National Symposium

on

"Biodiversity and Biotechnology: Research and Development Needs in Edible Mushrooms and Crop Disease Management"

TECHNICAL SESSION S_1

"Biodiversity and Biotechnology in Edible and Medicinal Mushrooms"

Venue	:	Committee Room, MRTC
Date	:	November 09, 2006 (14:30 hrs.)
Chairman	:	Dr. (Prof.) S. Kannaiyan, Chairman, NBA, Chennai
Rapporteurs	:	Dr. S.K. Singh (NRCM, Solan) Dr. K.K. Mishra (GBPUA & T)

S_1.1 Exploration of Biodiversity of Edible Mushrooms in Rajasthan

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Biodiversity of mushrooms plays a very important role in any ecosystem functioning. Mushrooms found to occur in soil, humus plant litter, herbivore dung, termite hill and also on living plants. Most of the mushrooms are cosmopolitan occurring both in tropical and temperate region of course some of them like *Lentinus* are more confined to tropical and subtropical regions and some like *Hygrophorus* to the temperate regions. It was studied that the agaric diversity of East Africa and the affinities of different species to different forest type. In 1970s hardly anyone worried about the effect of mass collecting some popular edible mushrooms. In 1980 report started appearing about the prominent decrease of some groups of mushrooms in some countries the use of mushroom as food and in medicine by the people of India has been age old. References to there occurrence and utilization are found in religious books such as Vedas and Bible and medical treatments of India, Samhita of Atrayacharak period dating back to 3000 ± 500 B.C. However, the utilization has definitely increased with the acquisition of more knowledge about the edible and poisonous species and technological advancement of its cultivation. India is a vast country with people in different regions having diverse food habits. The Use of mushroom as food and medicine in the present day is widespread with tribal people all over the Country. In view of their intimate association with nature, knowledge of mushroom species of human value is maximum with these people. This information remains to be documented fully.

Some of the species utilized by tribals of India as food are: *Agaricus campestris, Cantharellus spp; Coprinus spp; Lentinus subnudus ,Termitomyces spp.,Tricholoma spp; Pleurotus spp ;Boletus spp. Phellorina inquinans; Podaxis pistallaris* and *Tuber* spp. In Switzerland, France and Germany up to 78 distinct wild varieties of mushroom are offered for sale in the market. In India, too sale of wild mushrooms in cities during rainy seasons is not uncommon now, particularly in hill regions *Morchella* spp. (Guchhi) and *Pleurotus* spp. (Dhingri). It was reported that the collection and consumption of 123 tons of 8 wild edible species in West Bengal. Rajasthan is the second largest state of India and represent the 10 % of the land area of the country. However, 57% of the state consists of the great Thar Desert, which has been designated as one of the biosphere reserve and as the representative of the desert ecosystem in India is especially important from the conservation angle. Occurrence of edible mushroom like *Agaricus, Pleurotus* and *Coprinus, Termitomyces eurhizus* on termite nests, some new and additional hosts of *Pleurotus* species and some species of Agaricales from Rajasthan have described. Later on in1992 Sharma et.al. *Auricularia auricula judae, A. mesentrica , Phellorinia inquinans, Podaxis pistillaris, Boletus species, Termitomyces microcarpus, Termitomyces striatus, Pleurotus pulmoonarius, P.ostreatus, P.sapidus, P .sajor-caju, Agaricus campestris, Volvariella bombycina, V.speciosa* and *Lepiota* spp. Reported from Rajasthan. Ninety four species of macro-fungi belonging to 52 genera and seven families were reported in 1994. Among them 78 species are edible, eight species poisonous and 12 are not known for their edibility. Some of the gasteromycetes genera were reported in 1994. Occurrence and distribution of 173 species of mushroom coming under 95 genera were reported in 1997. All the collections carried out from the five major agro climatic zones of the state.

Host range of *Auricularia spp. Ganoderma spp.* and *Schizophyllum commune* was reported in 2001.

Further 225 wild mushrooms from different parts of Rajasthan was documented in 2005. Maximum 34 species were reported from poroid aphyllophorales followed by 32 species of Tricholomatacae and 26 from Gasteromycetes. Out of these *Podaxis pistillaris, Phellorinia inquinans* and *Phellorinia herculae* are collected in tones during rainy season and sold in the market fresh as well as in dry form at the rate of Rs 50-60/kg from zone I a,b, II a,b, III a,b, IV a,b, and V . Genera of poroid aphyllophorales were confirmed only in two zones that is zone IV a, b and zone V common are wood rotting fungi such as *Ganoderma lucidium, Ganoderma, tsugae, Ganoderma applantum, Polypores sulpheureus* and *Coriolous versicolour*

Tricholomataceae genera also confine to zone IV a, b and zone V, most of the genera are edible some important are Armillaria, Calocybe, Tricholoma, Collybia, Flammulina, Pleurotus and Pluteus.In Agaricales 12 species of predominant genera Agaricus are recorded and most of them are edible.

In Rajasthan only three mushrooms have been exploited commercially so far, there are non-conventional species such as *Phellorinia inquinans, Phellorinia herculae, Podaxis pistillaris,* which should also be tried. Further, wild strains of *Agaricus, Pleurotus, Auricularia* and *Calocybe* should be utilized for breeding purposes to develop temperature tolerant, disease / insect pest resistance and with higher yield and keeping quality strains.

S_1.2 Molecular Diagnostic Tools for Genetic Characterization of Edible Mushrooms

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Fungi are often microscopic, usually filamentous, spore bearing organisms that have few phenotypic markers that can be used to differentiate between individuals in a population. This limitation has hampered the studies of their population biology. In the past, pathologists had to rely on phenotypic markers such as vegetative compatibility, mating type, or specific avirulence genes to differentiate individuals Although, these markers proved very useful for differentiating certain groups in a species but lacked sufficient resolution to distinguish among all the individuals in a population.

True to the type mushroom stock cultures are not maintained as fruit body or as spore culture due to different sexuality patterns. Moreover, fruit body morphology within a given taxon is highly variable and microscopic characters are quite homogenous. Therefore, stock cultures are invariably maintained as tissue cultures. Further it is impossible to designate species to any mushroom culture without fruiting trials by conventional methods. Except for a few edible mushrooms, species level identification is still a challenge to the traditional taxonomists. This has led to a great taxonomic chaos in mushroom genera with large number of species.

The limitations of taxonomic identification of fungal strains based on phenotypic markers can be overcome by the use of DNA markers like Restriction Fragment Length Polymorphism (RFLP) and PCR based Random Amplified Polymorphic DNA (RAPDs), since they detect variation directly at DNA level and are not influenced by the environment (test conditions). Moreover, PCR based RAPD are fast, less cumbersome and very little template DNA is required for analysis and therefore, a large number of samples can be handled in a very short period of time. These properties make DNA markers very useful in studying genetic/ strain variability in fungi.

Sequencing of conserved genes: The use of modern methods such as RFLP analysis and direct sequencing to investigate the gene coding for production of 16S, 5.8S, 28S and 5S r DNA (r RNA) has allowed assessments and comparisons of phylogenetic relationship of organisms over a wide range of taxonomic levels. The breakthrough in amplification and direct sequencing of fungal ribosomal RNA gene for phylogenetics came with the description of nuclear ITS primers. Recent studies have demonstrated that polymorphism in the Internal Transcribed Spacer (ITS) regions of 5.8S rDNA (ribosomal DNA) has proved adequate to improve systematics of a wide range of fungi.

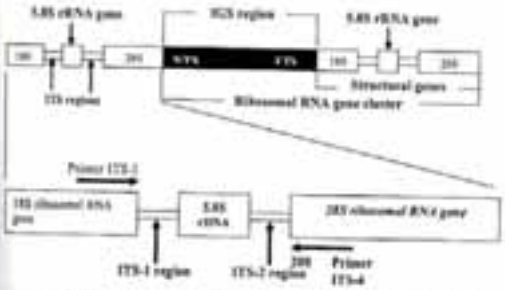

Fig. 1. Position of conserved genes in fungal genome.

Further, creation of National Centre for Biotechnological Information (NCBI), USA and other world data bases have not only allowed quick molecular identifications but also validated species level identifications of microbes. These recent advances in molecular taxonomy has addressed the basic question of edibility of mushrooms "Poisonous or edible" and can validate food poisoning related forensic examinations.

The 5.8S r DNA gene encompassing ITS-1 and ITS-2 regions can be amplified using ITS-1 (forward primer 5'- TCC GTA GGT GAA CCT GCG G- 3') and ITS-4 (Reverse primer 5'- TCC TCC GCT TAT TGA TAT GC-3') in a PCR. The amplified product appears as single band on 1% agarose gel in Tris-Acetic acid-EDTA (1 x TAE) buffer (Figs 2 &3).

Fig.2. Intra-species ITS Amplified products.

Fig.3. Inter-species ITS amplified products

PCR products can be sequenced directly by using universal ITS primers. ITS-4 reverse primer sequences are reverse complimented by using Gene Doc computer software programme. Nucleotide sequence comparisons are performed by using the Basic Local Alignment Search Tool (BLAST) network services against the National Centre for Biotechnology Information databases. The fungal species are designated to the sequenced based on similarity with the best-aligned sequence of the BLAST search.

RAPD analyses: The most popular method has been to use the product of RAPD to differentiate among individuals. In the procedure, an arbitrary 10 base pair nucleotide sequence anneals to complementary template sequences throughout the genome and act as a primer for extension by a heat stable Taq DNA polymerase using PCR. The results are a series of DNA fragments that are amplified in each individual. Each individual has a specific amplification pattern that may differ as a result

of mismatch between different primer and template sequence (Fig.4)

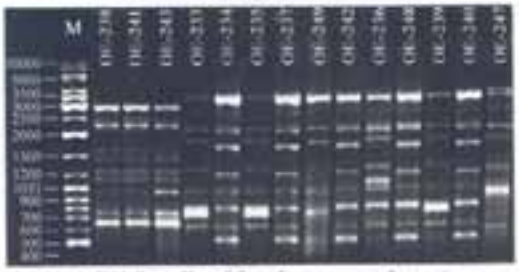

Fig.4. RAPD profiles of Ganoderma group of accessions.

In order to determine lengths of individual amplified fragment, 1Kb ladder of known fragment length is also run simultaneously on both the sides of the gel to score presence and absence of individual fragment at a particular base pair length in test samples.

The gel photographs are scored for presence and absence of scorable bands as 1 and 0, respectively, with the assumption of positional homology. To estimate the genetic distances between isolates, similarity coefficient are calculated and dendrogarm drawn using UPGMA algorithm (Unweighed Pair Group Method using Arithmetic Averages) of the NTSYS- Pc, Version 2.02h programme (Fig.5).

Restriction Digestion of ITS (ARDRA-Amplified Ribosomal DNA Restriction Analysis): In order to develop RFLP profiles of amplified ITS fragments, different restriction enzymes are used to develop very specific RFLP profiles. These profiles serve as a reproducible and reliable method of distinguishing individuals having similar ITS lengths. PCR product of ITS

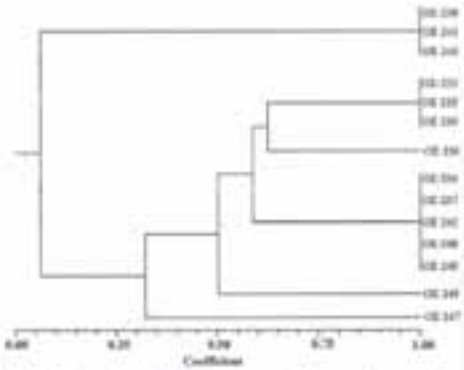

*Fig.5. **Phylogenetic relations among 14 accessions of Ganoderma species***

conserved gene is digested with restriction enzymes like Taq, Alu 1, Eco R-1,Bsu R-1, Hin f-1, Msp 1 etc. in presence of Buffer (specific to restriction enzyme) and water at suitable temperatures. The unique restriction fragment profiles generated by the restriction endonucleases enable us to identify marker fragment bands to distinguish individuals within and amongst species. This technique can be used for rapid identification of wild specimens as a cheap alternative to direct sequencing for mushroom germplaam cataloguing.

S_1.3 Biodiversity, Conservation and Utilization of Edible Mushrooms in Chhattisgarh Region

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Biodiversity refers to the variety and variability among living organisms and ecological complexes in which they occur. It plays a significant role in nature by enriching soil, maintaining water and climate cycle, humidity precipitation, conservation and recycling of waste materials into nutrients. Biological resources constitute a capital asset with great potential for yielding sustainable benefit. It is believed that the modern developmental activities are detrimental for biodiversity and it is felt that development is inversely proportional

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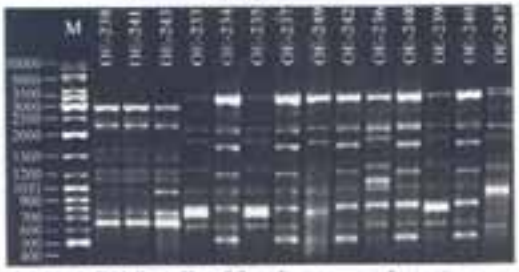

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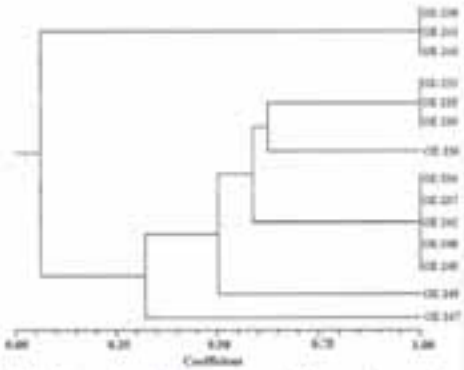

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to biodiversity. Rich biodiversity is an indicator of healthy habitat and its potential to sustain life.

India being the top ten megadiversity has ample species of wild mushrooms which occur during rainy season. The diversity of climatic conditions prevalent in India made this country a natural habitat for a number of mushrooms. There are about 2000 species of edible fungi known to man out of 10,000 species of macro fungi. India is richer in flowering plants than any other country of its size, the fungal wealth of India is also expected to be equally diverse. Lot of studies have been made to document mushroom wealth from East, West, North and South India but, the efforts made in Central India including Chhattisgarh and Madhya Pradesh States received very limited attention.

Chhattisgarh State of India is highly rich in biodiversity including mushroom diversity. The climatic conditions prevailing in Chhattisgarh made the natural habitat for a large number of mushroom flora. The detailed information for the presence of natural agaric flora in different agroclimatic zones of Chhattisgarh was started with the inception of All India Coordinated Mushroom Improvement Project in 1988 at IGAU, Raipur. Prior to 1988, no systematic efforts were made for collection, identification and preservation of fleshy fungi of this state. But, thereafter, the systematic work was started to explore the kind of wild mushroom flora available and exploit their production potential in a commercial scale.

Chhattisgarh is the third state in the country having largest area under forests. It has several wild life sanctuaries which have enormous mushroom diversity which is still conserved and unidentified. Most of the mushroom wealth in these parts remain untouched, while these parts are highly rich in mushroom diversity due to vast forest covers of 43.1% in C.G. and 37 percent in M.P. Conservation, identification and creation of awareness regarding such mushroom diversity was highly emphasized by a Quinquinnial Review Team (Constituted by ICAR for reviewing the Mushroom work all over the country) visited Raipur on September, 2005. The team constituting Dr. S. Kannaiyan as Chairman, QRT on Mushroom and Chairman, National Biodiversity Authority Chennai suggested to hold a two days workshop on "Awareness creation on Biodiversity and Conservation of Mushrooms". Accordingly, a two day workshop on above theme was held at IGAU, Raipur on December 1-2, 2005.

The forest cover of Chhattisgarh is predominantly inhabited by tribals whose livelihood is dependent on forest produce, mushroom being one of them. They collect the mushroom from these forest areas, consume them and bring them to the local markets, towns and cities for sale during monsoon period. Many species are traditionally consumed since the ages and mistaken identity is a problem in many areas which caused casualties. In Chhattisgarh 10-15 causalities from poisonous mushroom is reported every year in daily newspapers.

The work on survey, collection, isolation and preservation of naturally growing fleshy fungi during monsoon season was initiated in 1988. Nearly 50 tons of naturally growing edible fleshy fungi is collected from Bastar alone during monsoon season. A survey for the presence of naturally occurring fleshy fungi was conducted from 1989 to 1995. The forest area of Chhattisgarh was surveyed in 2001 and collected edible and wild mushrooms. Enormous wild mushroom flora existing in Chhattisgarh was reported in 2005 and some of the mushroom flora was isolated, identified and characterized. Various species of mushroom from Bilaspur division and some mushrooms have antitumour, antibacterial, antifungal and antiviral properties. The species of *Agaricus, Russula, Termitomyces* are being used for treatment of goitre, wound and small pox respectively. During survey, most commonly available mushrooms encountered were *Russula* spp, *Volvariella* spp., *Termitomyces* spp., *Tuber* spp. and *Cantherellus* spp., *Astreatus hygrometricus* etc. The fleshy fungi were collected from different areas of Chhattisgarh with respect

to habitat, season, distribution and ethnic information. Most of these fungi were identified using standard monographs.

Due to increasing stresses of population, urbanization and human greed, there is a rapid decline in the great mushroom diversity present in this part of the country. In spite of mounting efforts over last 2-3 decades, the loss of world's biodiversity from habitat destruction (like deforestation), over harvesting, pollution and other manmade problems like introduction of exotic species has continued. It necessitates the conservation of biodiversity *in situ*. An awareness programme is required to conserve wild mushroom flora particularly mycorrhizal mushrooms prevailing not only in Chhattisgarh but in other parts of the country so as to conserve them effectively and protect them from erosion. It needs to be isolated, identified, characterized and catalogued using conventional and biotechnological techniques. A need is also felt to monitor and map the mushroom diversity of forest ecosystem using Satellite Remote Sensing, GPS and GIS techniques.

Mushroom growing has proved as an important activity for generation of employment in rural areas. Mushroom is very much liked by the rural people and form an important ingredients in their daily diet. Mushroom growing in rural areas/ weaker sections of the society can improve the financial and nutritional status of the people who are malnourished and below the poverty line. It will be a boon to uplift them. Chhattisgarh being the rice bowl produce about 50 lakh tons of paddy straw and other substrate every year, and part of this straw made into heaps are exposed to rains produce lot of paddy straw mushrooms. As per one of the estimate 800-1000 tonnes of naturally occurring mushroom are being sold in C.G. market including 5-6 tons per day in Raipur city alone during rainy season from July to September. Most of this flora some time fatch very low price when available in abundance during favourable monsoon period. The people of this area are well aware with different varieties of edible mushrooms which they collect from forest areas growing naturally on wood logs, decumposed leafy materials, sandy loam soils, termite mounds and decomposed paddy straws.

Cheap and easy mushroom production technology needs to be evolved for the people who are mainly dependent on forest produce including mushrooms. Mushrooms being highly nutritious and having potent medicinal attributes, it should be made essential in mid day meals to school children, in daliya, in Dal Bhat Centres, in Aaganbadi programme and other programmes run by the Department of Women and Child Development and in Govt. Canteens and Hospital Wards. A policy framework and programme is required to blend mushroom powder in Atta, dalia, or dal, papad, badi, murku to improve the nutritional qualities of food particularly in the state of Chhattisgarh where 88% of the children and 70% of the women are anemic. The potential of medicinal mushroom in India is untapped and need to evolve into a successful biotechnological industry for the benefit of the mankind. Good quality mushroom spawn laboratory, cold storage facility, and small scale processing units in a district place need to be established to facilitate mushroom production, processing and marketing.

S_1.4 Recent Advances in Molecular Breeding of Button Mushroom *Agaricus* species

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The mushroom industry has a continuing need for new and improved varieties with well-defined characteristics. The application of molecular techniques to the genetic improvement

programmes has introduced innovative DNA based markers and provided much-needed fillip to the on-going research in button mushrooms. Genetic diversity is limited in *Agaricus bisporus*, so improvements can best be made by exploiting the diversity present in the genus *Agaricus* or by using molecular breeding. The term 'molecular breeding' has been used to characterize breeding programmes that are supported by the use of DNA-based technology. DNA markers are heritable differences in nucleotide sequences of DNA from two individuals, which follow a simple Mendelian pattern of inheritance. These markers are detected by employing two basic techniques: i) Southern blot hybridization and ii) Polymerase Chain Reaction (PCR). The molecular analysis of species relatedness, cloning and characterisation of genes are the frontier areas of research.

Although current breeding practices have been very successful in producing a continuous range of improved hybrids and strains in button mushrooms, recent developments in the field of molecular genetics and biotechnology can be employed to enhance mushroom breeding efforts and to speed up the creation of improved cultivars. The mushrooms are mainly characterized on the basis of variations in the fruiting body, spore morphologies and mycelium characteristics. The paucity of morphological markers, stage specific expression and the influence of ecological factors on the expression on these traits are some of the limitations of the morphological markers. However, the recent use of DNA markers has circumvented these limitations and has led to the precise identification and classification of wild collections upto species level in quick time. DNA markers have the potential to aid the mushroom breeding through a number of ways including DNA fingerprinting, reliable assessment of genetic variation and increasing the efficiency of selection of difficult traits. Enhanced understanding of genetic controls and identification of molecular markers closely linked to the genes/QTLs associated with important traits would help breeders to design more effective and targeted breeding strategies. DNA markers such as RFLP, RAPD, repetitive DNA sequences, ITS sequencing and more recently AFLP have been utilized in molecular breeding of button mushrooms. The recent advances in molecular breeding have influenced the following areas related to the genetic improvement in button mushrooms:

Assessment of genetic diversity: A low level of genetic diversity amongst commercial white pileus strains of *A. bisporus* was detected using RFLP, RAPD and repetitive sequences. In contrast to commercial lines, the wild collections from *Agaricus* Resource Programme were found to be genetically highly variable using nuclear and mitochondrial RFLPs, and repetitive sequence analyses. Recently AFLP markers were used for assessment of genetic relationship and variability in *A. bisporus* and *A. bitorquis*.

Identification of homokaryotic breeding lines: Genetic analysis and hybrid breeding of cultivated mushrooms require the isolation of homokaryons from heterokaryotic parental lines. This is difficult in *A. bisporus* because of its unusual life cycle. Now, with a combination of isozymes, RFLPs and RAPDs the identification of homokaryons is a routine process in *Agaricus* breeding programmes. Hybridizations in *A. bisporus* can be confirmed by the use of DNA markers - RFLPs and RAPDs. RAPD analysis has provided reliable basis for selection of parents and identification of hybrids.

Genomic inter-relationship: The interspecies consanguinity between *A. bisporus* and *A. bitorquis* was not found as close as expected with RAPD markers. The genomic relationship studies using ITS-II and a portion of 28S rRNA gene in the genus *Agaricus* revealed that the species *A. bitorquis*, *A. campestris* and *A. devoniensis* are more closely related to *A. bisporus* than the *A. xanthoderma* and *A. arvensis*. Restriction analysis of IGR and ITS regions have indicated genomic diversity in *Agaricus*. Thus, the molecular studies on genomic inter-relationships among species within a genus or between genera are of paramount importance for introgression

of genes from related species and establishing phylogenetic relationship.

Construction of linkage map: The genetic linkage map of *A. bisporus* has been constructed using molecular markers and more than 99 loci have been placed on 13 different chromosomes. Among the important genes mapped are *MAT*-mating type gene, *BSN* - gene determining basidial spore number are located on chromosome 1 and cap colour-determining locus *PPC1* on chromosome 8.

Breeding behaviour and sexuality: The characterization of breeding systems and inter-species relationships within the genus *Agaricus* are useful to (i) identify potential wild species for direct cultivation and (ii) to provide sources of novel variation for introgression into *A. bisporus*. The RAPD and ITS sequencing were used to elucidate the breeding system in the species of genus *Agaricus*. Heterothallism in *A. campestris* was confirmed by RAPD analysis of single spore progenies and heterokaryons, whereas, *A. subfloccosus* was shown to be homothallic based on the homogeneity of RAPD profiles. ITS sequence analysis revealed unifactorial heterothallic life cycle that permit both inbreeding and homokaryotic fruiting in the species of section *Arvensis*.

DNA fingerprinting and mushroom variety protection: Morphological markers have provided the descriptors for registration and identification of novel varieties and hybrids in field crops. While in the mushrooms, the failure of D.U.S. based on morphological data, has prompted breeders to use DNA fingerprinting profiles for mushroom variety protection. Molecular markers could successfully be used for legal protection of our indigenous mushroom genetic resources at national and international level and the new mushroom hybrids and varieties can be fingerprinted and registered.

S_1.5 Organic Button Mushroom Production with Innovative Methods of Pest and Disease Control During Cropping

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Crop of button mushroom *Agaricus bisporus* was grown organically, following the Organic laws of US Department of Agriculture 1990. Under this law the crop has to be grown without the use of chemicals / pesticides / fertilizers and non use of genetically engineered germplasm or sewage water. The compost was prepared with modified method of composting developed at the Centre, ensuring the elimination of pesticides detected from composting ingredients, during high temperature outdoor fermentation in phase-1 of composting . The pest exclusion was ensured with use of steam for pasteurization of substrate / casing and exclusion of the pests from the cropping cycle by non chemical means. The physical traps were used for controlling the flying insects like mushroom flies and diseased fruit bodies appearing on crop beds were isolated physically by inserting ice cream cups over the diseased mushrooms or burying the affected fruit bodies with powdered common salt . Use of clean portable water for spraying on the crop beds and use of clean water spraying equipment was ensured . Use of pesticides is strictly prohibited at any stage of crop raising under Organic laws . The pesticide residue analysis of all base materials used for composting , compost before and after steam pasteurization , casing material , spawn , water and the fruit body was done to verify the freedom of fruit body from pesticides below permissible levels under international standards for food crops . The fruit body harvested was evaluated for quality characteristics like whole mushroom weight , mushroom length , cap dia , cap thickness , cap weight , stipe length, stipe

dia , stipe weight, cap- stipe ratio , fruit body whiteness , fruit body dry weight , N-content of mushroom and others. These parameters were compared with mushroom fruit body harvested from non- organic substrates used normally for growing button mushrooms.

Extensive use of pesticides/chemicals over longer periods of time for obtaining higher crop yields has rendered agricultural practices pesticide/chemical dependant, resulting in ingestion of pesticides on a large scale by human populations causing serious ailments, disease and suffering to mankind. It has been a tradition and a common practice from time immemorial in India to grow agricultural crops organically. It is only in recent times that there has been pressure of population on the limited area of farming land forcing the farmers in India to adopt to extensive use of fertilizers and pesticides to harvest increased quantities of farm produce, at the cost of the quality of the produce. This created a disturbance in the natural cropping pattern/normal succession of crops for maintenance of soil fertility and soil health. Besides, this resulted in tons of pesticides/ harmful chemicals gaining entry into the soil body, water body and the total environment system supporting the life on mother earth. The crops grown in these soils with use of contaminated water for irrigation resulted in the transport of these harmful chemicals into our food materials and all agricultural crops grown on such soils showed toxic chemical residues on analysis.

For button mushroom production, agro byproducts like cereal straws are used as base materials for substrate preparation, which are supplemented for nitrogen with animal manures, legume seed cakes, wheat/rice bran, brewer's grain and other such agro-wastes for recycling of these agro-byproducts. Spawn (mushroom seed) is prepared on wheat grain, which again carries pesticide residues into the growing cycle. Then the casing layer of 3-4cm thick necessary for mushroom growth on top, basically derived from agro-wastes like Farm Yard Manure/Spent Mushroom Substrate/Coir Pith and other such agro-byproducts, again were found to carry the pesticide residues into the growing cycle. Water from streams, springs, underground sources used at various stages of crop raising carry the pesticide residues into the crop. These all ingredients that go into production of button mushroom show pesticide residues in various concentrations and ultimately some of these travel to the fruit body. Controlling pesticides/ chemicals usage for cereal crop production, fodder cultivation for animals, grain cultivation for poultry feed and other such activities that contribute by-product materials for mushroom production is a difficult task. It is the farm economy that guides the farmer for use of chemical fertilizers/pesticides to harvest economic crop yields, and control on entry of pesticides at this stage seems to be a difficult task. The second option with the researcher is to eliminate those harmful chemicals/pesticides that gain entry at various stages of crop raising, through improved substrate fermentation process/cultural practices. It is this second option that has been addressed in present investigation and experimentation, with a systematic approach following a three pronged strategy of : i) improvement in substrate fermentation process, ii) use of potential degrading microbes in composting process, iii) elimination/avoiding entry of pesticides/chemicals during the growing cycle.

The first country to come out with Organic Food Production Act (1990) was the Deptt. of Agri., USA, adopted as part of 1990 Farm Bill. This requires USDA to develop national standards and regulations for organically produced agricultural products. The guidelines for organic food production have also been evolved by various other international agencies like "International Federation for Organic Agriculture Movement (IFOAM)", European Union and Codex Standards. In India APEDA (Min. of Commerce) has been given the responsibility for promotion of organic

agriculture in India and this organization formulated rules for National Programme for Organic Production (NPOP) in May, 2001.

Project on Organic Mushroom Production was initiated at NRCM in the year, 2001 in collaboration with Deptt. of Entomology, UHF, Nauni, Solan, HP (Residue analysis). Seven trials have so far been conducted under the project and data on yield and quality of the latest trial conduct in March-June 06 period is presented in this paper. The compost was prepared organically without the use of chemical fertilizers and C:N ratio of the compost balanced with use of organic materials only. The composting ingredients and techniques were modified and standard package of practices followed for raising of the button mushroom crop (*Agaricus bisporus*) under controlled environmental conditions. Coir pith (coir industry waste) and FYM were used as casing materials and wheat grain spawn of strain A-15 (Sylvan) was used in the trials. Steam was used for pasteurization of compost and casing, crop raised organically without the use of pesticides. Mushroom yield of 18.5kg / 100kg compost was obtained in about four weeks of cropping. Mushroom flies were controlled by use of light traps and diseased mushrooms, when detected, were isolated physically with use of ice cream cups / sprinkling of common salt over effected fruit bodies (and its removal subsequently). Excellent quality mushrooms were harvested with average fruit body weight of about 14g, as compared to 10-11g in non organic crops.

Samples were drawn for all the ingredients used in compost making, composts at various stages, casing, spawn, water and finally the mushroom fruit body for pesticide residues analysis. In the initial trials, the pesticides were detected from all the ingredients, composts, casing, water and the fruit body. But with the improvement in composting technology/ prolonging of high temperature fermentation, the pesticide residues were either eliminated or detected at very low concentration. The organic mushroom production threw up some interesting finding on the food value of the mushroom. It was found by analysis that protein content of the mushroom increased by growing on substrates prepared organically, and there was improvement in the quality characteristics of the fruit body.

S_1.6 Biodiversity and Utilization of Medicinal Mushrooms

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In 1990, the magnitude of fungal diversity, that is, the actual number of species worldwide, was estimated conservatively to be at least 1.5 million species. Of the 1.5 million estimated fungi, it has been estimated that 14,000 species produce fruiting bodies of sufficient size and suitable structure to be considered macro-fungi, which can be called mushrooms. Of these, about 50% or 7,000 species are considered to possess varying degrees of edibility and more than 3,000 species from 31 genera are regarded as prime edible mushrooms. To date, only 200 of them are experimentally grown, 100 economically cultivated, approximately 60 commercially cultivated and about 10 have reached an industrial scale of production in many countries.

Of the 14,000 species of mushrooms in the world, around 700 have known for medicinal properties. Thus, mushrooms have vast prospects as sources of medicines. The early herbalists were more interested in the medicinal properties of mushrooms than in their basic value as a source of food. Humankind has constantly searched for new substances that can improve biological functions and thereby make people fitter and healthier. About 3.5 billion people

worldwide, all over half of the world populations rely on plant-based medicines and dietary supplements for their primary health care. Of the plant materials involved in medicines or health tonics, quite a few are fungi. The practice of using fungi as herbal medicines can be traced in different early records of the 'materia medica'. The earliest book on medicinal materials the "Shen Nongs Herbal" recorded the medicinal effects of several fungi such as *Ganoderma lucidum, Poria cocos, Tremella fuciformis Polyporus umbillatus* and other unidentified fungi. The most famous of all work on the traditional medicines "Pen Ts'ao Kang Mu" which was compiled by Li Shi-Zhen of the Ming Dynasty recorded the medicinal fungi totaling more than twenty species, including *Ganoderma lucidum, Poria cocos, Polyporus umbellatus, Lentinus edodes, Termitomyces albuminosus, Auricularia auricula, Pleurotus ostreatus, Armillaria mellea* etc. A very unique insect-infecting fungus, *Cordyceps sinensis*, was for the first time taken as a medicinal fungus in the book "Essentials of Materia Medica". There have been new discoveries and developments in the field of medicinal fungi, which have greatly enriched the treasure house of our traditional medical and medicinal sciences. As a result of large number of scientific studies on medicinal mushrooms in the past three decades, the traditional uses of many mushrooms have been confirmed and new wider uses found. Some traditionally important and leading medicinal fungi in the Oriental medicines are presented below:

Ganoderma lucidum: *G. lucidum* (Lingzhi in Chinese; Reishi, Mannentake or Sachitake in Japanese and Youngzi in Korean) is a species of Basidiomycetes which belongs to Ganodermataceae of Aphyllophorales. Commonly it is known as wood-decaying fungus; it causes white rots on a wide variety of trees and can thus be described as phytopathogenic fungus. Because of its perceived health benefits, its fruiting body has gained wide popularity recently 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 herb is due to the variety of its biological activities, such as anti-tumor, immunomodulating, cardiovascular, respiratory, anti-hepatotoxic and antinociceptive effects. The diversity in the beneficial values and medicinal effects may be attributed to the fact that *Ganoderma* is composed of a vast number of bioactive compounds. The major compounds with significant pharmacological activities appear to be triterpenes, polysaccharides and adenosines although bioactive proteins, nucleic acids and other substances have also been identified. Of particular interest are a group of fungal immunomodulatory proteins-Fips, which have been isolated from *G. lucidum*.

Uttaranchal is gifted with a rich flora of *Ganoderma*. Some of the forest areas adjacent to Pantnagar, Haldwani and Ranikhet of Uttaranchal were surveyed to collect the fruit bodies of *Ganoderma*. The substrates wheat straw, coir pith, saw dust chickpea straw, mustard straw and three isolates of *Ganoderma lucidum* designated as Pantnagar isolate (G-1), Haldwani isolate (G-2) and Ranikhet isolate (G-3) were used under experimentation. There was significant difference to one another in terms of yield. Wheat straw gave significantly higher yield among all the substrates evaluated and minimum yield was given by coir pith. Among the supplement, rice bran supplemented @ 5 per cent gave significantly higher yield over all the substrates.

Among the different isolates, isolate G-1 gave significantly higher yield (84.40 g/500 g dry substrate) over wheat straw supplemented with rice bran @ 5 per cent. Minimum yield was obtained from isolate G-3 over all the substrates. The lowest yield was recorded from the isolate G-2 on the substrate coir pith (14.25 g/500 g dry substrate). All the isolates of *Ganoderma lucidum* were also evaluated for the presence of ganoderic acid which is responsible for immunomodulating effect. Experimental results showed that this mushroom contained ganoderic acid and

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possessed anti-oxidative properties such as reducing power and chelating activity on Fe^{2+}.

Auricularia auricula: It is commonly known as Juda's ear, wooden ear, tree ear. The fruiting body is gelatinous, elastic, semi-translucent, cup or ear shaped, red brown in colour and rubbery in texture but becoming hard when dry. In traditional Chinese medicines, the wooden ear is mild and sweet in nature. It activates the blood and stops the pain. It is considered specific for bleeding, especially excessive uterine bleeding, abdominal and tooth pain. It is often used to treat hemorrhoids, to stimulate bowel movements, to help build up energy and to act as an anti-coagulant as well as a source of dietary fibre for the prevention of geriatric disorders.

A few villages of Tarai, Bhabhar and foot hill of U.S. Nagar and Nainital districts of Uttaranchal were surveyed to collect the fruit bodies of *Auricularia*. Substrate wheat straw, maize stalk, rapeseed straw, paddy straw was used for the yield of local isolates of Auricularia. Significantly higher yields of isolates Auricularia from oak (AO) and Auricularia from Neem (AN) were obtained on paddy straw supplemented with wheat bran (3 per cent). The supplementation of substrate at the rate of 3-7 per cent resulted in at par yield of isolates Auricularia light brown from mango (AMLB) and Auricularia dark brown from mango (AMDB) which did not differ from the check (*A. polytricha*) of these isolates.

Lentinula edodes: *L. edodes* (common name: black forest mushroom; Chinese name: Shiang-gu; Japanese name: Shiitake) is the second most important edible mushroom in the world from the stand point of production. For a long time, this mushroom has been valued for its unique taste and flavour and as a medicinal tonic. It is indicated in medical treatments of colds, measles in children, bronchil inflammation, stomachache, headache, dizziness, dropsy, smallpox and even mushroom poisoning. It is also valuable in stimulating the immune system to increase the body's ability to ward off cancerous tissues, viral infections and chronic fatigue syndrome. It is claimed to accelerate vital energy, wards off hunger feeling and defeats body fluid energy. It is the source of a well known anti-tumor polysaccharide, lentinan, from the fruiting bodies or mycelia. Lentinan is a highly purified, high molecular weight polysaccharide which does not attack cancer cells directly but produces its antitumor effect by activating different immune responses in the host. It is often used as an adjuvant to support immune system function in cancer patients during chemotherapy and may prolong the survival times.

The cultural characters of two strains of *L. edodes* were studied at Mushroom Research & Training Centre, Pantnagar and they were cultivated over different substrates to study the yield performance and biological efficiency. The biochemical makeup of both of the strains was also studied. The findings indicated that the strains vary extensively in their cultural, morphological and biochemical characteristics. Wheat straw supplemented with 10 per cent wheat bran resulted in maximum yield and biological efficiency in case of strain L_1. The total sugars, reducing and non-reducing sugars content were at par and found to be 22.38, 0.03 and 22.35 per cent respectively, in the fruiting bodies of *Lentinula edodes*.

Cordyceps sinensis: These species are unique mushrooms that feed on insects. It is parasitic on the larva of Lepidoptera. Chinese herbal doctors often use this fungus as a tonic and tranquilizing medicine. It is used for treating general debility after illness, weakness, splitting of blood caused by tuberculosis, chronic coughing and asthma caused by senility, night sweating, spontaneous sweating, anaemia and malignant tumor. It has inhibitory effect on the growth of bacteria, such as *Streptococcus, Bacterium mallei, Bacillus antracis, Pasteurella susseptica* and *Staphylococcus*. *C. sinensis* has been reported to have anti-tumor activity against certain cancers mediated through the host enhanced immune function. The dried material composed of the

fungal fruiting body and the insect body has been used as a medicine for alleviating spasm especially childhood convulsion or palpitation, coughing and swollen throat with inflammation. It has also been reported that it has strong vitalizing effect.

Mushroom Research & Training Centre, Pantnagar has already initiated the research work on this medicinal mushroom using its own resources. Our survey teams have made several collections of the fruiting bodies of the fungus from Dharchula and Munsiyari zones. We have succeeded in generating mycelial culture of this fungus under laboratory condition. So far, we have been able to develop hyphal tip cultures from wild collections. These cultures are being compared for their cultural and morphological variability. Entomopathogenic nature of the fungus is to be evaluated against larvae of different insects. The fruiting bodies of this fungus were evaluated for anti-oxidative properties. The findings clearly indicated that this fungus have anti-oxidative properties such as reducing power and chelating activity on Fe^{2+} but the anti-oxidative properties of this mushroom was found to be slightly lower than that of *Ganoderma lucidum*.

Grifola frondosa: In Japan it is called as maitake, which means "dancing mushroom". It is considered a first rank edible mushroom when young, which has a good taste, a crisp texture and an excellent aroma. It has been frequently used for improving spleen and stomach ailments, calming nerves and mind and treating hemorrhoids. It has shown significant blood-pressure lowering effect and cholesterol-lowering and hepatoprotective activities. Various isolated polysaccharides and polysaccharide-protein complexes from *G. frondosa* exhibit strong anti-tumor activity. It may be effective for treating breast and colorectal cancers as well as therapeutic active against AIDS.

The research achievements in medicinal mushroom during last two decades give the impression, and the confidence, that medicinal mushrooms have much to offer to health care system for humans in the 21st century. In cases where modern medicines may not provide a complete remedy, complementation by mushroom nutriceuticals may augment the success of the treatment. Prevention of diseases is beneficial to everyone and deserves the same attention that is given to the curing of diseases. Efforts directed to disease prevention can have positive financial and social impact and on the individual basis can maintained or even improve quality of life and human dignity.