11. SUMMARY

The present study was undertaken to explore the diversity and distribution of lepiotoid and termophilous mushrooms from North West India along with evaluation of their nutritional, nutraceutical and sociobiological aspects.

In the present work, mushroom surveys were conducted during the rainy season from 2007 - 2011 within the altitude ranging from 250 - 3300 m above MSL from a variety of natural habitats in North West India. Several fruit bodies of these fungi were collected and taxonomically worked out. The identified edible species were analyzed for their proximate composition with respect to their nutritional and nutraceutical components.

During the mycofloristic survey of the region, about 75% of the mushrooms collected were lepiotoid belonging to family Agaricaceae while about 25% of them belong to Lyophyllaceae family.

During the present study 152 collections have been worked out for their morphological, anatomical and chemical reaction details. As many as 41 taxa of lepiotoid mushrooms belonging to eight genera of family Agaricaceae namely Clarkiendra, Macrolepiota, Chlorophyllum, Chlorolepiota, Leucoagaricus, Leucocoprinus, Lepiota, Cystolepiota and 14 taxa of termophilous mushrooms belonging to genus Termitomyces of family Lyophyllaceae have been taxonomically described. Among these five species namely Leucoagaricus albidus sp. nov., Cystolepiota indica sp. nov., Chlorolepiota indica sp. nov., C. brunneotincta sp. nov. and Lepiota attenuiispora sp. nov. and 3 varieties, namely Leucoagaricus barsii var. bulbobasilarus var. nov., Leucoagaricus tener var. brevispora var. nov., and Chlorophyllum sphaerosporum var. macrossora var. nov. are new to science while 17 taxa, namely Leucoagaricus melanotrichus var. melanotrichus, L. crystallifer, L.
sublittoralis, L. rhodocephalus, Cystolepiota icterina, Lepiota castaneidisca, L. humei, L. boudieri, L. atrodisca, L. fl oralis, L. roseifolia, L. plumbicolor, L. truncatispora, Macrolepiota fuligiosa, M. excoriata, M. heimii and Termitomyces reticulatus are new records for India. Termitomyces schimperi, Leucocoprinus squamulosus, Macrolepiota puellaris, Leucocprinus bresadolae and Clarkeinda trachodes, which are already reported from India have been documented for the first time from the study area.

All collections have been deposited under PUN in the Herbarium of Botany Department, Punjabi University, Patiala (Punjab), India. Pure cultures of 7 taxa namely Macrolepiota dolichaula, M. rachodes, Lepiota humei, Leucocprinus cepaestipes Termitomyces radicatus, T. heimii and T. mammiformis has been raised and maintained for the purpose of conservation and further investigations. The cultures were deposited in the GenBank of Directorate of Mushroom Research (ICAR), Chambaghat, Solan.

ITS region of genomic DNA of five different fruit bodies of lepiotoid and termitophilous mushrooms, namely Lepiota humei, Macrolepiota dolichaula, Chlorolepiota brunneotincta sp. nov., Termitomyces heimii and T. mammiformis was subjected to sequencing so as to confirm their identity by employing molecular tools. PCR was performed to amplify the ITS 1 and ITS 2 region with the specific primers ITS-1 (5’TCC GTA GCT GA ACCT GCC G-3’) and ITS 4 (5’- TCC TCC GCT TAT TGA TAT GC-3’) for the taxonomical identification. The purified PCR product showed bands from 459- 708 bp in agarose gel electrophoresis. The sequenced data revealed that the ITS region of T. mammiformis had 99% similarity for 98% coverage, T. heimii with 97% similarity for 98% coverage, Lepiota humei with 99% similarity for 100% coverage and Macrolepiota dolichaula gave 99% similarity with 100%
coverage with the sequences of NCBI database. The ITS sequence arrangement of *Chlorolepiota brunneotincta* sp. nov. is reported for the first time. All the sequences obtained were submitted to the GenBank and accession numbers obtained. The evolutionary relationship of the ITS - I and ITS - IV regions of selected mushrooms was studied by construction of phlogenetic tree with closely related species. Molecular evolutionary software, Multialign and MEGA have been used for construction of dendrogram.

During the present investigation lepiotoid and termitophilous mushrooms were found growing on sandy soil, humicolous soil, on termitaria heaps, subterranean termitaria, in the vicinity of conifers, various angiospermous trees, shrubs, herbs, hedges, on wood, on trees, grasses, etc. and majority of the mushrooms were available from May to October which coincides with the monsoon period in North West India. *Lepiota castaneidisca, Leucocoprinus birnbaumii* and *L. cepaestipes* of family Agaricaeaeae worked out presently were noted to form close association with *Grevillea robusta, Pinus roxburghii* and *Dalbergia sissoo*, respectively and species of *Termitomyces* were found growing on or near the termite nests or on subterranean termitaria the during the survey of different localities of Himachal Pradesh and Punjab plains. Out of these *Lepiota castaneidisca* formed putative mycorrhizal association with *Grevillea robusta*.

Distribution wise *Termitomyces, Leucocoprinus, Clarkienda, Lepiota, Chlorophyllum, Macrolepiota, Cystolepiota, and Leucoagaricus* are reported to be cosmopolitan in distribution (Pegler, 1977; Singer, 1986). Presently *Leucoagaricus crystallifer, L. melanotrichus* var. *melanotrichus, L. rubrotinctus, Lepiota cristata, L. truncatispora, L. humei, L. boudieri, L. atrodisca, L. roseifolia, Macrolepiota fuliginosa, M. dolichaula, M. heimii, M. puellaris, Leucocoprinus fragilissimus* were
collected from the Chir Pine and Deodar forests from 850 - 3000 m. Only few of the species of the genus Termitomyces namely Termitomyces microcarpus, T. radicatus, T. umkowaani and T. clypeatus were found growing in the Himachal Pradesh. As compared majority of termitophilous mushrooms, namely Termitomyces mammiformis forma albus, T. heimii, T. microcarpus, T. radicatus and lepiotoid mushrooms, namely Lepiota humei, Leucoagaricus rubrotinctus, Macrolepiota rhacodes and Chlorophyllum molybdites were very common in plains and shiwalik region extending from 250 m to about 850 m.

The experiments were conducted to evaluate the nutritional, nutraceutical and enzymatic profile of edible lepiotoid and termitophilous mushrooms by employing standard biochemical techniques. For this purpose fruit bodies of 8 species of Termitomyces species viz., T. microcarpus, T. radicatus, T. badius, T. medius, T. heimii, T. striatus T. mammiformis and T. reticulatus, 3 species of Macrolepiota namely, M. procera, M. rhacodes and M. dolichaula and one species of Lepiota humei were analyzed for their nutritional properties. The results of their chemical composition, the estimated energy value and the macronutrient profile of the evaluated mushrooms in general revealed that the wild mushrooms are rich sources of proteins and carbohydrates and possesses low amount of fat. Simultaneously, experiments were also conducted for the evaluation of the nutraceutical composition viz. phenolic compounds, flavonoids, alkaloids, carotenoids, etc. Qualitative and quantitative evaluation for laccase and extra-cellular protein was also carried out on some selected species.

Biochemical analysis of fruit bodies of these mushrooms confirmed the presence of nutritionally important components in them. The results on dry weight basis demonstrated significant amount of proteins which ranged from 12.95 - 46.2%,
Vitamin A 0.01 - 0.16 mg/100 g, Vitamin B 0.13 - 0.8 mg/100 g, Vitamin C 0.18 - 1.45 mg/100 g, ash 2.5 - 8% and moisture 5.7 - 7.7%. Furthermore crude fibers ranged between 1.9 - 15.6% and carbohydrates 33.3 - 68.18%. All the species were very low in fat content, which ranged from 1.65 - 3.3% and energy value calculated ranged from 306.5 - 364.7 kcal/100 g. As in other edible mushrooms, proteins constituted one of the greatest fractions of the mushroom dry matter.

Because of the high ash content mushrooms are quite rich in minerals. In case of these mushrooms, out of the seven macro and micro minerals (Ca, Mg, Fe, Zn, Cu, Se and Mn) estimated, Fe (82 - 673 mg/100 g) was found in larger amount as compared to Mg (6 - 330 mg/100 g), Ca (5 - 204 mg/100 g), Zn (0.4 - 0.09 mg/100 g), Se (0.05 - 0.12 mg/100 g), Mn (2 - 13 mg/100 g) and Cu (4 - 11 mg/100 g). The results of mineral values of these edible species of mushrooms clearly indicate the potential for their use as source of good quality food.

The mushroom samples were also evaluated for the presence of heavy metals (Hg, Cd and As). Amongst these the amount of Hg varied from 0.018 - 0.10 mg/100 g, Cd 0.014 - 0.048 mg/100 g and As 0.00002 - 0.10 mg/100 g (As). However, the net amount of heavy metals in these mushrooms is below the range of permissible limits of toxicity for human consumption as per FAO/WHO norms.

The present study was also focused on the qualitative screening and extracellular ligninolytic enzyme activity in seven mushrooms at optimum temperature and pH 6.5. Qualitative assay was done by ABTS-oxidizing activity for laccase. Fungal strains oxidized ABTS to the dark green ABTS cation radicals (ABTS+) indicating the production of extracellular oxidoreductases in the form of colour reaction. *Macrolepiota rhacodes* and *Lepiota humei* gave positive reaction immediately after inoculation and formed dark green zone around the mycelial bit
while *Leucocoprinus cepaestipes* formed light green zonation on 3rd day after inoculation, followed by *Macrolepiota dolichaula* on 12th day after inoculation while others formed very light green zonation after 15th days of inoculation. Laccase activity was not observed in *Termitomyces radicatus* and *Termitomyces mammiformis*.

The study was also conducted for quantitative screening of edible mushrooms for their laccase activity under varied media and incubation conditions. All the studied taxa were able to produce high titers of laccases in wheat straw broth in comparison to other media. Laccase which is an extra-cellular enzyme was found to have mol. wt. of 60±1kd. Hence quantitative estimation of the protein was carried out using Bradford method. The study carried out on 5th, 10th and 15th days, respectively confirmed that laccase enzyme was found in maximum amount in *Macrolepiota rhacodes* and *Lepiota humei*.

The present study also gives an account of vast wealth of ethno-mycological and traditional knowledge system including information about the mushrooms of this group which are frequently hunted by local people during monsoon season, their local names, and different types of recipes prepared by them in North India. Ethnomycological information with respect to each one of them was collected using questionnaires, personal observations and interviews with the old and experienced persons and local informants. Amongst the interviewees, about 30 - 40 % were aware about the ethno-mycological uses out of which, majority (80%) were the elders (above 60 years of age). They collected wide variety of mushrooms including those inhabiting termitaria for consumption and earning revenue. Only few of the medicinal uses like use of mushroom soup for persons suffering with indigestion, malnutrition and anemia were documented.
The present study significantly contributes to the nutritional and chemical characterization of edible lepiotoid and termitophilous mushrooms growing in different localities of North West India. Furthermore, as they are a source of important antioxidants, these can be used in the diet as nutraceuticals and/or functional foods for maintaining and promoting health, longevity and life quality.