mm and 8 mm, respectively. It is concluded that *H. ulmarius* has better nutritional content and *P. eous* medicinal properties.





# **Session-X**

## **Economics, Social, IT and Marketing Issues**



## Keynote Presentations

### X-K-1. India on the threshold of a Non-Green Revolution

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References of mushrooms are quite frequent in the Indian ancient epics like **Samveda, Charak-Samhita etc.**, yet India was a late starter as far as Mushroom Farming is concerned. It was only in the first half of 20<sup>th</sup> century that paddy straw and oyster mushrooms were first artificially cultivated in India, while white button mushroom was successfully grown in the later part of that century under various projects launched by National and International Research organizations like, FAO, ICAR, CSIR and Agriculture Universities/ Depts. of Horticulture etc. Almost at the same time, an Indo-German Agricultural Development (IGADA) project was also operating to initiate mushroom production in the then UP Hills. To further strengthen Mushroom research in the country, the ICAR established the National Centre for Mushroom Research & Training (NCMRT) as well as an All India Coordinated Mushroom Improvement Project (AICMIP) in the year 1983 at Solan (HP). Again, in the year 2008, the Solan Centre was upgraded as the Directorate of Mushroom Research (DMR), while the AICMIP was enlarged and renamed as All India Coordinated Research Project on Mushroom (AICRPM) with 14 Coordinated and 2 Cooperating Centers covering different climatic zones in 15 States of the country. Also the Govt. of India, during the VIIIth 5-year Plan (1993-97), launched a massive drive to create 30 Composting Units and 29 Spawn Laboratories in 21 States of the country, besides an allocation of Rs. 1.36 crores (ca.US \$ 0.27 millions) for training 27,300 potential mushroom growers. This was in addition to the modern facilities created to produce pasteurized short method compost (SMC), casing soil and quality spawn under an International Cooperation for Indo-Dutch Mushroom Project in the year 1988 at 4 places located at Palampur (HP), Srinagar (J&K), Jeolikote (UP) and Bangalore (Karnataka).

With such an excellent R&D infrastructure and human-resource available across the country, mushroom farming obviously got a real boost during the last 3 decades or so, it has made considerable growth both in terms of seasonal as well as climate controlled growing of mushrooms. Of course, climate control is still restricted to button mushrooms, while seasonal farming is being practiced for button as well as specialty mushrooms. In fact, climate controlled farming of button mushroom got a real fillip, when Govt. of India launched a scheme to encourage entrepreneurs and Business houses to set-up organized Hi-tech Mushroom Farms as Industrial ventures. The scheme called as 100% Export Oriented Units (EOUs) attracted 4 dozens of EOUs to register with an installed production capacity of over 0.1 million tons of button mushroom per annum. However, the number of such units actually installed and continued was much less, hence the current annual production of half a dozen existing EOUs today is still short of touching that targeted figure. Of course, a good number of smaller climate control Farms have come up across the country, which along with the seasonal units are not only contributing to raise the total annual production of button mushroom to >0.11 million tons, but are also catering to the increasing demands & per capita consumption of mushrooms in India from a meager 25g to 40g. Of course, a variety of specialty mushrooms being produced in the country today has helped to reach this delicacy to the middle & lower middle class also and their role in enhanced consumption of mushrooms in India cannot be underestimated.

Among the specialty mushrooms, Oyster is leading the group by reaching almost the entire country, being grown mostly by seasonal growers. However, appearance of some organized Oyster Farms, viz. i) Zuari Agro-Chemicals, Goa, ii) KR Mushrooms Vijaywada (AP), iii) Sri Karpaga Vinayagar Mushroom, Farms at Coimbatore and Chennai (TN) iv) Ghai's Mushrooms, Raipur, etc and a large scale cooperative Farming

in NEH States involving 3000 small farmers producing 600 tons of Oyster mushrooms p.a. under the auspices of Mushroom Development Foundation, Guwahati are such milestones, which seem to open new vistas in the growth of this mushroom in India.

Second in the queue is the fastest tropical mushroom *Volvariella volvacea*, which has attained commercial status in some States. Odisha, the eastern coastal state of India has adopted this mushroom for low cost cultivation for 9 months of the year, either outdoors in shades of bamboo plantation, coconut grooves & various orchards, or in low-height huts made up of coconut leaves etc., with spawn made locally on paddy grains in makeshift village labs. Even such a simple farming practice helps the rural farmers to produce over 10,000 tons of Paddy Straw Mushroom per year. Next is the Kerala State, which grows this mushroom round the year due to its very conducive climate for *Volvariella*. Tamil Nadu, West Bengal, Punjab and some NE states are the other important players for this mushroom.

*Calocybe indica*, locally called as Milky mushroom is a new entrant, but its attractive snow-white color as well as stout & fleshy appearance have helped its fast acceptance in India. Moreover, being a tropical mushroom, its cultivation method is suiting to plain areas with hot climate without any cost to environment control. Its farming began in Tamil Nadu, but it has already spread to some Northern States also and has crossed 10000 tons yearly production figure and is poised to enter the export-market sooner than later.

The mushroom wealth of India has a major share of a large variety of indigenous edible species, many of which have high export potential also. *Morchella* spp. abounding in states of J&K, Himachal Pradesh and Uttarakhand are considered as most delicious mushrooms, and till a decade ago, they comprised the highest quantity of dried mushrooms exported from India. Though reported from Shillong also, the high Hills of NEH States of Meghalaya, Arunachal, Nagaland, Manipur and Sikkim are yet to be explored for morels. Same is the status of wild Kabul-Dhingri (*Pleurotus eringii*) common in border-areas of Kashmir with Afghanistan. In recent years, export of *Cordyceps sinensis*/*C. militaris*, the medicinal mushrooms collected from Himalayan ranges in Uttarakhand and Arunachal Pradesh have gained much importance and they need urgent attention for survival and sustained growth. Rajasthan, a desert State, has also two excellent wild mushrooms occurring in large quantities in sand dunes. These species are *Phellorinia inquinans*, *P. herculae* and *Podaxis pistillaris*, which are still being consumed in nearby States and are yet to be exported. The NEH States are also rich in wild edible fungi, the most important being *Lentinula edodes* and *L. laterata*, which along with *Auricularia* spp. are collected in quintals and sold in dried form in large Indian cities, and probably outside also. Other delicious mushrooms native to NE region are *Cantherellus cibarius*, *Tricholoma giganteum*, *Termitomyces* spp, *Laccaria amethystea*, *Gomphus floccosus* and *Lactarius queticolor*. In fact, *Termitomyces* spp. are very widely distributed in India and sold in large quantities in local markets of Chhattisgarh, Jharkhand, Odisha, West Bengal and Kerala also, and if processed well can be a good export commodity. Two very popular and common edible mushrooms sold in Jharkhand are i) Puffballs (*Lycoperdon*/*Scleroderma* spp) and ii) *Macrolapota* sp, which fetch very high prices. Many more species of edible and medicinal mushrooms have been identified from various parts of India and it is heartening to note that the wild mushrooms are also receiving attention for their proper conservation, characterization and evaluation for future utilization for human welfare in the coming years.

It is apparent from above that the R&D efforts going into the Cultivated and wild edible mushroom species of India and the international cooperation received so far from various agencies, have led Mushroom Farming in India to the door-step of a "Non-Green Revolution", which is poised to augment the benefits of the Green Revolution in giving India not only 'Food Security' but also the "Nutritional Security" to the toiling masses of this ancient nation. What is required is a persistent, concerted and focused effort by all dealing with this curious but responsive creature of the third kingdom.



## **X-K-2. Leveraging ICT for mushroom research and farming**

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Information communication technology (ICT) is a contemporary term that describes the combination of computer technology (hardware and software) with telecommunications technology (data, image, and voice networks). It empowers both people and machines with information, which is transformed into knowledge and intelligence. Appropriate use of the knowledge by both people and machines contributes to sustainable development. Informed and empowered people know their role as citizens in an environmentally sustainable society where as empowered machines have the knowledge to minimize energy and material use, wastes, and pollutants. Internet facilitates people from across the globe to co-operate and perform various activities of human life and agriculture is not an exception. Mushroom being a commercial crop with elite appeal, rich source of nutrients along with its relatively high immunity to climate change, and drought puts it in better place for harnessing potential of ICT revolution than other field crops. Mushroom researchers and farmers can take leverage of processing, storage, transmission, and sharing of knowledge generated by Directorate of Mushroom Research in electronic form, without any spatial or temporal constraints. ICT tools can, not only support mushroom extension or advisory services but can play the role of game changer. In the current scenario, most of the research/knowledge flow is from top to bottom (scientists to farmers) but with the new avenues opened by the use of ICT tools it can be changed to demand driven research/ knowledge flow for solving problems originating from farmer's field, seeking solutions in real time. ICT based virtual trainings can enhance the mushroom production and processing skills of more number of farmers in few courses as compared to the number of farmers trained till now by the Directorate. Farmers can not only get the market prices but can also connect to consumers directly through ecommerce applications. On the other hand, government and traders can accurately estimate the inflow of produce in the market on daily basis. ICT can also help in organizing farmers and producer groups and facilitate adoption of technologies that promotes sustainable natural resource management practices. With the upcoming intelligent systems like web enabled expert systems (AGRIDaksh), recommender systems, multi agent systems, analytics based on big data and cloud computing, internet of things powered by the IPv6 protocols, sensor based networks, bioinformatics infrastructure for quickly developing new improved varieties, the present role of ICT in mushroom farming and research is like a tip of iceberg and can only grow with a more faster speed.

## Oral Presentations

### X-O-1. Changing global scenerio in mushroom industry

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Mushroom industry globally has expanded both horizontally and vertically, meaning that the expansion has been in production and addition of newer types of mushrooms for commercial cultivation, both edible and non edible mushrooms. Today China is leading in global mushroom production both in cultivation of edible and non edible types. China produces approximately 70 percent of world mushroom production and mushroom is their sixth economically important crop as far as country's revenue generation is concerned. The second highest mushroom producing country is USA, followed by some European countries. European production is confined to France, Germany, Holland, Italy and other countries in western-Europe. There is a matching contribution in mushroom production in Eastern European countries like Hungary and Poland where mushroom production has received a boost as can be seen from the production figures available and mushroom activity in these countries.

### X-O-2. Regional mushroom mela and kisan goshtih: enabling farmers to access technology and advisory services

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*Melas* (Field days) and *kisan goshtih* (Farmer-expert interactions) have been key activities of agriculture research institutes to spread awareness about the technology generated at institute among their clients and to address their problems. The Directorate of Mushroom Research, Solan initiated the concept of regional mushroom mela and kisan goshtih during 2010. Unlike the national mushroom mela, the regional mushroom melas were organised among the cluster of mushroom growers in these states with three key objectives of spreading the advanced technology among the rural farmers, addressing the problems encountered by mushroom growers in cultivation by expert advises and promoting mushroom consumption among masses. Five such melas were organised during 2010-11 with prior planning, preparation and publicity. About 200-350 mushroom growers other farmers, entrepreneurs, several officials, scientists and technical staff from different government organisations, financial institutions, Krishi Vigyan Kendras took part in the mela. It was observed that, less than 10 percent of women participated in the mela except one place (Khanpura, Punjab) where nearly 18 percent women attended. With respect to the age group, the young people aged between 15-35 years outnumbered the other age groups in all the five melas. Though, the participation of young aged people is a welcome trend, the lesser participation of women needs to be addressed as mushroom cultivation offers significant employment and livelihood opportunities for women folk. The event was organised to synchronise with the seasonal mushroom growing activities in the area. The majority of the farmers indicated that, either DMR staff or officials of state department of agriculture and horticulture were the chief source of information about the mela. The majority of the participants at all the venues expressed happiness and approval about the timing of the event, benefits for participating, participation in future events, facilities and arrangements during the mela and recommending to others for participating in future events. Continuation of such regional melas focusing the location specific issues and problems can be highly beneficial to the farming community. Further innovations and amendments to suit to other enterprises, stakeholders, region, constraints prevailing etc would bring higher impact among the farmers in the rural areas.

**X-O-3. Status and scope of mushroom cultivation in Haryana- constraints and future outlook****Surjeet Singh<sup>1\*</sup> and Ajay Singh<sup>2</sup>**<sup>1</sup>CCS Haryana Agricultural University, Hisar, Haryana, India<sup>2</sup>HAIC R&D Centre, Murthal, Sonapat, Haryana, India

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*Agaricus bisporus* (white button mushroom), being a temperate mushroom was initially grown only in the hilly regions of India, particularly Himachal Pradesh and Kashmir, under seasonal conditions. Seasonal cultivation of this mushroom was introduced in North-Indian plains including Haryana in 80s. Since, then the state has achieved a tremendous increase in mushroom production with current production of more than 10,000 tonnes per annum. Though the production technologies for *Agaricus bisporus*, *A. bitorquis* (button mushroom), *Pleurotus* spp. (oyster mushroom), *Calocybe indica* (milky mushroom) and *Volvariella volvacea* (paddy straw mushroom) have been developed, yet all these mushrooms could not achieve the commercial status except white button mushroom (*A. bisporus*). This mushroom is cultivated in India under both controlled and natural conditions. Thatched structure made of locally available material like stalks of pearl millet, paddy straw; cotton sticks, etc. have been found superior to pucca brick structure under low cost technology. These structures are cheap and provide natural ventilation, which is required in mushroom houses. Regarding of fresh mushrooms, the growers do not face any difficulty and they like to cultivate mushrooms because this venture is very less land dependent and also escapes the natural vagaries like rain, hailstorms, etc. Even though, centralized facilities have been developed in some parts of the state to provide spawn and pasteurized compost, the quality of spawn, low and variable productivity and lack of industries involved in post-harvest processing/value addition are the problems faced by many growers.

**X-O-4. Evaluation of mushroom types suited for Kuttanad through participatory technology development****Reeny Mary Zacharia\*, Dhanya Das, Suma Divakar, Thomas George and Leenakumary Subhadra**

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Kuttanad is a special agro ecological zone representing the water logged lands spread over 69 panchayaths of Alappuzha, Kottayam and Pathanamthitta districts of Kerala state. Large area of this land is below, at, or just above mean sea level. Climate is tropical humid monsoon type with a mean annual temperature of 27.6 °C and rainfall 2746 mm. Humidity in general is very high. Wet lands of Kuttanad, the rice bowl of Kerala stretch over an area of 35,500 ha, where rice is cultivated. Total production of rice vary from 1.6 lakh tons to 1.75 lakh tons and straw from 2.25 lakh tons to 2.5 lakh tons. Usually this large quantity of straw is left in the field for incorporation in the soil. A viable alternative for Kuttanad farmers for recycling of agro waste into protein rich food is mushroom cultivation. Hence, the present study was undertaken with a view to evaluate mushroom types best suited for cultivation in Kuttanad during rainy and summer seasons based on Participatory Technology Development (PTD), nutrient analysis and consumer survey. PTD trial was conducted at 10 farmers' fields in a completely randomized design in factorial form. During rainy season mean maximum temperature inside the shed was 24±2 °C and mean relative humidity was 90%. Total rainfall during the period was 595.3 mm. Results confirmed that pink oyster, *Pleurotus eous* (875.5 g/bed) was significantly superior in mean yield over *Pleurotus florida* (786 g/bed) and *Pleurotus sajor-caju* (671.7 g/bed). During the rainy season (July- August 2012), highest biological efficiency (87.5%) was exhibited by *Pleurotus eous* compared to *P. florida* (78.6%). During summer season (April-May 2013) mean maximum temperature inside the shed was 34±2 °C and mean relative humidity was 68%. Total rainfall during the period was 168 mm. Mean yield of pink oyster (790 g) was on par with milky mushroom *Colocybe indica* (800 g). Nutrient analysis of different types of mushroom harvested from

different types of substrate done at Central Institute of Fisheries Technology, Kochi, revealed that pink oyster was nutritionally better as it contained lower carbohydrate, higher protein and highest dietary fibre compared to *P. florida*, *Hypsizygus* sp. and *P. sajor-caju*. Mineral content was also optimum when compared to other mushrooms under study with a good amount of potassium (2800 ppm). In the comparative analysis of nutrients of different mushroom types grown on steam sterilized paddy straw, concentration of proximates like carbohydrate (15%) and fibre (3.4 %) was highest in pink oyster when compared to *P. florida* and *P. sajor-caju*. With regard to mineral content also, *P. eous* showed highest concentration of magnesium (174 ppm) and zinc (16.9 ppm). In consumer survey, pink oyster ranked second (56%) and was mainly due to its less availability compared to *P. florida*. Based on the PTD trials, nutrient analysis and consumer survey, pink oyster- *P. eous* can be recommended for cultivation in Kuttanad region of Kerala for both rainy and summer seasons. *P. florida* also can be recommended for rainy season and *P. eous* and *Calocybe indica* for summer season.

## Poster Presentations

### X-P-1. An insight into mushroom: myths, facts and reality about commercial cultivation

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Mushrooms have been valued throughout the world as both food and medicine for thousands of years. They are a rich source of nutrition and form a major chunk of health foods. Fats occur in mushrooms in minor amounts, especially compared with protein and carbohydrates, and the fatty fraction consists predominantly of unsaturated fatty acids such as linoleic acid, they may be the perfect food for maintaining a healthy heart and cardiovascular system. Earlier Mushroom eating was restricted to specific regions and areas of the world but due to globalization, interaction between different cultures, growing consumerism has ensured the accessibility of Mushrooms in all areas. Mushrooms are increasingly gaining acceptance in different cuisines and in everyday consumption. They have created a space in a common man's kitchen. Also, current trend of consumption conveys the opportunity that lies in the area of mushroom exports. The current study is a review report of mushroom cultivation in commercial scale by small farmers and growers. The most alarming part of the research was that every year 8 out of 10 mushroom growers stop their operations either due to lack of marketing or inability in handling cultivation. Mushroom growers do not have standard operational protocol for cultivation and hence, they were found to be misled and misguided into cultivation leading to increase in production cost, irregularity in cultivation, huge contamination and thus lack of market. Another observation made was the growing number of training institutes (Private) and consultants in mushroom cultivation who pass on misleading information and incorrect operational methods which costs the growers a heavy investment. This study was conducted on more than 70 growers and their major concern was found to be the marketing strategy for selling mushroom and handling the cultivation. Due to increasing cost of production, the small growers are not able to sell their produce at competitive prices and thus lose their market share. Also, these growers were found to be having little or no knowledge regarding the disposal or usage of the spent mushroom substrate. The study proposes the following solution to the alarming situation among the growers. First, there should be an association of all growers at district level, these district level associations can join together to form an association at state level and so on. Second, the consultants & training institutes should be registered with the state agricultural universities or bodies like NRC, PAU, TNAU, IIHR and so on. Third, workshops should be jointly organized by these institutes along with the growers and discuss their issues and sort them at commercial level. Fourth, by-products like pickle, *papad*, cookies, instant mix, and soups should be promoted to increase consumption of mushroom.

### X-P-2. Continuous production and processing of *Pleurotus florida* and *Calocybe indica* utilizing locally available lignocellulosic substrates for additional income generation in rural area

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Agricultural wastes are generated in rural areas can effectively be utilized for production of mushroom as a source of alternative food supplements as well as for income generation. Unexplored locally available lignocellulosic substrates such as reeds, banana stem, sugar cane bagasse milled and crushed, sugar cane leaves, coir pith, sorghum husk, sun flower stem, pine tree leaves and paddy straw were utilized at small scale, pilot scale and field scale production of *P. florida* and *C. indica*. *P. florida* was recorded



maximum bioefficiency 100.30% (small scale), 95.81% (pilot scale) and 81.00% (field scale cultivation) whereas *C. indica* recorded maximum bioefficiency 134.16% (small scale), 115.74% (pilot scale) and 91.97% (field scale). The field scale cultivation was carried out by 127 farmers utilized 14,646 kg of substrates produced 12,565 kg of mushroom generated an additional income and also consumed as nutritional supplement. The harvested fruit bodies of these mushrooms were analyzed for carbohydrates (49.28-51.27 g), protein (26.94-28.73 g), fat (0.37-0.73 g), crude fibre (38.46-40.52 g), total ash (8.80-9.11 g), folic acid (35-57 µg), vitamin B2 (0.35-0.58 mg) and vitamin B3 (16.28-22.94 mg). *P. florida* and *C. indica* were cultivated continuously by using different locally available substrates generated to rural people.

### **X-P-3. Exploring the mushroom farming as entrepreneur for low income group community through ensuring the strategy on market opportunities**

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Marketing analysis is the key for any successful product. Direct data collection based on personal interviews with stakeholders was the approach in the study. The growers marketing strategy is broadly related to four aspects - Marketing method: deciding where to market, Product decision: deciding what and when to produce, Price strategy: realizing the potential, Merchandising: making the most of the marketing payoff. Proposed model for mushroom marketing has mission to improve the livelihood of villagers through offering new enterprise to bring new income, encourages the need for cooperation among enterprises and training and facilitation. Embedded in this marketing strategy is the crucial concept that livelihood is not about money, but about empowerment. Through cooperative approach villagers become confident as individuals in making well-informed decisions and are willing to look beyond competitiveness in marketing to the common good.

### **X-P-4. Impact assessment of training programme on mushroom cultivation in Pali district of Rajasthan, India**

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Mushroom cultivation is a women friendly profession. Mushroom growing is one agricultural activity in which farmers can play a vital role without sacrificing their household responsibilities. Mushroom cultivation is simple, low cost and suitable for rural areas, is labour intensive and can provide employment in both the semi-urban and rural areas. Mushroom cultivation will improve the socio-economic condition of farmers, families and solve employment problems of both literate and illiterate especially women. The study was carried out in Pali district of Rajasthan. Out of ten blocks, one block namely, Sumerpur was purposely selected because of participation by larger number of trainees in the training programme. Sumerpur block comprises of 144 villages. Out of these, 6 villages were selected purposely on the basis of dense population of the trainee participants in the training on mushroom cultivation organized by CAZRI, KVK, during the preceding year that is 2011-12. The list of the trainees participated in the training programme was obtained from the KVK, Pali. Total 160 trainees participated in training during 2011-12. Out of these 160 trainees were from Sumerpur block. A sample of 90 trainees was drawn by simple random sampling method, which constituted the sample for the present study. The findings revealed that that 51.09 percent of the respondents were belonged to age group between 26 to 35 years. Whereas, 55.43 percent of the respondents had education upto college level education. In case of occupation 52.17 percent respondents had an occupation of farming. Thus, it is inferred that mostly educated farmers were interested in participation in the training programme. The investigation revealed that about 69.00 percent of the topics

of mushroom training programme was perceived to be either relevant or highly relevant. Also about 75.07 percent of the respondents rated the topics of the programme either as most useful or useful. The topics such as cultivation of oyster mushroom, bed setting and spacing, and sterilization of straw received a high mean score for relevance and utility. About 80.00 percent of the respondents perceived the programme either effective or highly effective. About 59.80 percent of the trainees were confident enough to start mushroom cultivation after the training.

### **X-P-5. Information needs, technical efficiency and interactive system for mushroom stakeholders**

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In the present study, a survey was carried out to determine the information needs of the mushroom farmers of four states, namely, Himachal Pradesh, Punjab Haryana and Uttarakhand. The information was collected personally as well as through mailed questionnaires. The information requirement of the farmers was ranked using Summary Cards which were based on the CRC (Class-Responsibilities-Collaboration) Card modeling technique. The most preferred information was related to “Loans and Subsidy schemes of the government” followed by “Marketing of mushrooms” and “Post harvest management and value addition”, respectively. Information related to “Cultivation of mushrooms for exports” was desired by 23 farmers as first preference, 86 farmers as second preference and 91 farmers as third preference. It shows that a good number of the farmers are well aware of the standards expected by the consumers at national and international levels. Some educated farmers mentioned that they have to depend on trickling down of decision inputs from conventional sources which are slow and unreliable. They desired that more and more information/alerts should be made available on mobiles in local language so that they can derive maximum benefit from latest information technology creations. The priority of information requirements mentioned by mushroom farmers would help in modifying the present research policies. The extension personnel could also transform their approach accordingly so that the latest technologies could reach the end users in an efficient manner.

Technical Efficiency (TE) is the effectiveness with which a given set of inputs are used to produce an output. An operation is said to be technically efficient if it produces the maximum output from the minimum quantity of inputs, such as labor, capital and technology. Given the increasing importance of mushrooms in our diet due to shrinking agricultural land space and changing agro-climatic conditions, it becomes important to study how improvements can be made in the productivity of this sector. The present study attempts to measure the TE of mushroom farm operations of farmers in Himachal Pradesh. It employs the stochastic frontier function which is a method of economic modeling. As compared to other mushroom countries, mushroom productivity in India is relatively low. One important reason for low productivity is that many farmers with low literacy rates and inadequate physical infrastructures face difficulties in understanding new technologies and, therefore, fail to fully exploit these technical opportunities.

The investigation revealed that the compost preparation elasticity is the highest followed by spawn production. The mean efficiency of raising agricultural output is 0.6875 without additional resources, which shows that there is 31.25% scope for improvement so that the TE could be converted into economic efficiency and farmers experience increased returns. A majority of the regions stand to gain the most from policy interventions towards improving technical efficiency.

It was the need of the hour that a facility should be developed where a mushroom farmer could have online interaction with experts of different fields who could guide him/her during the various stages of the cultivation of different mushrooms. The advantage of a two way communication process is that there is quick response from the experts which leads to a speedy answer/solution to the queries/problems of the farmers/users. Indian Agricultural Statistics Research Institute (IASRI), New Delhi and Directorate of Mushroom Research (DMR), Solan have developed such a system using a tool called *AGRIDaksh*, and is available at <http://agridaksh.iasri.res.in/mushroom.jsp>. In addition to the information related to mushroom cultivation, economic analysis and disease management, it has a farmer's feedback/expert's response window through which queries can be sent online. The expert responds back with a suitable answer to the query. The system developed is new in the way that it provides an interactive environment where the queries of the mushroom farmers/users are replied by the experts. The IDs and passwords provided to the domain experts enable them to update the information available in the system. The System is expected to facilitate better decision making ability among the mushroom entrepreneurs and farmers, thereby minimizing losses due to diseases and pests infestation and improved productivity. Other typical tasks that would be fully integrated into the system in due course are classification, diagnosis, monitoring, design, scheduling, and planning for specialized endeavors. Nevertheless, such systems remain supplements, rather than replacements, for human experts.

#### **X-P-6. Issues and opportunities in spread of mushroom enterprise In Odisha**

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In India, mushroom farming is popular in coastal states like Odisha, Andhra Pradesh, Tamil Nadu and West Bengal. In Odisha, mushroom production is widely spread in all 30 districts producing 8000 tonnes of straw mushroom per annum. Along with straw mushroom during hot and wet months, preferably oyster is produced during winter and button mushroom in sporadic patches of the state. The catch of technology is so fast because of certain advantages like better taste, labour intensiveness, short production period, easy and simple cultivation method and higher profit and potentiality of the enterprise to provide gainful employment to small, marginal, landless, women farmers and unemployed youth. In spite of the accelerating factors like willingness, ability, interest and existing conducive environment to support spread of mushroom enterprise, certain social, financial and marketing issues have been found to limit its spread. A study was undertaken in the state of Odisha covering 300 mushroom growers from three districts namely Bhadrak, Dhenkanal and Puri with two blocks from each district with the objective to assess the impact of the enterprise on the socio-economic status of the mushroom growers. The key major issues identified during the study are comparatively poor resource base of the farmers to take it up in commercial scale, lack of specific project or programme to promote the enterprise, lack of ownership of line departments in spread of the technology, unmatched support of banking and insurance institutions, reducing yield potential of spawn, non availability of quality spawn in desired quantity, high perishability of the produce, lack of cold chain, unstable market price, presence of traders and middlemen in the supply chain in both management of input and produce, yield fluctuation due to climate fluctuation, reducing yield due to continuous cropping, substandard marketing facility and absence of fixed price for mushroom as opined by majority of the respondents. Apart from socio- personal and marketing issues, lack of proper cataloguing of mushroom growers, spawn producers and established input agencies were identified to be the major issue in technology dissemination attempts. These issues have been broadly categorized in consultation with selected sample and experts in the field. The majority of the respondents (44.77%) perceived limited access to finance as the prime issue closely followed by limited availability of production technology, inadequate schematic support for production and post harvest, inadequate market support, limited applicability of available mushroom production technology and poor availability of inputs. In the context of spread of the enterprise the major opportunities identified from this studies are abundant availability of substrate found to be the

most important reason in continuing mushroom production with mean score of 8.08 followed by growing demand for mushroom, increasing number of agencies and promoters to promote the mushroom enterprise, emerging market channels and diversifying food habit from non-vegetarian. to vegetarian. More specifically Government strategies and future programmes will be the major support in enhancing the enterprise in the state. Mushroom farming in our country may flourish like other mushroom dominating country in the coming years if the issues & opportunities identified in the study are taken into consideration while formulating strategy for future growth of the enterprise.

### **X-P-7. Mushroom acceptability and consumption intention for the main mushrooms produced in Brazil**

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Mushrooms are known for their functional and nutritional importance. However, there are few works about their acceptance as food by Brazilian consumers, which is important as directives for mushroom producers. The objective of this study was to evaluate *Agaricus bisporus*, *Pleurotus ostreatus*, *Agaricus blazei* and *Lentinula edodes* mushroom acceptability and consumption intention. A dish with rice and *A. bisporus*, *P. ostreatus*, *A. blazei* or *L. edodes* mushrooms was prepared. The dishes (samples of 20 g) were given to 192 randomly-chosen untrained panelists. The acceptability was determined in a 5-point hedonic scale, and the habit of consumption and purchase intention were evaluated. The mushroom global score acceptability was 3.61 for *A. bisporus*, 3.48 for *A. blazei*, 3.24 for *P. ostreatus* and 2.89 for *L. edodes*. For *L. edodes* the color was the main rejected characteristic. There are no differences to the mushroom acceptability according to the panelists' socioeconomic characteristics. Although most of the panelists do not have the habit of buying mushrooms, the majority (90.6%) was willing to purchase mushrooms and 38.5% were willing to pay as much as US\$ 80 per dried kilogram of mushrooms. Due to the high commercial prices of mushrooms in Brazil (at least US\$ 100 per dried kilogram) considered exotic and are likely bought as functional food, because of healthy benefits, than sensorial characteristics.

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### **X-P-8. Seasonal mushroom growing in north Indian plains**

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Mushrooms are being cultivated in India both on commercial scale and also as a seasonal activity. In latter case cultivation is done in temporary huts during winter taking one extended crop during winters in bamboo-paddy straw huts in north Indian plains. The advantage of growing at a new place with no farm around is there. As the farm and site gets older, the spores of green mould, yellow mould, plaster mould and others accumulate in the soil, the vegetation around, on bamboos which are used repeatedly. They attack on compost not made "selective" which is often the case with long composting. Then mushroom flies and bubble are there to attack as soon as severe winter is over and warming up start by beginning of March. It is not possible to maintain any standard of hygiene in these temporary huts erected along the paddy fields. There are many reasons for low production. Right formulations and right procedures can not only ensure good production, but also eliminate need of adding chemicals to the compost. More efforts are required to impart technical knowledge to the farmers. Being a seasonal activity, farmer get only two months for composting and three to four months for growing, it is a race against time and "skill" is difficult to acquire, be it the moisture content in compost in relation to structure, or picking speed or



watering regime during cropping etc. Time is changing fast. Seasonal growing is highly labor intensive. Unskilled labor is available but not as easily as three to four years ago and getting too expensive unlike prices of mushrooms. It is not easy to get a bank loan for mushroom growing, we do need a strong cooperative which could get us a better price.

### **X-P-9. Technological and marketing fissures of white button mushroom at traditional and scientific know-how in mid hills of Uttarakhand state, India**

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Four mushroom growers (MGs) each from Pauri, Khirsu, Kaproli and Thailisain of mid hills of Uttarakhand State of India, were randomly selected for white button mushroom cultivation and to evaluate the period of spawn run, days consumed between casing and 1<sup>st</sup> harvest, cropping pattern yield, number and average weight of fresh mushroom fruit and net income in relation to that of main centre of experiment situated at Vir Chandra Singh Garhwali, College of Horticulture, Bharsar. Alongside, tech-fissure and market-fissure of MGs was also calculated using formula:  $\{(value\ of\ selected\ parameter\ of\ Bharsar - value\ of\ selected\ parameter\ of\ MG) / value\ of\ selected\ parameter\ of\ Bharsar\} \times 100$ . It was found that all mushroom growing farms were inferior as they took longer period of spawn run and longer period between casing and 1<sup>st</sup> harvest, and were less in yield, numbers of mushroom and net income against to that of parameter recorded at Bharsar centre. The entire 7 week cropping pattern on yield and numbers of fruits of each trial was proved to be undifferentiated when compared with Bharsar centre in which maximum fruit yields and numbers were obtained within first two weeks then declined gradually till the last cropping week. Out of total yield and numbers obtained during whole cropping period, 44.83-58.79% share of yield and 36.54-57.62% share of numbers of white button mushroom were taken out within first 2 cropping weeks from all trials, in corresponding to 60.13% share of yield and 56.79% share of numbers obtained from Bharsar for the same period of time. There were negative tech-fissures with all MGs when calculated against period of spawn run, days consumed between casing and first harvest, yield, number and average weight of fresh mushroom fruit. All MGs were sold out their fresh mushroom in local market at local prevailing rates against fixed invested cost of ₹ 992.18. MG1 was sold mushroom relatively at much higher rates of ₹ 120/kg and earned highest net income of ₹ 1355.02/q compost with 8.15% positive market-fissure and 1:2.36 cost: benefit ratio over to that of Bharsar centre, in which ₹ 1252.82 was earned as a net income with 1:2.26 cost: benefit ratio. The technology and market-fissure was recorded more negative in the trial located in Village Kaproli with very poor net income and cost: benefit ratio in comparison to the rest trials located in urban and semi-urban towns like, Pauri, Khirsu, Thailisain and Bharsar.

### **X-P-10. Windows based applications for mushroom growers**

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Windows based applications are most suitable wherever concurrent online data is not required and are suitable to local users for solving specific issues. Input selection, profit prediction, know-how about mushroom cultivation, identification and management of pests and diseases, etc. are some of the specific issues important for mushroom cultivation in India which hinder expansion of mushroom industry. These issues can be addressed with the help of windows based computer application as all these are structured problems. This paper elucidates the windows based applications developed at DMR under the SERB project entitled "Development of spatial decision support system for mushroom choice for round the year cultivation and its popularization in India". It includes mushroom information system, profit calculator,



compost N calculator, database on Indian weather at district level, Indian wild mushroom database, information sources, etc. in detail. The module on mushroom information system is suitable for guiding the mushroom growers in detail about the mushroom cultivation whereas profit calculator informs the grower whether he will be able to get desired profit from the selected mushroom cultivation in his own region. Compost N calculator directs the farmer for selecting different organic waste inputs for ideal substrate preparation for mushroom cultivation. The information on input and machinery suppliers, spawn production units, KVKs, Agricultural Universities and ICAR institutes can be obtained easily from module on information sources while local weather information can be accessed from Indian weather data base. In nutshell, these windows based applications will promote the mushroom growers to produce more mushroom through proper guidance and extension functionaries for popularizing mushroom cultivation using the given necessary information.

### **X-P-11. Economic impact of mushroom production and value addition on marginal farmers of Almora district of Uttarakhand in India**

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The agro-climatic conditions are suitable for mushroom cultivation in hills of Uttarakhand. To enhance the livelihood security of marginal and landless farmers, mushroom farming is better option to such farmers along with other agricultural activities and it requires limited resources such as any spare room, paddy/ wheat or cereal straw, minimum labour and capital. Mushroom is highly perishable and low shelf life commodity and it requires proper post harvest and value addition techniques to enhance commercial production and increase productivity. Therefore, the present investigation was done in Almora district of Uttarakhand and economic impact of production and value addition in button mushroom has been worked out. Capital budgeting technique was used to analyze the impact of technology on marginal farmers and results found that before adopting the technology, farmers were getting the return of ₹ 39.1 thousand annually on their investment in agriculture, which increase up-to ₹ 43.7 thousand after adoption of mushroom cultivation practices. It indicates that the farmers started using their resources in more efficient manner after adoption of new technology. It was also revealed that the significant contribution of value addition activities has been found to reduce the post harvest losses and increase shelf life as well as generated additional incomes of ₹ 9.3 thousand per annum by farmers who are doing value addition activities in button mushroom. On an average, mushroom production and value addition activity were generated 10 to 15 days of full time employment and 16-20 % of additional income per annum. The adopted technology has been found successful for economic growth of marginal farmers as well as enhancement of commercial production and increase productivity.

