Chapter 1

Introduction

1.1 General Background

1.1.1 Glimpse on Mushroom

Mushrooms are the eukaryotic spore bearing organisms under macrofungi that derive their nutrients through their mycelia and grow on dead decomposed matter as saprophytes. The term “Mushroom” is derived from many languages- 1. Latin ‘Fungo’ ‘to flourish’ i.e. growing out from ground or from trees, 2. Greek ‘Sphonggos’ or ‘sphogges’ means ‘sponge’ i.e. species having sponge like structure and 3. French ‘Mousseron’(muceron), ‘Mousse’ or ‘Moss’. Other variations are ‘Muscheron’ and ‘ Mouscheron’(Gogoi *et al*, 2006).

According to Ramsbottom (1954), the term mushroom is derived from French word ‘mousseron’ (mucerum) which, in turn, arises from another French word ‘Mousse’ Moss meaning ‘fast growing’.

Rinaldi and Tyndalo (1972) stated that the structures which are commonly known as mushrooms are nothing else but the fruiting bodies of those organisms are developed which are called the mycological higher fungi. Mushrooms are generally termed as the edible or poisonous gill bearing fleshy agarics.

Generally, mushrooms grow everywhere in the world, either on ground or on organic substrate under the humid condition. They are generally found amongst grasses, fallen leaves, on logs, tree stumps or woody debris, on dung or enriched soil, on burnt grounded wood, on other fungi and even on living trees (Shrestha, 2008).

1.1.2 Essential Parts of a typical Mushroom

A typical mushroom generally has the umbrella like fruiting body representing the reproductive part of the organism. This umbrella-like fruiting body, known as pileus or cap, with central stalk, known as stipe or stem. The arrangement of pileus is usually central often eccentric or lateral. On the central side or lower side of pileus, there are numerous radially arranged gills, while in some, there is a spongy tissue pierced by numerous holes. At the basal end of stipe, there is somewhat bulb-like structure called 'volva', the remnant of universal veil. Most of the mushrooms posses a ring called 'annulus', the remnant of partial

veil towards the upper end of the stipe. The development of fruiting body takes place through the binding of mycelia in availability of favourable condition. These fruiting bodies may vary greatly in shape and size (Shrestha, 2008).

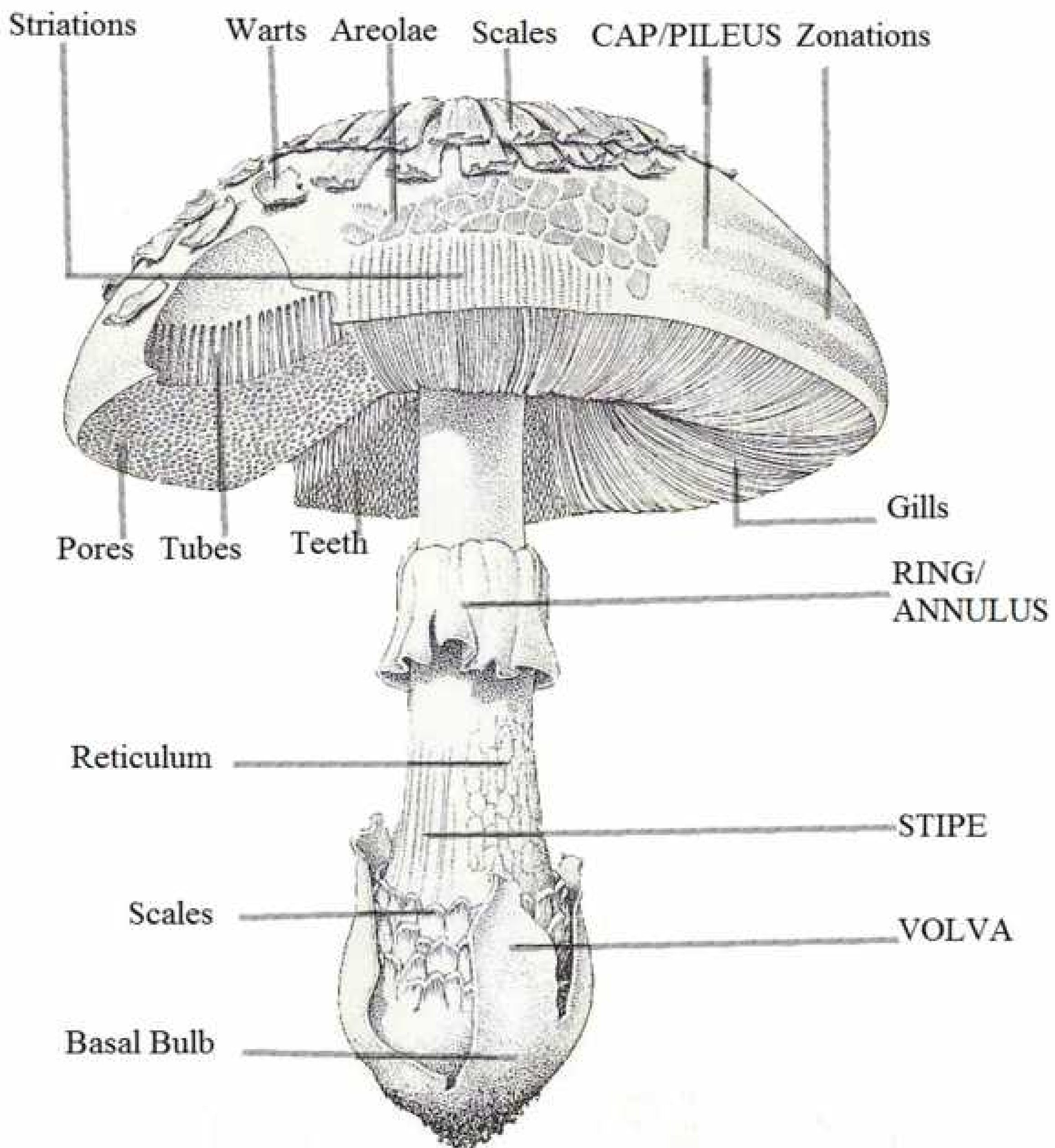

Fig: Structure of a Typical Mushroom with Different Parts

Thus the important characters used in mushroom taxonomy are the presence or absence of pileus, stipe, annulus and volva, along with their shape, size, colour and texture. Thus the classification of mushrooms depends on their morphological structure, hymenial surface and nature of fruiting body. The shape, size and colour of the spores are also an important character for the identification of mushrooms. Spore colour is particularly important and is determined by making a spore print. The spore print is necessary, since the colour of the spore is not always the same as the colour of the gills (Shrestha, 2008).

1.1.3 *Amanita* and *A. chepangiana*

The genus *Amanita* belongs to class basidiomycetes, order Agaricales and Family Amanitaceae (Alexopolous and Mims, 1993). It contains about 600 species of agarics including some of the most toxic known mushrooms found worldwide. This genus is responsible for approximately 95% of the fatalities resulting from mushroom poisoning, with *A. phalloides* (death cap) accounting for about 50% on its own. The most potent toxin present in these mushrooms is amanitin. However, the genus also contains many edible mushrooms, but mycologists generally discourage amateur mushroom hunters from selecting these for human consumption. Nonetheless, in some cultures, the larger local edible species of *Amanita* are mainstays of the markets in the local growing season. Examples of this are *Amanita zambiana* and other fleshy species in central Africa, *A. basii* and similar species in Mexico, *A. caesarea* in Europe, and *A. chepangiana* in South-East Asia. Other species are used for colouring sauces, for example, *A. jacksonii*, with a range from eastern Canada to eastern Mexico, is used to impart red colour to various sauces. Many species are of unknown edibility, especially in countries such as Australia where many fungi are little-known. Understandably, this is not a genus that lends itself to safe experimentation (http://en.wikipedia.org).

Figure 1.1: *A. chepangiana*

Amanita chepangiana Tulloss and Bhandary is the typical wild edible mushroom in Nepal (Tulloss and Bhandary, 1992). Its' cap is 130 - 190 mm wide, often pure white, sometimes with a slight greyish, brownish, or yellowish tint over the center. This species is one of the 'Slender Caesar group' that has a cap that is not umbonate. The gills are free, close to sub crowded, white to very pale pinkish in mass, and up to 15 mm broad; the short gills are truncate, of varying length, adjacent to margin or stipe or neither. The stem is 150 - 180 mm long and about 20 mm wide (more or less); white; with a copious, skirt-like white annulus; and with a large, membranous, white, sack-like volva at the base. The external surface of the 50 - 60 mm 35 - 50 mm, tubular, sack-like volva is white, sometimes with pale yellowish or pale tannish (Yellowish Brown Colour) tints. The spores measure (6.5-) 9.2 x 12.5 (-16.8) μm (5.8-) 8.0 x 10.8 (-12.3) μm and are subglobose to broadly ellipsoid (occasionally globose or ellipsoid) and are in amyloid. Clamps are present at bases of basidia. Like other members of the 'Slender Caesar group' (e.g., *A. jacksonii* Pomerleau), the present species has a substantial felted extension to inner limb of the volval sac. This limb may be carried up in its entirety by the edge of the annulus during stipe expansion. In the original description of this species, the spores were incorrectly described as amyloid (http://www.amanitaceae.org).

According to Tulloss and Yang (2005) *A. chepangiana* occurs with members of the Fagaceae and Dipterocarpaceae from China and peninsular south East Asia to Nepal (http://www.amanitaceae.org).

Many woodland mushrooms are essential to good growth, and even survival of trees. Such mushrooms, known as mycorrhizal mushrooms, establish a relationship with roots of living trees that is mutually beneficial. *A. chepangiana* is mycorrhizal mushroom which grows mainly in the rhizosphere of *Shorea robusta* trees.

1.2 Concepts of Poisonous and non-poisonous mushroom

1.2.1 General Concept

The growth and development of mushrooms are directly or indirectly influenced by the diversity of soil, variation in substrate (rotten logs, tree stumps and fallen leaves), climatic condition (temperature, rainfall, humidity) and other factors. In Nepal, the wild edible species are collected from the forest and fields but it is very difficult to identify the edible from the poisonous ones as there is no hard and fast rule to differentiate edible mushrooms from toxic ones. As a matter of fact, mushroom hunting is an art where trial and error and 'Do it yourself' methods do not count. Moreover this is a skill where experience and patience prevails (Bhandary, 1991).

1.2.2 Poisonous Mushroom

Some species of mushrooms are known as toxic and in some countries many cases of mushroom poisoning are reported every year. In the year 1998 in France 1,675 cases of intoxications by mushrooms were reported, and in this country alone, it is estimated that 8-10,000 cases are expected to be registered every year. Most of these accidents are due to incorrect identification of species that is often made by empirical and traditional knowledge. A wide variety of toxic mushrooms belong to different genera that are discussed bellow. The family Amanitaceae (genus *Amanita*) is well known as having many toxic species. Amatoxins are present in species of *Amanita* genus such as: *A. phalloides*, *A. virosa*, *A. verna*, *A. ocreata*, *A. bisporigera* ,*A. suballiacea*, *A. tenuifolia* and *A. hygroscopica*. The family of amatoxin comprises a neutral component designated as alpha-amanitin, an acidic one called betaamanitin, gamma and delta-amanitin and the nonpoisonous component amanullin from *A. phalloides* and amaninamine from *A. virosa*. Other toxins also found in Amanita genus belong to the family of phallotoxin that includes phalloin, phalloidin,

phallisin, phallacidin, phallacin and phallisacin. Virotoxin is also found in this genus and are closed related the phallotoxins. The species *A. phalloides* is responsible for the majority of the fatalities caused by mushroom poisoning. The toxic effects are caused by phallotoxin and amatoxin. Phallotoxin causes alterations of enterocytes cellular membrane, while amatoxin inhibits protein synthesis at a transcriptional level within enterocytes, hepatocytes and proximal renal tubular cells. After ingestion of *A. phalloides*, amatoxin causes necrosis of liver cells with mortality rates ranging from about 10 emergency liver transplantation. Species with hallucinogenic effects are also found in *Amanita* genus. *A. pantherina* and *A. muscaria* are well known toxic mushrooms that have been mistaken for the edible mushroom *A. rubescens*. Two dissociative constituents such as ibotenic acid (IBO) and muscimol (MUS) are responsible for the hallucinogenic effects. IBO is a powerful agonist of N-methyl-D-aspartic-acid (NMDA) receptor and MUS is a potent GABAA agonist. The intoxications caused by *A. muscaria* for long time were believed to be due to muscarine, but it was demonstrated that this substance is present in small amounts. *A. muscaria* and *A. pantherina* grow in North America, Europe, Africa and Japan, in recent year it has been reported that young people in several countries have intentionally eaten *A. muscaria* to evoke hallucinations. The most common symptoms of intoxication are motor depression, ataxia, changes in mood, perception and feelings, dizziness, euphoria, drowsiness, gastrointestinal disturbances and muscle twitches. The *pantherina muscaria* syndrome is atropine-like and in the number and severity of poisoning cases fatality is rare. In most cases recovery is complete after 24 hours. The treatment is mainly symptomatic cholinestarase inhibitors may be recommended as it counteracts the effects of poisoning, benzodiazepinics or phenobarbitone can be used in case of seizures. The treatment of patients intoxicated with species containing amatoxins includes detoxification, careful monitoring and sometimes liver transplantation is necessary.

1.2.3 Edible (Non-poisonous Mushroom)

Some mushrooms are poisonous and some are edible and delicious when properly prepared. The edibility of the majority is either not known or they are not considered for food because of their small size or poor flavour or texture.

Even though not everyone is interested in collecting mushrooms to eat, it is important to understand most have an important and beneficial role in the environment. They grow in a wide variety of habitats. Most of the mushrooms seen on a walk through

woods are beneficial. Many species are quite specific about their food source and will be found only under or near certain kinds of trees-some under pines, others under oak, etc. Some are important as decay organisms, aiding in the breakdown of logs, leaves, stems and other organic debris.

Wild mushrooms are important Non-Timber Forest Products (NTFPs) from the forests and are being used as food since time immemorial. Collection of wild mushrooms is very common in Nepal and is important for livelihoods in rural areas (Adhikari, 2000; Christensen and Larsen, 2005; Devkota, 2006; Devkota, 2008).

1.3 Literature Review

Ayodele (2008) studied that Glucose was the best carbon source for the growth of *Psathyrella atroumbonata* Pegler. That was followed by maltose, starch and mannitol in that order. The least growth was observed in lactose and cellulose. Yeast extract which was organic nitrogen proved to be the best nitrogen source. *P. atroumbonata* utilized organic nitrogen better than inorganic nitrogen. Thiamine was the best vitamin among the vitamins tested. This was followed by nicotinic acid riboflavin. The least growth was observed in folic acid. Asparagine proved best among the amino acids tested followed by aspartic acid. The least stimulatory amino acid was glycine. Carbon to nitrogen ratio of 4:1 and 1:4 stimulated good mycelial growth.

Pokharel *et al.* (2004) observed that most favourable carbon source for the mycelial growth of *Lyophyllum decastes* was glucose followed by fructose, sucrose and maltose respectively and lactose was poor carbon source. Polypeptone was the most suitable for the stimulation of favourable growth in mycelium followed by yeast extract, ammonium tartarate and ammonium nitrate but ammonium phosphate was least suitable for the mycelial growth of *Lyophyllum decastes* (Pokhrel *et al*., 2004). According to Pokhrel *et al.*, glutamic acid was the best amino acid source for the mycelial growth of *L. decastes*.

According to Ashokkumar (2012) a *Pleurotus sp*. showed the pH optimum for Laccase production was 5.5, where the enzyme retained more than 50 productions in 4.0-6.0 at 50°C. Glucose and peptone were the most suitable carbon and nitrogen source for laccase production.

The best mycelial dry weight was obtained during cellulase production in *Termitomyces globulus* at pH of 6.8. *Termitomyces clypeatus* had the highest significant mycelial yield of

117 mg/30ml when the medium was supplemented with carboxymethylcellulose (CMC). Similarly, high mycelial yield of 119, 116, and 114 mg/30ml were obtained for *Pleurotus tuberregium*, *Coriolus versicolor* and *Agaricus sp*. respectively when the medium were supplemented with yeast extract at 28^0C and pH of 6.8(Jonathan and Adeoyo, 2011).

The most suitable carbon and nitrogen sources for mycelial biomass and EPS production by *Pleurotus citrinopileatus* were fructose and yeast peptone powder (Wu *et al*., 2008).

Kim *et al.* (2003) reported that the optimum temperature for mycelial biomass was 20^OC and optimum initial pH was found to be 9 while high level of mycelial biomass was obtained when celobiose, fructose, maltose or sucrose was used as carbon source in the same way all the inorganic nitrogen sources gave rise to poor mycelial growth and EPS production and amongst organic nitrogen sources, polypeptone yielded the best EPS.

Wu *et al.* (2003) studied that Nitrogen utilization of yeast extract was better than that of peptone when monosaccharide was used as the sole carbon source.

In terms of mycelium growth rate and mycelial characteristics, the most suitable culture media were potato dextrose agar and biotin aneurin folic acid agar for *Lactarius pyrogalus* and L. *controversus*. Although *L. pyrogalus* had higher mycelial growth rate than *L. controversus,* they showed similar trend at different temperature. 25^OC and 4.5 to 6.0 pH values gave the best results for mycelial growth of these mushrooms. Mannitol, glucose, dextrose and maltose were the most suitable carbon sources for *L. pyrogalus*, while mannitol and lactose were the best carbon sources for *L. controversus* and significantly enhanced mycelial growth. Malt extract, peptone, yeast extract and $Ca(NO_3)_2$ were found to be the most suitable nitrogen sources for both *Lactarius* species, except for peptone in *L. controversus*. The lowest mycelial growth was determined in xylose among carbon sources, and NH_4NO_3 and $(NH_4)_2HPO_4$ among nitrogen sources for both *Lactarius* species (Kibar and Peksen, 2011).

The temperature suitable for the mycelial growth was found to be 25^OC and optimal range of temperature was $20\text{-}30^OC$. Mushrooms have a broad pH range (5-9) for their mycelial growth but mostly favorable pH was between pH 6 and 7. Hamada, Lilly, PDA and YM were the most appropriate nutrient media whereas Czapek Dox, Hoppkins and Hennerberg were the most unsuitable for radial mycelial growth of mushrooms. Among 10 different carbon sources, dextrin, fructose and sucrose were the best while lactose and galactose were the

most unfavorable ones. In carbon sources, mycelial density was found to vary from compact to somewhat compact. The most suitable nitrogen sources for mycelial growth were glycine, ammonium acetate and calcium nitrate whereas the most unsuitable were histidine and ammonium phosphate. In case of glycine, mycelial density was found to vary from nearly compact to compact but in the rest of nitrogen sources the mycelial density was rather thin (Imtiaj *et al*., 2008-2009). *Pleurotus ostreatus* (oyster mushroom), an edible mushroom was able to grow optimally at a temperature of 28°C and pH of 9. It utilized various carbohydrate sources such as glucose, fructose, maltose, ethanol, starch, sucrose and lactose, with glucose the most utilized and this significantly enhanced mycelial growth. The least utilized carbohydrate was lactose. Peptone supported the greatest mycelial growth as a nitrogen source followed by yeast extract while inorganic nitrogen sources had no appreciable effect. Evaluation of agro-industrial wastes like corn cob, cotton waste, paperwaste and sawdusts of Mansonia altissima and Irvingia as to their suitability for mushroom production gave corn cob as the best substrate for the mycelia growth of the organism.

Fasidi and Olorunmaiye (1994) who reported that glucose was the most stimulatory carbon source for *Pleurotus tuberregium*. Kadiri and Fasidi (1994) reported that the best utilizable carbon sources for *Lentinus subnudus*) were fructose, maltose, dextrin and glucose. The most suitable carbon and nitrogen sources for mycelial biomass and EPS production by *Pleurotus citrinopileatus* were fructose and yeast peptone powder (Wu et al. 2008).

The optimal temperature that supported the best vegetative growth (113 mg/30 ml) during amylase production in *Agaricus sp.* was 25°C. *Agaricus blazei* , *Coriolus versicolor* and *Termitomyces globulus* also produced mycelial biomass of 106, 104 and 103 mg/30ml respectively at 25°C. Likewise, all the fungi were able to produce mycelia at pH range of 3.8 and 7.8.

Bears *et al*. (1994) found that *Agaricus bisporus* showed good growth in a defined buffered medium on glucose as a carbon source and a number of organic nitrogen compounds or ammonia as a nitrogen source. No growth was observed using nitrate as a nitrogen source. *A. bisporus* was not able to use organic nitrogen containing substances as a sole nitrogen and carbon source. Specific activities of the ammonia assimilating enzymes showed some variation when mycelia were cultivated on different nitrogen sources. Highest specific activities for glutamine synthetase, NAD-dependent glutamate dehydrogenase and NADP-dependent glutamate dehydrogenase were found when mycelia were grown on glutamate as a

nitrogen source. Lowest values were found when the mycelia were grown on glutamine.

Luo (1993) also reported that fructose,glucose and maltose were the most suitable carbon sources for *Auricularia auricula*. Different carbon sources were used to monitor the most advantageous mycelia growth. The suitable mycelial growth was found in dextrin and fructose. Glucose, sucrose and xylose showed moderate mycelial growth of *Shizophyllum commune*. The lowest growth of mycelium was obtained in lactose, mannose and sorbitol. Most of the carbon sources showed compact mycelial density. Considering mycelial phenotype, dextrin and fructose were the best among 10 carbon sources (dextrin, fructose, galactose, glucose, factose, maltose, mannose, sorbitol, sucrose and xylose).

1.4 Objectives, Hypothesis and Justification

1.4.1 Objectives:

General Objectives:

To identify the best nutrient sources for mycelial growth of *A. chepangiana*.

Specific Objectives:

1. To determine the best carbon source for the mycelial growth of *A. chepangiana*.
2. To determine the best nitrogen source for the mycelial growth of *A. chepangiana*.
3. To determine the best vitamin source for the mycelial growth of *A. chepangiana*.
4. To determine the best amino acid source for the mycelial growth of *A. chepangiana*.
5. To determine the best carbon:nitrogen source for the mycelial growth of *A. chepangiana*.

1.4.2 Research Hypothesis

1. There is significant difference in mycelial growth pattern in different nutrient basal media within same source.

2. There may be better new ideas for the beneficial commercialization that help for the conservation of the species.

1.4.3 Rationale of Study

Wild edible mushrooms are widely consumed in many countries. Their culinary and commercial value is mainly due to their organoleptic properties, such as aroma and flavour, and also due to their richness in carbohydrates, fibres, vitamins, minerals and unsaturated fatty acids (Ribeiro *et al.*, 2009). Nepal is rich in mycodiversity, including mushroom diversity (Rana and Giri, 2006). Overall, it was confirmed that 228 edible mushroom species are collected in Nepal for consumption (Christensen *et al.*, 2008). Among them 21 species are commonly used in Nepal which are also commonly used all over the world (Christensen *et al.*, 2008).

Species of *Amanita* Pers. form a significant component in wild mushroom commerce in many countries around the world. There are amanitas especially, *Amanita chepangiana* Tulloss & Bhandary, on which whole populations of the Chepang people of Nepal depend for sustenance during certain periods of the year (Tulloss, 2005). Preference of *A. chepangiana* for consumption by chepangs in comparison to other mushrooms, it has high nutritive and also potential culinary value. Thus, this species has been selected for the current study. Analysis of nutrient requirement for the mycelial growth of mushroom is necessary to identify the suitable substrate for its domestication. In market places there are amanitas species of *Amanita* Pers. form a significant component in wild mushroom commerce in many countries around the world. In Nepal, amongst the few studies on mycoflora, there are no detail studies in their individual species. There is no strict rules and regulations i.e. national strategy for the collection of wild ecological important mycoflora and their sustainable use including other biological resources. Due to overexploitation, these are going to be the vulnerable condition. The beneficial commercialization of the highly nutritive wild edible mushrooms species is necessary that helps for the conservation of the mycoflora. Therefore, I could say that the study will be helpful for the knowledge of significant upon the growth pattern of mushroom mycelia in different basal nutrient media within the same source.

Chapter 2

Materials and Methodology

2.1 Materials

2.1.1 Apparatus

Following apparatus were used during the research work

Petri-Plates

Test Tubes

Microscope

Slides/cover slipes

Cotton

Conical Flask

Cork Borer

Beakers Needles

Sprit/Sprit lamp

Electric Balance

Micrometer

Digital P^H Meter

Autoclave

Other Requirement for Inoculation, Culture and observation in lab

2.1.2 Chemicals

Following chemicals were used during the research work

KH_2PO_4

$MgSO_4$

Agar

Sucrose

Fructose

Dextrose

Manitol

Malt extract

Lactose

Thiamine hydroclohloride

Calcium Nitrate

Sodium Nitrate

Ammonium Nitrate

Peptone

Yeast extract

Urea

Ascorbic acid

Nicotinic acid

Folic acid

Thiamin

D-prolic acid

Leucine

Valine

Arginine

Glutamic

Aspartic Acid

Serine

2.2 Methodology

Collection, preservation and Identification of specimen:

Amanita Chepangiana is collected from the Udayapur Community forest, Northern-Western part of the Shaktikhor and Jutpani VDCs of Chitwan district in June 2011. It was collected around the rhizosphere of *Shorea robusta*. The specimen was photographed in natural habitat and dried well. The morphological characters including spore prints were noted down. The Altitude, Latitude and forest type of study area were noted. Information about the species was taken from local people.

Isolation of pure culture of A. chepangiana

For isolation, a young healthy-looking (with recently opened pileus) fructification of *A. chepangiana* was selected. It was surface sterilized by soaking in 90% alcohol for 1 minute and was washed thrice with sterilized water. With the help of a sterilized blade, stipe was detached from the pileus. Then tissue was taken from the attachment point of pileus and stipe and was transferred to PDA Media supplemented with streptomycin (200 μg/l). It was incubated for 7 days at 22±2^{O}C.

In laboratory, pure culture form was obtained from subculture of mycelia in PDA media. The pure culture of *A. chepangiana* was maintained for further study at Plant Pathology and Mycology Laboratory of CDB, T.U.

Assessment of Vegetative growth patterns of mycelia in different nutrient sources

Effect of different nutrient sources (Carbon, Nitrogen, Vitamins, Amino acids, C:N) on vegetative mycelial growth patterns were observed in the Plant Pathology and Mycology Laboratory of CDB, TU. The mycelial growth was determined by a mycelial diametric

growth method. Modified form of the basal medium (solidified form) was used as described by Chandra and Purkayastha (1977) and Fasidi and Olorunmaiye (1994).

The basal medium and supplementary compounds with agar were dissolved in 1 litre of distilled water and its pH was adjusted to 6 with 1 N NaOH or HCl. The medium so formed was sterilised by autoclaving at 121°C temperature and 15 lb(pound) per inch square pressure for 30 min. Nine cm diametric petriplates were sterilized at 160°C in hot air oven for three hours. Then 20 ml media was poured into each petriplate. After the solidification of the media in petriplates, they were each inoculated at the center with a 5 mm diameter agar block taken from the growing edge of a seven-days-old mycelial colony of *A. chepangiana* grown on PDA media. Then the plates were sealed with parafilm (tape) and covered with aluminium foil. The inoculated petriplates were incubated for 7 days at 22±2^{O}C temperature. Each experiment was replicated five times. After 7 days, the growth of the mycelium was observed and the maximum and minimum growth diameters were recorded.

Carbon compounds:

Carbon sources selected were Sucrose, Fructose, Dextrose, Mannitol, Malt extract and Lactose. The basal medium used consisted of peptone 2 g, KH_2PO_4 0.5 g, Mg $S0_4MgSO_4.7H_20$, 0.5 g. Five gram each of the carbon sources were incorporated into the basal medium and distilled water added to make 1 litre. Basal medium alone without the carbon source was used as control.

Nitrogen compounds:

The following nitrogen compounds were selected: calcium nitrate, sodium nitrate, ammonium nitrate, peptone, yeast extract and urea. Two gram of each was added to the basal medium containing fructose 10 g, KH_2PO_4 (0.5 g), $MgSO_4.7H_20$ (0.5 g), Thiamine hydrochloride (500 μg) and made up to 1 litre with distilled water. Basal medium alone without the nitrogen source was used as control.

Vitamin sources:

Vitamins selected were nicotinic acid, riboflavin, ascorbic acid, folic acid, Pyridoxine and thiamine. The basal medium used was the same as the one used for the determination of nitrogen compounds. Each vitamin was added 500 μg to the basal medium and made up to 1 litre. The setup was treated the same way as for carbon and nitrogen. Basal medium alone

was used as control.

Amino acid sources:

The amino acids selected were glycine, aspartic acids, glutamic acid, asparagine and leucine. The basal medium used was the same as that of Nitrogen source. 500 μg of each amino acid was added to the basal medium and made up to 1 litre and dispensed into the petriplets which were treated the same way as for carbon, nitrogen and vitamin sources described above. Basal medium alone without amino acid was used as control.

Carbon to nitrogen ratio (C:N):

The basal medium was similar to that used for nitrogen compounds but the amount of glucose was varied with yeast extract as sources of C:N. A concentration of 0.15 g/litre each of glucose and yeast extract in the basal medium serve as 1:1 ratio. Other ratio was prepared proportionately.

2.3 Statistical analysis.

For analysis of data statistical packages, SPSS version 16 was used. Statistical parameters like Means and standard error (SE) of the existing average mycelial growth. One way anova test was applied to compare the means of the different nutrient sources to know significant differences mycelia growth. Statistical software Microsoft Excel was used for the calculation of various parameters. For the presentation of results by table, graphs, and figures SPSS version 16 and Microsoft Excel was used.

Chapter 3

Result

Table 3.1: Effect of various carbon sources on mycelia growth of *Amanita chepangiana*

Carbon Source	ColonyDiameter(cm)*	ColonyDensity**
Dextrose	5.64± 0:30[b]	*t*
Fructose	4.60± 0:21[a]	*t*
Lactose	8.14± 0:16[c]	*t*
Malt Extract	8.60± 0:00[c]	*t*
Manitol	5.70± 0:30[b]	*t*
Sucrose	5.69± 0:25[b]	*t*
Control	8.27± 0:02[c]	*t*

* Values are the mean ± SE of mycelial growth measurements and small alphabet letters indicate the same letters are not significantly different according to Tukey HSD (P<0.05).

**t indicates the thick mycelial mass in the basal media.

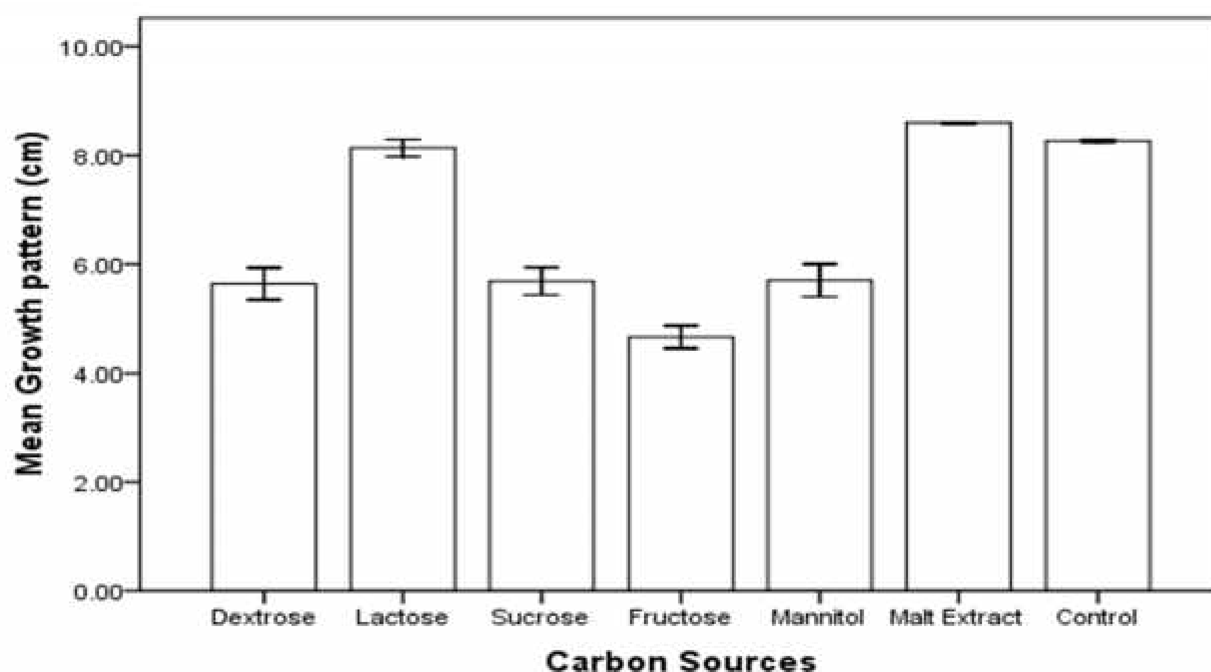

Figure 3.1: Effect of Carbon Sources

Among the different carbon sources, the optimum growth of the *A. chepangiana* was found in the malt extract, control and lactose while moderate in manitol, glucose and sucrose. The lowest mycelial growth was recorded in fructose (Table and Figure 3.1). Tukey HSD test showed that the maximum mycelial yield in the malt extract. The growth patterns of the mycelium in malt extract, control and lactose have no significant difference. Similarly, in mannitol, glucose and sucrose have no significant difference. But it is different in Fructose as compared to mannitol, glucose and sucrose which is more significantly different as compared to malt extract, control and lactose. In the same way, it is significantly different in Malt extract as compared to mannitol, glucose and sucrose which is more significantly different as compared to fructose.

Table 3.2: Effect of various Nitrogen Sources on mycelia growth of *Amanita chepangiana*

Nitrogen Source	ColonyDiameter(cm)*	ColonyDensity**
Ammonium Nitrate	3.75 ± 0.24^{a}	t
Calcium Nitrate	7.57 ± 0.16^{cd}	t
Peptone	6.38 ± 0.21^{b}	t
Sodium Nitrate	8.36 ± 0.02^{e}	t
Urea	8.26 ± 0.03^{de}	t
Yeast Extrate	7.75 ± 0.26^{cde}	t
Control	7.34 ± 0.09^{c}	t

* Values are the mean ± SE of mycelial growth measurements and small alphabet letters indicate the same letters are not significantly different according to Tukey HSD ($P<0.05$).

** t indicates the thick mycelial mass in the basal media.

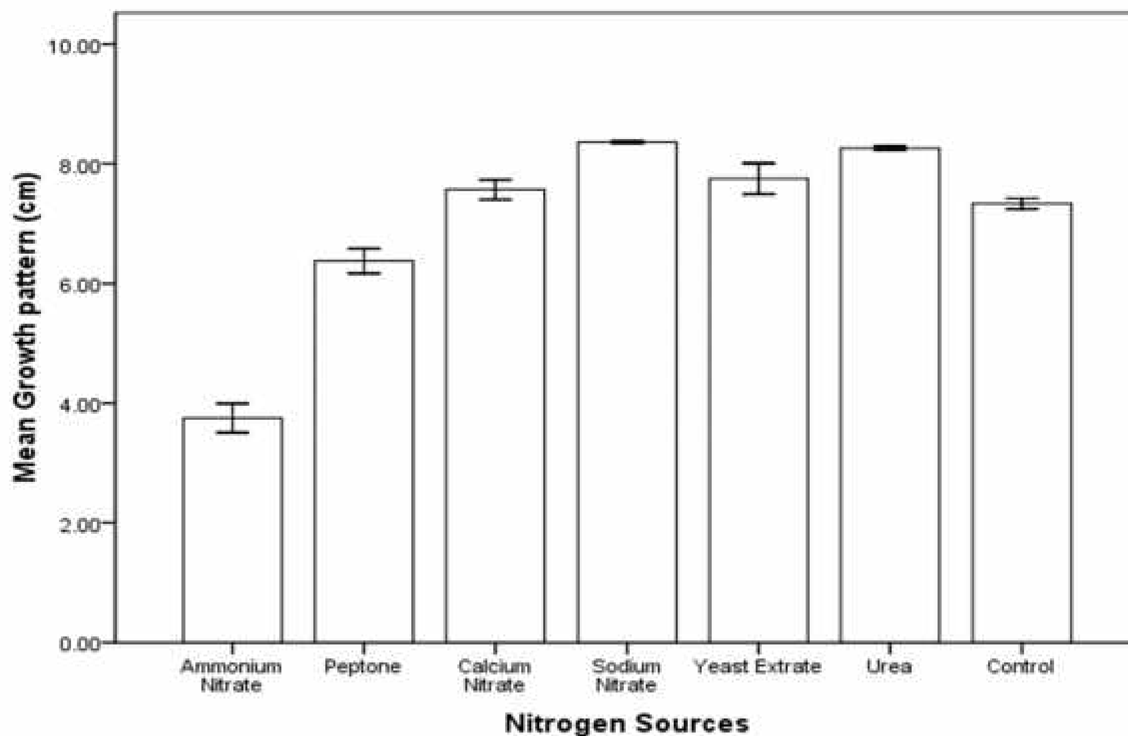

Figure 3.2: Effect of Nitrogen Sources

Among the different Nitrogen sources, the optimum growth of *A. chepangiana* was found in Sodium Nitrate while the lowest mycelial growth was recorded in Ammonium Nitrate (Table and Figure 3.2). Tukey HSD test showed that the mycelial growth pattern in seven different Nitrogen sources including Nitrogen control had significant differences. In Sodium Nitrate, Urea and Yeast extract, the mycelial growth pattern had no significant difference. Similarly, in Urea, Yeast Extract, Calcium Nitrate as well as Yeast Extract, Calcium Nitrate and control had no significant difference. But they were significantly different as compared to the Peptone and Ammonium Nitrate.

Table 3.3: Effect of various Vitamin sources on mycelia growth of *Amanita chepangiana*

Vitamin Sources	ColonyDiameter(cm)*	ColonyDensity**
Ascorbic Acid	8.49 ± 0.18^{d}	*tn*
Nicotinic Acid	6.10 ± 0.23^{a}	*tn*
Folic Acid	6.50 ± 0.13^{ab}	*tn*
D-Prolic Acid	6.99 ± 0.05^{bc}	*tn*
Thiamine	6.72 ± 0.09^{abc}	*tn*
Control	7.34 ± 0.09^{c}	*tn*

* Values are the mean ± SE of mycelial growth measurements and small alphabet letters indicate the same letters are not significantly different according to Tukey HSD (P<0.05).

** tn indicates the thin mycelial mass in the basal media.

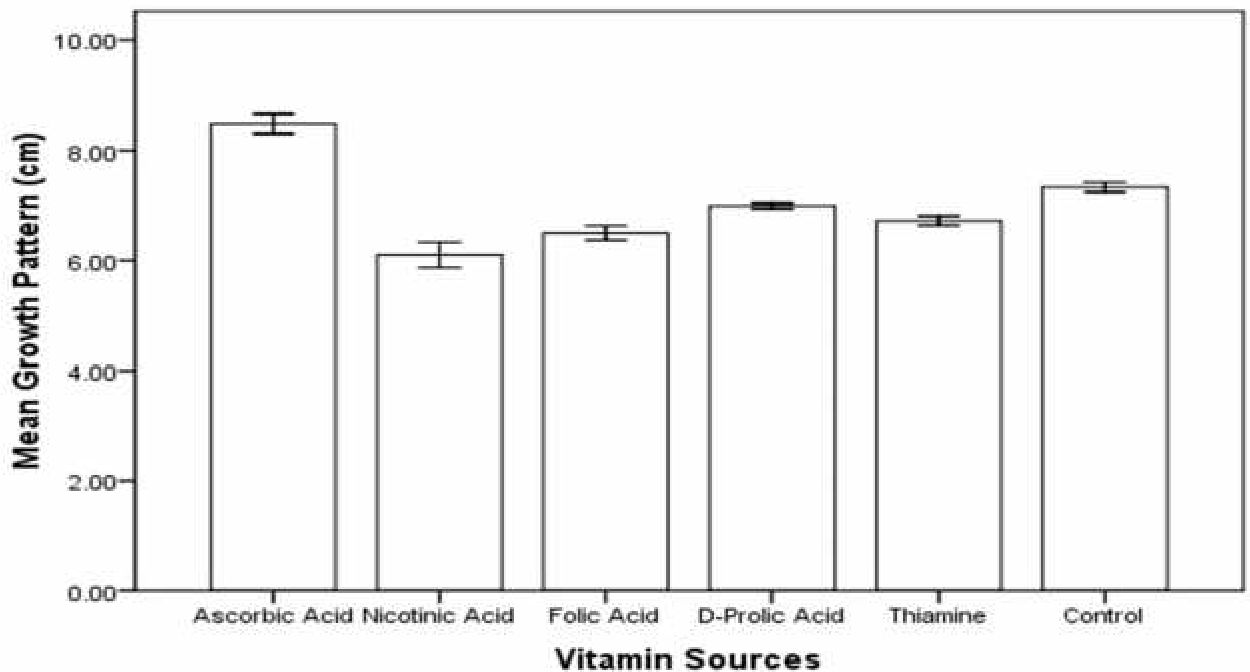

Figure 3.3: Effect of Vitamin Sources

Among the different Vitamin sources, the optimum growth of *A. chepangiana* was found in Ascorbic Acid while the lowest mycelial growth was recorded in Nicotinic Acid (Table and Figure 3.3). Tukey HSD test showed that the mycelial growth pattern in six different Vitamin sources including Vitamin control had significant differences. In Ascorbic Acid, the mycelial growth pattern had significant difference as compared to all the tests. While D-prolic acid, Thiamine, Vitamin control and folic acid, D-prolic acid Thiamine as well as Nicotinic acid, Folic acid, Thiamine had no significant difference.

Table 3.4: Effect of various Amino acid sources on mycelia growth of *Amanita chepangiana*

Amino acid sources	ColonyDiameter(cm)*	ColonyDensity**
Aspartic acid	8.40 ± 0.07^{c}	tn
Arginine	8.89 ± 0.06^{d}	tn
Glutamic acid	8.31 ± 0.14^{bc}	tn
Leucine	8.83 ± 0.05^{d}	tn
Serine	8.49 ± 0.09^{c}	tn
Valine	8.02 ± 0.11^{b}	tn
Control	7.34 ± 0.09^{a}	tn

* Values are the mean ± SE of mycelial growth measurements and small alphabet letters indicate the same letters are not significantly different according to Tukey HSD ($P<0.05$).

** tn indicates the thin mycelial mass in the basal media.

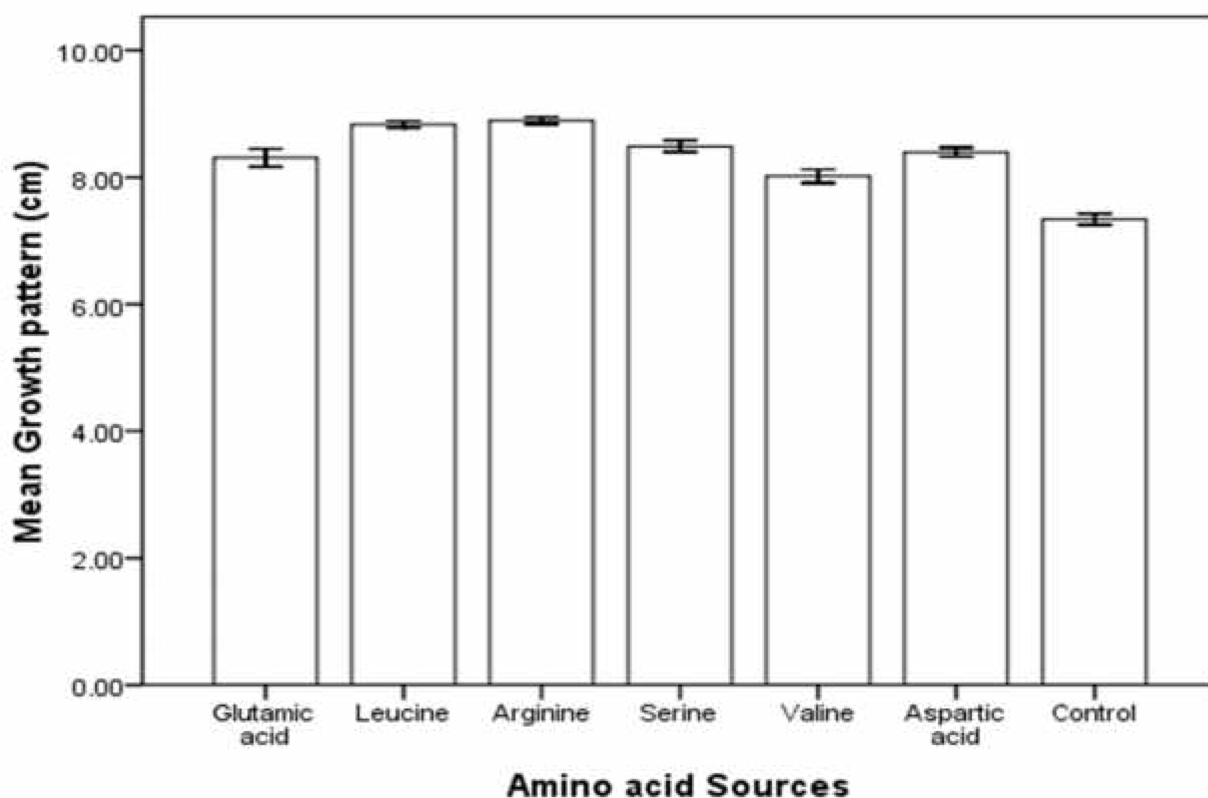

Figure 3.4: Effect of amino acids

Among the different Amino acid sources, the optimum growth of *A. chepangiana* was found in Arginine while the lowest mycelial growth was recorded in Valine (Table and Figure 3.4). Tukey HSD test showed that the mycelial growth pattern in seven different Amino acid sources including its control had significant differences. In Arginine, the mycelial growth pattern had no significant difference as compared to Leucine while significant difference as compare to Aspartic acid, Glutamic acid and Serine where as more significant difference as compare to Valine and highly significant difference as compared to its Control among the tests.

Table 3.5: Effect of various C:N sources on mycelia growth of *Amanita chepangiana*

C:N Sources	ColonyDiameter(cm)*	ColonyDensity**
1:1	4.61± 0.09[a]	tn
1:2	5.95±0.06[c]	tn
1:3	7.37±0.15[d]	tn
1:4	8.76±0.09[f]	tn
1:5	7.98±0.07[e]	tn
2:1	5.26±0.12[b]	tn
3:1	5.68±0.09[c]	tn
4:1	8.93±0.04[f]	tn
5:1	7.21±0.12[d]	tn

* Values are the mean ± SE of mycelial growth measurements and small alphabet letters indicate the same letters are not significantly different according to Tukey HSD (P<0.05).

** tn indicates the thin mycelial mass in the basal media.

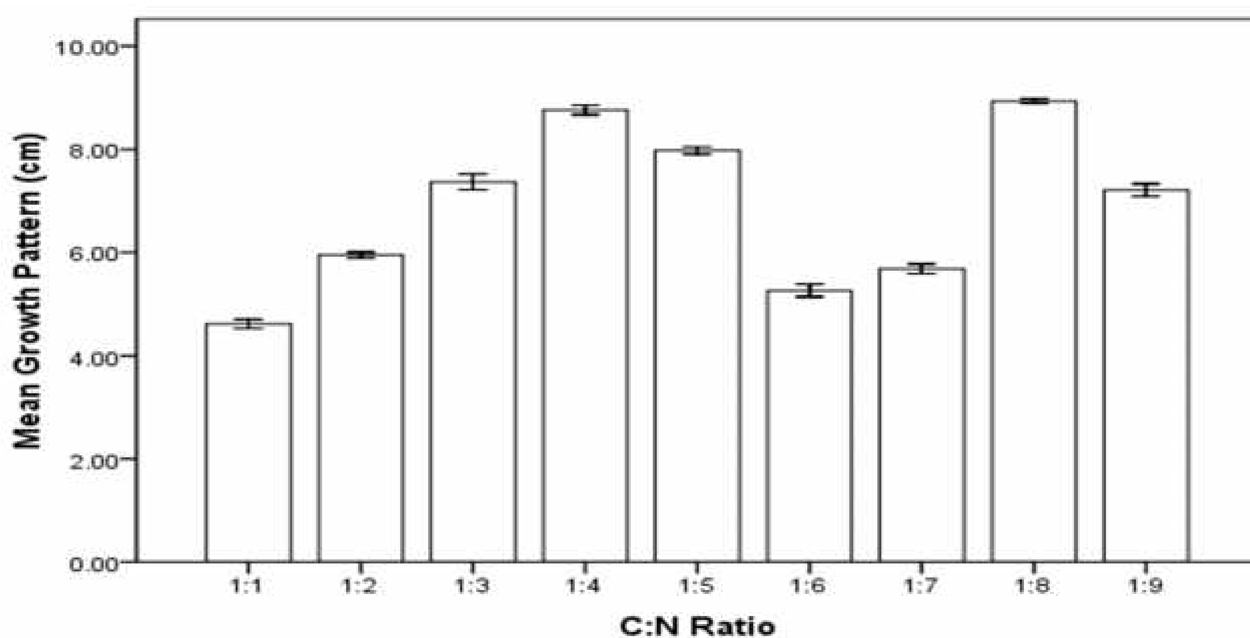

Figure 3.5: Effect of C:N

Among the different C:N sources, the optimum growth of *A. chepangiana* was found in 4:1 sources while the lowest mycelial growth was recorded in 1:1 sources (Table and Figure 3.5). Tukey HSD test showed that the mycelial growth pattern in nine different C:N sources had significant differences. In 4:1 sources, the mycelial growth pattern had no significant difference as compared to 1:4 sources while significant difference as compared to other C:N sources.

Chapter 4

Discussion

4.1 Carbon Source

During this research work, it was found that different carbon sources might have different effect on mycelial growth. Malt extract and lactose were best carbon sources for the *A. chepangiana*. The diametric mycelial growth of *A. chepangiana* also better in carbon control. The mycelial growth was found to be poor in fructose.

Ayodele (2008) reported that *Psathyrella atroumbonata* showed different preferences for carbon sources for its metabolism in which Glucose was best carbon source. *P. atroumbonata* produce enzyme that utilize glucose better than any other carbon source which was in the line with the work of Fasidi and Olorunmaiye (1994) who reported that glucose was the most stimulatory carbon source for *Pleurotus tuber-regium*. In the same vein, Kadiri and Fasidi (1994) studied that the best utilizable carbon sources for *Lentinus subnudus* were fructose, maltose, dextrin and glucose. According to Kim *et al*. (2005) the optimal combination of the media constituents for mycelial growth of *Agrocybe cylindracea* was as maltose, Martone A-1. Xu *et al.* (2003) reported that glucose was identified to be the most suitable carbon source for the mycelia growth of *Paecilomyces tenuipes.* Kaur, (2011) observed that the most favourable carbon source for the mycelial growth of *Termitomyces striatus* in order of effectiveness were D (+) glucose, D (+) sucrose, maltose and D (+) raffinose. This fungus showed poor growth with lactose. Adebayo-Tayo and Ugwu (2011) found that xylose and sorbitol supported the highest biomass yield by *Trametes versicolor and Coprinus sp.,* respectively. Gbolagade *et al.* (2006) reported that among the monosaccharides, glucose stimulated the best biomass production (186.7 mg/30 cm3) of *Pleurotus florida* followed in order by fructose mannose, and sorbose (P 0.05). In the series of complex sugars and sugar alcohols, mannitol supported the highest biomass mycelial yield followed closely by dextrin while the least value was obtained with arabitol. Manjunathan and Kaviyarasan (2011) reported that dextrose was the best carbon source for the optimum mycelial growth of *Lentinus tuberregium.* Bae *et al*. (2000) reported that Maltose was the most suitable carbon source for the optimum mycelial growth of *Paecilomimyces japonica*. Park *et al*. (2001) reported that the sucrose was best carbon source for the mycelia growth of *Cordyceps militaris.* Pokharel *et al.* (2004) observed that most favourable carbon source for *Lyophyllum decastes* was glucose followed by fructose, sucrose and maltose respectively but lactose was

poor. However, in this, it was better source for mycelial growth of *A. chepangiana*.

Manjunathan and Kaviyarasan (2011) reported that dextrose was the best source of carbon for *Lentinus tuberregium*. He also mentioned that lactose was the poor carbon source. Akata *et al.* (2012) reported that much higher mycelium growth *Lactarius deliciosus* were observed in solid sucrose media whereas his same report mentioned that fuctose was the best carbon source for *Infundibulicybe geotropa.* But glucose was the best carbon source for mycelial growth of *T. anatolicum* in liquid and solid culture samples. Liao (1990) observed that glucose and mannose were superior carbon sources for the mycelial growth of *Coriolus versicolar*. According to Kim *et al.* (2010), glucose was the optimal mycelium growth in *Tricoloma matsutake*. Luo (1993) also mentioned that fructose, glucose and maltose were the most suitable carbon sources for *Auricularia auricula*. The suitable mycelial growth was found in dextrin and fructose. Glucose, sucrose and xylose showed moderate mycelial growth of *Schizophyllum commune*. The lowest growth of mycelium was obtained in lactose, mannose and sorbitol. Most of the carbon sources showed compact mycelial density. Considering mycelial phenotype, dextrin and fructose were the best among 10 carbon sources (dextrin, fructose, galactose, glucose, factose, maltose, mannose, sorbitol, sucrose and xylose). Shim *et al.* (2005) observed that maltose was best carbon source for *Macrolepiota procera*. Shim *et al.* (1997) studied 19 carbon sources and reported that *Grifola umbellata* was favourable to use as carbon sources except salicin, cellobiose and lactose. According Shim *et al.* (2003), dextrin was screened as suitable carbon source for mycelial growth of *Paecilomyces fumosoroseus*. According to Lai *et al.* (2011), Glucose yielded significantly larger colony diameter in *Lignosus rhinocerus*. Additionally, fructose, and mannose also recorded a high radial mycelial growth which is parallel to our findings. But they showed that in all carbon sources, mycelial density is thin where our result contradicts. Kibar and Peksen, (2011) reported that mannitol, glucose, dextrose and maltose were the most suitable carbon source for the *Lactarius pyrogalus* while mannitol and lactose were the best carbon sources for the *L. controversus* and significantly enhance the mycelia growth. The lowest mycelial growth was determined in xylose among carbon sources, for both *Lactarius* species. Imtiaj *et al.*, (2008-2009) recorded that among 10 different carbon sources, dextrin, fructose and sucrose were the best while lactose and galactose were the most unfavorable ones. In carbon sources, mycelial density was found to vary from compact to somewhat compact. Bears *et al.*, (1994) found that *Agaricus bisporus* showed good growth in a defined buffered medium on glucose as a carbon source.

The higher preference given by *A. chepangiana* to malt extract as a carbon source might be due to the production of enzyme that utilize malt extract better than any other carbon source by this fungi. Similar conclusions have been made by Ayodele (2008) in *Psathyrella atroumbonata*, and Fasidi and Olorunmaiye (1994) in *Pleurotus tuberregium*. According to Ayodele (2008), glucose is the best carbon source for the mycelial growth because of the better potentiality of producing enzymes utilizing this carbon source. Similarly, Fasidi and Olorunmaiye (1994) have concluded that *Pleurotus tuberregium* shows best growth in fructose due to its ability to produce enzymes for its metabolism.

4.2 Nitrogen Source

The study showed that the optimum growth of *A. chepangiana* was found in Sodium Nitrate while the lowest mycelial growth was recorded in Ammonium Nitrate. However polypeptone was the most suitable for the stimulation of favourable growth in mycelium followed by yeast extract, ammonium tartarate and ammonium nitrate; but ammonium phosphate was least suitable for the mycelial growth of *Lyophyllum decastes* (Pokhrel *et al*., 2006). While his another study in liquid media of the same species showed that yeast extract yielded the highest mycelia while Manjunathan and Kaviyarasan (2011) mentioned that yeast extract yielded the greatest mycelial growth in *L. tuberregium*. Shim *et al.* (2005) screened that glycine was the best nitrogen source for the mycelial growth of *M. procera*. Lai *et al.* (2011) reported that potassium nitrate yielded the largest colony diameter in *L. rhinocerus*. In the study of shim *et al*. (2003) histidine was found as a suitable nitrogen source for the mycelial growth of *P. fumosoroseus*. Adebayo *et al.* (2011) recorded that urea was best nitrogen source for exobiopolymer and biomass production by *Pluerotus ostreatus*. In the same way Liao (1990) reported that ammonium chloride was the best nitrogen source for the mycelial growth of *Coriolus versicolar*. Xu *et al* (2003) found that KNO3 was best nitrogen source for the Paecilomyces tenuipes C240. Kaur (2011) reported that Sodium nitrite served as the best inorganic nitrogen source for the growth of *Termitomyces striatus* (Beeli)Heim. Ammonium acetate, ammonium phosphate, ammonium oxalate, potassium nitrate and sodium nitrate supported fairly good growth of the fungus. Gbolagade *et al.* (2006) reported that yeast extract was the best complex nitrogen sources for the vegetative growth of *Pleurotus florida* (mont.) Singer. Manjunathan and Kaviyarasan (2011) found that yeast extract was the best nitrogen source for the effective mycelial formation for *Lentinus tuberregium* (Fr.). Bae *et al.* (2000) found that yeast extract was the best nitrogen source for the effective mycelial formation for *Paecilomyces japonica.* Park *et al.* (2001) reported that corn steep powder was

the most suitable nitrogen source for both mycelial growth of *Cordyceps militaris.* Hamzah *et al.* (2012) found that Peptone was the better nitrogen source than urea, potassium nitrate (KNO_3), yeast extract, ammonium sulphate ($(NH_4)_2SO_4$) and ammonium chloride (NH_4Cl) for the mycelial growth of *Trichoderma virens*. High mycelial yield of 119, 116, and 114 mg/30ml were obtained for *Pleurotus tuber-regium*, *Coriolus versicolor* and *Agaricus* sp. respectively when the medium were supplemented with yeast extract at 28^0C and pH of 6.8 as recorded by Jonathan and Adeoyo, 2011. Kim *et al*. (2003) reported that polypeptone was the best organic nitrogen source while all the inorganic nitrogen source gave rise to poor mycelial growth. Kibar and Peksen, (2011) reported that peptone yeast extract and $Ca(NO_3)_2$ were the most suitable nitrogen sources for both Lactarius species. The lowest mycelial growth was determined in NH_4NO_3 and $(NH_4)_2HPO_4$ among nitrogen sources for both *Lactarius* species. Kadiri and Fasidi (1994) reported that the best utilizable carbon sources for *Lentinus subnudus*) were fructose, maltose, dextrin and glucose. Imtiaj *et al*., (2008-2009) recorded that the most suitable nitrogen sources for mycelial growth were glycine, ammonium acetate and calcium nitrate whereas the most unsuitable were histidine and ammonium phosphate. In case of glycine, mycelial density was found to vary from nearly compact to compact but in the rest of nitrogen sources the mycelial density was rather thin. Baars *et al*. (1994) found that *Agaricus bisporus* showed good growth in a number of organic nitrogen compounds than the inorganic nitrogen compound as nitrogen source.

4.3 Vitamin Source

The above result on the vitamin sources showed that ascorbic acid was the favourable source for the mycelial growth of *A. chepangiana* due to the rich vitamin source, while Nicotinic Acid was poor source for the mycelial growth. However Ayodele (2008) reported that thiamine was the best vitamin source for the mycelial growth of *P. atroumbonata*. According to Pokhrel *et al.*, glutamic acid was the best amino acid source for the mycelial growth of *L. decastes*. Manjunathan and Kaviyarasan (2011) reported that thiamine was the best vitamin sources for the mycelial growth in *L. tuber-regium*. Adebayo 2011 observed that ascorbic acid and folic acid had the highest stimulatory effect on mycelia yield *Pluerotus ostreatus*. Adenipekun and Gbolagade (2006) observed that the most suitable vitamin for the mycelial growth of *Pleurotus florida* (Mont.) Singer was thiamine followed by pyridoxine while the least was cobalamine. Manjunathan and Kaviyarasan (2011) reported that the most suitable vitamin for the mycelia growth of *Lentinus tuberregium* (Fr.) was thiamine.

4.4 **Amino acid Source:**

The above result on the amino acid sources showed that the optimum growth of *A. chepangiana* was found in Arginine while the lowest mycelial growth was recorded in Valine/Amino acid control gave the lowest mycelial growth. However Ayodele (2008) reported that asparagine was the best amino acid source for the mycelial growth of *P. atroumbonata*. According to Pokhrel *et al.*, glutamic acid was the best amino acid source for the mycelial growth of *L. decastes*. Manjunathan and Kaviyarasan (2011) reported that glycine was the best amino acid source for the mycelial growth of *L. tuberregium*. Kaur (2011) reported that the among different amino acids tested, maximum average mycelial growth of *Termitomyces striatus* (Beeli) Heim was obtained with L-arginine followed by glycine and DL-tryptophan. The fungus showed poor growth with L-amino-n-butyric acid, L-cystine, L-cysteine HCl and DL-serine. Gbolagade1 *et al.* (2006) tryptophan and alanine enhanced moderate vegetative growth of *Pleurotus florida* (mont.) Singer. Leucine was found to be the least amino acid for biomass production in this fungus. Manjunathan and Kaviyarasan (2011) reported that the glycine was the best amino acid for the optimum mycelial growth of *Lentinus tuberregium (Fr.).* Adebayo-Tayo and Ugwu (2011) reported that the least stimulatory amino acids were alanine and glutamate respectively while the best amino acids for mycelial growth of *Trametes versicolor* and *Coprinus* sp by the isolates were aspartic acid and asparagines, respectively.

4.5 C:N Ratio:

The above result on the C:N Ratio, the optimum growth of *A. chepangiana* was found in 4:1 sources while the lowest mycelial growth was recorded in 1:1 sources which was same as the study of Ayodele (2008) for the *P. atroumbonata*. Pokhrel *et al.* (2006) reported that best mycelial growth of *L. decastes* in media with the ratio 1:1 conversely least growth occurred at the ratio of 1:4. Manjunathan and Kaviyarasan (2011) reported that the C: N of 1:3 and 1:5 supported best growth of the *Lentinus tuberregium*, growth was reduced above or below this level. Shim *et al.* (2005) reported the optimum C/N ratio suitable for favourable growth of *Macrolepiota procera* was 30:1. Lai *et al.* (2011) reported that the most favourable mycelial growth of *Lignosus rhinoceros* was C/N ratio of 10:1. According Shim *et al.* (2003), 40:1 was C:N ratio suitable for a favourable mycelial growth of *Paecilomyces fumosoroseus*. Adenipekun and Gbolagade (2006) reported that the carbon nitrogen ratio of 5:1 was found to be the most suitable for the mycelial growth of *Pleurotus florida (Mont.)* Singer followed by 4:1 ratio and the least was 1:5.

Chapter 5

Conclusion and Recommendation:

Conclusion

The preferred species of wild edible mushrooms may reach vulnerable condition. The modern semi-artificial cultivation and commercialization of such highly nutritive wild edible mycorhizal mushroom species which has potentiality, will help not only to conserve the valuable species itself but also to maintain the wild habitat.

From the above study, it can be concluded that the vegetative growth patterns for a particular mushroom mycelium is different in different carbon sources e.g *Amanita chepangiana* has optimum growth having dense mycelium in malt extract while lowest growth in fructose. It can also be concluded that growth pattern of mycelium is different in different mushroom species. Thus, this research work shows that the vegetative growth patterns of different mushroom species is different in different carbon sources.

Among the different Nitrogen sources, the optimum growth of *A. chepangiana* was found in Sodium Nitrate while the lowest mycelial growth was recorded in Ammonium Nitrate. In Sodium Nitrate, Urea and Yeast extract, the mycelial growth pattern had no significant difference. Similarly, in Urea, Yeast Extract, Calcium Nitrate as well as Yeast Extract, Calcium Nitrate and control had no significant difference. But they were significantly different as compared to the Peptone and Ammonium Nitrate.

Among the different Vitamin sources, the optimum growth of *A. chepangiana* was found in Ascorbic Acid while the lowest mycelial growth was recorded in Nicotinic Acid .In Ascorbic Acid; the mycelial growth pattern had significant difference as compare to all the tests. While D-prolic acid, Thiamine, Vitamin control and folic acid, D-prolic acid Thiamine as well as Nicotinic acid, Folic acid, Thiamine had no significant difference.

Among the different Amino acid sources, the optimum growth of *A. chepangiana* was found in Arginine while the lowest mycelial growth was recorded in Valine. In Arginine, the mycelial growth pattern had no significant difference as compared to Leucine while significant difference as compared to Aspartic acid, Glutamic acid and Serine where as more significant difference as compared to Valine and highly significant difference as compared to its Control among the tests.

Among the different C:N sources, the optimum growth of *A. chepangiana* was found in 4:1 sources while the lowest mycelial growth was recorded in 1:1 sources. In 4:1 sources, the mycelial growth pattern had no significant difference as compared 1:4 sources while significant difference as compared to other C:N sources.

Recommendations

- Necessity of proper management of community forest in order to avoid the extinction of this species.
- Afforestation strategies of *Shorea robusta* should be emphasized for conserving their habitat.
- Semi-artificial cultivation (domestication) by seedling infection method for beneficial commercelization.
- Sustainable utilization of *Shorea robusta* and *Amanita chepangina* to fulfill the requirement of the local people and to maintain them for future generation.
- Public awareness programmes should be conducted to make them aware of mushroom poisoning and proper identification of this mushroom.
- People should be educated about the controlling of the invasive alien species of the community forest.
- Further research work should be carried about the nutritional value of this species.

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Annex

Table A.1: Average data reading per replica of various Carbon Sources on mycelia growth of *A. chepangiana*:

S.N.	Replica	Carbon Sources	Colony Growth (cm)				
1	5	Dextrose	5.35	5.60	6.50	4.75	6.00
2	5	Lactose	7.70	8.00	8.50	8.50	8.50
3	5	Sucrose	6.30	6.30	5.10	5.35	5.40
4	5	Fructose	4.75	5.10	4.20	5.10	4.15
5	5	Manitol	6.00	6.75	5.25	5.35	5.15
6	5	Malt extract	8.60	8.60	8.60	8.60	8.60
7	5	Control	8.20	8.30	8.30	8.30	8.25

Table A.2: Average data reading per replica of various Nitrogen Sources on mycelial growth of *A. chepangiana*:

S.N.	Replica	Nitrogen Sources	Colony Growth (cm)				
1	5	Ammonium Nitrate	3.45	3.35	4.00	3.35	4.60
2	5	Peptone	5.70	6.85	6.70	6.15	6.50
3	5	Calcium Nitrate	7.45	7.50	7.15	8.15	7.60
4	5	Sodium Nitrate	8.30	8.30	8.40	8.40	8.40
5	5	Yeast Extract	8.50	7.25	8.20	7.60	7.20
6	5	Urea	8.20	8.25	8.35	8.30	8.20
7	5	Control	7.50	7.45	7.35	7.00	7.40

Table A.3: Average data reading per replica of various Vitamin Sources on mycelial growth of *A. chepangiana*:

S.N.	Replica	Vitamin Sources	Colony Growth (cm)				
1	5	Ascorbic Acid	8.50	8.00	8.20	8.75	9.00
2	5	Nicotinic Acid	5.75	6.00	5.5 0	6.50	6.75
3	5	Folic Acid	6.40	6.20	6.70	6.30	6.90
4	5	D-Prolic Acid	7.10	7.00	7.00	7.05	6.80
5	5	Thiamine	6.70	6.50	6.60	6.80	7.00
6	5	Control	7.50	7.45	7.35	7. 00	7.40

Table A.4: Average data reading per replica of various Amino acid Sources on mycelial growth of *A. chepangiana*:

S.N.	Replica	Amino acid Sources	Colony Growth (cm)				
1	5	Aspartic acid	8.50	8.40	8.20	8.60	8.30
2	5	Arginine	8.90	8.70	8.85	9.00	9.00
3	5	Glutamic acid	8.25	8.00	8.75	8.50	8.05
4	5	Leucine	8.75	9.00	8.80	8.70	8.90
5	5	Serine	8.25	8.60	8.35	8.75	8.50
6	5	Valine	7.80	8.30	7.75	8.20	8.05
7	5	Control	7.50	7.45	7.35	7. 00	7.40

Table A.5: Average data reading per replica of various C:N Ratio Sources on mycelial growth of *A. chepangiana*:

S.N.	Replica	C:N Ratio Sources	Colony Growth (cm)				
1	5	1:1	4.85	4.70	4.65	4.35	4.50
2	5	1:2	6.05	6.00	5.90	6.05	5.75
3	5	1:3	7.20	6.95	7.50	7.85	7.35
4	5	1:4	8.50	8.75	8.90	9.00	8.65
5	5	1:5	8.15	8.05	7.75	7.90	8.05
6	5	2:1	5.05	5.65	5.35	4.95	5.30
7	5	3:1	5.40	5.55	5.85	5.90	5.70
8	5	4:1	9.00	8.85	9.00	8.80	9.00
9	5	5:1	7.35	6.85	7.05	7.55	7.25

Note: The full form of abbreviation of the Thesis are given in the abbreviation chapter.

PHOTO PLATE

Photo 1: Lab work by researcher

Photo 2 : Mycelial growth in carbon source

Photo 3 : Mycelial growth in nitrogen source

Photo 4 : Mycelial growth in vitamin source

Photo 5 : Mycelial growth in amino acid source

Photo 6 : Mycelial growth in C:N ratio