NUTRITIONAL STATUS OF CULTIVATED MUSHROOMS OF KATHMANDU VALLEY

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Submitted to
CENTRAL DEPARTMENT OF BIOTECHNOLOGY
Tribhuvan University
Kirtipur, Kathmandu, Nepal

A dissertation submitted as the partial fulfilment of the requirement for M.Sc. degree in Biotechnology

Dilli Raman Devkota

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ABSTRACT

This investigation provides the proximate screening of nutrients and minerals present in the four cultivated mushroom species sold in the markets of Kathmandu valley. The cultivation practices include all the exotic mushroom species introduced from India, Japan and China. They are *Lentinula edodes, Pleurotus ostreatus, Agaricus bisporus* and *Pleurotus djamor.* All these mushrooms are cultivated in the corners of Kathmandu valley and brought to the vegetable markets such as Asan, Kalimati, Balkhu and Lagankhel for sale.

The result of the analysis indicated that the mushrooms are good sources of Crude Protein (23%to 46.3%), Carbohydrate (28% to 50%), Fat (2.4% to 4.2%), Fiber (11.9% to 17.9%), Ash (6.3% to 18.3%) and moisture (79% to 93.3%). The nutrient contentvaries widely among the mushroom species. The result showed that the mushrooms are good source of nutritionally important minerals including Phosphorus (100.7mg to 837mg), Calcium (11.82mg to 165mg) and Iron (6.07mg to 52mg) per 100g on dry weight basis.

GC- MS result analysis suggested that mushroom samples are free of pesticides. Based on the result of this study, it is suggested that these mushroom species are nutritionally good without any human hazard.

Key words: Edible mushrooms, proximate composition, Mineral composition, nutrient supply, GC-MS analysis.

TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

LIST OF PHOTOGRAPHS

ABBREVIATION

AAS	Atomic Absorption Spectrometry
AM	Atomic Mass Unit
AOAC	Association of Official Analytical Chemists
BHC	Benzene Hexa- Chloride
CAT	Centre for Agriculture Technology
CDBT	Central Department of Biotechnology
DDT	Dicholoro Diphenyl Tricholoroethane
DDE	Dichloro diphenyl dicholoethylene.
DADO	District Agriculture Development Office
DBCP	Dibromo chloropropane
DFTQC	Department of Food Technology and Quality control
eV	Electric volt
FAO	Food and Agriculture Organisation
FAAS	Flame Atomic Absorption Spectrometry
FS	Flame Spectrometer
GC-MS	Gas Chromatography Mass Spectrometry
HCH	Hexachlorocyclohexane
HCl	Hydrochloric Acid
hrs	hours
ICMBMP	International Conference on Mushroom Biology and Mushroom Products
NASS	National Agricultural Statistics Service
NARC	National Agricultural Research Centre
nm	Nanometer
NAST	Nepal Academy of Science and Technology
mm	millimeters
µl	microliters
PMRA	Pest Management Regulatory Agency
PSPC	Polysaccharide Protein Complex
ppm	Parts per million
PSA	Primary Secondary Amines
USAID	United States Agency for International Development
US	United States
UGC	University Grant Commission

CHAPTER I

INTRODUCTION

1.1 Background

The word "mushroom" is most often applied to Basidiomycetes, Agaricomycetes that have a stem (Stipe), a cap (Pileus) and gills (Lamellae) or pores on the underside of the cap. But it encompasses both the genera of Ascomycotina (without lamellae or pores) and Basidiomycotina (Oliveira *et al*., 2008). Mushrooms are the fruiting bodies of macrofungi. They include edible, medicinal and poisonous species. However, originally the word “mushroom” was used for the edible members of macrofungi. Mushrooms are the freshly, spore bearing fruiting body of a fungus, typically produced above ground on soil or on its food sources (Heleno *et al.,* 2010).

Edible mushrooms have been used to maintain health (Maize *et al*., 2000). Nowadays, they are undoubtly consumed much more for their texture and flavor than for their nutritional and medicicinal properties. More than 2000 mushroom species exist in nature, but only approximately 22 spcies are intensively cultivated (Maize *et al*., 2001). In most countries there is a significant consumer acceptance of cultivated mushrooms such as *Agaricusbisporus*, *Pleurotus spp., Lentinulaedodes, Volvariellavolvacea* and *Auriculariaspp* (Diez*et al*., 2001). Mushroom has been part of a normal human diet for thousands of years and, its recent times, amounts consumed have risen greatly, involving a larger number of species. Because of increasing mushroom consumption, developments in cultivation techniquies, mushroom production has 1ipette1o which in turn affect the nutrient contents in mushrooms (Zakhary*et al*., 1983).

1.1.2 Morphology of Mushroom

A mushroom develops from a nodule, or pin head, less than two millimeters in diameter, called the mycelium, the mass of thread like hyphae that make up the fungus. The primordium enlarges into a roundish structure of interwoven hyphae roughly resembling an egg, called a "button". The button has a cottony roll of mycelium, the universal veil that surrounds the developing fruit body. As the egg expands, the universal veil ruptures and may remain as a cup, or volva, at the base of the stalk, or as warts or volval patches on the cap. Many mushrooms lack a universal veil and therefore do not have either a volva or volval patches. Often there is a second layer of tissue, the partial veil, covering the blade like gills that bear spores. As the cap expands, the veil breaks, and remnants of the partial veil may remain as a ring, or

annulus, around the middle of the stalk or as fragments hanging from the margin of the cap (Tomako *et al*., 2009). The ring may be skirt-like as in some species of *Amanita*, collar-like as in many species of *Lepiota*, or merely the faint remnants of a2ipette (a partial veil composed of filaments resembling a spiderweb), which is typical of the genus *Cortinarius* (Nonaka *et al*., 1995). Mushrooms that lack a partial veil do not form an annulus (Stuntz*et al.*, 1978). The stalk (also called the stipe, or stem) may be central and support the cap in the middle, or it may be off-center and or lateral, as in species of *Pleurotus* and *Panus* (Larrea*et al*., 1996).

In other mushrooms, a stalk may be absent, that form shelf-like brackets (Marazullo *et al*., 1995). Puffballs lack a stalk but may have a supporting base. Other mushrooms, like truffles, jellies, earthstars, bird's nests, usually do not have stalks, and a specialized mycological vocabulary exists to describe their parts. The way that gills attach to the top of the stalk is an important feature of mushroom morphology (Burns & Dick, 2002).

Mushrooms in the genera *Agaricus, Amanita, Lepiota*and*Pleurotus*, among others, have free gills that do not extend to the top of the stalk. Others have decurrent gills that extend down the stalk, as in the genera *Omphalotus* and *Pleurotus*(Alvarez *et al*., 1998a). There are a great number of variations between the extremes of free and decurrent, collectively called attached gills. Finer distinctions are often made to distinguish the types of attached gills: adnate gills, which adjoin squarely to the stalk; notched gills, which are notched where they join the top of the stalk; adnexed gills, which curve upward to meet the stalk and so on (Kaur, 2002). Scientifically cap is known as pileus, gills as lamellae, ring as annulus, and stalk as stipe and roots as mycelia strands. The typical basidioma has these structures, while asco fruiting bodies lack lamellae and annulus.

1.1.3 Concept of Mushroom and Toadstool

According to the "Dictionary of Fungi" toadstool is an agaric, basidioma, especially edible one, macrofungus with distinctive fruit body, which can Seither be hypogenous or epigenous, large enough to be seen by naked eye and to be picked by hand (David &Stalpers, 2004; Kirk *et al.,* 2008). Different authors define the mushrooms and toadstools in their own languages and included in the same group "Agaricale" i.egill fungi (Fries, 1838; Rea, 1992; Singer, 1986). The word "toadstool" comes from the German word "toad stuhl", which means death-stool causing sickness and even death. The poisonous members are however popularly known as toadstools. Even closely related species of the same genus are not all poisonous or edible for instance *Lepiotamorgani* and *Amanita muscaria* are poisonous while *Macrolepiotaprocera* and

Amanita caesarea are edible (Adhikari, 1976). Kibby, (1979) defined "Toadstool" as the inedible or poisonous group.

The "toadstools" are also gill bearing agarics but are mostly inedible and poisonous. The "*Dictionary of Botany*" defined the term "Toadstool" as an essentially synonymous with the mushroom in both the narrow and broad senses, among them very few are edible but is more often used in inedible species. Pacoini, (1985) made a clear distinction between "Mushroom" considering only the edible species and "Toadstools" as inedible or poisonous species. Krieger, (1967) defined toadstool as " all the fleshy umbrella shaped fungi and to a small number of the best known edible forms".

1.2 Historical account on cultivation

Mushrooms have been a widely used as food and food supplements for millennia. Edible mushrooms were first collected by a man in China, which dates back to 4000-5000 BC (Zhanxi&Zhanhua, 2000). It is estimated that the first cultivation of edible mushrooms was done in China in the early 7^{th} century, with the species *Auriculariaauricu-judae* (Chang & Miles, 1987). China is a country with a long tradition in cultivation and consumption of mushrooms and it has more than ten species of fungi which are currently cultivated in several countries of the world(Zhanxi& Zhanhua, 2001). China is a pioneer in the cultivation and consumption of edible and medicinal mushrooms, followed by Japan, Europe and The United States (Urben*et al*., 2001).

Edible mushrooms were described as the "food of the Gods" and as such, confirmed by Roman gourmets, who appreciated them as a kind of spice. The Chinese considered them as the "elixir of life". The Greeks believed that the mushrooms were able to give strength to warriors in battles and the Egyptian pharaohs also nourished themselves on these spices (Chang & Miles, 1984). Mushrooms had a wide acceptance, and some species are considered as "Kings of the dining table" or "kitchen diamonds" (Zhanxi&Zhanhua, 2001).

The Greeks Euripides, Theophrastus and Plinio have described the consumption of edible mushrooms in their time (Guzmán*et al*., 1993). In some societies, the mushroom was a royal food, probably by its pleasant flavor and texture (Miles & Chang, 1997).

The Romans knew several edible and poisonous fungi. There is a story about the Emperor Julius Caesars who was very fond of *Amanita caesarea* mushroom, whose scientific name was a homage to him and for that reason, it became known as "Mushroom of the Caesars" (Guzmán*et al.,* 1993). According to Molena (1986), the species *Polyporustuberoster* (fungaie stone) and *Polyporus corilinus* are among the first

cultivated mushrooms, collected from the wood of hazels and *Eucalyptus*. These fungi were consumed in 4-5cm slices, and their production demanded about six months, yielding sometimes one or two mushrooms at a time (Volka T., 2001). There was neither any knowledge about their nutritional requirements, nor about their growth cycle. The only thing that was known was that rubbing a mature mushroom on those woods, and leaving them in a wet environment during a particular period of the year could produce appreciable mushrooms (Molena, 1986). During the Roman Empire the fungae stone (stone that produces the mushroom) appeared in Italy, which was composed of a cluster of humus, leaves, twigs and limestone rocks, forming a compact mass, which was cut in blocks in the form of bricks and transported to the royal palaces (Stuntz *et al*., 1978). They were kept in a damp place and irrigated daily until harvest time to serve the senators and other members of the Roman aristocracy (Molena, 1986). In France, the mushroom cultivation began during the reign of Luis XIV according to Molena, (1986). However, the cultivation of *Agaricusbisporus,* the "Champignon de Paris", the most widely cultivated and commercialized species, has been produced since about 1650AD (Delmas, 1978; Chang & Miles, 1984). With the advances of knowledge and technology of mushroom cultivation, commercial production of dozens of species became viable in several countries in recent decades (Guzmán*et al.*, 1993; Stamets, 1993, 2000; Eira, 2000), reaching a production of approximately 4.3 million tons of edible mushrooms in 1991 (Miles & Chang, 1997). The world production is around 6.2 million tons (Chang, 2003). Production of mushroom has increased to 11 billion tons (Mondal *et al*., 2011). Mushroom is an important food item concerning human health, nutrition and disease prevention (Chang, 1996). There is a common saying that “medicines and foods have a common origin” (Kaul, 2001).

The consumption of edible fungi as food and drug is closely related to history of mankind. Even the early men knew the special properties of mushrooms. They called them God's flesh. Mushroom was first cultivated in France 1650AD. The method was first developed by Gardner in 1700AD (Matsumoto *et al*., 2011). It was taken to USA was introduced in later part of 19^{th} century. In Eastern, mushroom began to be grown on a commercial scale in the people's republic of China, South Korea and Taiwan (Chang, 1997). The Chinese mushroom has very old history. This mushroom has been used as food for human beings since Cho dynasty about 3000 years ago in China (Kaul, 2001). It was introduced in South east Asian countries by overseas Chinese, (Baker, 1934 and Benemertio,1936), Since then, its cultivation has been conducted in various countries outside the China like Philippines, (Clora, 1937; Go, 1959), Malaysia (Baker 1934; Sands 1935), Burma (Seth, 1944) and Thailand (Jalavicharama,1950; Hashioka, 1962). The history of the Chinese mushroom cultivation is very old. As far as its artificial

cultivation is concerned it is believed that, it was begun in Nanhua temple of Chaohsi, Kwantung province in Southern China, almost 200 years ago, (Chang, 1977).

After World War II, the edible mushroom industry grew from 350,000 tons in 1965 and in the early 1980s, only *Agaricusbisporus*(Champignon de Paris) and other species of this genus and "Shiitake" (*Lentinusedodes*, currently named *Lentinulaedodes*) had a modern technology for commercial production, where 70% of the world production was represented by *Agaricus*and 14% by *Lentinula*(Chang & Miles, 1984). Special technologies are being developed in several countries allowing the cultivation of: *Volvariellavolvaceae* in China, Taiwan, Japan, Philippines and Indonesia; *Kuehneromycesmutabilis, Flammulinavelutipes*, *Hypholomacapnoides* and *Coprinuscomatus* in some countries of Europe and Asia; *Pleurotusostreatus* in Italy, Hungary, West Germany, Mexico and Brazil (Chang & Miles, 1984; Guzman *et al*., 1993; Zhanxi&Zhanhua, 2001; Urben*et al*., 2001). Through there are over 300 genera of mushrooms and related fleshy basidiomycetes, only a few species of these fungi are cultivated commercially (Chakravarty*et al*., 2011). From which 3.4 million tons belong to the six most worldwide important genera: *Agaricus, Pleurotus, Lentinula, Auricularia, Volvariella*and *Flammulina* (FAO, 2004). But many saprophytic species have been amenable to cultivation. some of the more common cultivated species are the button mushroom, *Agaricusbisporus* which was widely cultivated in Europe before being exported to North America by the settles; the shiitake mushroom (*Lentinulaedodes*) which is grown for centuries in China and other oriental countries and oyster mushroom (*Pleurotusosteratus)* which was collected as wild species from forests in Florida and later actively cultivated in several around the world (Chakravarty, 2011). The paddy straw mushroom (*Volvaariella volvaceae*) and fungus (*Auricularia auricular*) have great medicinal value. Other cultivated mushrooms are the Reishi mushroom(*Ganodermalucidum*) which is used as an alternative medicine and also as flvoring agent in Japan; the Nameko (*Pholiotamicrospora* = *Pholiotanameko*) grown in the orient and *Tremela fusiformis* or white jelly fungi that is grown for use as food supplements in Taiwan. Verities of *Agaricusbisporus* that are grown commercially include the crimini and portabllo. They are considered a food delicacy and rated as one of the most expensive natural food in the world (Trappe *et al*., 2007). This might be due to the fact that many of them are mycorrizal and may not sporulate in the absence of host. The increase in the number of species on a commercial scale might be due to development of cultivation techniques using plastic bags, which allowed many wood decomposers edible fungi to be grown on lignocellulosic residues, preferably the cultivation on logs, reducing considerably the cultivation time; due to the marketing techniques

highlighting the nutritional merits of mushrooms as an important part of the diet (Miles & Chang, 1997).

1.3 World Mushroom Market

Although mushrooms have been collected from the wild and cultivated artificially for human food and for medicine uses for hundreds and thousands of years. The world market for mushrooms industry in 2001 was valued at over US$ 40 billion. The mushroom industry can be divided into three categories: edible mushrooms valued about US$ 30 billion; medicinal mushroom products were about US$ 9-10 billion and wild mushroom, USA 4-5 billion (FAO, 2004). Production of mushroom has been increasing steadily, mainly due to contribution from developing countries, such as China, India, Poland, Hungary and Vietnam.

The large numbers of mushroom species are not edible and nutritional but also possess tonic and medicinal qualities (Wasson, 1968). However, some mushrooms are lethally poisonous and one should eat mushrooms only if one knows their names and their properties with considerable precision. Technologically development on the mushrooms industry in general has seen increasing production capabilities. This is due to innovations in cultivation technologies, improvement in final product, capitalizing on mushrooms nutritional and medicinal properties, and utilizing mushrooms' natural for environment benefits.

The mushroom industry in UK and in some other western countries is often overwhelmingly focused on one mushroom species, *Agaricusbisporus*. These industries are nearly 100% dominated by *Agaricusbisporus* (Graze, 2005). In the USA *Lentinulaedodes* for 1% and *Pleurotus*for only about 0.5% and remaining production was from *Agaricusbisporus* (Barnett *et al*., 2006).Spain was the third largest mushroom producer in the EU. In 2004, mushroom production in Spain was 110000 tons compared with 26,512 tons in 1992, increasing 315 %. The production consists of 80% of *Agaricus* mushroom, 15% *Pleurotus* mushrooms and 5% *Lentinula*mushrooms (National Agricultural Statistics Service (NASS); 2005, USA).

On the other hand, Mushrooms in East Asian countries are far more popular than *Agaricusbisporus*. *Agaricus* accounted for 12.8% of total mushroom production in China in 2003, 11.6% in South Korea and in Japan (Chang and Miles 2006). Furthermore, while the production of three important mushrooms. *Agaricus, Lentinula* and *Pleurotus*mushrooms together make up nearly 100% of mushroom industry in USA and Spain (Chang 2006; Cui 2004; Ho &Peny, 2006).

1.4 Nutrients found in mushroom

The greatest difficulty in feeding man is to supply a sufficient quantity of the body-building material, the protein. The other three nutritional categories are the source of energy food, carbohydrates, fats and accessory food factors like vitamins and inorganic compounds which are indispensable to good health (Fasidi*et al*., 1990).

In terms of the amount of crude protein, mushrooms rank below animal meats, but well above most other foods, including milk, which is an animal product (Chang & Miles, 1989). Furthermore, mushroom protein contains all the nine essential amino acids required by man (Manjunathan*et al*., 2011). The moisture content of fresh mushrooms varies within the range 70-95% depending upon the harvest time and environmental conditions. In addition to their good proteins, mushrooms are a relatively good source of the following individual nutrients; Fat, Phosphorus, Iron and vitamins including thiamine, riboflavin, ascorbic acid, ergosterine and niacin (Hall *et al*., 1998). They are low in calories, carbohydrates and calcium. Mushrooms also contain a high proportion of unsaturated fat (Schultes, 1940). In recent years, there has been a trend toward discovering ways of treating mushrooms so as to give them added value. For example, Wermer&Beelman (2002) have reported on growing mushrooms enriched in selenium. The desirability of a food product does not necessarily bear any correlation to its nutritional value. Instead, its appearance, taste, and aroma, sometimes can stimulate one's appetite (preference). In addition to nutritional value, mushrooms have some unique color, taste, aroma, and texture characteristics, which attract their consumption by humans (Ertrug, 2000).

The Egyptian regarded them as food for pharaohs. The Greeks and Romans described them as "food for Gods", and were served only on celebrations. Reference to mushrooms is found in Vedas (Chube, 1995; Adhikari, 2000, 2003). Mushrooms can be used for the food to solve the malnutrition problem (Manandhar, 2003). Man has been hunting for the wild mushrooms since antiquity (Cooke, 1977). Thousands of years ago, fructifications of higher fungi have been used as a source of food (Mattila*et al.*, 2001) due to their chemical composition which is attractive from the nutrition point of view. During the early days of civilization, mushrooms were consumed mainly for their palatability and unique flavors (Rai, 1994, 1997). Present use of mushrooms is totally different from traditional because, lot of research has been done on the chemical composition of mushrooms, which revealed that mushrooms can be used as a diet to combat diseases. The early history regarding the use of mushrooms in different

countries has been reviewed by number of workers (Buller, 1915; Bano*et al.*, 1964; Jandaik&Kapoor, 1975; Houghton, 1995).

1.5 Medicinal value of mushroom

Mushrooms contain functional or medicinal properties, which may be used as a source of biologically and physiologically active substances where its estimate is about 50% of the annual harvests of 5 million metric tons of mushrooms (Cheung *et al*., 2003). According to Mizuno (1995), Wasser (1995) and(Wasser*et al*., 1999) approximately 700 species of higher basidiomycetes have been found to possess significant pharmacological activities. The second major attribute of mushrooms, their medicinal properties, e.g., hypotensive and rental effects (Tam *et al.*, 1968; Yip *et al*., 1987), immunomodulatory and antitumour activities of polysaccharide-protein complex (PSPC) from 8ipette cultures (Liu *et al.,*1996; Wang *et al.*,1996, Morin *et al*., 2013), immunomodulatory and antitumour activities of lectins from edible mushrooms (Wang *et al*., 1996), isolation and characterization of a Type I Ribosome-inactivation protein from *Volvariellavolvacea* (Yao *et al*., 1998), and medicinal effects of *Ganodermalucidum* (Chang &Buswell 1999; Chang & Miles, 2004).Mushrooms have been found to have potential biological activities such as anti-bacteria, anti-fungi, antitumor, anti-inflammatory, anti-hepatotoxic activity, cardio-tonic activity, cholesterol level lowering activity, antiviral and immune-modulatory activity (Miles & Chang, 1997; Quang*et al*., 2006; Petrova*et al*., 2007; and Nyigo*et al*., 2009).

1.6 Neutraceuticals and dietary supplements

The recent upsurge of interest in traditional remedies for various physiological disorders and the recognition of numerous biological response modifiers in mushrooms have led to the coining of the term “mushroom neutraceuticals” (Chang &Buswell, 1996). A mushroom neutraceutical arefined/partially defined mushroom extractive which is consumed in the form of capsules or tablets as a dietary supplement (not a food) and which has potential therapeutic applications (Fan *et al*., 2003). A regular intake may enhance the immune responses of the human body, thereby increasing resistance to disease and in some cases, cause regression of a disease state.

1.7 Mushroom Biotechnology

Mushroom biotechnology is concerned with mushroom products (mushroom derivatives) and encompasses the principles of mushroom biology/ Microbiology biotechnology, Fermentation technology and Bioprocess (Harkonen, 1989). Mushroom biotechnology, both as technology and as the basis for new mushroom products,

requires industrial development. It is like many bioscience industries operates at the cutting edge of science and involves numerous regulating issues.

The three components of applied mushroom biology include mushrooms Science; mushroom Biotechnology; and mushroom Mycorestoration (Chang &Buswell, 2008). It has been pointed out that mushroom biotechnology is concerned with the investigation of chemical compounds, the tools used, technologies involved and preparation of their products. It also involves the genetic study, recombination and or engineering to produce viable and new strains of commercially important products for meeting the daily consumption of human needs. Moreover it encompasses the principles of involvement of fermentation technology and the microbiological or and bioprocess for any desired or undesired by products. Mushroom or their products have a good nutritive and medicinal (antioxidant, therapeutic) values, which in some cases may act prophylactically by increasing resistance to disease in humans from the balancing of nutrients in the diet and the enhancing of the immune systems (Barnett *et al*., 2001). Some have mild or strong biotoxic values, which can be used as selective biochemical to control unwanted pests or diseases (Zahid*et al*., 2012).

1.8 Pesticide

Most pesticides are chemicals that are used in agriculture for the control of pests, weeds and plant diseases. These chemicals may be extracted either from plants organic origin or may be "synthetic" made from of Sulphur, Copper, Lime, Murcurial, Quinene components and especially DDT, HCN (Lindan), Acepphate, Eudosulpfan, Fenthion, Malathion, Phorate and Dieldrin Kim *et al*., 2015.

FAO (1986a) defined a pesticide as any substance or mixture of substances intended forpreventing, destroying, or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during, or otherwise interfering with. The production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animals for the control of insects, arachnids or their pests in or on their bodies is the vital to control the quality of food. Similar definition has been adopted by the Codex Alimentarius Commission (Cod, 1984).

1.9 Use of Pesticides in World

Pesticides are classified by target organism (e.g. *herbicides, insecticides, fungicides, rodenticides* and *pediculicides)*. Biopesticides include microbial pesticides and biochemical pesticides. Plant-derived pesticides have been developing quickly. These include the pyrethroids, rotenoids,nicotinoids and a fourth group that includes strychnine and scilliroside.

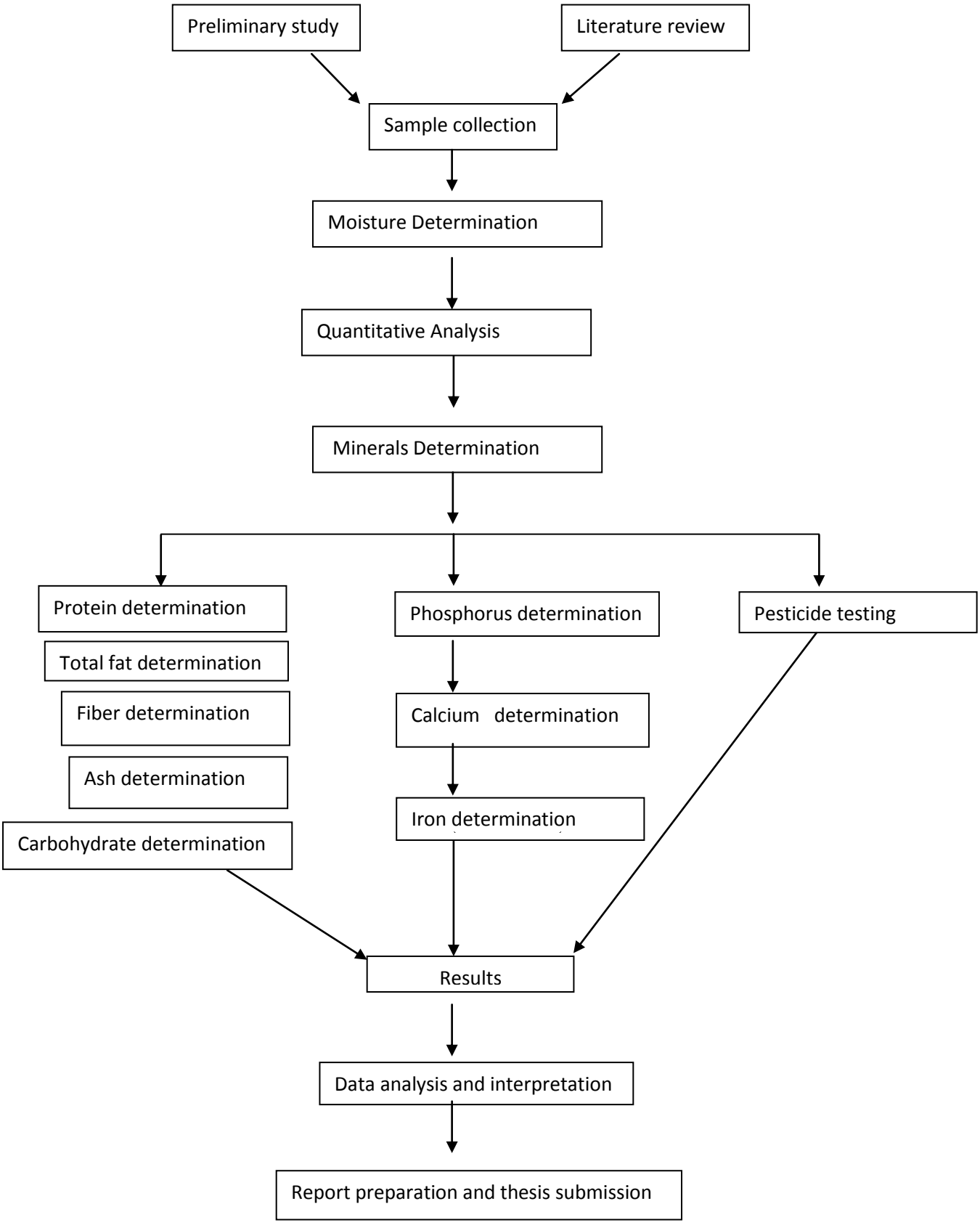
However, DDT and other Organochlorine pesticides have been banned in most countries because of their persistence in the environment and human toxicity. DDT use is not always effective, as resistance to DDT was identified in Africa as early as 1955 and by 1972 nineteen species of mosquito worldwide were resistant to DDT.

Table 1. The fifteen most used pesticides in Bangladesh, India, Republic of Korea, Nepal, Pakistan, Philippines and Thailand.

S.N	ame of Pesticide
1	Carbary | (I)
2	Malathion (I)
3	Parathion- methyl (I)
4	Diazinon (I)
5	Monocrotophos (I)
6	Endosulfan (I)
7	Carbofuron (I)
8	Mancozeb (I)
9	Paraquat (H)
10	Aluminium phosphide (I)
11	Oxy demeton- methyl (I)
12	Phospamidon (I)
13	2,4-D (H)
14	BPMC(2-sec-butylphenylmethylcarbamate) (I)
15	Zinc phosphide (I)

I= Insecticide, F= Fungicide, H= Herbicide(Source: FOA, 2015)

1.10 Experimental Overview



1.11 Hypothesis of investigation

In Nepal, near about 1300 species of mushrooms are found growing naturally (Adhikari, 2014), which enlodge both edible and poisonous forms. Among them about 147 species are found to be edible. None of these edible species have been taken for commercial cultivation. About 10 exotic cultivars have been introduced in the country. Till this date no screening of nutrient present in these species has done in Nepal. So their nutrient constitutes have been visualized necessary to be screened for edibility to avoid human health hazards. Hence, this research or investigation will highlight on the percentages of screened nutrients present in the cultivars found sold in various well known markets of Kathmandu valley.

This research will provide a significant result and add vista for other researches in future. This investigation will certainly make aware the mycophilian and mycophagus society, cultivators or producers and consumers about the edibility status of mushrooms. Till now, there is no any policy and acts legislated or put forward by the government to control or promote the mushroom growing industry in Nepal. Hence, this will provoke the Nepalese government to pass the bill about edible mushrooms to be sold in the market, be strict in certifying the cultivated edible species, and the producers or growers to cultivate the mushrooms in a slandered method.

1.12. Research Objectives

1**.** General objectives:

- To gather the different species of cultivated mushrooms sold in the markets of Kathmandu valley.
- To determine the percentages of nutrient constituents present in different cultivated species of mushrooms sold mainly in Kathmandu Valley of Nepal.
- To quantify the amount ofresidual pesticidein mushrooms if present.

2. Specific objectives:

- To determine the proximate composition including moisture content, carbohydrate, protein, fat, ash and fiber.
- To determine the mineral content such as Phosphorus, Calcium and Iron.
- To compare the nutrient composition present in different species of mushrooms gathered from Asan, Lagankhel, Kalimati and Balkhu markets.

- To determine the presence of Organochlorine pesticides in mushrooms

1.13 Significance

Academic significance:

The research is performed by the cumulative knowledge on morphology, identification, Botany, Biochemistry, Statistics and Biotechnology. The knowledge of the study reveals the nutrient condition, method of detection of nutrients on the basis of biochemical aspects. The outcome of the study will be associated to the information and building the knowledge related to the subjects, proving the prevailing information on the topics of nutrients analysis and adding new information to the pool of the knowledge of ingredients mushroom contain and presence of pesticide.

Applied significance:

The research has huge impact on the applied aspects such that agricultural genetics. It is helpful for the establishment of the various nutrients present in different cultivated species of mushrooms as well as nutrient profiling of mushrooms found in Kathmandu Valley. Truly speaking, it will make aware for the growers for production of better quality to use specialized or advanced technique. Use different media and natural biopesticides for control of invading enemies of mushrooms during production.

1.14. Limitations

1. The present investigation is limited to screening of mushroom samplessold insome vegetable markets of Kathmandu valley; it may not accurately depict the whole nationwide scenario.

2. Unavailable of standard compounds, Chemicals and lack of skilled and trained manpower in the field of Analytical Chemistry.

3. Lack of budget to buy expensive chemicals and equipments.

4. Duration for the completion of thesis was limited as a result of which I was unable to collect our native species.

CHAPTER II

LITERATURE REVIEW

2.1 Historical review of mushroom cultivation in Nepal

Nepal is rich in biodiversity due to its complex variation in geomorphology and phytogeograhpy (topology, climate and altitudinal) (Adhikari, 2000, 2014). Nepal occupies 0.09% of total surface of world's (Jha, 1992).

Nepal is situated on the southern slopes of the Central Himalayan and occupies total areas of 1,47187sq .Km. The country is located between latitudes 26°22' and 30°27'N and longitudes 80°12' E. The altitudes vary from some 60m above sea level in the terai to Mount Everest (Sagarmatha) at 8848m (the highest point in the world. According to Hagen (1971), Nepal has seven physiographic divisions viz: Terai, Siwalik, Hill zone, Mahabharatlekh, Midland Himalaya, Inner Himalaya and Tibetan Marginal Mountain.

Nepal, known as treasure house of mushrooms diversity (Adhikari, 2014) and has been well known on the use of wild mushroom since time immemorial. The collection and survey on mycoflora of Nepalese himalayan belt was at first started by Berkeley (1838) and followed by Lloyd (1908) and (Adhikari, 19995).

Mushroom diversity of Nepal is a reflection of unique geographical position and altitudinal variations. Its affinity is the transitional zone between the Eastern and Western Himalayan elements. It incorporates the Palaeoaryantic and Indo-Malaysian, Western and Central Asiatic, Southeast Asiatic and African elements creating a unique and rich terrestrial biodiversity (Adhikari, 2009; Wang *et al*., 2000).

Nepal is considered as a crossroad of migration in the Himalayan region, overlapping between eastern and western Himalayan elements. Total mushroom species reported from Nepal is as follows, which includes both wild and cultivated species.

ASCOMYCOTA					
Class	**Order**	**Family**	**Genus**	**Species**	**Variety**
Pezizomycotina					
Geoglossomycetes	1	1	2	10	2
Leotiomycetes	3	6	20	32	1
Pezizomycetes	1	10	32	65	1
Sordariomycetes	3	6	18	58	3
BASIDIOMYCOTA					
Class	**Order**	**Family**	**Genus**	**Species**	**Variety**
Utilaginomycotina					
Exobasidiomycetes	1	1	1	7	
Agaricomycotina					
Dacrymycetes	1	1	3	5	
Tremellomycetes	1	4	5	9	
Agaricomycetes	18	79	276	1085	21
Total	**29**	**108**	**357**	**1271**	**27**

(Source: Mushrooms of Nepal (Adhikari, 2014))

Although, the collection and study of fungi started with works of Berkeley (1838), the detail study fungi in and around the15ipette15o valley came to light since 1966 (Singh, 1966). While talking of the wild edible species it encounters around 147 species and the poisonous species are recently recorded to reach near about 100 species (Adhikari, 2008c, Adhikari*et al.,* 2008, 2012). The medicinal are 73 species, while 20 species can be utilized in decoration (Adhikari, 2008a). The daily consumption of wild mushroom is found in tropical forest areas, midlands, temperate and subalpine region of Nepal, where the local inhabitants dwell near by the forest (Adhikari and Adhikari, 1996-97, 2000). The high consumption is found in temperate region in comparison to others. This mishappening always occurs in the areas where there is near to forest and or scarcity of food.

Till now amount of collection, consumption and the mortality rate due to the consumption of wild mushroom is unknown. The mortality rate due to the consumption of wild mushrooms encircles around 30 to 35 (Department of Forestry, 2015). The mortality rate is high where there is poor awareness of prompt communication and first aid services. The collectors are mostly traditional mycophagus society such as Tamang, Gurung,Sherpa, Tharu, Magar, Danuwar, Kami, Newar, Damai and Sharki and directly connected with the collection and consumption of mushrooms historically due

to mushroom's delicious taste. The age of collectors ranged between 10 to 45 years. The victims are mostly infants and old persons. It's due to their necessity to earn money for livelihood and serve hand to mouth. They are illiterate and ignorant to differentiate between edible and poisonous mushromms (Adhikari, 2004). Some important wild mushrooms of very high commercial values are:

1. *Boletus edulis* (Cep or Boletus)
2. *Cantharellusciberius* (Chantharelle)
3. *Ophiocordycepssinensis* (Yarsagumba in Nepali)
4. *Ganoderma lucidum* (Ganoderma, Ratochayau)
5. *Craterellusconnucopides*(Horn of plenty)
6. *Morchellaconica*(Morel)
7. *Morchellaesculenta* (Morel)
8. *Tricholomamatsutake* (Matsutake)

Among these wild mushrooms of very high commercial value, *Ophiocordycepssinensis* has become significant sources of income for a lot of people in hilly region. Due to variation in altitude of Kathmandu valley a lot of potential mushrooms grow naturally and assist in cultivation of mushrooms. For example: *Cortinariuscallisteus* is found in Pine and *Quercus* mixed forest of Chandragiri and Thankot (2,220m). *Gymnopilusspectabilis* grows on soil in root crevice of *Graveliarobusta* tree in open moist place of Thankot (1,450m). *Pezizapetersii* is found growing in *Quercussemecarpifolia* forest of Chandragiri (2220m). *Psathyrellapiluliformis* (= *Psathyrellahydrophila*) grows on decayed stump of mixed forest at Kakani (2040m) (Adhikari, 1986, 2014).

2.2 Cultivated mushrooms in Nepal

2.1.1 Experimental cultivation

The experimental cultivation mushrooms in Nepal started since 1972 with the works of Singh &Nisha (1973) at Department of Botany Tribhuwan University, Kritipur. They reported the successful cultivation of Nepalese *Pleurotus* species (*P. nepalensis*) using various substrates (*Qurecus, Pinus, Euphorbia*). In 1991, National Herbarium and Plant Laboratory (NHPL) at Godawari, carried the experimental cultivation of cultivation of *Lentinula edodes* was also started in the promises of Khumaltar.

2.1.2 Commercial cultivation

In 1976, the cultivation of button mushroom (*Agaricus bisporus*) it was started in Plant Pathology Division, Khumaltar under the supervision of Ms. Bunu Devi Pandey. Later, in 1977, it was started by farmers (Ahikari, 2004). Initially, cultivation technology was transferred in different locations in Kathmandu Valley like Balambu, Chapagaun, Harisiddhi, Koteshwor etc. and then to various places outside the Kathmandu Valley including Chitwan, Ilam, Sunsari, Jhapa, Dhankuta, Bara, Makawanpur, Nawalparasi, Pokhara, Mustang, Dang and Dhading district (Adhikari, 1997). Plant Pathology Division in NARC began distribution of spawn. Oyster mushroom was introduced to farmer in 1984. In the beginning a few numbers of farmers started. After successful production of oyster mushroom, the number of farmers increased to 50. At present there are about 5000-6000 mushroom farmers in Kathmandu alone (Department of Agriculture, 2015). The researches for other species started from 2001, by NARC as well as private organization Centre for Agriculture Technology (CAT) under Dr. KeshariLaxmiManandhar and Agro Business Center (ABC) Kalimati.

At present, most of the farmers started to produce the spawn and fruiting bodies of mushrooms (*Agaricus bisporus, Pleurotus ostreatus, Lentinula edodes, Lentinus sajor-caju* and *Ganoderma lucidum*) in commercial scale.

In Nepal, the annual productions of mushrooms are as follows. In 1974 the production of fruit bodies were 30kg, while in 1978, 6 metric tons of fruit bodies of *Agaricus bisporus* were produced, in the year 2005, 700 metric tons of fruit bodies of *Agaricus bisporus, Pleurotus ostreatus* and *Lentinus sajor-caju* were produced. In the last fiscal year the production of *Lentinus* spawn production in 2008 exceed of 2 lakhs bottles of *Agaricus bisporus* while, those of *Lentinus sajor-caju* was limited to 1.3 lakhs bottle (Pers. 17ipet. H.C. Bastola, NARC).

The average production is about 8000-10000 kilogram per day (Adhikari, 2014). Pokhara and Chitwan are other major mushroom producers. Other districts also produce these but in very less amount, which is not enough to meet local demand.

There are two seasons of cultivation of *Agaricus* mushroom. *Agaricus* harvested from Falgun to Baisakh, if spawn is inoculated in compost at Paush to Magh and *Agaricus*can be harvested from Ashwin toMaghif spawn is inoculated at the month of Ashad to Shrawn(Manandhar. 2006).

The main areas, where mushrooms cultivation is being done in Nepal are:

District:	Areas:
1. Kathmandu:	Balambu, Kakani, Thankot, Gokarna, Sundarijal, Budhanilkantha, Sankhu
2. Lalitpur:	Chapagaun, Lamatar, Lakuri, Bhanjyang, Lele, Godawari
3. Bhaktapur:	Sirutar, Balkot, Janagal
4. Kavre:	Dhulikhel, Panauti, Nala
5. Chitwan:	Padampur
6. Kaski:	Pokhara

Farmers produce white bottom mushroom and oyster mushroom in tunnels made of bamboo framework and covered by plastic and straw, producing about 300-400kg in a season and 150-250 kg during an off season (Adhikari, 2006). The farmers choose mushroom farming because of the more profit within a short period of time. According to the farmers they could make profit up to 4 times their investment in average.

2.1.3 Market and selling price

The cultivated as well as wild edible mushrooms are found sold in Kathmandu valley markets especially at Kichapokhari, Asan, Balkhu and Kalimati vegetable markets (Kathmandu) and Lagankhel (Lalitpur). They are even found sold in various road sides of Kathmandu valley (Adhikari, 2004).The cultivated mushrooms are sold at different rates. The rate fluctuates in between Rs.150-200 for *Pleurotusostreatus* and *Lentinussajor-caju*. While the rate of *Agaricusbisporus*is in between Rs.200-350 per kg. "Shiitake" is in between Rs.700-1000 per kg [Source: Vegetable markets in Kathmandu, 2073].No international trade is found done at large scale. There are 10 cultivable species found in Nepal but in our local markets it has been observed only 5 species for daily consumption. There are different agencies, entrepreneurs and growers some of them are:

1 Chapagoan Mushroom production services, Lalitpur: Grows *Agaricusbisporus, Pleurotusostreatus*and*Lentinussajor-caju.* Its annual production is 2 tons per day. Near about 175 persons are involved in the production of mushrooms.

2 Ago Business Centre for Research and Development, Kalimati: Grows *Lentinussajor-caju, Lentinulaedodes, Pleurotusostreatus, Pleurotuserygii, Ganodermalucidum.* It sells 20-50kg or even 100kg of *Pleurotus* spawn to farmers every day. Experimentally, it has grown *Ganodermalucidum*on wood

logs, wood powder and paddy straw. *Lentinulaedodes* on sawdust and wood log based media *Pholiotamicrospora*(*Pholiotanameko*) in sawdust based media and *Flammulinavelutipes* in sawdust based media. It also produces spawn in plastic bags for selling purpose.

3 Balambhu mushroom cooperatives Ltd. Grows *Agaricusbisporus, Lentinussajor-caju* and *Pleurotusostreatus*. It is cooperative of 230 growers.

4. R.R ChyauUdhyog, Lagankhel, and Lalitpur: Grows *Agaricusbisporus*.

5. Center for Agricultural Technology (CAT), Imadol, Grarko, Lalitpur; Produces spawns, grows mushrooms and provides training for cultivation to the growers.

6. Tika Ram Aryal mushroom production, Pokhara; Grows *Pleurotus* and *Lentinula* (Shiitake). It distributes near about 15,000-20,000 of plugged bottles of *Pleurotus* and 1500 to 3000 bottles of Shiitake. It also provides training for mushroom production in Pokhara.

2.3 Taxonomic information

'Chyau' (Chiyau= to peep) in Nepal, 'Mmhukam/ Bammhukan' in Newari and "Shyamo, Shyamu" (Shya= meat) in Tamang and Sherpa languages denote generally for all species of mushrooms. (Shing = wood in Tamang; Shin = Newari; Shi = wood, Take = mushroom in Japanese (Adhikari, 2014).

Recently (Kirk *et al*., 2008; Kuo, 2012) "*Dictionary of fungi"*classified Basidiomycota (ba, sidi, omi, koota) into *Agaricomycotina, Pucciniomycotina* and *Ustilaginomycoes Agaricomycotina* includes *Agaricomycetes,* which embraces the order like *Agaricales* and *Polyporales.* The order *Agaricales* comprises of the families like *Agaricaceae, Lyophyllaceae, Pleurotaceae* and *Marasmiaceae*. The order *Polyporales* includes the family *Ganodermataceae.*

The molecular phylogenetic investigations have bought drastic changes in systematic position and nomenclature of fungal taxa. Morphologically similar taxa are placed to morphologically quite dissimilar families, genus and species (Adhikari, 2014). All the information incorporated here is adopted after Adhikari (2014).

All the species cultivated in Nepal fall in the order *"Agaricales*" and "*Polyporales*" of the class *Basidiomycotina. Ascomycota* are not grown in Nepal. The family *Agaricaceae* Chevall (1860) includes the genus *Agaricus* L. (1753), while the family *Lyophyllaceae*

includes *Calocybe* (Khuner & Donk, 1962). The genus *Pleurotus* (Fr.) Kumm, (1871) has been included in the family *Pleurotaceae* and the genus *Lentinula* Earle (1909) has been placed under the family *Mrasmiaceae* (Adhikari, 2014). The genus*Lentinula* Fr. (1825) and *Ganoderma* P. Karst, (1881) have been placed under the family *Polyporaceae*.

The concept on edibility of the taxa are adopted from the following literature like; Lincoff (1981), (Chaumaton *et al*., 1985),(Imazeki *et al*., 1988), (FAO, compiled list published as edible species of different countries), (Courtecuisse & Dunem, 1994), (Courtecuisse, 2000), (Phillips, 2006), (Okuzawa, 2007) and (Eyssartier & Roux 2011).

The common names are provided in English and Nepali (Nepali, Tamang and Newari) are adopted after Adhikari (2000, 2007-12, 2014). Similar names are seen in common languages to denote different species.

Agaricus bisporus (Lange): Imbach-Cultivated mushroom, Buttom mushroom, Gobre chyau, Dalle Chyau. *Agaricus bisporus*is available in various vegetable markets of Nepal especially in Kathmandu (Singh, 1966; Adhikari, 1987; Bhandary 1985; Pandey & Budhathoki, 2002). It is distributed in Europe, Japan, China, N.America, India and Nepal.

Ganoderma lucidum Curt: Fr.) Karst. - Panacea polypore, varnished conk, Ling club, Ganoderma luisant, Lingzhi, Reishi, Dadu chyau, Dhishyamu (Adhikari & Pandey 1985). Kanchatak reported from Kenja Likhu khola (Ryv., 1979); on rotten trunk, Bakhri Kharka (north of Pokhara) (Balfour-Browne, 1968); on tree trunk, Lele (Kathmandu valley) (Singh & Nisha, 1976) and on trunk of *Rhododendron 20ipette20o*and *Quercus*, Manichur (Adhikari, 1988); on stump between Seti khola Bagar and Agra goan (Bajhang District (1700m); Adhikari, 1988); in root crevices of stump, Phulchowki (1800m); on tree stump (Thapa, 1990); Suryvinayak (1540m) (Adhikari *et al*., 1996; Adhikari & Durrieu, 1996); very common in *Dalbergia sissoo* and *Acacia catechu* plantations of Tarai belts (Hetauda, Chitwan,Bara, Parsa, Rautahat, Siraha, Saptari, Dhanusha, Mahotari, Udayapur, Rajbiraj etc. (between 70 and 500m), found infecting mango plantations. Daman (Adhikari & Manandhar, 2004); Shivapuri, (HRB &NHM, 2005); on *Quercus semecarpifolia* trunk, Thulakharka, Lumle (2190 m; (Devkota, Tiwari, Manandha & Adhikari, 2005); on dead stumps and logs of *Dalbergia s*issoo and *Acaccia* sp., Lumbini (Adhikari *et al.,* 2006); place not metioned (Pandey & Budhathoki, 2002); listed as ornamental (Pandey *et al.,* 2006); Nagarjoon (Pandey, 2008); Phulchowki, Chitlang, Suryabinayak (Adhikari *et al.*, 2011) Saljhandi to Peepaldanda community forest Lumbini (Aryal, Budathoki & Adhikari, 2012); on rotten trunk, Karhiya community forest, western Terai, (Aryal & Budathoki, 2012); on *Bombax ceiba* trunk, Sankarnagar Community forest and Rupandehi (Aryal & Budhathoki, 2013). Recently cultivated (exotic and indigenous strains) in Nepal. The distribution of *Ganoderma lucidum* is

worldwide. Wide range of hosts and wide spread in between tropical and temperate belts of Nepal (Adhikari, 1996a; Parajuli *et al.*, 1999ab).

Lentinula edodes (Berk) Pegler: [= *Lentinus edodes* (Berk) Singer]-Shiitake, Mirge Chyau, Tangi Shyamo. *Lentinula edodes* cultivated are sold in various markets of Nepal. *Lentinula edodes* were found cultivated in various places of Nepal (Bhandary, 1985; Adhikari, 1987; Adhikari & Durrieu, 1996); Shivapuri, Daman (Cotter, 1987), cultivated at Godawory (Adhikari & Manandhar, 1993b). However, substrate and districts are not mentioned, listed as edible in Chritensen *et al*.(2008); recently gathered from Phulchowki forest and Crystal hotel , Lele (NHM, 2011). *Lentinula edodes* is distributed in Japan, China, India and Nepal (Adhikari, 2012).

Lentinus sajor-caju(Rumph: Fr.) Fr**.** [*Lentinus sajor-caju* (Fr.) Fr.][**=** *Pleurotus sajor-caju* (Rumph.) Fr.; *Pleurotus sajor-caju* (Fr.) Singer]– Kande chyau, Kanya chyau, Parale chyau, Marmo shyamo Bauddha Nath, cultivated (Cotter, 1987) growing on *Quercus* tree, Phulchowki (collection- Adhikari, 2012). Distribution of *Lentinula sajor- caju* is in Japan, China, N. America, India and Nepal (Singh & Upadhya, 1978).

Pleurotus djamor(Rumph;Fr.) Boed: (= *Pleurotus flabellatus* Berk and Br.; *Pleurotus ostreatus* (Berk & Br.). This species has been recently cultivated in Kathmandu valley. It is distributed in Europe, Japan, N. America and Nepal (Kaneko *et al*., 1996).

*Pleurotus eryngii(*DC. Fr.): Quell. Spawn imported from China and cultivated in Nepal. It is distributed in Europe, Africa and most of countries of Asia except Korea and Japan. Wide spread in temperate, subtropical and tropical zones (Pandey, 1976).

Pleurotus ostreatus(Jacq.Fr.): Kumm**-**Oyster, Kande chyau, Kanne chyau, Kanya chyau, Pate chyau, Parale chyau, Marmo shyamoNagarjoon (Pandey, 1976); market and Manichur (Adhikari, 1976; Adhikari & Adhikari, 1996-97; Adhikari & Durrieu, 1996); Gorepani (Bhandary, 1991); listed as edible in Pandey *et al.* (2006); Asan and Shivapuri. (HRB, NHM, 1991); listed as edible in Chritensen *et al*. (2008); growing on Quercus on wood log, Phulchowki, (Adhikari, 2012) and on log, Manaslu area. Culture spawn bought from abroad and cultivatedin Nepal. *Pleurotus ostreatus* is distributed in Europe, Japan, China, N.America, India and Nepal (Bhandary, 1987).

Calocybe indica: Dudhe chyau recently cultivated in Kathmandu valley. Spawn bought from India and it has not been reported from Nepal(HRB, NHM, 2012).

2.4. Brief phytogeography of Kathmandu valley

Kathmandu is the capital city of Nepal.The valley includes Lalitpur, Bhaktapur and Kathmandu districts. Kathmandu valley lies between 27°34'- 27°46'N latitude and 85°10'- 85°52'E longitude with its unique physiography (altitude ranging between 1350 – 2760m) covering an area of about 650 sq.km. The valley is drained by the rivers Bagmati, Bishnumati and their tributaries. The valley is surrounded by mountains and peaks ranges (Siwaipuri, 1910m; Nagarjun, 2,500m; Pulchowki, 2,765m; and Chandragiri, 2,220m) which is surrounded with scenic natural beauty (ever green oak-laurel forest) contain different religious areas and picnic spots of botanical interest (Singh &Nisha, 1976).

It is characterized by typical monsoon climate with rainy summer and dry winter. Pre-monsoon during March to May is mostly dry and warm during this season blows dusty winds. At the end of the season thunder storm and rain occurs. During monsoon period from early June and ending by late September mostly raining. Start of September to November sunny days. During winter from January to February it receives little bit shower of rain (Malla et *al*., 1986).

According to the record (2015) of the Meteorological Station, Kathmandu, the maximum temperature on August reached 33.52°c, while the average minimum temperature on January was 1°c. The average precipitation was highest during July (1392.9mm) whereas lowest precipitation was recorded during December (2.3mm).

Kathmandu valley has mixed type of vegetation due to diverse altitude (1350 – 2760m) and climatic conditions. The area (Nagarjun, Shivapuri, Manichaur, Nagarkot, Suryavinayak, Phulchowki, Lele, Dakshinkali and Chandragiri) surrounding the valley consists of subtropical to temperate type of forests. The sub- tropical vegetation predominates at lower elevation, while temperate forest species dominates towards the top of mountains.*Schima-Castonopsis* is found on the floor of the valley, while *Pinusroxburgii* predominate on the lower hills slopes.*Quercussemecarpifolia*and *Quercuslanata*predominate at upper region.

Hence, a lot of potential mushrooms grow naturally (see Adhikari, 2014) in different forest from tropical to Alpine region. For few examples; *Cortinariuscallisteus* is found in Pine and *Quercus* mixed forest of Chandragiri and Thankot(2,220m). *Gymnopilusspectabilis* grows on soil in root crevice of *Graveliarobusta* tree in open moist place, of Thankot (1,450m). *Pezizapetersii* is found growing in *Quercussemecarpifolia* forest of Chandragiri (2220m). *Psathyrellahydrophila* grows on decayed stump of mixed forest at Kakani (2040m) (Adhikari, 1986).

2.5. Pesticide condition in Nepal

Nepalese society in terms of pesticide related health expenses, environment pollution, crop losses due to pest resurgence and spending extra costs both to farmer and country as whole (Thapa, 2003).

The chemical pesticides introduced first time in Nepal was DDT and Pyrethrin in 1950 from USA exclusively for Malaria control for Gandaki hydropower project(Manadhar*et al.,* 2000). Subsequently, in November 1952, DDT became the chemical pesticide to be introduced in Nepal by Ministry of Health / His Majesty's Government of Nepal this marked the introduction of pesticides in Nepal (Wieland, 2009). Not only this but also in 1955, Paris green, Gammexene and Nicotine sulfate were imported for the same purpose of eradicating malaria (G.C, 2011).The different groups of pesticides introduced in Nepal in chronological order are as: Organochlorines – 1950s; Organophosphates-1960s, Carbamates-1970s, Synthetic pyrethoids – 1980s (Manadhar*et al*., 2006).

In Nepal the principal pesticides used are Parathion-methyl,Carbofuran, Malathion, Fenitrothion, and Demeton-methyl (Thape, 2003). In Nepal chlordane and Heptachlor (for use only in termite control) and DDT are banned (Annual report of Agriculture, 2010). Basicallyfor Pesticide tests sulphur and chlorine tests are done. Under chlorine tests, Alpha lindane, Beta lindane, Gama lindane, Delta lindane,Heptashor,Aldrin, Heptachlor, Epoxide, Gama-Chordane, Endosulfan II, Alpha-chlorden, P,P'- DDE, Dieldrin, Endrin, Endosulfan II, P,P'- DDD, Endosulfan sulfate, P,P' DDT, Endrin ketone and Methyoxychlor are checked (DFTQC, 2015). In Nepal "Oranochlorines" are the mostly used insecticides. Most of the insecticides of this group possess an innate capacity to persist for longer period in the environment. Coupled with their solubility in fats and liquids, their use in agriculture always accentuates the risk of accumulation of traces of these insecticides in the adipose tissues of mammals when they consume contaminated foods (Manadhar*et al*., 2006).To evade the health hazard caused by chlorinated hydrocarbons, most of the countries have given up their use. But some insecticides of this group are still in use in the South East Asian Region including Nepal (Plant Protection Directorate, 2010).

From various researches done they suspected that mushroom have been mixed with various pesticides in it such as Nuvan (2ml/L: on bed), Ethyl alcohol, Formalin (0.2 ml/L– 2ml/L, 500 ppm- before and after), Derosal (0.5 gm/L– 2ml/L), Diathane (0.25), Malathion (2ml/L:casing), Entophil (0.15ml/L), Bovistan (0.15ml/L), NP Carbodazin, Bovistin, Nemagon, Thionazin [Reference – Anonymous (2069) Byabasai Kit

Nirdeshanalaya, HariharBhawan; (Bastola, 2073),*Byabasaichyaukheti*; (Raut, 2070),*Aadhunicchyaukhetiprabidhi*; (Neupane, 2068),*Nepalmachyaukheti*] most of them are highly poisonous.

However, till date none has tested pesticides in cultivated mushrooms available in Nepal [District Agriculture Development Offices (DADOs), 2014].

CHAPTER III

MATERIALS AND METHODS

3.1. Study area

Lalitpur and Kathmandu districtsof Central Development region of Nepal were selected as the study area as these districts consumed a lot of Mushroom. The cultivated sample were collected and carried to Central Department of Biotechnology (CDBT), Tribhuvan University for laboratory work.

3.2. Sample collection

In Nepal manyspecies of mushrooms are cultivated such as [*Agaricus bisporus, Flammulina velutipes, Ganoderma lucidum, Lentinula edodes, Lentinus sajor-caju, Pholiota microspora, Pleurotus erygii, Pleurotusostreatus* and *Pleurotus djamor*]. But only few of them are sold in markets.

Among these cultivated mushrooms (*Agaricus bisporus, Pleurotusostreatus, Pleurotus djamor* and *Lentinula edodes*) were collected from vegetable markets such as Asan, Balkhu, Kalimati and Lagankhel. From four vegetable markets wet samples of*Agaricus bisporus, Pleurotus ostreatus, Pleurotus djamor* and *Lentinula edodes* were collected early in the morningin plastics bags during November & December, 2015.

3.3. Place of laboratory work

After collection of samples from vegetable markets, they were directly bought to CDBT laboratory for moisture determination. Calculation of Protein, Fat, Crude fiber, Ash, Carbohydrate, Calcium and Iron were done inCentral Food laboratory (DFTQC), Babarmal. Determination of Phosphorus was done in laboratory of Nepal Bureau of standard and Metrology Balaju. Pesticide tests were done in National Forensic Science Laboratory (NAFOL).

3.4. Methodology

3.4.1 Determination of Moisture

Moisture calculation was based on American Society for testing and materials in publication ASTMD 4442 (2010). 100gm of mushroom was taken in Petri- dish and kept in hot air oven at 60°c for about 9 hours. Then it was cooled in desiccator and weight was taken. The samples were heated again in the oven for about one hour and the process was repeated, till a constant weight was obtained. The moisture content was

calculated by reducing weight of fresh mushroom samples before drying and weight of dried sample divided by weight of sample multiplied by 100.

3.4.2. Determination of protein by Micro Kjeldahl's method.

All the materials, reagents used were from HiMEDIA Company 2014 and they are listed in appendices alphabetical order A to L.

The protein determination involves three steps which were based on Micro Kjeldahl method, (2004).

3. 4.2.1 Digestion

0.5g dried mushroom was taken in the digestion flask. To this 2.5g of the digestion mixture (copper sulphate and potassium sulphate (1:5) and 15ml of concentrated sulphuric acid was added.The solution was heated for 2 hours at 400°c until it became clear and glass beads were added in order to prevent bumping. It was then cooled. After the digestion was completed, the tubes were allowed to cool down until the distillation process.

3.4.2.2 Distillation

The distillation apparatus used was an automatically operated one.The tubes were placed in the distillation chamber. Distilled water and 40% NaOH required for the distillation were stored in gallons and connected to the distillation unit. A program was set in the distillation unit. 25ml of boric acid solution was taken and placed at receiving end of the unit. The distillate (NH_3) obtained in the flask containing boric acid, was then titrated.

3.4.2.3 Titration

The Boric acid having trapped ammonia was titrated with 0.1 N Sulphuric acid using methyl red as indicator. The color of boric acid having ammonia changed to pink. The percent of protein was calculated by

$$\% \text{ protein} = \frac{(V_1 - V_2) \times N}{W} \times \frac{14 \times 6.25 \times 100}{100 - mc}$$

Where,

V_1 = Sample titrant V_2 = Blank titrant

N = Strength of H_2SO_4 6.25= Conversion factor

14= Molecular weight of Nitrogen W = Weight of sample

$\frac{100}{100-mc}$ = dryweight *mc*= moisture content

3.4.3. Determination ofFat

Fat determination based on protocol of Food Safety and Inspection Service, office of public health (2009) of India. The dried sample was taken and crushed. 2.0g of the sample was taken in the paper thimble and connected to a Soxhlet extractor (Cat 09-551B, Fisher). 150ml of petroleum ether was poured on the flask and refluxed for 8 hours with a heating mantle. Crude fat was extracted on the flask kept over for 2hrs. The flask was cooled in a desiccators and the weight was taken. The percentage of crude fat content was determined by weight of flask with fat residue minus weight of empty flask divided by weight of original sample multiplied by hundred.

3.4.4 Determination of Crude Fiber

Crude Fiber in mushroom was determined based on Food Analytical methods, (2009) Volume 2, pp 110-115. Two and half grams of the defatted mushroom sample (W) was taken in a beaker and boiled in 200ml of 1.25% sulphuric acid for 30 minutes. The content was then filtered and washed with distilled water to neutralize the content. The content was transferred again to the beaker and boiled in 250ml of 1.25% sodium hydroxide for 30minutes. The crucible having crude fiber was weighted (W_1) and crucible was set for heating at 550°c for 3 hours and weight was taken (W_2). The percentage of crude fiber was determined from the difference in the weight (W_1-W_2) divided by weight of sample multiplied by 100.

3.4.5. Determination of Ash

Ash determination was based on Journal of AOAC International 923.03 (2000). Firstly, 5 g dried mushroom sample taken in a crucible. The sample charred over a low flame 80°c for 3 hours and kept in a muffle furnace set at 550°c for 5 hours until white ash was obtained. The crucible was taken out and kept in dessicator and weighted. The percentage of ash was calculated as weight of sample before heating in muffle furnace minus weight obtained afterash formed divided by weight of sample taken multiplied by 100.

3.4.6 Determination of Carbohydrate

Determination of carbohydrate was based proximate composition, phytochemical screening and antioxidant activity of ten selected wild edible Nigerian mushrooms(Egwin *et al*., 2011). The percentage of carbohydrate content in mushroomwere determined by taking sum of percentage of Protein, Crude Fat, Crude Fiber and Ash content subtracted from hundred percentage.

3.4.7 Mineral estimation

Sample preparation for Iron and Calcium were based on the Optimised protocol of DFTQC, (2014) which followed Food Analytical Methods published in 2009. Total Ash was taken for the analysis of mineral contents. Two ml of conc. HCl was added to the ash and heated for 2 minutes. One drop of hydrogen peroxide was added into the solution to remove turbidity. The solution was then transferred into a volumetric flask and total volume was made 100ml by adding deionized water. This was then used to analyze the contents of Iron (Fe), and Calcium (Ca).

3.4.7.1 Phosphorus estimation

Phosphorus determination was done based on AOAC962.02 (2014) by Gravimetric method. The sample preparation was done by taking one gram of sample dissolved in 30ml conc HNO_3 and 5 ml conc.HCl and then boiled until organic matter was destroyed. It was then cooled and filtered by whatman 41 filter paper. An aliquot of the filtrate (containing not more than 20mg of P_2O_5) was 28ipette into 500ml conical flask diluted with 100ml of distilled water. 30ml of citric molybdic reagent was added and boiled gently for 30 min and was removed from heat and swirled carefully. Then 10ml of quinolone solution was added immediately with continuous swirling first 3 to 4ml drop wise and then steady stream. Then, it was cooled to room temperature, swirled carefully 3 to 4 times during cooling and passed through Gooch filter with paper previously dried at 250°c and weighed and washed five times with 25ml of H_2O. The residue was kept in hot air oven at 550°c for about 3 hours. It was cooled in desiccators, weighted and calculation was done as the total phosphate (P_2O_5)

$$\% (P_2O_5) = 3.207 \times \frac{\text{(mass of residue with sample – Mass of residue obtained in blank)}}{\text{Mass of prepared sample present in the aliquot taken}}$$

Where, 3.207= Conversion factor

3.4.7.2 Iron estimation

Iron determination was based onmineral contents in *pleurotus* (oyster mushroom): association ofcookingmethod(Parisa *et al*, 2014) by using AAS.An amount of 5g homogenized sample was dried inhot air oven at 105°c for 3 hours.The dried sample was next charred until it ceased to smoke. The charred sample was then ashed in a muffle furnace at 550°c until whitish or grayish ash was obtained. The ash was treated with concentrated hydrochloric acid, transferred to a volumetric flask and made up to 50ml. For each mushroom sample studied, two ash solutions were prepared i.e duplicate analysis carried out. An aliquot of each ash solution was used for the determination of Iron by AAS method. For analysis of AAS method, Flame Atomic Absorption Spectrometer (Perkin-Elmer model 175) and wave lengthwas set to 248.3 nm. Ferric nitrate solution was used as standard to prepare a standard calibration curve of Iron within the analytical range. Concentration of Iron in test solution was calculated from the standard curve prepared. For each ash solution, at least three readings were taken and the average was calculated.

3.4.7.3 Calcium estimation

Calcium determination was based on Nutritional Metals in Foods by AAS (Mary Millican, 2010). First of all ash solution was prepared as done for iron determination then an aliquot of each ash solution was used for the determination of calcium by the AAS method.For analysis of calcium, Agilent Technologies 200 seriesPerkin-Elmer model 175was set to wavelength 422.7nm.Calcium Carbonate was used as standard to prepare a calibration curve.To eliminate phosphorus interference in course of calcium determination Lanthanum carbonate solution was used. Concentration of calcium in test solution was calculated from the standard curve obtained. For each ash solution, at least three readings were obtained and the average was calculated.

3.4.8.0Sample collection for pesticide (Feb & May, 2016)

Hari Manandhar grew most of the commercial mushrooms in his farm located in Kritipur(Nagaun) that are supplied to various vegetable markets such as Vegetable market of Kalimati and Balkhu. *Agaricus bisporus, Pleurotus ostreatus* and *Lentinula edodes* were collected in Febrary and May. The samples were collected in plastic bags and brought to CDBT laboratory. Mushrooms were dried in hot air oven at 55°c for about 9 hours. After the constant weight was observed, the mushrooms were powdered.

3.4.8.1 Extraction preparation

Pleurotus ostreatus, Agaricus bisporus, Pleurotus djamor and *Lentinula edodes* powder extracts were processed in National Forensic Laboratory in Khumaltar. Extraction procedure was based on optimized QuEChERS mini-multi-residue method, (2015). Two

grams of powered mushroom extract was mixed with 10ml of water. Next day 5N NaOHand 300µl Calcium was added further 10 ml of Acetonitrile was added and shaken by hand for about 1 minute. 4gm $MgSO_4$ and 1gm NaCl was furtheradded and shaken for 1 minute. Tube was centrifuged at 3500rpm for 5 minutes. Aliquot was taken and 150mg anhydrous $MgSO_4$ was added. 50mg primary secondary amines (PSA) were added per ml of extract. Again, Tube was shaken for 30s and centrifuged at 3500rpm for 5minutes. An aliquot was taken and acidified to P^H- 5 with 5% formic acid in Methyl cyanide. The labeled nicotine was added here assuming 1gm sample per extract. Extract was transferred into clean tube and analysis was done via Gas Chromatography Mass Spectrometry.

3.4.8.2 Gas Chromatography Mass Spectrometry (GC/MS) analysis

GS-MS analysis of the mushrooms was performed using AOAC 20i QC 2010 plus model. For GS-MS detection; electron ionization energy of 70eV was used. Helium gas (99.99%) was used as the carrier gas constant flow rate of 1ml/min and an injection volume of 2µl wereemployed (split ratio 1:0). Injection temperature and Ionsource temperature were set to 240°c and 200°c respectively. The oven temperature was programmed from 120°c (isothermal for 2 min), with an increase of 10°C / min to 200°Cthen 5°C / min to 240°C.Mass spectra were taken at 70eV; a scan interval of 0.30 seconds and fragments from 35 to 400 Da. Total GC runningtime was 25 minutes. The relative percentage of each component was calculated by comparing its average peak areas to the total areas. The peak available were divided into two phases which means peaks obtained in the month of Febrary and Second phase means pesticide tests done by taking samples from month of May 2016.

CHAPTER IV

RESULTS

4.1 Nutrient screening findings

Table 4.1.1 Nutrient status of cultivated mushrooms bought from Asan market (Source; Chapagau)

Nutrient Content	**Name of Species**				
	Unit	*Lentinula edodes*	*Pleurotus ostreatus*	*Agaricus bisporus*	*Pleurotus djamor*
Moisture	%	88.13	87.7	90.69	86.6
Protein	%	23.0	24.8	45.0	23.0
Fat	%	3.1	2.9	4.2	2.9
Ash	%	11.1	9.18	6.2	10.3
Fiber	%	11.1	13.9	16.6	14.2
Carbohydrate	%	51.7	49.22	28.0	49.6
Phosphorus	mg/100 g	520	307	117	613
Iron	mg/100 g	48	17.24	7.68	56
Calcium	mg/100 g	89.18	157.68	56	47

Table 4.1.2 Nutrient status of cultivated mushrooms bought from Kalimati market (Source; Balembu, Chapagau, Kritipur)

Nutrient Content	**Name of Species**				
	Unit	*Lentinula edodes*	*Pleurotus ostreatus*	*Agaricus bisporus*	*Pleurotus djamor*
Moisture	%	85.6	88.06	91	86
Protein	%	26.32	27.2	46.3	25.8
Fat	%	3.4	3.2	4.0	3.3
Ash	%	10,9	11.2	12.1	16.8
Fiber	%	12.3	11.6	12.1	16.8
Carbohydrate	%	47.09	46.8	30.3	43.6
Phosphorus	mg/100 g	769	307	153	667
Iron	mg/100g	52	21	8.7	33
Calcium	mg/100 g	93	153	59	47

Table 4.1.3 Nutrient status of cultivated mushrooms bought from Balkhu market (Source; Kritipur, Balembu).

Nutrient Content	**Name of Species**				
	Units	*Lentinula edodes*	*Pleurotus ostreatus*	*Agaricus bisporus*	*Pleurotus djamor*
Moisture	%	88	89	93	91.7
Protein	%	28.2	24.4	43.7	23.7
Fat	%	3.0	2.8	4.0	2.4
Ash	%	16	9.0	7.0	9.0
Fiber	%	12.9	16.9	16.0	15.4
Carbohydrate	%	39.9	46.9	29.3	49.5
Phosphorus	mg/100 g	812	318	222	740
Iron	mg/100 g	44	24	6.07	36
Calcium	mg/100 g	97	158	56	49

Table 4.1.4 Nutrient status of cultivated mushrooms bought from Lagankhel market (Source; Chapagau, Bhaktapur)

Nutrient Content	**Name of Species**				
	Units	*Lentinula edodes*	*Pleurotus ostreatus*	*Agaricus bisporus*	*Pleurotus djamor*
Moisture	%	93	85	93.3	88
Protein	%	25.0	21.0	41.7	24.0
Fat	%	4.2	2.6	4.0	3.0
Ash	%	10.3	18.3	7.0	11.0
Fiber	%	11.9	14.2	11.7	12.0
Carbohydrate	%	48.6	43.9	35.6	50.0
Phosphorus	mg/100 g	837	310	100.7	802
Iron	mg/100 g	9.8	23	7.63	12.43
Calcium	mg/100 g	101	156	60.13	45

4.2 Findings

A. First phase

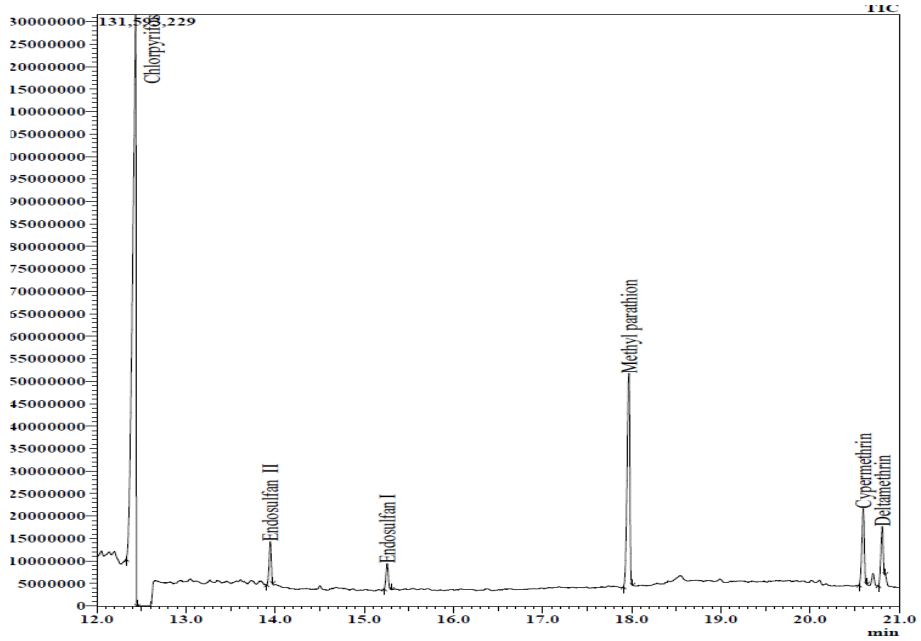


Figure 4.2.1 GC-MS analysis showing control: Peak 1 Chlorpyrifos, Peak 2 Endosulfan II, Peak 3 Endosulfan-I, Peak 4 Methyl Parathhion, Peak 5 Cypermethrin and Peak 6 Deltamethrin.

Peak	R.Time	Area%	Name of compound	Base m/z
1	10.834	0.82	Chlorpyrifos	73.05
2	13.275	6.32	Endosulfan II	55.05
3	13.547	1.87	Eddosulfan I	71.10
4	15.123	94.07	Methyl Parathion	67.05
5	16.513	0.64	Cypermethrin	56.05
6	5.89	0.21	Deltamethrin	43.0
	Total	100.00		

Table 4.2.1.1 Peak reports showing Retention time (R.Time), Area %, Name of compound and Base (m/z) in control sample.

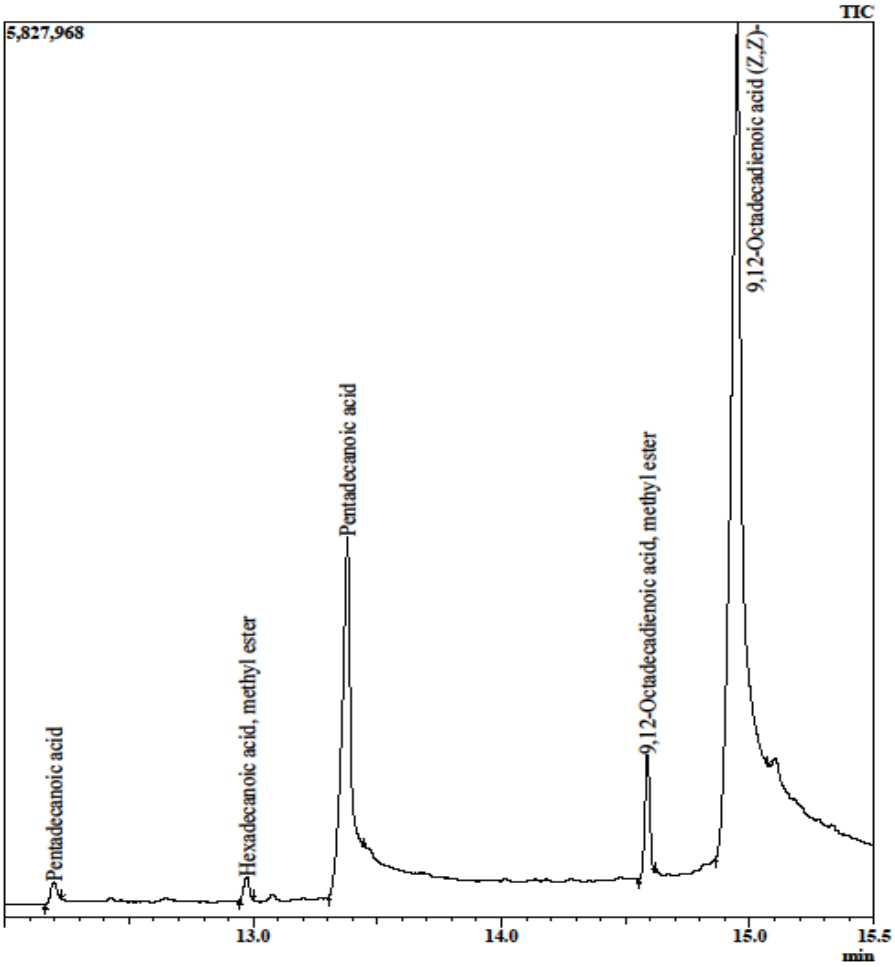


Figure 4.2.2 GC-MS analysis of *Lentinula edodes*: Peak 1 Pentadecanoic acid, Peak 2 Hexadecanoic acid, methyl ester, Peak 3 Pentadecanoic acid, Peak 4 9,12- Octadecadienoic acid, methy ester and Peak 5 9,12- Octadecadienoic acid (Z,Z).

Peak	R.Time	Area%	Name of compound	Base(m/z)
1	11.759	0.72	Pentadecanoic acid	43.15
2	11.277	0.32	Hexadecanoic acid, methyl ester	55.00
3	13.983	18.87	Pentadecanoic acid	73.10
4	14.610	0.67	9,12- Octadecadienoic acid	67.05
5	15.15	69.21	9, 12- Octadecadienoic acid (Z, Z).	65.0
	Total	100		

Table 4.2.2.1 Peak reports showing Retention time (R.Time), Area %, Name of compound and Base (m/z) in *Lentinula edodes.*

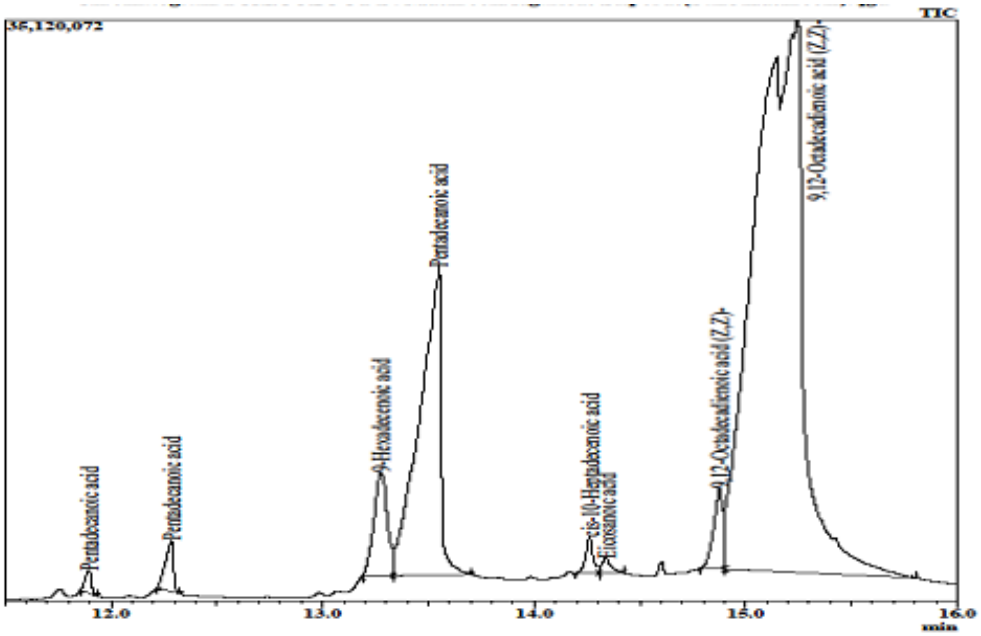


Figure 4.2.3 GC-MS analysis in *Pleurotus ostreatus*: Peak1 Pentadecanoic acid, Peak 2 Pentadecanoic acid, Peak3 9-Hexadecenoic acid, Peak 4 Peantadecanoic acid, Peak 5 Cis-10-Heptadecenoic acid, Peak 6 Eicosanoic acid, Peak 7 9,12- Octadecadienoic acid (Z,Z) and Peak 8 9,12-Octadecadienoic acid (Z,Z).

Peak	R.Time	Area%	Name of compound	Base (m/z)
1	11.759	0.32	Pentadecanoic acid	73.05
2	11.899	0.52	Pentadecanoic acid	57.10
3	13.277	4.87	9-Hexadecenoic acid	149.15
4	18.438	4.23	Peantadecanoic acid	55.05
5	15.15	403	Cis-10-Heptadecenoic acid	37.00
6	14.67	4.87	Eicosanoic acid	132.0
7	13.55	0.48	9,12- Octadecadienoic acid	45.21
8	15.05	69.07	9,12-Octadecadienoic acid	67.05
	Total	100.00		

Table 4.2.3.1 Peak reports showing Retention time (R.Time), Area %, Name of compound and Base (m/z) in *Pleurotus ostreatus.*

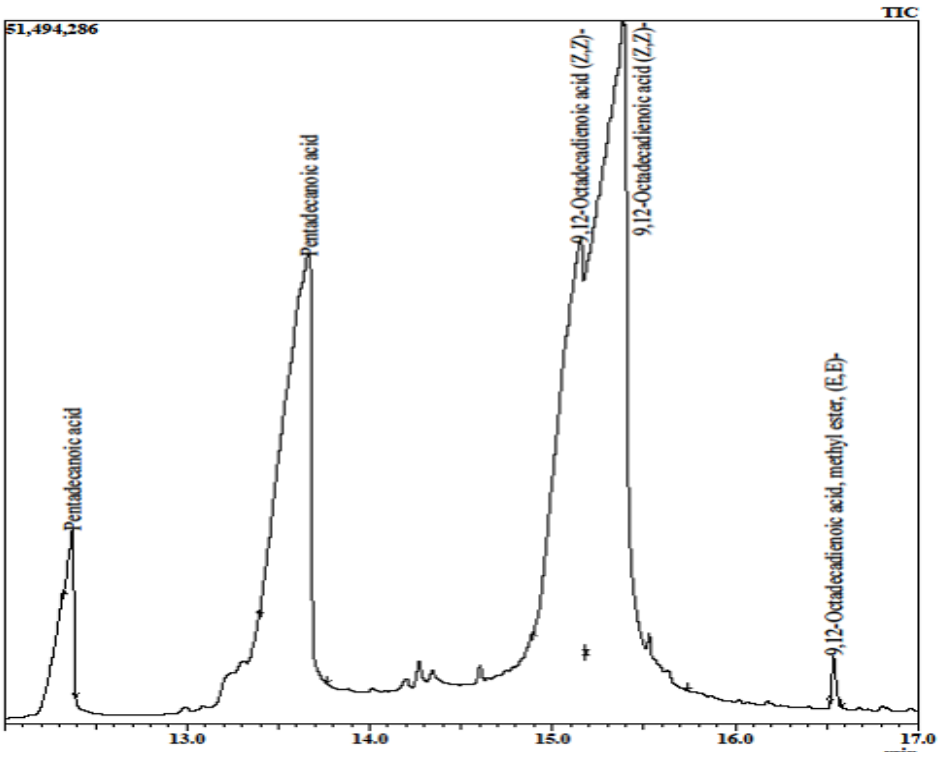


Figure 4.2.4 GC-MS analysis in *Agaricus bisporus*: Peak 1 Pentadecanoic acid, Peak 2 Pentadecanoic acid, Peak 3 9,12-Octadecanoic acid(Z,Z), Peak 4 9,12-Octadecanoic acid(Z,Z), Peak 5 9,12-Octadecanoic acid, methyl ester,(E,E).

Peak	R.Time	Area%	Name of compound	Base (m/z)
1	11.755	0.29	Pentadecanoic acid	43.05
2	11.866	0.11	Pentadecanoic acid	57.05
3	12.372	4.07	9,12-Octadecanoic acid(Z,Z)	73.15
4	19.382	60.28	9,12-Octadecanoic acid(Z,Z),	67.00
5	17.15	4.53	9, 12-Octadecanoic acid, methyl ester,(E,E).	34.10
	Total	100.00		

Table 4.2.4.1 Peak reports showing Retention time (R.Time), Area %, Name of compound and Base (m/z) in *Agaricus*

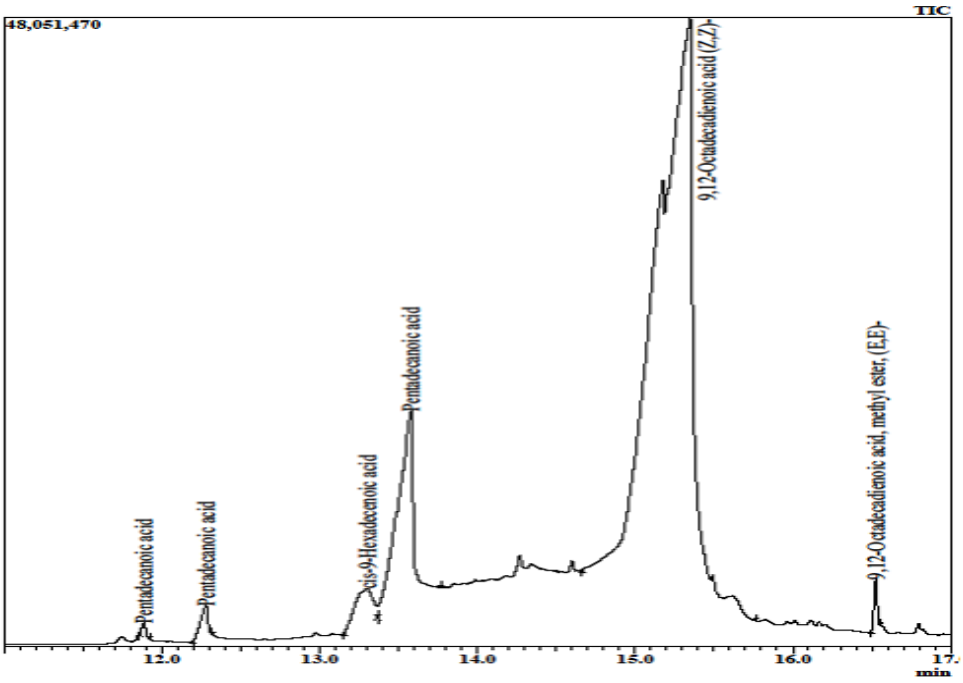


Figure 4.2.5 GC- MS analysis in *Pleurotus djamor*: Peak1 Pentadecanoic acid, Peak2 Pentadecanoic acid, Peak3 Cis-9-Hexadecenoic acid, Peak 4 Pentadecanoic acid, Peak5 9,12-Octadecanoic acid(Z,Z) and Peak 6 9,12-Octadecadienoic acid,methyl ester(E,E).

Peak	R.Time	Area%	Name of Compound	Base (m/z)
1	11.743	0.11	Pentadecanoic acid	43.05
2	13.306	2.15	Pentadecanoic acid	55.05
3	14.372	5.04	Cis-9-Hexadecenoic acid	73.00
4	13.302	10.28	Pentadecanoic acid,	55.00
5	21.15	66.31	9, 12-Octadecanoic acid(Z,Z)	67.10
6	13.00	1.730	9,12-Octadecadienoic acid,methyl ester(E,E)	36.25
	Total	100.00		

Table 4.2.5.1 Peak report showing Retention time (R.Time), Area %, Name of compound and Base (m/z) in *Pleurotus djamor.*

B. Second phase

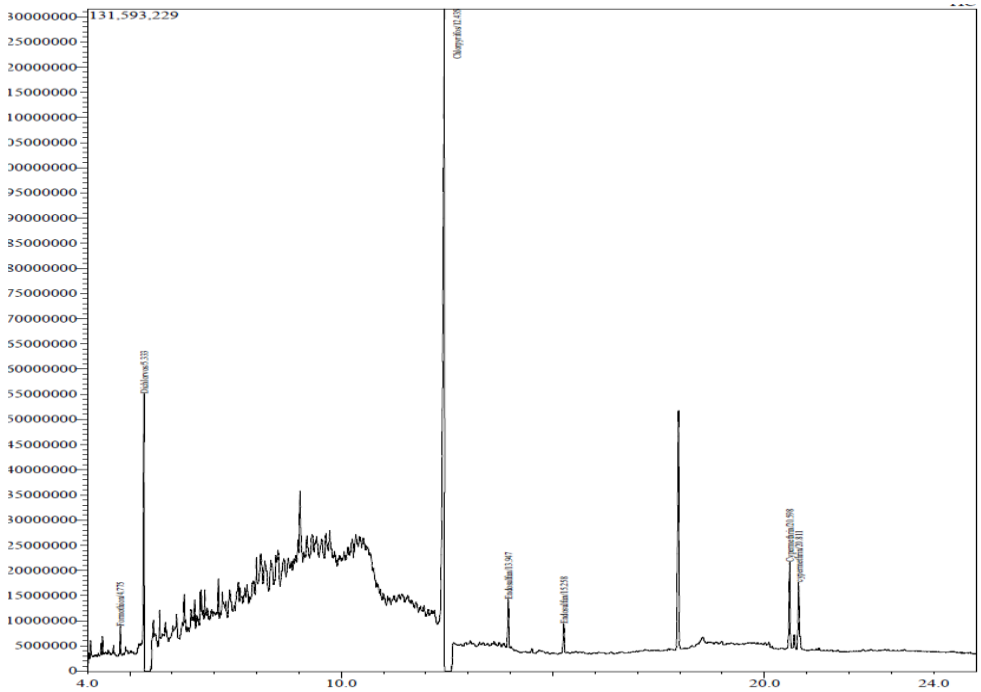


Figure 4.2.6 GC - MS analysis showing in Control sample: Peak 1Formothion, Peak 2 Dichlorvos, Peak 3 Chlorpyrifos, Peak 4 Endosulfan, Peak 5 Endosulfan, Peak 6 Cypermethrin and Peak 7 Cypermethrin.

Peak	R.Time	Area%	Name of compound	Base (m/z)
1	4.775	1.02	Formothion	171.95
2	5.333	11.14	Dichlorvos	109.15
3	12.435	70.17	Chlorpyrifos	96.95
4	13.947	3.22	Endosulfan	194.90
5	15.258	2.12	Endosulfan	194.90
6	20.598	6.55	Cypermethrin	163.00
7	20.811	5.79	Cypermethrin	163.00
	Total	100.00		

Table 4.2.6.1 Peak report showing Retention time (R.Time), Area %, Name of compound and Base (m/z) in control sample.

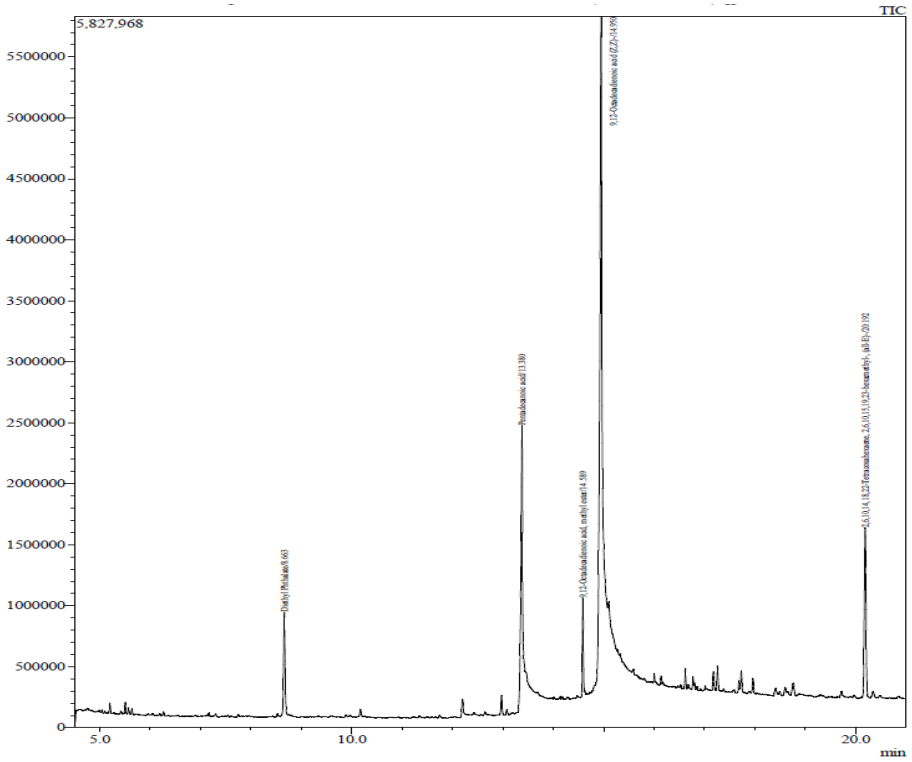


Figure 4.2.7 GC-MS analysis in *Lentinula edodes*. Peak 1 Diethyl Phthalate, Peak 2 Pentadecanoic acid, Peak 3 9,12-Octadecadienoic acid, methyl ester, Peak 4 9,12-Octadecadienoic acid (Z,Z) and Peak 5 2,6,10,14,18,22-Tetracosahexaene.

Peak	R.Time	Area%	Name of compound	Base (m/z)
1	8.663	5.86	Diethyl Phthalate	149.10
2	13.380	18.62	Pentadecanoic acid	43.05
3	14.589	2.21	9,12-Octadecadienoic acid, methyl ester	67.05
4	14.950	61.35	9,12-Octadecadienoic acid (Z,Z)-	67.05
5	20.192	11.95	2,6,10,14,18,22-Tetracosahexaene.	69.05
	Total	100.00		

Table 4.2.7.1 Peak report showing Retention time (R.Time), Area %, Name of compound and Base (m/z) in *Lentinula edodes.*

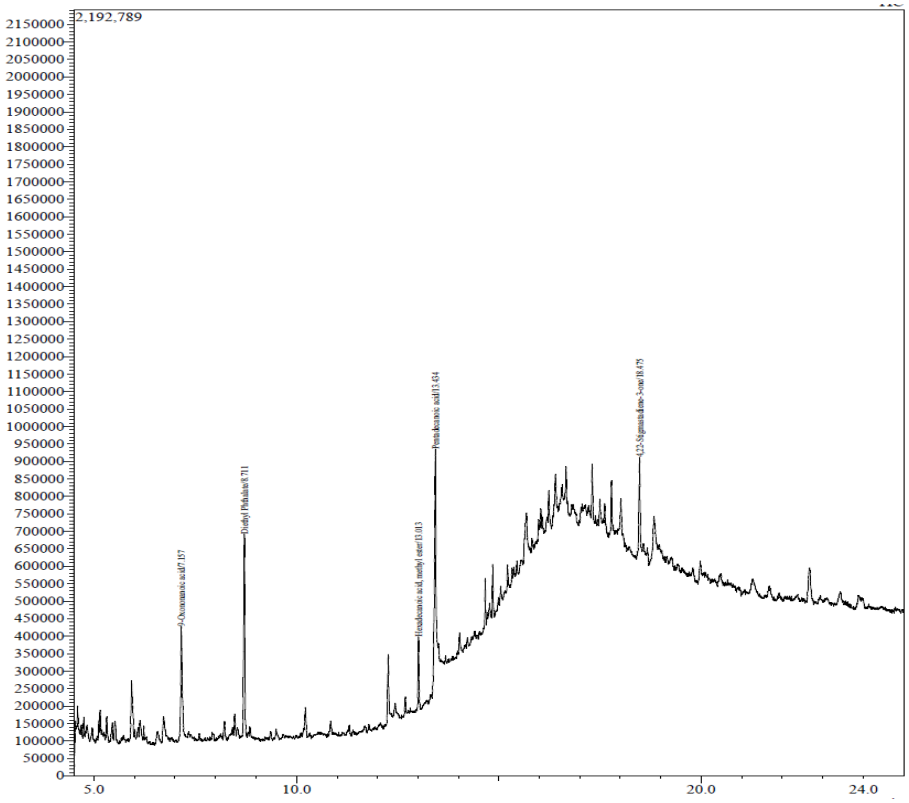


Figure 4.2.8 GC-MS analysis of *Pleurotus Ostreatus*: Peak 1 9-Oxononanoic acid, Peak 2 Diethyl Phthalate, Peak 3 Hexadecanoic acid, methyl ester, Peak 4 Pentadecanoic acid and Peak 5 4,22-Stigmastadiene-3-one.

Peak	R.Time	Area%	Name of compound	Base m/z
1	7.157	17.73	9-Oxononanoic acid	55.00
2	8.711	25.79	Diethyl Phthalate	149.10
3	13.013	8.05	Hexadecanoic acid, methyl ester	74.00
4	13.434	36.00	Pentadecanoic acid	43.05
5	18.475	12.44	4,22-Stigmastadiene-3-one	69.05
	Total	100.00		

Table 4.2.8.1 Peak reports showing Retention time (R.Time), Area %, Name of compound and Base (m/z) in *Pleurotus ostreatus.*

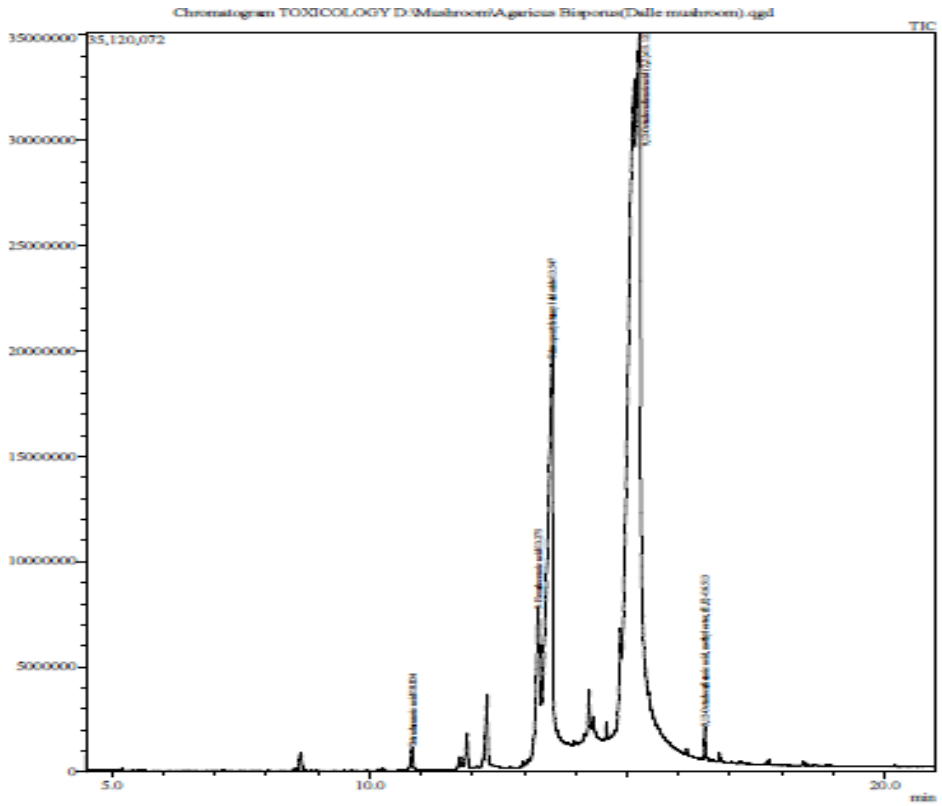


Figure 4.2.9 GC-MS analysis of *Agaricus bisporus*: Peak 1 Tetradecanoic acid, Peak 2 9-Hexadecenoic acid, Peak 3 Trineopentylstannyl chloride , Peak 4 9,12-Octadecadienoic acid (Z,Z) and Peak 5 9,12-Octadecadienoic acid, methyl ester, (E,E).

Peak	R.Time	Area%	Name of Compound	Base(m/z)
1	10.834	0.85	Tetradecanoic acid	73.05
2	13.275	6.32	9-Hexadecenoic acid	55.05
3	13.547	1.87	Trineopentylstannyl chloride	71.10
4	15.123	94.07	9,12-Octadecadienoic acid (Z,Z)	67.05
5	16.513	0.64	9,12-Octadecadienoicacid, methyl ester, (E,E)	67.05
	Total	100.00		

Table 4.2.9.1 Peak reports showing Retention time (R.Time), Area %, Name of compound and Base (m/z) in *Agaricus bisporus*.

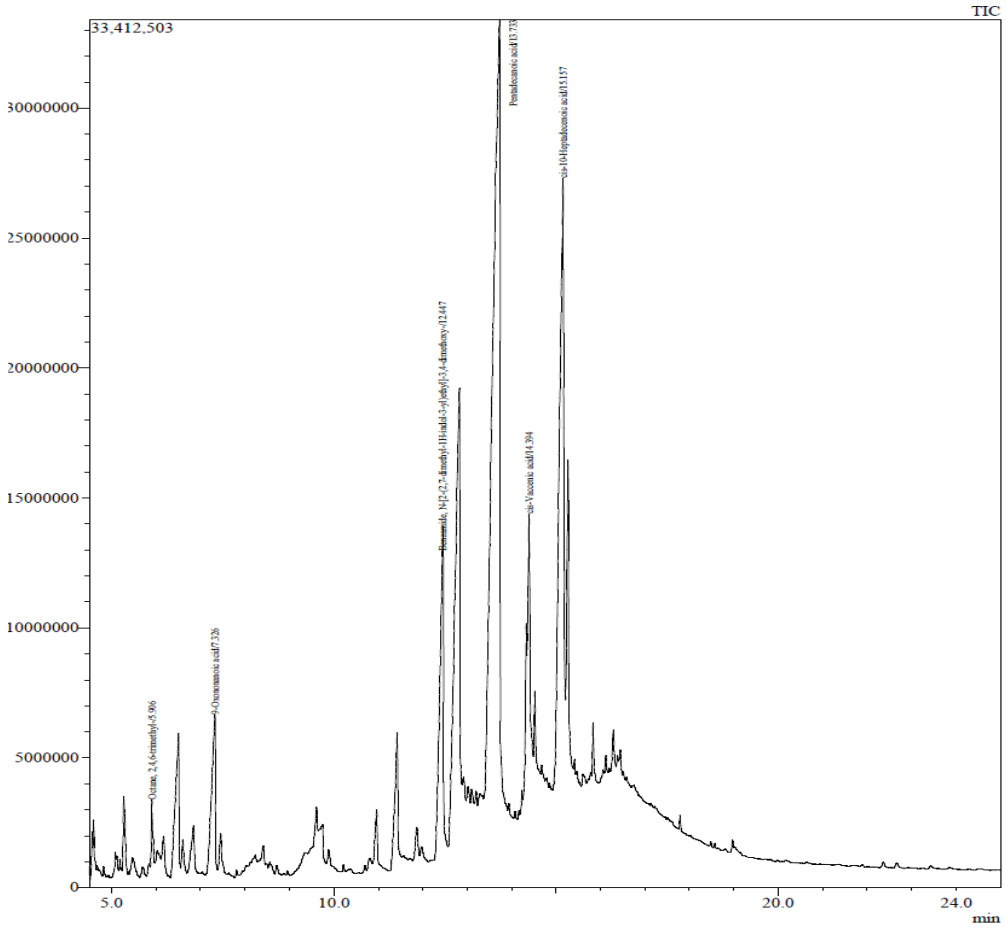


Figure 4.2.10 GC - MS analysis of *Pleurotus djamor* :Peak 1 Octane, 2,4,6-trimethyl, Peak 2 9-Oxononanoic acid, Peak 3 Benzamide, N-[2-(2,7-dimethyl-1H-indol-3-y, Peak 4 Pentadecanoic acid, Peak 5 cis-Vaccenic acid and Peak 6 cis-10-Heptadecenoic acid.

1	5.906	1.00	2,4,6-trimethyl- Octane	57.00
2	7.326	6.71	9-Oxononanoic acid	55.00
3	12.447	0.43	Benzamide, N-[2-(2,7-dimethyl-1H-indol-3-y	171.15
4	13.733	49.25	Pentadecanoic acid	72.95
5	14.394	7.90	cis-Vaccenic acid	55.00
6	15.157	35.57	cis-10-Heptadecenoic acid	55.00
	Total	100.00		

Table 4.2.10.1 Peak reports showing Retention time (R.Time), Area%, Name of compound and Base (m/z) in *Pleurotus djamor*.

CHAPTER V

DISCUSSION

The mushroom screening result of Moisture, Crude Protein, Crude Fat, Crude Fiber, Carbohydrate, Phosphorus, Calcium and Iron has been presented on above tables 1, 2, 3, and 4. All nutrient and minerals contents have been presented on dry basis except moisture content. Comparison of different nutrients among the mushroom samples were made based on mushrooms genera and species because all the four cultivated samples used in the study belonged to different species except *Pleurotus ostreatus* and *Pleurotus djamor.*

5.1 Moisture content

The result of the nutritional values obtained for the studied edible mushrooms were carrrid out in triplicate their results are listed in table 1, 2, 3 and 4.

The moisture content in *Lentinula edodes* was found highest (88.13%) in the sample bought from Asan market and lowest (85.6%) in Kalimati market. These values are not significantly different from those previously reported moisture content in the same species: 78.63% (Yuen*et al*., 2014) to 89% (Reis *et al*., 2011).

The moisture content in *Pleurotus ostreatus* bought from Asan, Kalimati, Balkhu and Lagankhel was 87.7%, 88.06%, 89% and 85% respectively. Moisture content in the similar range (82.7% to 91.27%) has been previously reported by several authors (Garcha *et al*., 2007; Shah *et al.*, 1997; Reia*et al*., 2011; Bandopadhayay., 2013).

The moisture content in the cultivars of *Agaricus bisporus* was 90.69%, 91%, 93%, 93.3% sold in Asan, Kalimati, Balkhu and Lagankhel markets respectively. These values are consistent with those (90.4% to 93.08%) reported in the previous researches (Soni*et al*., 2008), Masamba & Kazombo (2010; 91.6%), Simon *et al*. (2010; 92.7%) and Owaid, (2015; 93.08%).

Pleurotus djamor sold in Asan, Kalimati, Balkhu and Lagankhel market had moisture content of 86.6%, 86%, 91.7% and 88% respectively. Highest moisture content was found in sample from Balkhu market and the lowest was found in the sample from Kalimati market. (Khan *et al*., 2013; Maftoun *et al*., 2015) reported the moisture contents of 84.5% and 82.21% respectively in *Pleurotus djamor*.

The result of moisture content of various mushrooms collected from four vegetable markets of Kathmandu valley ranged between 85 and 93.3%. In comparison of moisture content *Agaricus bisporus* had shown highest moisture content and it was found in the sample bought from Lagankhel vegetables market. Moisture content was similar among *Pleurotus ostreatus, Pleurotus djamor* and *Lentinula edodes*. These results are consistent with the results reported by Ragunathan & Swaminathan (2006), Chang *et al.*(2007) and Bisaria *et al*.(2008). *Lentinula edodes* and *Pleurotus djamor*had less moisture content and among four species collected from four vegetable markets. Mushroom bought from Asan vegetable market contained less moisture.

Mushrooms contain a high moisture percentage depending on the mushroom species and other parameters related to harvest, growth, culinary and storage conditions (Guillamon *et al.,* 2010). The higher moisture content of some of the mushrooms obtained in this work is an indication that fresh mushrooms cannot be kept for longer time, as water activity enhances microbial growth (Aletor, 1995).

5.2Protein content

The highest (28.2%) protein content was observed in Balkhu cultivar and lowest (23%) in Asan cultivar. Variations in the protein content (23.21% to 32.83%,) have been found reported by Chang & Miles(2004),Asadi *et al. (*2014) and Dulay*et al.(*2015) in *Lentinula edodes.*

The protein content in *Pleurotus ostreatus* washighest in the sample bought from Kalimati (27.2%), followed by the samples from Asan market (24.8%), Balkhu (24.4%) and Lagankhel (21.0%). These values were slightly higher than those (15.4% and 16.35%) reported by Garcha *et al*.(2008) & Soni *et al*.(2008), however marginally lower than those (32.31% and 33.31%) reported by Shah *et al.*(1997) and Adejumo *et al*.(2015) in *Pleurotus ostreatus*.

Protein content of *Agaricus bisporus*ranged from 41.7% to 46.3%. The highest protein content was observed in the sample bought from Kalimati (46.3%) whereas least protein content was found inLagankhel (41.7%). Soni *et al*. (2008), by Consensus document (USA 2008), Pushpa &Purushothama (2010), Tekit (2015) and Adejumo & Awosanyn, (2015) reported a protein content of 26.8%. 28.4%, 41.06%, 41.08% and 37%, respectively.

*Pleurotus djamor*collected from Kalimati had maximum protein (25.8%). Lowest protein (23%) was present in the sample collected from Asan market.Edible mushrooms are

good source ofproteins and their protein contents usually range from 21% to 46.3 % Dharmaraj *et al*. (2011, Khan *et al*. (2013; 21.89%), Parah *et al.* (2014; 26.34%).

A wide variability was observed in the protein content based on the type of mushroom species. Among these four species cultivated in Kathmandu valley, the maximum protein content was found in *Agaricus bisporus*, which was almost double the protein content of other species.

The protein contents of mushrooms have been reported to vary according to the genetic structure of species, the physical and chemical differences of the growing medium (Sanme *et al.,* 2003). According to FOA, (2004) the protein content of edible mushroom has been claimed to be twice as that of onion (14%). The protein content found in mushrooms are much more than that of vegetables and fruits (Cabbage, 1.4%; Potatoes, 1.6%; oranges, 1.0%; and apple, 0.3%; Annual report of FAO, 2012). Therefore, in terms of the relative amount of crude protein, mushroom ranked above the above mentioned vegetables and cereals foods [Crisan & Sands, 1978; Chang & Miles, 1989].

5.3 Fat content

Fat is one of the important macronutrients. Fat content ranged between 2.4% and 4.2 %. The highest fat content in *Lentinula edodes* was found to be 4.2%, which was bought from Lagankhel vegetable market and lowest fat content was found in Balkhu sample (3%). Fat content in *Lentinula edodes* has been reported to range from 1.19% (Reis *et al*. 2011) to 2.31% (Dulay *et al*., 2015). However, overall *Lentinula edodes* grown in Kathmandu valley had slightly more fat content than other parts of world.

Fat content in *Pleurotus ostreatus* from Asan market was 2.9%, while it was 3.2%, 3.0% and 4.2%in the samples bought from Kalimati, Balkhu and Lagankhel markets respectively. This species has been reported to have a fat content ranging from 0.9% (Mehmet *et al.*, 2008) to 15.28% (Adejum *et al.*, 2015).

The fat content in *Agaricus bisporus* bought from Asan market was 1.2 % and that of the samples of Kalimati, Balkhu and Lagankhel was 2.13%, 1.4% and 0.8% respectively. Masamba (2010) reported 2.12%, Tekit (2015) reported 2.13% and 1.4% by Colak *et al*. (2009).

The *Pleurotus djamo*r strain bought from Kalimati market had maximum percentage (3.3%) of fat, while sample from Langankhel market had 3.0% and Asan sample had 2.9%. The lowest(2.4%) fat content was found in Balkhu market. These values are

within the range of fat contentpreviously reported (0.80% - 3.9%) by Oyetayo *et al.*(2007),Khan *et al*.(2013), Asadi *et al*.(2014) andJohari *et al*.(2015).

Among four mushroom samples *Lentinula edodes* ranked highest in fat content (4.2%) and *Agaricus bisporus* was lowest (0.8%). In comparison with four vegetable markets *Lentinula edodes* species from Lagankhel had maximum (4.2%) fat content followed by the samples from Kalimati market (3.4%), Asan (3.1%) and Balkhu market (3.0%).

5.4 Fiber content

The fiber content obtained in *Lentinula edodes* were 11.1%, 12.3%, 12.9%, and 11.9 %, in the samples that were bought from Asan, Kalimati, Balkhu and Lagankhel markets respectively. Fiber content was significantly higher from than reported byYuen*et al.* (2014; 3.77%). However, these values were between the range (12.49% to 20.4%) of fiber contents reported by multiple other researches (Mattil *et al*., 2002; Miles, 2004; Celestine *et al.,* 2015).

Pleurotus ostreatus is one of the most easily available cultivated mushrooms found in the market. The fiber contents in *Pleurotus ostreatus* were 9.0%, 11.6%, 16.9% and 14.2% in the samples bought from Asan, Kalimati, Balkhu and Lagankhel markets respectively. There was no significant difference in fiber content of *Pleurotus ostreatus*when compared to the previous studies. Soni *et al*. (2007) reported a fiber content of 12.0% and Oyetayo *et al.* (2013) reported a content of 11.8%. However, the fiber content obtained in this study was fairly higher that that (7.05%) reported by Dulay *et al.* (2015).

In case of *Agaricus bisporus*cultivar of Asan market had the highest fiber content (16.6%), followed by the cultivars in Balkhu (16.0%), Kalimati (12.1%) and Langankhel (11.7%). The fiber content in *Agaricus bisporus* was lesser than that reported by Tekit *et al,*(2015) who had reported a content of 18.23%, however were similar to that reported by Pushpa *et al*.(2010) and Akindahunsi *et al*.(2007) who had reported a vaule of 16.56% and 16.32% respectively.

Pleurotus djamor of Lagankhel had minimum fiber content (12.0%), while the specimen from Kalimati was found to have maximum (16.8%). These values were consistent with those (8.99% to 17.2%) reported in previous studies (Khan *et al*., 2013; Minteshot *et al*., 2014 and Johari *et al*., 2015).

5.5 Ash content

Ash contents in *Lentinula edodes* were 11.1%, 13.9%, 16.6% and 14.2% in the samples collected from Asan, Kalimati, and Balkhu and Lagankhel vegetable markets respectively. In the previously published studies the values in the range of 9.42% to 16.63% have been documented (Reis *et al*. (2011, Adejumo*et al.* (2015); Awosanya *et al.* (2015); andDulay *et al*. (2015).

Ash contents present in *Pleurotus ostreatus* were 13.9%, 11.6%, 16.9% and 14.2% in the samples bought from Asan, Kalimati, Balkhuand Lagankhel vegetable markets respectively. A wide vaiability (1.41% to 18.5%) in the ash content has been reported in literature Khurshidual *et al*. (2014) and Bandopadhyay, (2013)). In average the values in this study were higher than those reported by Khurshidual *et al*.(2014); Shah *et al.*(1997) and Soni *et al.* (2008) who respectively reported the values of 1.41% 4.05% and 7.9% and similar to reported by Chang Ragnunathan (2008 ; 9.7%), Akyuz*et al*.(2008; 13.7%), Sevda *et al*.(2008; 12.7%), Patil *et al.*(2010 ; 13.90%) and Dulay Bandopadhyay (2013 ; 18.5%).

The ash content of *Agaricus bisporus* in the sample from Asan vegetable market had the highest (16.6%) ash content. The sample from Kalimati market showed 12.1%, 16.0% from Balkhu and 11.7%from Lagankhel.Various reports show quite a difference in ash content; Hanif *et al.* (2006; 11.01%), Garcha *et al*.(2008; 10.9%),Celestine *et al.*(2015; 16.13%), Pushpa *et al*.(2010; 7.07%), Simon *et al*.(2010 ; 9.80%), Asadi *et al.* (2014; 12.18%) and Owaid *et al*. (2015; 7%).

The ash content in *Pleurotus djamor* bought from Asan, Kalimati, Balkhu and Lagankhel markets were 14.2%, 16.8%, 15.4% and 12.0% respectively. The previous researches done reported by Oyetayo *et al.* (2007; 10.37%), Khan *et al.* (2013; 7.65%), Johari *et al.* (2015; 5.83%) and Minteshot *et al.* (2014; 6.92%).

5.6Carbohydrate

Carbohydrate is the major macronutrient content found in the mushrooms. Carbohydrate content in *Lentinula edodes* ranged between 34.9% and 45.7%. The highest (45.7%) carbohydrate content was found in specimen bought form Asan market and lowest (34.9%) was from the Balkhu market. Carbohydrate content in *Lentinula edodes* from other researches ranged from 13% (Dulay *et al*, 2015) to 17.62 % (Ranjinni *et al*., 2012).

In *Pleurotus ostreatus* sample bought from Kalimati, Balkhu, Asan and Lagankhel carbohydrate content were 46.8%, 46.9%, 49.22% and 43.6% respectively. Shah *et al*. (1997) reported similarcarbohydrate content (44.41%) from south India. In comparison to this study, relatively higher carbohydrate contents were reported by several other authors. Such as the contents of 61.12% (Dulay *et al*., 2015), 66.1% (Yabani *et al*., 2008), 55.12 % (Patil *et al*., 2010), 61.9%, (Malik *et al*., 2015) and 54.4% (Garcha *et al*., 2015) have been reported.

Carbohydrate content in *Agaricus bisporus* were 28.0%, 30.0%, 39.9% and 35.6% from vegetable markets collected from Asan, Kalimati, Balkhu and Lagankhel respectively. The researches done previously showed the carbohydrate content ranging from 28.8% (Tekit, 2015) to 62% (Adejumo *et al.*, 2015).

Pleurotus djamor species are commercially less grown species of Kathmandu valley. Carbohydrte contentof *Pleurotus djamor* bought from Lagankhel market ranked higest (50%) followed by sample bought from Balkhu (49.5%), Asan vegetable market (48.9%) and Kalimati (43.6%). *Pleurotus djamor* from Kalimati had found almost same result reported as 43% by Bernardi et *al*. (2008). Lowest carbohydrate content (30.43%) has previously been reported by Oyetayo *et al*. (2007) and highest (64%) by Adejumo *et al*. (2015) in case of *Pleurotus djamor*.

5.7Mineral content

Mineral are essential for human health. The amount or concentration of the mineral plays an important rolein the different organs and cellular mechanisms to maintain the physical structure and strength of the body. Mineral status must be known before using them because it is necessary to know levels of toxicity. The presence of high and lowamount of minerals showed typical disorders in physiological activities and construction of the body.

5.8 Phosphoruscontent

Phosphorus is an important element. Phosphorus content in *Lentinula edodes* were 520mg, 769mg, 812mg and 837mg samples bought from Asan, Kalimati, Balkhu, and Lagankhel respectively. Records of phosphorus content screened from previous researches reported Hernandez *et al*. (2003; 814mg), Pedneault *et al*. (2007; 527mg), Oyetayo *et al*. (2007; 769.9mg), Motato *et al*. (2007; 783.4mg), Ranjini *et al.* (2012; 769.9 mg) and Owaid *et al.* (2015; 345mg). Phosphorus content from the research ranged from 520 mg to 83 mg which matched the phosphorus content from other researches between 345mg to 814mg.

In*Pleurotus ostreatus* samples the phosphorus content were found to be 307mg, 307mg, 318mg and 310mg from Asan, Kalimati, Balkhu and Lagankhel markets respectively. Thescreening results reported for phosphorus content from previous researches ranged from41.13mg (Zahid *et al*., 2013) to 308mg (Patil *et al*., 2010). It has been found that phosphorus content from the study was similar to other researches.

Agaricus bisporus is one of the most common and important mushrooms available in our markets. Phosphorus content found in samples bought from four vegetable markets ranged 100.7mg (Lagankhel) to 222mg (Balkhu). However, the various researches done previously reported 81.07mg (Adejumo *et al*., 2011) to 100.5mg (Mallikarjuna *et al*., 2013). The values obtained for phosphorus content were more to that of previous researches.

In *Pleurotus djamor* phosphorus content were 613mg, 667mg, 740mg and 802mg from Asan, Kalimati, Balkhu and Lagankhel vegetable markets respectively. The various researches done were 737mg (Khan *et al*., 2013), 763mg (Mallikarjuna *et al*., 2013), 713mg (Motato *et al*., 2006)and 663mg (Marizi *et al*., 2015). From the study it has been noticed that the phosphorus content were similar to that of other researches.

Phosphorus content from four speciesobserved were mixed proportion that might be directly related to the bacteria available around that mushroom cultivation areas and which could be able to convert free available phosphorus to soluble phosphate that could be used by mushrooms.

5.9 Iron content

The Iron content in *Lentinula edodes* were 48mg, 52mg, 44mg, and 9.8 mg in the strains gathered from Asan, Kalimati, Balkhu and Lagankhel vegetable markets respectively. The screening results from other countries showed significantly less 16.3mg (Mattil *et al*., 2002), 18.2mg (Patricia *et al.*, 2007), 14.8mg (Rajini*et al*., 2012) and 14mg (Goncalves *et al*., 2014).). However,Kalac *et al*., 2014 reported 116.23mg and 85mg (Adhikari, 2000) reported iron content from in wild edible species found in Nepal content more iron content.

In*Pleurotus ostreatus* the iron content was 17.24mg, 21mg, 24mg, and 23mg in the specimens bought from Asan, Kalimati, Balkhu and Lagankhel market respectively. The researches done in other parts of world reported iron content between 15.25mg (Kordera *et al*., 2015) and 35.9mg (Zahid *et al*., 2013). The results of iron content were similar. But, in indigenous wild mushrooms species of Nepal reported by Adhikari,

(2000) was found to be 45.84mg which was higher than the results obtained from four cultivated mushrooms.

In case of *Agaricus bisporus* the iron content was found to be 7.67mg, 8.7mg, 6.07mg and 7.63mg, which were bought from Asan, Kalimati, Balkhu and Lagankhel respectively. Iron content obtained from previous researches ranged from 11.5mg (Shah *et al*., 1997) to 43.77mg (Alerandrino*et al.*, 2014). These values were found to be below other researches.

Pleurotus djamor content maximum (36mg) iron content in the sample bought from Balkhu followed by Kalimati, Asan and Lagankhel (33mg, 32.58mg and 12.48mg)respectively.The researches done under Global Biotechnology & Biochemistry in 2006 had found 20mg,Mallikarjuna *et al*., 2012 and Masamba *et al*., 2013 reported 14.8mg and Maihara, 2014; 24mg.

5.10Calcium

*Lentinula edodes*collected from Lagankhel market had maximum (101mg) calcium content followed by Balkhu (97mg), Kalimati (93mg) and Asan (89.18mg). Iron content obtained from previously done researchers found that valued ranged 113mg (Mattil *et al*., 2002; Dulay *et al*., 2015) to 174.9mg (Hermandez *et al*., 2003). The calcium content in *Lentinula edodes* from the study has been found to be lower than other researches.

In the species of *Pleurotus ostreatus,* calcium content found to be 157.68mg, 153mg, 158mg and 156mg bought from Asan, Kalimati, Balkhu and Lagankhel market respectively. The various researches done reported57.91mg (Zhang *et al*., 2002), 132.12mg (FAO, 2004), 114.91mg (Bilal & Wani, 2010), 386mg (Patil *et al.,* 2010), 8.87mg (Oyetayo*et al*., 2013) and 47mg (Goncalves *et al*., 2014). Calcium content is in the range done by other researches.

Calcium content in *Agaricus bisporus* was found to be 56mg, 59mg, 56mg and 60.13mg in the samples bought from vegetable markets of Asan, Kalimati, Balkhu and Lagankhel markets respectively. Adhikari, (2000) and Australian Department of Food, (2007) reportedcalcium content 71 mg. Various researches done reported 2.2mg (Masamba *et al*., 2010), 24.23mg Kalac *et al*., 2014), and 32.2mg (Asadi *et al*., 2014) and 8.27mg (Mallikanjuna *et al*., 2015). It has been observed that calcium content from the study were found to contained more calcium content than others researches.

Calcium content in *Pleurotus djamor* contained 45mg to 49 mg. Calcium content 59.3mg (Zhang *et al*., 2002), 44.7mg (Alerandrina*et al*.,2007), 34.2mg (Dharmaraj *et al*.,

2014) and 29.9mg (Maftoun *et al*., 2015) the above datas were found to be near to the range obtained from the study.

The highest calcium was in *Pleurotus ostreatus*. This might be due to the condition of soil if the soil had greater amount of calcium content it absorbed that calcium from soil to the mushroom species.The mixing of various calcium ions into the soil like Lime (Calcium carbonate powder) gets accumulated as a result of which calcium content was seen fluctuated.

The results on nutritionally valuable minerals showed that the four mushroom species were rich in phosphorus and calcium. This was in agreement with the reports of studies done in other parts of the world. Even low amount of minerals are enough for metabolic reactions, transmission of nerve impulse, rigid bone formation and regulation of water and salt balance in our cells (Mattil *et al.*, 2001).

5.11 Pesticide estimation

In this study, the use of pesticides on mushrooms such as *Lentinula edodes*, *Pleurotus ostreatus*, *Agaricus bisporus* and *Pleurotus djamor* was evaluated using GC-MS. GC-MS analysis of mushrooms of major edible cultivated mushroom found in Kathmandu valley did not reveal the existence of pesticides. Time and often it has been broadcast in our national news that vegetables have contaminated with pesticides which directly and indirectly affect the human health.

The extract of *Lentinula edodes*, *Pleurotus ostreatus*, *Agaricus bisporus* and *Pleurotus djamor* were subjected to GC-MS analysis. All the examined mushroom samples were found to be free of pesticides that have harmful effect on human health. From GC-MS analysis five compounds were in *Lentinula edodes*, five compounds observed in *Pleurotus ostreatus*, eight in first phase and five compounds in second phase for*Agaricus bisporus,* and five compounds in *Pleurotus djamor.* The main intention to test pesticide at the interval of three months was to observe if mushrooms are contaminated with pesticides during the course of mushroom cultivation.

However, the GC-MS analysis revealed several peaks that corresponded to componentsof mushroom like 9, 12- octadecadienoic acid(RT); 9,12- Octadecadienoic acid methyl ester (E,E) (RT), Pentadecanoic acid, Diethyl phthalate and Cis-9 Hexadecenoic acids which have also been previously documented in literature (Priya *et.al.,* 2012; Janarie *et al*., 2012; Vijayalakhi *et al.,*2010; Zhau *et al*., 2015and Chen & Wu, 2015).

These components are alcohols, ketones, aldehydes, sulphur, acid and ester compounds. Alcohols have been considered as the main odorants of the mushroom aroma (Cho *et al*., 2003). It has been reported that alcohols are the characteristic flavor of mushroom which are formed by oxidation of linoleic or linolenic acyl in the presence of enzymes lipoxygenase and hydroperoxide lyase (Teo Feng *et al*., 2015).

CHAPTER VI

CONCLUSION

This is the first attempt in Nepal on screening of nutrients found in mushrooms which has sold in market of Kathmandu valley. Primarily four species were found (*Lentinula edodes*, *Agaricus bisporus*, *Pleurotus ostreatus* and *Pleurotus djamor*) in large amount. By adhering to the guidelinesof AOAC and AAS food safety methods (13^{th} edition), protocols received from America and Japan. The investigationsuggested that the content of Moisture (79% to 93.3%), Crude Protein (23% to 46.3%), Carbohydrate (28% to 50%), Fat (2.4% to 4.2%), Crude Fiber (11.9% to 17.9%), and Ash (6.3% to 18.3%) these resultswere observed in the same range as reported in variousliteratures around the world. However, Phosphorus (100.7mg to 837mg), Calcium (11.82mg to 165mg) and Iron (6.07mg to 52mg) per 100g were higher than those reported previously from other countries. This study will reflect condition of cultivation and the impact on human health created by mushroom consumed.

Most importantly GC-MS did not reveal any traces of pesticides in the mushrooms, suggesting the edibility of the mushrooms sold in the Kathmandu valley were safe to consume.

RECOMMENDATION

1. The mushroom can be used as a rich source of protein and minerals
2. Even though no pesticide contaminant was detected in the study, the possibility of use of pesticide cannot be completely ruled out. So additional study must be done.
3. All the cultivar found in market is the exotic so we must encourage indigenous species to cultivate commercially.
4. Salesman must be loyal to consumer and avoid of using water in course of sale.
5. Salesmen are not awareabout name of species,origin of specie and expiry date.
6. Cultivation of mushroom required continuous effort due to which many farmers lack these characteristics as a result of which cultivation of mushrooms in Kathmandu valley is decreasing.
7. The price of mushroom is high because of which poor people cannot consume all the species available in the market.
8. Government of Nepaldo not have any standard screening data of its own both in case of exotic as well as indigenous cultivars so policy maker must be aware to address this problem.
9. Unavailability of stand compounds on time.
10. Nutritional status determinatons of food items required continuous electricity supply which lacks in many laboratories.
11. After obtaining results for calcium and Iron it is hard to predict the correct result due to lack of Analytical Chemist.
12. Minimum observation limit of GC-MS is unknown.

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Appendix

A) **Required materials for protein estimation**:Conc.H_2SO_4, NaOH, Kjeldahl digestion and distillation apparatus, Kjeldahl tubes,250ml Erlenmeyer flasks, Glass beads, Digestion mixture (Potassium Sulphate and Cupric sulphate) and Indicator (Methylene red and bromocresol green).

B) **Required materials for Fat estimation:** Petroleum ether, Soxhlet extraction apparatus, Oven set at 105°c, Extraction thimbles and Desiccator (60°- 80°c).

C) **Required materials for Crude Fiber:**Soxhlet, Condenser, Buchner funnel and flask, Whatman filter no.41, Desiccators, and Gooch crucible with asbestos fiber, oven, Muffle, Petroleum ether, Sulfuric acid, and sodium hydroxide.

D) **Required materials for Ash estimation:**Porcelain crucible, Crucible furnace, Dryer, Desiccators.

E) **Required materials for Phosphorus estimation:**Citric-Molybdic acid reagent, Quinoline solution, Quimociac reagent, Citric acid, conc.HNO_3, conc.HCl, Sodium hydroide, Sodium molybdate, Acetone, 500ml volumetric flasks, 250ml volumetric flasks, burette, whatmans filter paper no.41, Gooch filter, Desiccators and Oven.

F) **Required materials for Iron estimation:** conc.HCl, AAS, Ferric nitrate solution as standard compound, 200ml volumetric flasks, micro pipette, deionized water

G) **Required materials for Calcium estimation:** conc.HCl, AAS, Lanthanum (0.4%, w/w), 200ml volumetric flasks, Falcon tubes (big and small), Calcium carbonate, Pipettes and deionised water

H) **Required materials for Pesticides estimation:** Primary Secondary Amines (PSA), conc. $MgSO_4$, 40% NaOH, NaCl, Pipettes, Methyl cyanide (MeCN).

I) **Preparation of 4% boric acid and Indicators**

The 4% boric acid solution was prepared by dissolving 40g of boric acid powder as added in volumetric flask. Hot water was added tp dissolve the boric acid powder. The indicators were added to solution in the ratio of 12:8 (bromocresol green: methyl red) and volume was made upto 1000ml.

0.1% indicators were prepared by mixing 0.1g of methyl red in 100ml of 95% alcohol and 0.1g bromocresolgreen in 100ml of 95% alcohol.

j)Reagent preparation for phosphorus estimation:

For preparation of Citric molybedic Acid reagent,54 gm of 100% molybdic anhydric (MoO_3) and 12 gm NaOH with stirring in 400ml hot water and cooled. 60gm of Citric acid in a mixture of 140ml HCl and 200ml water was added and cooled. It was then filtered and diluted to one liter.

Quinolone solution was prepared by mixing 50ml of synthetic quinolone, dissolving in a mixture of 60ml HCL and 300ml water by stirring. It was then cooled and diluted to 1 liter and then filtered.

Quimociac reagent was prepared by dissolving 70gm of Na molybdetate in 150 ml H_2O, 60gm of citric acid dissolved in the mixture of 85ml HNO_3 and 150 ml H_2SO_4 and cooled. Molybdate solution was gradually added to citric acid and HNO_3 with stirring. Percentage of synthetic quinolone was dissolved in mixture of 35 ml HNO_3 and 100ml H_2O, this solution was gradually added to molybdate citric acid. HNO_3 solution mixed and let it stood for 24 hours. It was then filtered, 280ml acetone added, it was diluted to 1 liter mixing with H_2SO_4.

k) Sample preparation for iron estimation:

Sample preparation was carried based on the optimized protocol (DFTQC, 2014). For sample preparation 200ml volumetric flask were used in which 200µl of Ferric Nitrate solution was kept as standard solution of Iron and 100ml of distilled water was added and made concentration of 20ppm. 10ml conc.HCl was added to new volumetric flasks. From standard solution of 20ppm, 5ml, 10ml, 15 and 20ml were drawn into respective volumetric flasks which contained 1ppm, 2ppm, 3ppm, 4ppm respectively. The final volume was made 100ml by adding distilled water to those working solutions. The blank was made which by adding 10ml conc.HCl and 90ml deionized **water.**

L) Sample preparation for Calcium estimation:

Sample preparation for Calcium was based on the optimized protocol (DFTQC, 2014). For sample preparation 200ml volumetric flasks were used in which 200µl of standard solution of Calcium carbonate was kept.1ml of Lanthanum carbonate solution was poured to each respective conical flask. Final volume was made to 100ml of deionized water having concentration of 20ppm. 9ml conc. HCl was added to new volumetric flasks. From standard solution of 20ppm, 5ml, 10ml, 15ml and 20ml were drawn into respective volumetric flasks which contained 1ppm, 2ppm, 3ppm, 4ppm respectively. The final volume was made 100ml by adding distilled water to those working solutions. The blank was made which by adding 9.5ml 1:1 HCl and 89.5ml deionized water. Two controls were made 1ml lanthanum, 9.5ml 1:1 HCl and 2ppm and 3ppm spiked calcium itself.

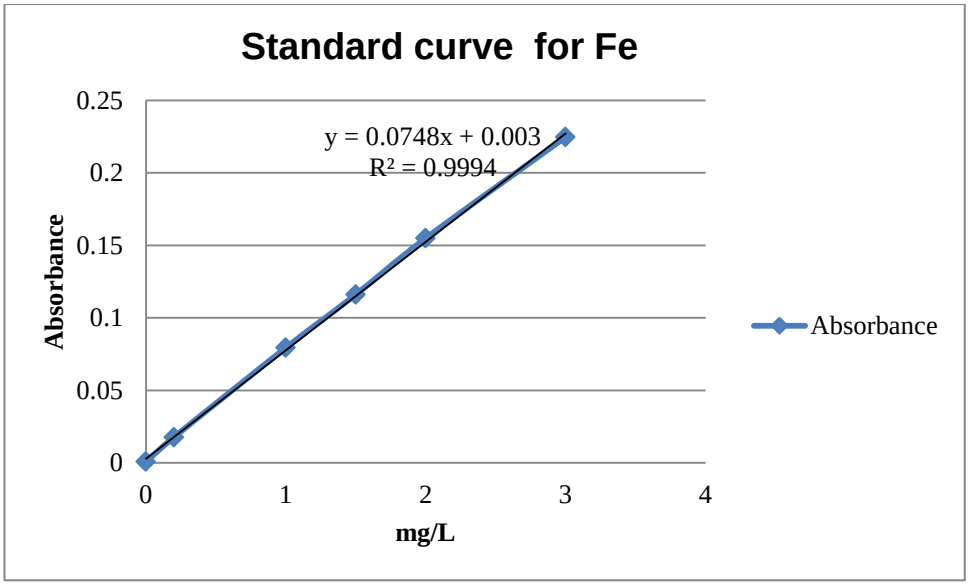


Figure a: Showing Standard Curve for Iron.

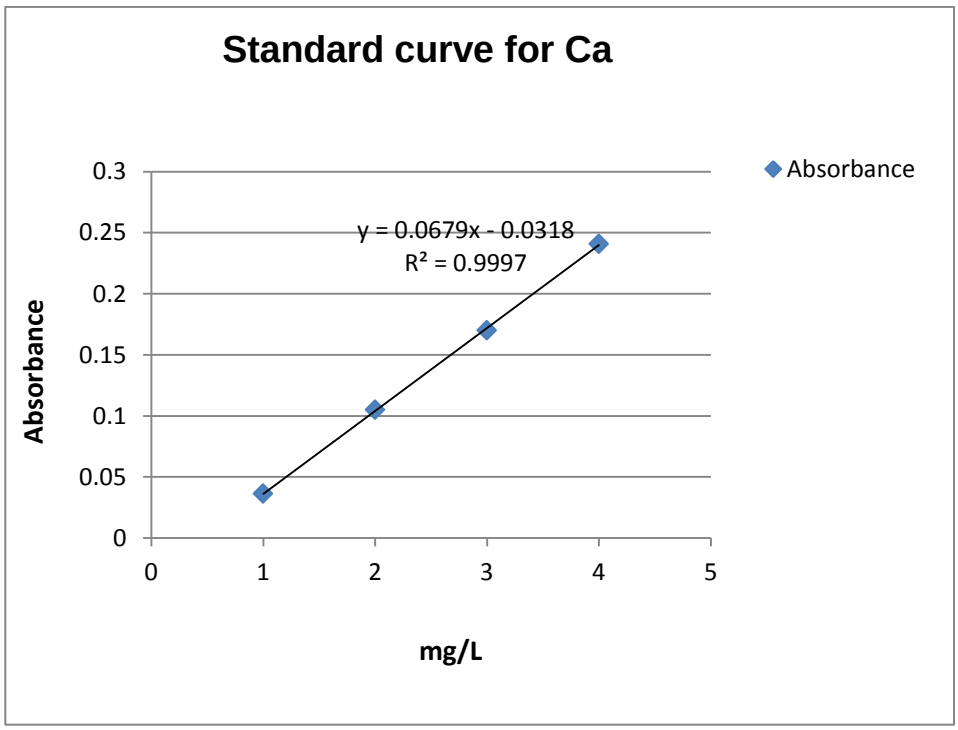


Figure b: Showing Standard Curve for Calcium

Photograph

1: *Agaricus bisporus* samples taken from four vegetables market Asan, Kalimati,Balkhu and lagankhel.

2: *Pleurotus ostreatus* taken from Asan, Kalimati, Balkhu and Lagankhel vegetable markets.

3: *Lentinula edodes* growing on woods, Pictures taken from Hair Maharjan's farm.

4: *Pleurotus djamor* samples taken from Asan, Kalimati, Balkhu and Lagankhel vegetable markets.

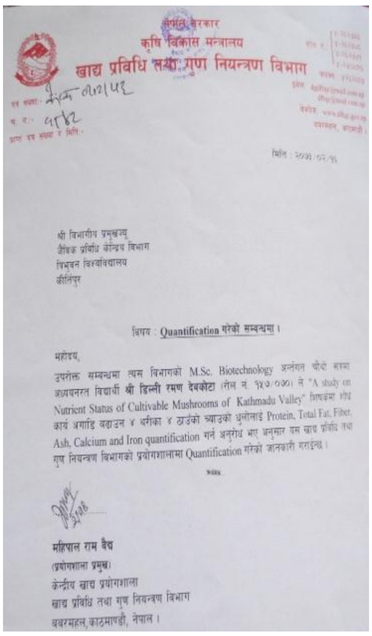
5: Protein determination(Digestion, Distillation, Titration)

6: Fat estimation by using Soxlet appratus

7: Sample preparation for Iron and Calcium estimation

8: Sample preparation for Phosphorus estimation

9: Mushrooms growing in Hari Maharjan's farm.



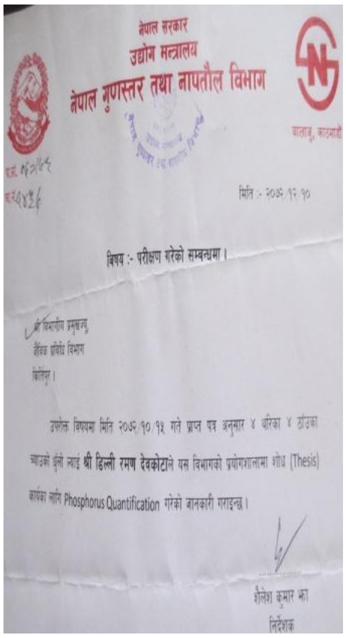
नेपाल सरकार
कृषि विकास मन्त्रालय
खाद्य प्रविधि तथा गुण नियन्त्रण विभाग

श्री विभागीय प्रमुखज्यू
जैविक प्रविधि केन्द्रिय विभाग
त्रिभुवन विश्वविद्यालय
कीर्तिपुर

विषय : Quantification गरेको सम्बन्धमा ।

महोदय,
उपरोक्त सम्बन्धमा त्यस विभागको M.Sc. Biotechnology अन्तर्गत चौथो सत्रमा अध्ययनरत विद्यार्थी **श्री डिल्ली रमण देवकोटा** (रोल नं. १५७/०७०) ले "A study on Nutrient Status of Cultivable Mushrooms of Kathmadu Valley" विषयमा शोध कार्य अगाडि बढाउन ४ थरीका ४ ठाउँको च्याउको धुलोलाई Protein, Total Fat, Fiber, Ash, Calcium and Iron quantification गर्न अनुरोध भए अनुसार यस खाद्य प्रविधि तथा गुण नियन्त्रण विभागको प्रयोगशालामा Quantification गरेको जानकारी गराईन्छ ।

महिपाल राम वैद्य
(प्रयोगशाला प्रमुख)
केन्द्रीय खाद्य प्रयोगशाला
खाद्य प्रविधि तथा गुण नियन्त्रण विभाग
बबरमहल,काठमाण्डौ, नेपाल ।



नेपाल सरकार
उद्योग मन्त्रालय
नेपाल गुणस्तर तथा नापतौल विभाग
बालाजु, काठमाडौं

विषय :- परीक्षण गरेको सम्बन्धमा ।

श्री विभागीय प्रमुखज्यू,
जैविक प्रविधि विभाग
कीर्तिपुर ।

उपरोक्त विषयमा मिति २०७२/१०/१४ गते प्राप्त पत्र अनुसार ४ थरिका ४ ठाँउका च्याउको धुलो लाई **श्री डिल्ली रमण देवकोटा**ले यस विभागको प्रयोगशालामा शोध (Thesis) कार्यका लागि Phosphorus Quantification गरेको जानकारी गराइन्छ ।

शैलेश कुमार झा
निर्देशक

राष्ट्रिय विधि-विज्ञान प्रयोगशाला

(Thesis) Pesticide test