SYNOPSIS

Studies on Macrofungal Diversity of Hamirpur Region, Himachal Pradesh

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Session: 2017-18

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Introduction:

Fungi are eukaryotic heterotrophs. These lack photosynthetic capacity and have saprophytic or parasitic mode of nutrition (Ainsworth et al. 1973; Webster and Weber 2007). Macrofungi include fungal taxa that produce macroscopic fructifications (ascomata in case of ascomycota and basidioma in case of basidiomycota) for their reproduction. These comprise about 10% of the total fungal diversity of the World (Rossman 1994). Macrofungi belong to Ascomycota, Basidiomycota and Zygomycota. Morphological and anatomical characters of ascomata and basidioma have decisive role in macrofungal taxonomy. Macrofungi are categorized into fleshy fungi (mushrooms), polypores, cup fungi, jelly fungi and puffballs etc. Most of these are edible, medicinally important and considered as one of the important non-timber forest products (Boa 2004).

Mushroom is the fleshy, spore-bearing fruiting body of a fungus (mostly belonging to Basidiomycota and Agaricomycetes). Most of the mushrooms are edible (e.g. *Agaricus bisporus*) and are known as the "meat" of vegetable World (Haas and James 2009). Puffballs are gasteroid fungi that produce gasterothecium (gasteroid basidiocarp) having an outer peridium that encloses a mass of spores. After maturation a large number of basidiospores are released through an apical pore or by disintegration of peridium (Pegler et al. 1995). Puffballs are generally saprobic, terricolous and humicolous; however, a few (e.g. *Morganella* sp.) grow on decomposing wood (Bates 2004). Jelly fungi belong to Tremellales, Dacrymycetales, Auriculariales and Sebacinales. These are having foliose, gelatinous and irregularly branched fruiting body. On drying these become hard and shriveled; when exposed to water, they return to their original form e.g *Auricularia auricula* and *A. polytricha*. Most of these are edible. Bracket fungi or shelf fungi belong to Basidiomycota (Kirk et al. 2008). They produce shelf or bracket-shaped fruiting bodies that lie in a close group of horizontal rows e.g. *Trametes versicolor*. Cup fungi belong to family Pezizaceae of Ascomycota. Its members produce cup shaped fruiting bodies e.g *Aleuria aurantia*, *Sarcoscypha coccinea* etc.

Fungi fruiting on woody substrata are usually either saprobes or plant pathogens. The wood inhabiting fungi include taxa belonging mainly to Agaricomycetes, Xylariaceae and Ascomycota. The wood inhabiting fungi that degrade the wood components are known as wood rot fungi (Mishra et al. 2015). There are three main types of decay fungi (white rot fungi, brown rot fungi and soft rot fungi)
which are distinguished by wood components that they degrade. White rot fungi have the ability to degrade cellulose, hemicellulose and lignin resulting in fibrous and bleached wood. Brown rot fungi degrade only the polysaccharide components and residual wood is a brown lignin framework that is characterized by cubical shrinkage and wood collapse. Soft rot fungi can degrade only cellulose and hemicellulose. They can tolerate very wet conditions and the rot is characterized by loss in mechanical strength and the wood becomes wet and spongy (Dix and Webster 1995).

Most of the macrofungi exhibit bioluminescence. Bioluminescent fungi emit a greenish light at a wavelength of 520–530 nm. The light emission is continuous and occurs only in living cells (Okané et al. 1990). Bioluminescence may occur in both mycelia and fruit bodies, as in *Panellus stipticus* and *Omphalotus olearius*, or only in mycelia and young rhizomorphs, as in *Armillaria mellea* (Wassink 1998). In *Roridomyces roridus* luminescence occurs only in the spores, while in *Collybia tuberosa*, it is only in the sclerotia (Moore et al. 2011).

Macrofungi play a central role in many microbiological and ecological processes, influencing soil fertility, decomposition, re-cycling of minerals and organic matter, as well as plant health and nutrition. Also they are the key in recycling dead vegetation. These make the nutrients available for the successive plant communities, provide a source of evolutionary pressure as plant pathogens and keep rampant monoculture plant populations in check. They form symbiosis with the vast majority of herbaceous and woody plants, allowing them to colonize poor soils and utilize otherwise unavailable nutrients from the soil. Macrofungi have great economic importance and are grown all over the World as a source of food as well as for medicinal purpose e.g. *Lentinula edodes* (Vane 2003). Most of the polyporoid fungi are medicinal and edible e.g. *Ganoderma lucidum*, *Trametes versicolor* and *Ganoderma applanatum* (Bishop et al. 2015).

**Literature Review:**

Macrofungi are among the most mysterious life forms (Sesli and Tuzen 1998). It is estimated that only 16-41% of macrofungi have been described in the World (Mueller et al. 2007). These still remains understudied over most of the parts of World including India (Winterhoff 1992).
The earliest studies on diversity of macrofungi in India pertain to floristic explorations by Berkeley. He studied fungi collected by Dr. Hooker and published flora of Sikkim-Himalayan fungi (Berkeley 1851). He has documented Sikkim and Khassyan fungi (Berkeley 1852) and consolidated account of fungi in India (Berkeley 1854). *Polyporus guhae* and *Trametes karii* were discovered as new species of polypore fungi from Bengal (Bose 1924). Banerjee has contributed to diversity of higher fungi of Sikkim Himalayas (Banerjee 1946). He also contributed to fungal flora of Calcutta and surrounding areas (Banerjee 1947). Long and Ahmad described the genus *Tylostoma* (Long and Ahmad 1947). Vasudeva in his study supplemented to account of fungi of India (Vasudeva 1952). Thind and Anand studied Clavariaceae of the Mussoorie Hills (Thind and Anand 1956a, 1956b). Corner et al. made additions to Clavariaceae of Mussoorie Hills (Corner et al. 1956, 1957, 1958). Thind and Dev modified the list of Clavariaceae of the Mussoorie Hills (Thind and Dev 1956, 1957a, 1957b). Thind and his associates documented Pezizaceae of the Mussoorie Hills (Thind and Batra 1957a, 1957b; Thind et al. 1957a, 1957b; Thind et al. 1958a, 1958b; Thind et al. 1959). Thind and Raswan studied Clavariaceae of the Mussoorie Hills (Thind and Raswan 1958). The larger fungi, the clavariaceae, the agarics and the Discomycetes received study from K. S. Thind and his group. He has done a lot of research work on Indian Clavariaceae (Thind 1961a, 1961b; Thind and Rattan 1967, 1972). Thind et al. studied Helotiales of Mussoorie Hills (Thind et al. 1961). Agnihothrudu and Barua have described *Trichoglossum hirsutum var. longisporum* from India (Agnihothrudu and Barua 1962). Thind et al. have described the diversity and distribution of Helotiales and Pezizales in India (Thind et al. 1964a, 1964b, 1965, 1966, 1967). Bakshi documented diversity of Polyporoid fungi in India (Bakshi 1966, 1971). Thind and Waraitch have done a lot of work on Xylariaceae of India (Thind and Waraitch 1969, 1976). Thind et al. published an account of Polyporaceae of India (Thind et al. 1969a, 1970). Thind and Rattan contributed to Polyporaceae of India (Thind and Rattan 1971). Bakshi documented Indian polypores and edible fungi (Bakshi 1971; Bakshi and Puri 1978). Pande contributed to the Xylariaceae of Western India (Pande 1973, 1976). An account of Aphyllophorales of India was published (Thind 1973). Kaul and Kachroo reported common edible mushrooms of Jammu & Kashmir (Kaul and Kachroo 1974). Diversity and distribution of *Scytinostroma* in India was documented (Rattan 1974). Ryvarden and Dhanda recorded two polypores from India (Ryvarden and Dhanda 1975). Thind and Dargan worked on Xylariaceae of
Saini et al. studied diversity of North Indian Agaricales (Saini et al. 1988, 1989). Fleshy fungi of North-Western Himalayas were documented (Sharma and Lakhanpal 1988). *Tuber himalayense* was recorded from North-West Himalaya as new species of *Tuber* (Zhang 1988). Wood rot fungi of Andaman and Nicobar Islands were documented (Quel 1989). Polypores of Indian Botanic Garden were documented (Sharma and Ghosh 1989). A new species of polypores was recorded from India (Sharma and Wright 1989). A new species of edible *Amanita* was recorded from India (Bhatt and Lakhanpal 1989). Family Russulaceae was reviewed (Atri and Saini 1989). Six new genera of Polyporaceae were recorded from India (Virdi 1989a, 1989b, 1990, 1991, 1992). Kumar et al. studied diversity of Amanitaceae in India (Kumar et al. 1990a). Gasteromycetous fungi of Eastern Himalaya were published (Sharma and Thind 1990). An account of fleshy fungi of North-Western Himalaya was published (Bhatt and Lakhanpal 1990). Quel published inventories of discomycetes of Mussoorie Hills (Quel 1991a, 1991b) and explored medicinally important agarics in Himalaya (Quel 2001). Rawla et al. revised checklist of discomycetes of Mussoorie Hills (Rawla et al. 1991a, 1991b). Kumar et al. conducted studies on ecology of macrofungi in the North-Western Himalayas (Kumar et al. 1990b) and subsequently recorded a new species of *Marasmius* (Kumar et al. 1991). Sharma and Sidhu revised Himalayan Geoglossaceae (Sharma and Sidhu 1991). An account of North-West Himalayan species of *Helvella* was published (Kaushal 1991). Fungal flora of Kashmir region of Jammu and Kashmir was documented (Abraham 1991). Mycoflora associated with pathological problems of some multipurpose tree species of Punjab was published (Dulat 1992). Fleshy fungi of North-Western Himalayas were documented (Lakhanpal and Sagar 1992, 1993). East Himalayan Coniophoraceae was studied (Dhingra 1992). Study on diversity and endemism among Indian *Helotioides* were published (Sharma and Kamal 1993). Status of conservation and significance of Himalayan forest mycoflora was reviewed (Rawla 1993, 2001). Corticiaceae of North-West Himalaya was published (Dhingra and Singla 1993). Role of *Schizophyllum* in forest ecosystem alongwith an account of wood-rot fungi of Haryana was published (Sharma and Gupta 1994, 2004). Three species of genus *Valsa* were recorded from Eastern Himalayas (Dargan and Sharma 1994). Diversity and distribution of *Polyporus* in India was documented (Roy et al. 1994). Sharma et al. recorded new edible species of *Leucocoprinus* from India (Sharma et al. 1994). Natarajan documented mushroom flora of South India (except Kerala) and presented a list of two hundred thirty agarics
and bolete species distributed among sixty seven genera from several states of South India (Natarajan 1995). Mushroom flora of Maharashtra was explored (Patil et al. 1995). New records of Polyporus were documented from India (Roy and De 1995). Distribution of Polyporaceae in India was published (Roy and De 1996a, 1996b, 1996c, 1997a, 1997b, 1998a, 1998b). Diversity of Phellinus in Bhimashankar forest was documented (Vaidya and Rabba 1995). Mushroom flora of North West Himalaya was compiled (Lakhanpal 1995). Mushroom flora of Punjab, Kerala and Western Himalaya was published (Lakhanpal 1996). A consolidated account of diversity of fungi in India was published (Sarbhoy et al. 1996). A detailed account of fungal wealth of India was published (Bilgrami 1997). Three rare species of macrofungi were recorded from Dhalhousie Hills (Dhingra and Singla 1997). Three hundred species of mushrooms and toadstools were recorded from North-Western Himalayas, of which seventy two species were found to be in mycorrhizal relationship with Abies pindrow, Betula utilis, Cedrus deodara, Picea smithiana, Pinus roxburghii, P. wallichiana, Rhododendron arboreum, Quercus incana and Q. semicarpifolia (Lakhanpal 1997). Studies on biodiversity and biosystematics of Agaricales of India were published (Sarbhoy 1998). A new species of Lachnum was recorded from Eastern Himalayas (Sharma et al. 1998). The distribution and diversity of Volvariella in Kerala (India) was documented (Pradeep et al. 1998). Edible fungi of Garhwal Himalayas were documented (Sharda et al. 1998). Observations on pathological problems and associated mycoflora of certain important multipurpose trees of Punjab were published (Dargan and Dulat 1998). The records on distribution and diversity of Morchella in India were revised (Lakhanpal and Shad 1999). Taxonomy of agarics of Punjab was studied (Atri et al. 2000). New species were added to mushroom flora of Assam (Gogoi et al. 2000). Mushrooms of the Garhwal Himalaya were enumerated (Bhatt et al. 2000).

Frequency of occurrence of mangrove fungi in East coast of India was documented (Sarma et al. 2001). Three new species (Lactarius abbotanus, L. subindigo and L. mayawatianus) of Russulaceae were recorded from Pindari glacier area of Nanda Devi Biosphere Reserve (Sharma and Das 2001, 2003). Medicinal agarics of Himalaya were documented (Rawla et al. 2001). Status of conservation of mycodiversity in India was reviewed (Kaul 2002). Pathological problems and mycoflora associated with Dalbergia sissoo plantations in Punjab were documented (Dargan et al. 2002). A new species of Lactarius was recorded from Kumaon
Himalaya, India (Das et al. 2003). Mycoflora associated with multipurpose tree species of North–West India was documented (Lalji 2003). Mycoflora of North-Western Himalayas was explored (Paul and Sharma 2003). A checklist of mangrove-associated fungi along with their geographical distribution was published (Schmit and Shearer 2003). Ecological distribution of *Lactarius* (Russulaceae) in Kumaon Himalaya was studied and a new of subgenus *Lactifluus* was recorded (Das et al. 2004a, 2004b). Diversity of *Lactarius* in Kumaon Himalaya was explored and *Russula compacta* was recorded for the first time from India (Das and Sharma 2004a, 2004b).

Seventeen species of macrofungi belonging to basidiomycota were documented from Binsar Wildlife Sanctuary, West Himalaya (Pant and Gupta, 2004). *Rhodocybe paurii* was recorded as a new species from Garhwal Himalaya (Moncalvo et al. 2004). The fungal diversity and its status of conservation in India was documented (Dargan et al. 2005). An account of fungal biodiversity along with its distribution, conservation status and scope of prospecting in India was published (Manoharachary et al. 2005). A checklist of Indian agarics and boletes was published (Natarajan et al. 2005a, 2005b).

Mycoflora associated with *Bauhinia purpurea* was documented (Dargan et al. 2006). Diversity of hyphomycetous fungi from North Eastern hill forests of Arunachal Pradesh, Assam and Nagaland was documented (Bhatt 2006). Macrofungal diversity in fragmented and disturbed forests of the Western Ghats of India was studied (Brown et al. 2006). Agarics of Eastern Ghats, South India were documented (Siva et al. 2006). Six new species belonging to Russulaceae were recorded from Himalaya (Das et al. 2006a, 2006b, 2006c). Eighteen species of polyporoid fungi were enumerated from buffer zone of Nanda Devi Biosphere Reserve (Prasher and Chander 2006). Studied on the diversity of *Niptera* with particular reference to Himalayan taxa were published (Prasher et al. 2003). Edible and medicinal macrofungi of Chandigarh were documented (Prasher et al. 2007). A new species of *Phellinus* was recorded from Maharastra, India (Foroutan et al. 2007). Checklist of the Clavariaceae of India/Himalayas was reviewed (Prasher and Porwal 2007). Checklists of Gasteromycetes of Indian Himalayas were published (Sharma et al. 2007). Diversity of macrofungi in semi-evergreen and moist deciduous forest of Shimoga district, Karnataka, India was explored (Swapna et al. 2008). Pyrenomycetous fungi of Punjab were documented (Dargan and Singh 2008). New basidiomycetous fungi were recorded from Ratanmahal Wildlife Sanctuary, Gujarat, India (Arya et al. 2008). *Bovista aestivalis* and *Calvatia craniiformis* were added to the list of Indian macrofungi (Syed et
al. 2008). Three macrofungi were enumerated from doda district of Jammu and Kashmir, India (Kumar and Sharma 2008). Macrofungal biodiversity of Kashmir forests was explored and new records of macrofungi were documented from Jammu and Kashmir, India (Ahmad et al. 2008). New species of macrofungi were recorded from India (Beig et al. 2008). Occurrence of macrofungi in the Coromandel coast of Tamil Nadu, Southern India was documented (Mani and Kumarsan 2009). Three new species of genus Sistotrema and one species of Hyphoderma was recorded from India (Dhingra et al. 2009a, 2009b). A checklist of resupinate, nonporoid Agaricomycetous fungi from North-East India was published (Dhingra et al. 2011). Taxonomy of Agaricomycetous fungi from district Roopnagar, Punjab was studied (Kaur 2012). A checklist of Aphylllophorales of Western Ghats of Maharashtra State, India was published (Ranadive 2011). A consolidated account of aphylllophorales of Himalaya was documented (Sharma 2012). The diversity of resupinate, non-poroid agaricomycetous fungi in the Himalaya and adjoining areas was published (Dhingra 2014). Radulodon acaciae was recorded as new species of agaricomycetes from India (Kaur et al. 2014a). Four new species of genus Hyphoderma were recorded from Punjab (2014b). Antrodiella indica was reported as a new species from India (2015a). Phlebiopsis punjabensis was published as new species from India (2015b). One hundred and six species of macrofungi belonging to sixty genera were enumerated from the Nanda Devi Biosphere Reserve and the Valley of Flowers National Park (Chander 2016a, 2016b). The diversity of genus Phanerochaete in Punjab and adjoining areas was documented (Kaur et al. 2016). New records of resupinate, non-poroid agaricomycetous fungi were made from India (Kaur et al. 2017). Phlebia brevibasidia was recorded as new species from India (2017a). Some new reports of resupinate non-poroid Agaricomycetous fungi from Punjab and adjoining areas were documented (2017b).

Earliest reports of macrofungi from Himachal Pradesh were made by Fries on the basis of explorations of macrofungi in North-Western Himalaya with particular emphasis on its diversity and distribution in Himachal Pradesh (1821, 1823, 1828a, 1828b & 1832). After him a lot of researchers have contributed to diversity and distribution of macrofungi in Himachal Pradesh. Sharma et al. revised mycoflora of Himachal Pradesh (Sharma et al. 1982). Sharma documented Polyporaceae of Himachal Pradesh (Sharma 1985). Dhingra and Rani documented new species of Pseudotomentella and Tomentella from Dalhousie hills (Dhingra and Rani 1991,
Dhingra and Sood recorded two new species of genus *Tubulicrinis* from Dalhousie hills (Dhingra and Sood 1992). Dhingra and Singla studied Corticiaceae of North-West Himalaya and recorded some interesting and rare species from Dalhousie hill (Dhingra and Singla 1993, 1997). Lakhanpal documented diversity of mushroom mycoflora in the North-West Himalaya with particular reference to Himachal Pradesh (Lakhanpal 1997).

Paul and Sharma documented mycoflora of Himachal Pradesh and recorded additional taxa of fungi from various hosts and substrata (Paul and Sharma 2003). Resupinate aphylloraceous fungi associated with some tree species of Himachal Pradesh was published (Singh 2007). Dhingra and Singh documented the diversity of resupinate Aphylloraceous fungi of Himachal Pradesh (Dhingra and Singh 2009). Prasher and Ashok documented wood rotting fungi (non-gilled Agaricomycotina) of Himachal Pradesh and polyporoid fungi of district Bilaspur of Himachal Pradesh (Prasher and Ashok 2011, 2013). Resupinate, non-poroid Agaricomycetous fungi of Himachal Pradesh was studied (Kaur 2012). Resupinate polyporales (Agaricomycetes) of Himachal Pradesh have been published (Priyanka 2012). Prasher et al. enumerated polyporoid fungi of district Mandi of Himachal Pradesh (Prasher et al. 2012). A checklist of resupinate, non-poroid agaricomycetous fungi from Himachal Pradesh, India was published (Dhingra 2014). Forty eight species of macrofungi including twenty species of coticioid fungi have been documented from Kangra district of Himachal Pradesh (Ritu et al. 2015). Chander et al. published preliminary studies on diversity of wood inhabiting macrofungi of Hamirpur and Sarkaghat region of Himachal Pradesh (Chander et al. 2017a, 2017b).

**Research Gap Identification:**

The detailed review of published records of macrofungi reveals that whereas, a lot of exercises to explore macrofungi of India, North Western Himalaya and Himachal Pradesh have been carried out by various workers, however diversity of macrofungi in Hamirpur is data deficient and understudied. Only twenty two species of macrofungi have been reported so far from Hamirpur district (Chander et al. 2017a, 2017b). There are least studies pertaining to diversity, mapping of distribution, status, substrate relationship, economic and ecological importance of macrofungi in the study area.
Objectives:

The main objectives of the study are:
1. To collect macrofungi from different localities and substrates.
2. To study morphology and anatomy of each specimen.
3. To identify species and preserve specimens for future reference.
4. To assess and document status (threatened, rare, endangered, common), diversity and distribution of each species.

Proposed Methodology:

Materials: The study area for the proposed research will be Hamirpur district of Himachal Pradesh. It lies in the subtropical climatic zone of Shivalik hills in Western Himalaya. Specimens of macrofungi and related field data concerning their distribution, status, economic and ecological values will be collected throughout the research period from different localities and substrates in and around the study area. Total geographical area of Hamirpur district is 1,118 km$^2$, of which 219 km$^2$ area (19.6%) is under forest. It is situated between 76°17'50" to 76°43'42" East longitudes and 31°24'48" to 31°53'35" North latitudes. It is located in the South Western part of Himachal Pradesh. It is covered by lower Himalayas (Shivalik hills) and the elevation varies from 400 m to 1,232 m. The maximum temperature of the district ranges from 37°C to 39°C during summer season and the minimum from 3°C to 5°C during winter season. Average temperature is 21.6°C. Annual precipitation is about 1572 mm. The driest month is November, with 14 mm of rainfall. Most precipitation falls in July, with an average of 478 mm. The warmest month of the year is June, with an average temperature of 30.3°C. In January, the average temperature is 11.7°C. It is the lowest average temperature of the whole year. The difference in precipitation between the driest month and the wettest month is 464 mm. The average temperatures vary during the year by 18.6°C. The main hill ranges of the district are known as Jakh Dhar & Sola Singhi Dhar. These ranges are covered with pine forests. The Jakh dhar runs in continuation of Kali Dhar range in the Kangra district. It enters in Hamirpur district near Nadaun and transverses it into South-Eastern direction. Hamirpur district is bounded in the North by river Beas which separates it from Kangra district. In the East, Bakar and Seer Khads separate it from Mandi district. In the South, It is bounded by Bilaspur district and in the west by Una district.
The forests of the study area are of two types (Champion and Seth, 1968):

1. **Shiwalik chil pine forests**: The chil (*Pinus roxburghii*) is the dominant species and occurs in the zone ranging from 600 m to 1,100 m height. It generally forms pure forest on the Western and South-Western slopes of the Jakh Dhar and in the Chabutra Dhar, the proportion of chil is reduced and scrubs of miscellaneous broad leaved species cover the ground.

2. **Northern dry mixed deciduous forests**: Khair (*Acacia catechu*) is the predominant species. However, besides it, other broad leaved species are also found. The altitudinal range is from 400 m to 850 m. The various species of plants and forest trees found in the district are Kikar, Amla, Neem, Karal, Taur, Bil, Kasmal, Khair, Ber, Chil etc.
Methods: The specimens will be collected randomly from various localities and substrates with the help of equipments such as a sharp knife, a saw etc. Efforts will be made to collect mature specimens. Hand lens (20X) will be used for examination of fructifications in the field. Important details concerning the specimen such as the size and type of fructifications, color of hymenial and abhymenial surfaces, annual/perennial nature, shape etc. will be recorded in the field note-book. Specimens will be photographed at the site of collection (wherever possible) or in the laboratory. Field data to be recorded during collection includes: collection number, details of locality, type of host/substrate, attachment of the fructification/s with host/substrate, name and approximate altitude (in meters) of locality and date of collection. The collected specimen will be sun dried and placed in paper packets/cardboard boxes of suitable size. A paper slip containing the field data will be placed/pasted in/on each paper packet/cardboard. Specimens will be brought to laboratory for further micro and macromorphological studies.

Spore print: A glass slide will be placed on a black chart paper and a portion of the selected fructification will be placed on a glass slide with poroid surface facing the slide. To avoid dust and other spores from the atmosphere, these will be covered with cellophane paper and kept at an undisturbed place overnight. Next morning spore prints will be checked and the slide with spore print will be covered with another clean slide, fixed with tape and packed in the butter paper envelop along with the folded black chart paper. After assigning the respective collection number, spore prints will be packed in cardboard box alongwith the specimen.

Laboratory observations: Microscopic observations will be recorded by using light microscope. All the measurements will be taken in 5% KOH solution. Common mountants/stains to be used for microscopic study include:

1. Cotton Blue: It is best suited for the study of spores. It stains the cytoplasm of fungal cells.
2. Phloxine: Phloxine imparts red colour to the fungal cortex. It makes the structures of the ascus wall and septation of the ascospores clearly visible. For this purpose, 1% aqueous solution of Phloxine will be used.
3. Glycerine: 50% aqueous solution of Glycerin will be as used as mountant to make permanent slides and to remove excess stain.
For the study of ascomycetous fungi, the dried specimens will be revived in 2% KOH and all the measurements will be recorded in this reagent. Different mountants will be used to study different parts of the fructifications such as thin sections of the ascocarp will be stained with Potassium hydroxide-phloxine-glycerine stain, the blueing of the ascus tip will be observed with Melzer's reagent (0.5g iodine + 1.5g potassium iodide + 20g chloral hydrate + 20ml distilled water), ascospore ornamentation cyanophilous of microscopic structures will be observed with cotton blue (the thin sections will be heated gently in a drop of this stain till the white fumes start coming out) whereas the congo red will be used to stain ascus wall, paraphyses, ascospores & excipular tissues.

For the study of basidiomycetous fungi, the sections of fructifications will be mounted in 2% KOH for measurements of various structures as basidiospores, basidia, cystidia, setae and stained with cotton blue (for determining the cyanophilous reaction), Melzer's reagent (for determining the amyloidity), 1% aqueous solution of congo red and phloxine (to determine the presence or absence of clamps and for measuring hymenial elements and hyphae) and sulfobenzaldehyde (for staining gloeocystidia).

**Identification:** The morphological and anatomical details will be compiled in the form of a description which will then be compared with the published literature, authenticated taxonomic keys and monographs.

**Drying and preservation:** Specimens will be sundried in the field and laboratory. Fresh fleshy fructifications will be put on blotting paper sheets with their hymenial surface facing upwards for drying in the bright sunlight. On rainy days these will be dried in an electric drier or in an oven at 40°C. Dried specimens will be packed in paper envelops/cardboard boxes of suitable sizes. For the preservation of dried specimens, 1,4-dichlorobenzene crystals will be put into each packet for protection from attack of worms, insects etc. The specimens will be allotted herbarium accession number and deposited in CPUH (Herbarium of Department of Bio-Sciences, Career Point University, Hamirpur).

**Thesis Outline:**

Thesis will comprise seven chapters as:
1. **Chapter 1** (Introduction): It will include general information about the diversity, growth forms, host-substrate relationship, economic importance, ecological importance, status and distribution of macrofungi. It will also include objectives of the study and the research gaps that originated the need for the present study.

2. **Chapter 2** (Literature Review): It will deal with the detailed review of the literature concerning earlier and recent finding by various mycologists on diversity and distribution of macrofungi in the World, India and Himalaya with particular reference to North Western Himalaya and Himachal Pradesh.

3. **Chapter 3** (Materials and Methods): It will provide the details about the general geography, climate and vegetation of the study area. It will also include methodology of collection, field studies/observations, morphological investigations, anatomical studies, identification and preservation of species.

4. **Chapter 4** (Observations): In this chapter all the species will be described morphotaxonomically and arranged alphabetically in the order: family-genus-species. The taxonomic key to families, genus and species will be presented for identification of macrofungi. The following data will be presented for each taxa:
   - Brief description of family along with authority, citation and distribution.
   - Taxonomic key to the genera.
   - Brief description of genus along with authority, citation, distribution, status, economic, ecological importance and remarks (if any).
   - Taxonomic key to the species.
   - Morpho-chemotaxonomic description of species along with authority, citation, herbarium accession number, substrate, locality, date of collection, distribution, status, economic, ecological importance and remarks.
   - Photographic image of species.

5. **Chapter 5** (Results and Discussion): It will include the results of analysis of diversity studies, macrofungi-substrate relationship, assessment of status (threatened, rare, endangered, common) of species, documentation of economic and ecological value of species.

6. **Chapter 6** (Conclusion and Summary): Comprises summary of entire study according to objectives along with conclusions. It will include inventory of
recorded species along with their distribution and threat status. The scope of further research will also be discussed.

7. Chapter 7 (References): The pattern of citing references will be according to guidelines of Mycologia (An official Journal of Mycological Society of America).

Research Plan Schedule:

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<td>Collection of macrofungi and field data from different habitats and substrates of study area</td>
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<td>Manuscript preparation for publication and thesis writing</td>
<td>Jan 2019 to June 2019</td>
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