TERMITOMYCES R. HEIM (TRICHOLOMATACEAE) IN NEPAL: DIVERSITY, NUTRIENTS AND GROWTH

A THESIS SUBMITTED TO THE

CENTRAL DEPARTMENT OF BOTANY

INSTITUTE OF SCIENCE AND TECHNOLOGY

TRIBHUVAN UNIVERSITY

NEPAL

FOR THE AWARD OF

DOCTOR OF PHILOSOPHY

IN BOTANY

BY

HARI PRASAD ARYAL

MARCH 2015

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DECLARATION

Thesis entitled "**Termitomyces R. Heim (Tricholomataceae) in Nepal: Diversity, Nutrients and Growth**" which is being submitted to the Central Department of Botany, Institute of Science and Technology (IOST), Tribhuvan University, Nepal for the award of the degree of Doctor of Philosophy (Ph.D.), is a research work carried out by me under the supervision of Prof. Dr. Usha Budhathoki.

This research is original and has not been submitted earlier in part or full in this or any other form to any university or institute, here or elsewhere, for the award of any degree.

.....
Hari Prasad Aryal

RECOMMENDATION

This is to recommend that **Mr. Hari Prasad Aryal** has carried out research entitled "**Termitomyces R. Heim (Tricholomataceae) in Nepal: Diversity, Nutrients and Growth**." for the award of Doctor of Philosophy (Ph.D.) in **Botany** under my supervision. To my knowledge, this work has not been submitted for any other degree.

He has fulfilled all the requirements laid down by the Institute of Science and Technology (IOST), Tribhuvan University, Kirtipur for the submission of the thesis for the award of Ph.D. degree.

……

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March 2015

LETTER OF APPROVAL

Date:

On the recommendation of Prof. Dr. Usha Budhathoki, this Ph.D. Thesis submitted by Hari Prasad Aryal, entitled "**Termitomyces R. Heim (Tricholomataceae) in Nepal: Diversity, Nutrients and Growth**." is forwarded by Central Department Research Committee (CDRC) to the Dean, IOST, T.U.

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March 2015

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I dedicate this work to my beloved parents, Late Ghanashyam Aryal and Late Hugat Kumari Aryal.

.....

Hari Prasad Aryal

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ABSTRACT

The termitophilous fungi are a monophyletic group of tropical gilled mushrooms belonging to the single genus *Termitomyce* R. Heim of the family Tricholomataceae under the class Basidiomycetes. These are good source of food and medicine. Nepalese mycoflora has been less explored compared to vascular plants. Therefore, exploration and documentation of these valuable mushrooms are highly imperative to understand diversity, distribution and their role in human welfare. Furthermore, studies on the pattern and conditions of growth, pattern of local uses and interaction between fungi and environment for the production of phytochemicals are prerequisite to develop conservation strategy and future potentiality of such mushrooms. The present work is mainly focused on three different aspects; systematics and distribution pattern, nutrient content and myceliall growth pattern of termitophilous mushrooms of Nepal. The field study was carried out during the year 2010-2012 throughout the country from tropical to temperate climatic regions up to the altitude 3000m asl.

Distribution and diversity of *Termitomyces* along three different ecological belts (Tarai, Siwalik and Midhill) within three phytogeographic divisions (East, Centre and West) were studied. Altogether 19 taxa have been recorded excluding non-termite mushrooms. Among them 17 taxa are new to mycoflora of Nepal including two taxa have been proposed to be new to the science. Cluster analysis was performed to trace out the interrelationships between studied taxa. Among the collected taxa of *Termitomyces*, the species richness was significantly variable within physiographic regions, land use types, locality and altitude. The study revealed that, physiography, land use, locality, altitude and air temperature were significantly influential for the distribution of *Termitomyces*.

The relationship between distribution pattern and edaphic factors were also studied for *T. albuminosus*. Soil samples from 27 spots were collected, of them four different locations (termite nest, casing soil, vicinity and forest) were fixed for each spot to observe nutrient status of soil and influence of surrounding soil nutrients in the growth of *T. albuminosus*. Analysis of soil nutrients (N, P, K, OM, Fe, Zn, Cu, Mn and B) revealed that the presences of organic matter and microelement content were apparently higher in most of the natural protected forest with comparisons to the agro-pastoral, plantation and managed forest. Most of the nutrients and their interaction

between soil source and eco-zone were significantly different at $p < 0.05$. The studied organic matter indicated highest in Tarai (5.40%) in termite nest (7.90%), comparative to the least contained as 2.47% in Midhill regions of casing soil (1.38%). The study observed that biomass yield of *T. albuminosus* was highest in Tarai, then that of the Siwalik and Midhill.

Comparative nutrients analysis of basidiocarps for carbohydrate, protein, moisture, ash, fiber, fat and dry matter and Cu, Fe, Mg, Ca, K, P, Mn, Na and Zn were analyses proximately of the four species of *Termitomyces* (*T. albuminosus, T. mammiformis, T. microcarpus* and *T. robustus*). Those were compared from different physiographic region among the species and individual of the species. They were significant at $p < 0.05$. Similarly, phytochemicals were analyzed qualitatively of the same four species, which were found in all the 27 study area. The screening revealed the presence of 16 phytochemicals in the etheral, methanolic and aqueous extracts. There were significant differences in the phytochemical composition of the samples collected from three ecological belts and three phytogeographic regions amomg the west Siwalik was the best. Its ethno mycological knowledge of different ethnic groups was also incorporated in this study.

Regading myceliall growth studies of *Termitomyces,* the research focused on optimizing the cultural media using different nutritional sources for the myceliall growth of four species of *Termitomyces*. For carbon and nitrogen sources, among the six the best growth was observed in maltose and yeast extract. Out of five vitamins and six amino acids tested thiamine and serine acts as the best for the mycelium growth. The combination of 'C' and 'N' in various proportion showed that the best growth was in C: N ratio of C_5: N_1 in both semisolid and submerged conditions.

This research provides in depth information for further studies in the field of systematics and physico-chemical properties of soil, which influence termite distribution and abundance and ultimately growth of *Termitomyces*. At the same time, due to high concentration of diverse primary and secondary metabolites they may be used in food drug development and paves the way for the upbringing new industries through articificial cultivation. Hence, this genus needs to be conserved to prevent from going to extinct.

LIST OF ACRONYMS AND ABBREVIATION

AGP	Agro pastoral
ANOVA	Analysis of variance
CDBP	Central Department of Botany Pathology Unit
CDB, TU	Central Department of Botany, Tribhuvan University
C: N	Carbon nitrogen ratio
DFRS	Department of Forest Research and Survey
DM	Dry matter
DPR	Department of Plant Resource
DsAcF	*Dalbergia sissoo-Acacea catechu* forest
DSM	Department of Soil Management/Directoriate of Soil Management
EPS	Exopolysaccharide
GPS	Global Positioning System
GoN	Government of Nepal
GNF	Government managed National Forest
GPF	Government managed Protected Area
IAAS	Institute of Agriculture and Animal Science
IOST	Institute of Science and Technology
NARC	Nepal Agricultural Research Council
NAST	Nepal Academy of Science and Technology
NHM	Natural History Museum
PDA	Potato Dextrose Agar
PD	Potato Dextrose
PF	Plantation Forest
Psi	Pound per square inch
SCF	*Schima-Castanopsis* forest
SPSS	Statistical package for Social Science
STDHF	Sub tropical deciduous hill forest
TDRF	Tropical deciduous forest
TESF	Tropical ever green Sal forest
VDC	Village Development Committee
VN	Voucher number
WETC	Water Engineering and Training Centre P. (Ltd.)

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CHAPTER 1

1. INTRODUCTION

1.1 Mushrooms: Morphology, Diversity and Distribution

Fungi singular: fungus is eukaryotic spores-bearing achlorophyllous organisms. They exhibit remarkable diversity in form, ranging from microscopic unicellular as in yeast to large macroscopic bodies as in Ascomycetes and Basidiomycetes (Alexopoulos & Mims, 1993). Fruiting bodies of fungi are mushrooms (Rinaldi & Tyndalo, 1972). The word mushroom have been derived from the French word "mousseron" (Mucerom), from mouses, moss (fast growing) but it is not used in quite same sense (Ramsbottom, 1954). They are macro fungus with a distinctive fruiting body; this can be either epigeous or hypogenous, and large enough to be seen by naked eyes. Most often, *Agaricus* or fleshy species of higher fungi under the order Agaricales of class Agaricomycetes (Kirk, *et al*., 2008) are recognized as mushrooms. Mushrooms are of various sizes, colours and shapes. The general form of an agarics fruiting body is umbrella shaped with a central stipe, supporting a cap or pileus with numerous radially arranged vertical downward plate, the gills or lamellae on the lower side of the cap (Denchev *et al*., 2013; Figure 1). Generally they are gill bearing, fleshy, achlorophyllous, macroscopic basidiocarpous or ascospors spore-bearing fungi (Phillips, 1981). However, fleshy species of other groups of fungi (Ascomycetes) bearing cap and gills on the underside and producing spores are also recognized as mushrooms, which may be edible, inedible, poisonous or non-poisonous (Miller, 1984).

Many authors (Kibby, 1979; Pacioni, 1985; Alexopoulos & Mims 1993) made a clear distinction between "mushrooms" considering only the edible species and "toadstools" as inedible or poisonous species. The belief is that toadstools are formed from the harmful substances of the earth and the venom of toads and that fungus always grow in places where toads abound and give shelter to them when they take the air (Ramsbottom, 1954). Krieger (1967) defined toadstool as "all the fleshy umbrella-shaped fungi and to a small number of the best known edible forms". Even closely related species of the same genus are not all poisonous or edible. For example, *Amanita muscaria* are poisonous, while *Macrolepiota procera* and *Amanita caesarea*

are edible (Adhikari, 2000). Depending upon the ecological factors or climatic variations, the above prejudices varies, for example *Scleroderma citrinum*, *S. cepa. S. aurantium* and *S. verrucosum* are edible in Nepal while these species are considered as poisonous in Europe and Japan (Adhikari, 1996). *Ramaria aurea* and *R. flava* are considered edible in Nepal but the same species have been reported poisonous in Japanese literature (Imazeki, *et al*., 1988).

Macro fungi generally contain two main groups, the Ascomycota and Basidiomycota. Although most of the Ascomycota are microscopic species, they also contain some "larger fungi'' such as cup-fungi, morels and truffles. Around 14000 to 16000 species of Basidiomycota are known (Hawksworth, *et al*., 1995). The order Agaricales comprises around 6000 species. Fungi in this order are commonly called mushrooms, toadstools, gill fungi, or agarics. They are terrestrial, lignicolous, saprobic, parasitic or mycorrhizal in nature. The members of order Agaricales are cosmopolitan in distribution and grow on variety of substrates including leaf litter, plant debris, tree trunks, and stems of herbs, shrubs, and bark of trees, on sandy soil, grassy soil, termitaria, clothings, ropes and even on other agarics (Singer, 1986). On the basis of their habitat they may be foliicolous, lignicolous, humicolous, coprophilous, termitophilous, terrestrial, etc. and appear mainly in the monsoon season (Atri, *et al*., 2010). The abundance of macro-fungus is low in hot and dry seasons while they are abundant in spring and autumn due to the humid climate (Sibounnavong, *et al*., 2008).

Macro-fungi have long been of interest to scientists for their significant roles in forest ecosystems, human life (in the form of their use in the pharmaceutical and food industries), as well as their vital role in biodegradation (De Boer, *et. al*., 2005). Their unique flavor and nutritional properties of mushroom have attracted the attention of mankind since times immemorial. Many studies have reported that the Basidiomycota accounts for most of the lignocelluloses decomposition in leaf litter thus plying significant role in forest ecosystem (Miyamoto, *et. al*., 2000). The phytochemical screenings of mushrooms have revealed that different species of mushrooms contain different chemicals (acids and alkaloids, amatoxin, psilocybine, coprin, helvellic acid, muscarine, ibutenic acid), which are toxic to human health. There are two types of intoxications caused by fungi, *viz*. mycetism (after the ingestion of higher fungi) and mycotoxicoses (after the ingestion of lower fungi) (Oliveira, 2009).

1.2 Historical Account

From the beginning of civilization there have been references to mushrooms in the ancient Greek, Roman, Chinese, Sanskrit and other literature. The mushrooms have been variously regarded as “earth’s excrescence” or “the evil ferments” of the earth (Ramsbottom, 1954). Greek regarded them as “strength food” and they believed mushrooms as the consequence of lightening; while Romans considered them as “food of Gods” and they believed that mushrooms sprang from ground in places struck by the lightening. Chinese regarded mushrooms as “elixir of life”; whereas the Aztecs of South America referred mushrooms as “God’s flesh” and worshiped a group of mushrooms as being divine (Wasson, 1969). The Guatemalan and Mexican Indian tribes correlate the appearance of the “Fly Agaric” to thunderbolt (Lowy, 1974). Similar are the beliefs held by Tamang ethnic group of Nepal, who also relates mushrooms with sky and thunder since they use the term “Muguding” for *Morchella* spp. (in Tamang language, *mu* means sky, and *guding* means ringing) (Pandey, 2008).

Mushrooms were mentioned in ancient Sanskrit literature. Rigveda (written in 1200-900 BC), for example, referred “somrus” as a decoction of mushroom known by the names “Ksumpa” in Sanskrit (Sensarma, 1998). The Manusmriti (written by Manu: the son of Brahma) also listed the names of various mushroom in Sanskrit and advised "not to eat the mushrooms growing anywhere" (Sensarma, 1996). In Shushruta-Samhita (400-200 BC), the mushrooms are considered as vegetables. Mandan Pal Nighantu (written by King Madanpal-1431 AD) state that the mushrooms to be sedative, heavy and may cause cough, *vayu* (air) and *pitta* (bile) in the human body (reviewed in Sensaarma, 1998).

The wild mushrooms seem to have been traditionally consumed by men since very early times, but these were then probably considered a food in wilderness which now has come to occupy a very popular place in the modern diet because of their nutritional value (Bano, 1976). In Nepali language, mushrooms are commonly known as “Chyau”. Similarly, they are variously named in Hindi language and associated dialects, such as "Kavak" (general name for mushrooms in Hindi, which refers to mushrooms occurring on trees); Bhauma (refers to fungi growing on soil), "Chhatrak" (refers to mushrooms with umbrella-shaped head), "Chhatrika" (refers to mushroom with small umbrella or cap), "Shilindhram" (refers to mushrooms which grow on organic materials), "Swedajam" (refers to mushrooms which grow on warm and humid place) and "Prithavikandam" (refers to mushroom which grows on soil) (Sensarma, 1998).

1.3 Termitophilous Fungi

Termitophilous fungi (termite's mushroom) are a monophyletic group of tropical gilled mushrooms belonging to the genus *Termitomyces* (Figure 1). They are unique fungi that grow in close and intimate association with termites. It was described by Heim (1942abc) for a group of termitophilous agarics. It comprises fungi that live in an obligate symbiosis with termites (commonly known as white ants) of the subfamily Macrotermitinae and order Isoptera. *Termitomyces* spp. was recorded from tropical and subtropical regions of the world (mainly from Africa, and South and South East Asia). However, samples were also recorded in Portugal of Europe and Ecuador of South America (Figure 2).

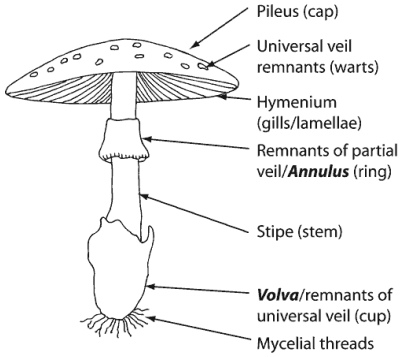
This genus is characterized by typical agaricoid fruiting body; carpophores pluteoid, usually fleshy, with large often sharply differentiated umbo; stipe central, with pseudorrhiza and simple veil; lamellae free to adnexed but with decurrent tooth; hyphae inamyloids, with a hilum of the open pore type; spores ellipsoid, smooth, spore-print pink; basidia normal; cystidia present; tramal hyphae inamyloids, without clamp connection, trame system monomitic, having lamellate hymenophore. The genus is represented by giants among the gilled mushrooms like *T. titanicus* (≥ 1 m cap diam.) as well as by small mushrooms, like *T. microcarpus* (≤ 2 cm cap diam) (Tibuhwa *et al*., 2010).

Growth of *Termitomyces* takes place through transport of spores by termites into the substrate made by them using plant material passing their guts (Frøslev, *et al.*, 2003). The fungal mycelium of termitomyces besides being food for termites, also absorb the excessive moisture so that the termites play a role in spreading the spawn in the moist soil, from which the fungal fruit bodies emerge on the termite mound making them bloom with basidiocarps. The mushroom fructification period is strongly seasonal. They emerge early in the rainy season, in places where termite colonies are about to swarm, and fruit bodies are on or near termites mounds (Harkonen & Mwasumbi, 2003). Termites cultivate the mycelium in their nest and fruit bodies are develop from the fungus combs on or near or within the nest of termites' mounds. Due to this special way of growth, the central stipe of *Termitomyces* prolonged into an underground pseudorrhiza, a root-like extention of the stipe, connected to the fungus comb in an underground termite nest (Figure 1).

Systematics of *Termitomyces*

Classification

Super kingdom	:	Eukaryota
Kingdom	:	Fungi
Division	:	Basidiomycota
Class	:	Basidiomycetes
Sub-class	:	Agaricomycetidae (=Holobasidiomycetideae)
Order	:	Agaricales
Family	:	Tricholomataceae/Lyophyllaceae
Genus	:	*Termitomyces* R. Heim



Structure of a typical mushroom with different parts

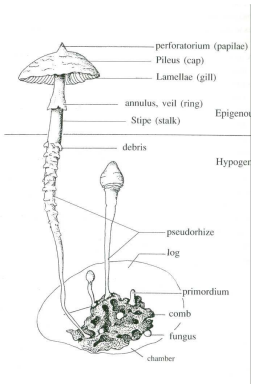
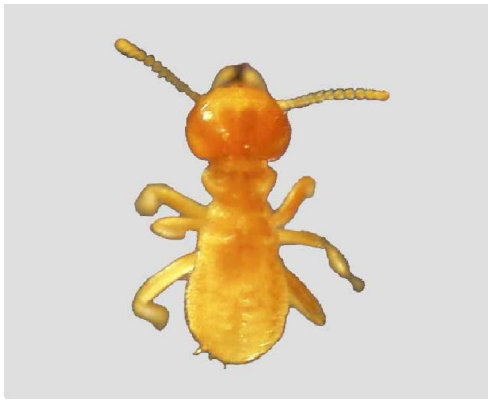


Illustration of *Termitomyces* sp. growing from termite's comb (epigenous and hypogenous). Source: Pichayangkul, 2004



Termite (*Hypotermes makhamensis* Ahmad; Family: Termitidae, Subfamily: Macrotermitinae) responsible for the growth of *Termitomyces* spp. Source: Ahmad, 1965.

Figure 1: Mushrooms and Termites

Aanen and Eggleton (2005) proposed that cultivation of *Termitomyces* by termites originated in the African rain forests as the main centre and migrated to other geographical regions, like Asia and Madagascar. (Frøslev, *et al.,* 2003) reported that the molecular studies have revealed no identical sequences among the *Termitomyces* between Africa and Asia, though it, demonstrating their geographical difference as well as a possible independent evolution. The life cycles and intricacies of coevolution between termites and *Termitomyces* (asexual and sexual phase) is still being debated (Rouland, 2002; Nobre, *et al.,* 2010). Recently, Nobre *et al.* (2010) argued that the termites harvest asexual spores of fungus along with lignocellulolytic enzymes of fungal and bacterial origin through faeces and foraged plant material to build the comb (fungus garden), which is comparable to an external rumen.

Until Heim (1942c) maintained the genus *Termitomyces*, early mycologists assigned termitophilic fungi to widely different genera like *Armillaria* (Fr.) Staude 1857, *Entoloma* P. Kumm. 1871, Lentinus Fr. 1825, and *Pluteus* Fr. 1836 (cited in Frøslev, *et al.,* 2003). The genus *Termitomyces* is now represented by 30 species in the tropical regions of the world (Frøslev, *et al.,* 2003; Kirk, *et al*., 2008). Out of which, 22 species have been documented in India (Natatajan, 1979; Jamalludin, *et al.,* 2001; Atri, *et al*., 2010) and 11 in China (Cheng *et al.,* 2010), and 20 species from Thailand (Kosakul, *et al*., 2007).

Termitomyces are an economically valuable natural resource serving as an alternative to plant and animal derived foods. They are highly priced for their nutraceutical utility. Fruiting bodies of *Termitomyces* spp. are a delicacy in different parts of the world, most importantly in Equatorial region of Africa (Nigeria, Zambia, Zimbabwe, Tanzania, Uganda and Cameroon), South Africa, Madagascar and South-east Asia (India, China, Nepal, Pakistan, Sri Lanka, Thailand, Taiwan, Philippines, Burma, Indonesia and Malaysia) (Heim, 1942c; 1977; Turnbull & Watling, 1999). One of the world's largest edible species is *T. titanicus*, which is found in Zambia and Tanzania. Several researchers have estimated the nutritional values of fruiting bodies of *Termitomyces* species from Africa and Asia and reported to be superior to all other edible mushrooms and common vegetables (Bother & Eicker, 1992). *Termitomyces* species are reported to contain higher dry matter, digestible lysine-rich protein, fiber, vitamins and minerals; but contain lower amount of fat and carbohydrates (Jiskani, 2001). They are also rich in important minerals, like phosphorus, potassium, calcium, copper and iron. In place of

starch, mushrooms contain sorbitol and linoleic acid (an unsaturated fatty acid). They are an excellent source of folic acid, thiamine, riboflavin, niacin, pantothenic acid and biotin (Manning, 1995). Besides nutritional value, many species of *Termitomyces* possess antioxidant properties and thus have high medicinal value (Tibuhwa, 2012a). They also produce a variety of enzymes in culture media useful as additives in food, in leavening of bread, in processing silage and in other industrial applications (e.g., clarification of non-citrus fruit juices) (Khowala, *et al*., 1992). Termitophilous mushrooms are always found in association with termites in the rainy season. They also play important roles in ecosystem replenishment and perform a wide variety of ecological roles.

1.4 Termitophilous Mushrooms in Nepal

The investigation on mushrooms of Nepal started since 19th century (Lloyd, 1808). So far, 812 species of mushrooms have been documented (Adhikari, 2009). Out of them, 228 species are edible (Christensen, *et al*., 2008a) and 66 are poisonous (Pandey, 2008; Adhikari, 2009). Similarly, 73 species of mushroom reported from Nepal are used as medicine, 12 species are used for decorative purpose, and 50 species are considered as mycorrhizal (Adhikari, 2009). Regarding the *Termitomyces* mushrooms, there are the records of only two species of *Termitomyces* from (Banepa) Central Nepal, *Termitomyces eurrhizus* at 1200 m asl (Singh & Shrestha, 1986), from Kathmandu valley at 1350 m asl (Joshi & Joshi, 2008), from Shaktikhor-Chitwan at 300 m asl (Rijal, 2011), from Twanrakhola-Makwanpur at 500 m asl Uprety, e*t al*., 2012) and *T. clypeatus* from Kathmandu valley at 1350 m asl (Adhikari, *et al.*, 2005); 1500 m asl (Pandey, 2008); and from Dolpa hill at 2954 m asl (Devkota, 2008). Except these no proper work has been made so far in Nepal.

1.5 Rationale and Objectives

In Nepal, the complex phytogeographic factors have played an important role in growth and distribution of fungi, considered as the homeland for mushroom floral diversity. However, very few studies are found related to Nepalese mycoflora as compared to higher plants. The species of mushrooms, used for cultivation in Nepal include [*Agaricus bisporus* (J.E. Lange) Imbach (button mushroom), *Pleurotus ostreatus* (Jacq.) P. Kummn (oyster mushroom), *Pleurotus sajor-caju* (Fr.) Singer (oyster mushroom), *Pleurotus floridanus* Singer, *Lentinus edodes* (Berk.) Singer

(Shiitake), *Auricularia polytrica* (Black mushroom), *Ganoderma lucidum* (Curtis) P. Karst. (Reishi mushroom) and *Volvarielia volvaceae* (Straw-mushroom) are being cultivated on Nepal on commercial scale (Singh, 2007), besides these *Calocybe indica* Purkay. & A. Chandra, also cultivated in the tropical belt of Nepal. However, most of the strains, and even species, currently used for cultivation are of exotic origin. Contrastingly, indigenous species of mushrooms of Nepal, having high culinary and medicinal value, are either being threatened in wild by over exploitation (*Morchella* spp., *Ophiocordyceps sinensis* (Berk.) G.H. Sung, J.M. Aung. Hywel-Jones & Satafora) or are being underappreciated due to low availability or anonymity (*Scleroderma* spp., *Termitomyces* spp., *Volvariella* spp.). It is necessary to identify and domesticate such valuable local species of mushrooms to protect from the disappearance of these taxa.

These indigenous mushrooms are one of such underappreciated group of fungi, which are reported elsewhere as having high medicinal and nutritional values (Shingh & Shingh, 2005). Exploration and documentation of such valuable mushrooms are highly imperative for understanding their diversity, distribution and their role in human welfare.

As Nepal has expressed its commitment for conservation and sustainable use of biological resources, there is a need to have detailed information and knowledge about its natural resources and potentialities. Evaluating conservation measures and future potentiality of such mushrooms need further information about their patterns and conditions of growth, pattern of local uses and fungal-environment interaction for production of phytochemicals. These are also considered as basic information for the domestication of mushrooms. Therefore this mushroom is potential in term of diversity, nutrients and growth has been studied. This research is focused on taxonomy and eco-physiological aspects of termitophilous mushrooms of Nepal, particularly aiming to understand their patterns of diversity, distribution, nutrient contents and growth. The specific objectives of this research are to:

- collect, identify and taxonomically describe the species from different parts of Nepal based on macro- and micro-morphometric analysis
- assess patterns of species diversity and distribution along the east-west and south-north gradients of Nepal Himalaya

- document local uses applying ethno-mycological approaches
- assess fungal-environment interaction for production of essential micro- and macro-nutrients and secondary metabolites
- assess patterns of myceliall growth under various nutritional supplement media

1.6 Limitations

Given the seasonal nature of mushroom growth, sampling during precipitation at a time or immediate precipitation could not represent the entire termite's mushroom from the studied sites. All the myco-chemical as well as preserved specimens is not being identify up to species level due to immature/over matured stage of specimens as well as due to consistency of time period to work. However, this study provides important information related to diversity, contents of nutrients, domestication and media optimization on culture media for the growth of mycelial.

1.7 Thesis Plan

The present study has been divided into seven chapters.

Chapter one provides general morphology, diversity, distribution and historical account of the *Termitomyces* species found in the world including Nepal, with their justification of the study and its limitations.

Chapter two is related to the systematics of Nepalese *Termitomyces*. In this chapter, 19 taxa were described on the basis of 'macro' and 'micro' morphological characters including 'chemical test' and 'identification key'. Intra-specific variations were found among the species collected from different ecological regions. Cluster analysis was performed to trace interrelationships among studied taxa. So that investigations based on as many as 19 taxa belong to single genus *Termitomyces* R. Heim of the family Tricholomataceae has been documented.

Chapter three deals with ethnomycology of *Termitomyces* in Nepal. *Termitomyces* spp. was documented for their socio-cultural, food and medicinal values in different parts of Nepal. Local use value and practices were documented by interviewing 300 people representing 63 ethnic groups of Nepal (CBS, 2001). Local knowledge provided enough background on *Termitomyces* to appreciate the diversity and their relevance in ecosystem maintenance in general and human welfare in particular.

Chapter four described with distribution and diversity of *Termitomyces* along ecological gradient in Nepal. In this chapter, richness of *Termitomyces* species was assessed with relation to elevation, aspect, forest type and other topographic variables, including elemental/chemical analyses of soil of termite's nest, casing soil, its vicinity and forest of the studied area. Edaphic factor, affect the land use potentials, vegetation pattern and hydrology, which directly affected on termites nesting latter it influence on, termites distribution and abundance. Analyzed biomass was highest in Tarai follows siwalik and Midhills

Chapter five referred with the explained about the comparative description of macro and micro nutrients (primary metabolites) analysis of four species in different physiographic region and analysed phytochemical (secondary metabolites) which are significantly different at 5% level of significance of *Termitomyces* in Nepal.

Chapter six concerns with the myceliall growth studies of *Termitomyces,* the research focused on optimizing the cultural media for the mycelial growth of four species of *Termitomyces,* this is a step for domestication of these edible species by pure culture technique on lab. Comparative studies on the myceliall growth of these were done to assess the effect of different nutritional factors.

Chapter seven showed the conclusion and Implication.

Termitophilous fungi are a monophyletic group of tropical gilled mushrooms belonging to the single genus *Termitomyces.* It contains higher amount of macro and micro nutrients. The research carried out revealed 19 taxa with food and medicinal significance, (except two species) with the (14 species and 3 forma) new addition of micofungal flora of Nepal and (2) newly proposed to the science. Physico-chemical properties of soil played as the major role on termites nesting, which influence on termite distribution and abundance. Under suitable in-situ conditions it could be maintained in termite gardens for expanding the natural cultivation.

This research provides in depth information for further studies on taxonomic field. Due to high concentration of diverse primary and secondary metabolites it is used in food drug development and paves the way for the upbringing new industries. It plays an important role for the process and product developments in biofuel and environmental biotechnology. More than 50% of the species of the genus *Termitomyces* recorded worldwide occur in Nepal, so it creates attraction to the mycologist, entomologist, food technologist and environmentalist to the research.

CHAPTER 2

2. SYSTEMATICS OF NEPALESE TERMITOMYCES

2.1 Introduction

The genus *Termitomyces* of the family Lyophyllaceae and order Agaricals is represented by 30 species in the world (Kirk, *et al*., 2008). Taxonomic determination of *Termitomyces* mostly based on basidiocarp structure resulted several synonyms for taxa found in Africa and Asia, but their morphological characters are quite different (Tang, *et al*., 2006; Wei, *et al*., 2006). Fries (1821 & 1832) first elaborated the classification of fungi using spore colour and various microscopic characteristics, the methods which are in use by taxonomists till today. Importance of spore colour in the taxonomy of mushrooms has been highlighted by many researchers (Saccardo, 1882 & 1931; Singer, 1986). This tropical genus has a symbiotic life together with termites.

Several species of edible *Termitomyces* are reported from Asia (Pegler & Vanhaecke, 1994), and from Africa (Heim, 1942a; Moriss, 1986 etc.). Molecular phylogenetic analysis revealed that both African and Asian *Termitomyces* species have evolved from the same ancestor (Sawhasan, *et al.,* 2011). No proper documentation of *Termitomyces* is made in Nepal. In Nepalese market, *Termitomyces* spp. are considered superior to all other mushroom in terms of nutritive values (field observation, 2012). This study aims to document *Termitomyces* spp. from Nepal and assess their relationships based on detailed macro- and micro-morphological characters.

2.2 Literature Review

The order Agaricales (Basidiomycetes) to which the termitophilous mushrooms belong was established by Underwood in 1899. Linnaeus (1753) also used the generic name *Agaricus* to include the entire gill bearing fungi. The phylogenetic classification of the major groups within the Basidiomycetes to which termitophilous mushrooms belongs still remains unsettled. It has been divided into Heterobasidiomycetes (jelly fungi) and Homobasidiomycetes (mushroom-forming fungi) based on the structure of the septal pore apparatus and the spindle pole body (Hibbett, *et al*., 2007). Termitophilous mushrooms are included in Homobasidiomycetes. A preliminary phylogenetic study of Homobasidiomycetes using NSSU (nuclear small subunit) and

mt-SSU (mitochondrial single subunit) rDNA sequences revealed eight major clades, which were Polyporoid, Euagarics, Boletes, Russuloid, Thelephoroid, Hymenochaetoid, Cantharelloid and Gomphoid-phalloid clade (Hibbett *et al.*, 2007). Termitophilous mushrooms fall under Hymenochaetoid clade.

Taxonomy: The world scenario

The genus *Termitomyces* was placed within Tricholomataceae by Singer (1986) on the basis of siderophilous granulation in basidia, which is close to Lyophyllaceae. Later, on the basis of the pluteoid habitat, densely crowded free lamellae and pink spore deposit, the genus was placed in Lyophyllaceae (Kirk, *et al.*, 2008). Heim (1942a) was the first to recognize *Termitomyces* as an independent genus with 10 species and 6 forma. As a matter of fact a number of species of this genus have been described mainly from tropical areas, particularly Southern Africa, South East Asia and throughout the equator (Pegler & Vanhaecke, 1994) (Figure 2).

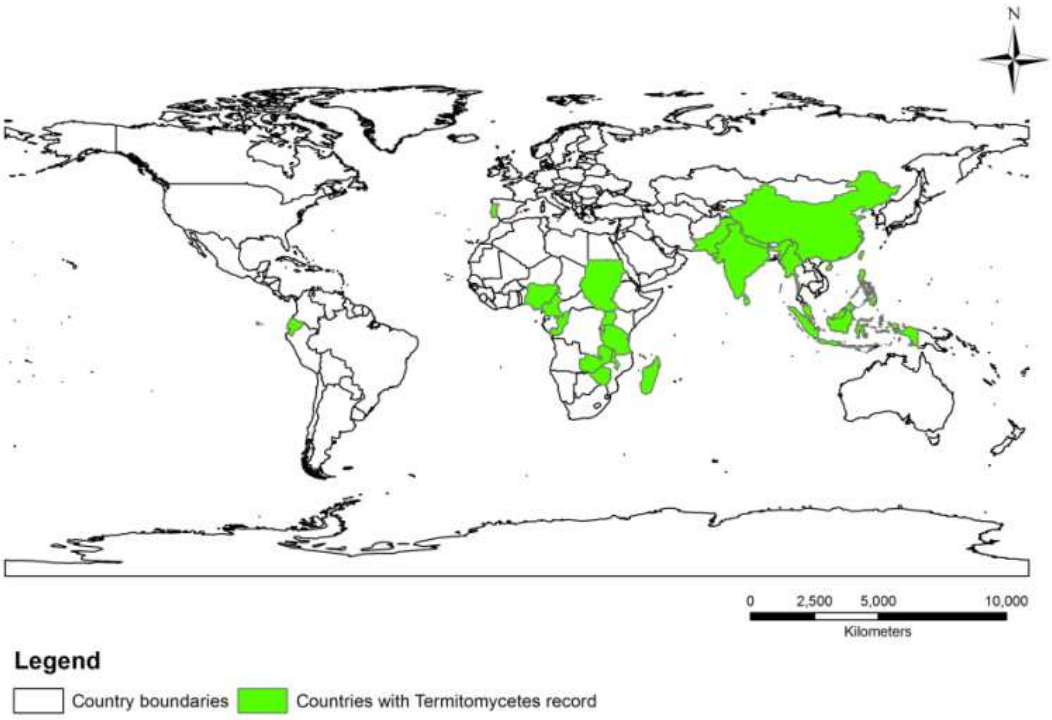


Figure 2: Distribution of *Termitomyces* in the world

Source: www.freeusandworlds.com, retrived: 16 September 2014

In addition, some other significant contributions on systematics which also included edibility, ecology and conservation, contributions by taxonomic or systematics perspectives have been made by Otieno, 1969; Bhavani, 1980; Vrinda and Pradeep,

2009; Vrinds *et al.,* 2002, and on evolutionary biology perspectives, by Aanen and Boomsma, 2005, a lot.

Batra and Batra (1966) worked on the subject of association of fungi with termites. Heim (1977) published a monograph with 28 taxa of these mushrooms covering Africa, Asia (Reid, 1975; Saarimäki, *et al*., 1994), Cameroon (Mossebo, *et al*., 2002) and South America (Gómez, 1995). In total, 68 taxa have been published in *Termitomyces,* with 81 names including combinations and autonames. According to *Index Fungorum* database, currently 55 names published in *Termitomyces* based on 53 types are reported to be distributed throughout the equatorial and Sothern Africa and widely in South East Asia.

Zang (1981, 1992) proposed a new Genus *Sinotermitomyces* including five species. Later on the genus *Sinotermitomyces* was merged with *Termitomyces* by Wei *et al*. (2006). In China 25 taxa of *Termitomyces,* including three new species have been reported from 17 provinces within China (Wei & Yao, 2003). Tang et al. (2005) revised three *Termitomyces* species from India and concluded *T. longiradicatus, T. quilonensis* and *T. poonensis* to be synonyms of *T. heimii* and *T. eurhizus*. Altogether from China as many as 11 species of termitophilous mushrooms have been documented in "A revised checklist of edible fungi in China" (Cheng, *et al.,* 2010).

While working on termitophilous mushrooms in India, (Bose & Bose, 1940) prepared a list of about 28 varieties of edible species of *Termitomyces.* Atri and Kour (2003) and Atri *et al.* (2005) documented 12 species of *Termitomyces* and one species of *Sinotermitomyces* and gave a key to their determination. Purkayastha and Chandra (1975) described *Termitomyces eurhizus,* Chakaravarty and Khatua (1979) described *Termitomyces microcarpus,* and Natarajan (1975, 1977, & 1979) described number of species of these mushrooms including some new species. Manjula (1983), providing a very exhaustive list of Agaricoid and Boletoid fungi from India and Nepal. This is so far the best comprehensive list, which enumerates 538 genera and 20 families in the order Agaricales. Similarly, Rawla *et al.* (1983) and Leelavathy *et al*. (1983) also described genus *Termitomyces* from India. Other Indian contributors are Bhavani *et al*. (1980) described *Termitomyces robustus*; Atri *et al.* (1995) published taxonomic studies on the genus *Termitomyces.* Vrinda *et al*. (2002) described *Termitomyces umkowaani*. Vrinda and Pradeep (2009) described an edible species *T. sagittiformis,* a lesser known edible mushroom.

Taxonomy: Nepalese scenario

The Investigation and study on mushrooms of Nepal started since the contribution of Lloyd (1808). It was Hooker (1854), who at first gathered plant diversities in Nepal. Since then several botanical investigations have been done. The major literatures concerned with the mushroom flora are: *Termites Et Champignons Boubee,* Heim (1977). *Biodiversite des Basidiomycetes au Nepal: etude systematique et biogeographique, Specialite Ecologie-Mycologique,* (Adhikari, 1996); *Mushrooms of Nepal* (Adhikari, 2000); *Researches on the Nepalese mycoflora,* (Adhikari, 2009). The family Sclerodermataceae: ectomycorhizal fungi from Nepal (Manandhar & Adhikari, 2009). In Basidiomycotina, Pragmobasidiomycetes class, (4) genus, (11) species, transitional group, (5) genus, (11) species and Homobasidiomycetes, (171) genus, (585) species, among them (1) monotypic taxa and (38) new species were recorded by (Adhikari, 2009). In this way Nepal represent a rich repository of macro-fungal resources and has been explored for larger fungi by various workers (Bhandary, 1980; Manjula, 1983; Bill & cotter, 1989; Tulloss, *et al*., 1992; Devkota, *et al*., 2005b; Christensen, *et al*., 2008b etc.)

Taxonomy: A molecular approach

Molecular techniques are becoming increasingly important as means for obtaining characters for studying taxonomic and phylogenetic relationships among fungi (Zambino & Szabo, 1993). The limitation of identification of mushroom strains based on a few morphological characters can be overcome by the use of DNA based techniques like RAPD, AFLP, RFLP or DNA sequence analyses (ITS, nLSU, nSSU, mtSSU and RPB2). Any of the molecular methods could be combined with morphological methods to make identification of fungal species reliable (Khush, *et al*., 1992; Calonje, *et al*., 1997). Little information is available on the use of ITS in the taxonomy of some of these mushroom isolates. Rouland (2002) reconstructed a phylogeny of some termitophilic fungi from sequences of South African data and postulated with evidence *Termitomyces* to be a monophyletic group. Frøslev *et al.* (2003) found phylogenetic relationship of *Termitomyces* and related taxa and sequenced portions of nuclear LSU rDNA and mitochondrial SSU rDNA from fruitbodies of termitophilous mushrooms representing the total range of morphological variation and geographical distribution. Later on Wei *et al.* (2006)

revised taxonomic status of *Sinotermitomyces* and merged the genus with the representatives of known *Termitomyces* species on the basis of phylogenetic studies. Hence the morphology and molecular study of these mushrooms revealed that both the African and Asian *Termitomyces* are evolved from the same ancestors (Sawhasan, *et al*., 2011).

2.3 Materials and Methods

2.3.1 Study Area

Location

The studies was focused on natural as well as community managed forest, though agriculture field and barren land also included on the basis of information found from the local informant and on the basis of availability of the species. The number of sites were supervised 81 forests, among them 45 forests were randomly chosen by RBD (Randomised Block design) method. Elliott (1971) has suggested that whenever possible, the stations should be selected at random. Alternatively the sampling area is divided into different biotopes and stations are seleted at random in each biotope. In the preliminary study field work was conducted during the month of May, the actual information of these 45 forests were noted from the interaction with 300 members of the individuals of ethnic groups of Ahir, Badi, Barai, Bantar, Bing, Bhujel, Bote, Chamar, Chhantel, Churaute, Darai, Damai, Danuwar, Dhanuk, Dhimal, Dom, Gangai, Gurung, Harijan, Jhagar, Jirel, Kahar, Kamar, Kami, Kanu, Khash-Bramhan, Khash-Chhetri, Khuna, Koiri, Kumal, Kurmi, Lama, Limbu, Lodh, Magar, Majhi, Mallah, Musahar, Muslim, Newar, Pasi, Praja, Pathrkot, Rai, Rajbansi, Raute, Santhal, Sanyasi, Sarki, Sherpa, Sudi, Tajpuriya, Tamang, Teli, Tharu, Thakali, Thakuri, Thami, and Yakkha. Herein, participatory rural appraisal (PRA) technique (Frendenberger, 2011) was adopted with the local people to get information on nutritional as well as medicinal aspects. There was also focusing on the forests dominated by species. The studies area was divided into three categories East, Centre and West (on the basis of vegetational zones) and three sub categories Tarai, Siwalik and Midhill including Dune valley (on the basis of ecological zones) of Nepal. From each sub categories they were again narrowing down by the selection of random sampling of three forests. On the basis of the forest area, the number of spots to be sampled was determined so as to represent 10-20 % of the studies sites (Elliott, 1971).

The total of 3 spots was sampled from each topography of Eastern, Central and western region of Nepal. Altogether a total of 27 spots were sampled from the 27 forest (including agricultural and barren land) occupies area.

Phytogeography

According to Good (1953) Nepal serves as a transitional zone of 4 broad phytogeograpical provinces (Sino-Japanese, western and central Asiatic, North East American, partially North African and mostly Indian sub continental). The eastern sector shows some of the flora characteristic to South-East Asia while the rest show their affinity with the North-West Himalayan elements of India. Thus the Himalayas stand as centre for the origin of biological diversity and distribution between high and low altitude forms.

Vegetation

Forest types starting from the evergreen tropical sal forest to Nival zone covered with snow on the north. Hagen (1961) divided the country topographically into six physiographic regions: Tarai, Siwalik, Mahabharat Lekh, Midland Himalaya and Tibetan plateau. Stearn (1960) divided on the basis of three important rivers: Karnali, at west, (between 80°5′ and 86°4′ and 83°0′ E), Gandaki, at central (between 83°0′ and 86°5′ E) and Koshi, at east (between 86°5′ and 88° 12′ E longitude). Stainton (1972) divided the country phytogeographically into seven major divisions: Tarai, Foot-hill, Midland, Humla-Jumla region, dry river valley, inner valley and Dolpo-Mustang region. Similarly Dobremez (1972) divided into four divisions: eastern (from east to the Arun river), central (between the Arun river and Dhaulagiri), western (between Dhaulagiri and west frontiers) and north-western (Saipal, Hiunchuli Dhaulagiri, Annapurna and Manaslu) regions. Likewise Swan and Leviton (1962) classified into seven vegetational zones: Lower monsoon, Middle monsoon, Upper monsoon, Deciduous and Rhododendron, Conifer and Rhododendron, Wet alpine and Dry alpine.

Table 1: Geographical, physiographical and ecological regions of Nepal.

Geographical Region	Physical Feature	Ecological Zone	Physiographic Region	Elevation (m)
Mountain (Himal)	Tibetan Marginal Mountain	High Himalayas	High Himalayas	4000
	Inner Himalayas			
	Himalayas			
Hill (Pahar)	Midlands	Transition zone	High Mountain	
	Mahabharat Lekh		Middle Mountain	
Plain (Tarai)	Siwalik zone	Siwalik	Siwalik	2000
	Tarai	Tarai	Tarai	

Source: Joshi & Joshi, 2005.

Table 2: Physiographic zone with corresponding bio-clamatic zones and sub zones.

Physiographic Zone	Bio-Climatic Zones	Sub zone (Altitudinal range)
High Himal	Nival	Above 5000 m
High Mountain	Alpine	Upper (from 3501-4000 m)
		Lower (from 3001-3500 m)
	Sub-alpine	Upper (from 4501-5000 m)
		Lower (from 4001-4500 m)
Mid Hills	Temperate	Upper (from 2501-3000 m)
		Lower (from 1501-2500 m)
		Lower (from 1001-1500 m)
Siwalik	Tropical	Upper (from 501-1000 m)
Tarai	Tropical	Lower (below 500 m)

Source: Dobremez, 1972.

Climate

The country is affected by the monsoon which arises from the Bay of Bengal and moves from the east to North West belt. So Nepal falls within the monsoonal system of the Indian subcontinent on the South (Shankar & Shrestha, 1999). The volume of rainfall varies from east to west and north to south with wet summer and dry winter. The heavy rain fall occurs in mountains rather than foot hills and Tarai belts. June to September is the main monsoon period. The rainfall decreases in the west. The eastern region receives more rainfall than the western region (HMG/MFSC, 2002). The climate is controlled by the monsoonal winds and altitudinal variations. The rainfall pattern reflects local differences, ranging from $\leq$ 2.5 cm/yr to 600 cm/yr (Dobremez, 1976). The southern slop of the Mahabharat receives maximum annual rainfall, (5500mm at Lumle), and minimum (295 mm at Jomsom) in the inner

Himalayan valleys (Stainton, 1972). Snow fall down to the elevation of 3000 m much heavier in western region during pre-monsoon and post monsoon period of (Jan-May and October-December). December and January are the extreme cold followed by bright sunny days. March and April are the pleasant and cool, while May and June Tarai belts are tropically hot and humid. The western regions are hotter and drier whereas central and eastern sectors are humid. September to November is also cool but semihumide with brilliant sunlight.

Temperature

The maximum temperature recorded 44.1°C in June 1979 (Dhangardhi) and minimum -17.9°C in January (Tengboche) (HMG/PCSCBS, 1988). The tropical belt in summer is hot (35°C) in the west (Dailekh, Dhangadhi and Nepalgunj), remaining below 35°C in the central (Pokhara and Hetauda) and 30°C in the east (Biratnagar and Ilam). The winter is cool the mean maximum temperature ranging between 6 and 15° C on west (Dailikh, Dhangadhi and Nepalgunj and between 3 and 8° C in the central and eastern region (Pokhara, Biratnagar and Ilam). The sub-himalayan range (1000-2500 m) in summer is cool, pleasant and moist with the average maximum temperature in between 16 and 25°C (Jiri). The winter is cold with the mean minimum temperature dropping to -1 and -3°C followed by occasional frost. In summer season the lower or midland Himalayas (2500 to 3500 m) is cooler and shorter than lower belts. The winter is cold with the night temperature running below the freezing point. In summer rainfall predominates and winter follows with snow fall. The mean maximum temperature does not exceed 20°C in summer and drops down to -5°C during winter. The alpine region (4000-5000 m) is cold and semi humid. The summer days are cool. The winter is severe with plenty of snow fall and strong wind. The Himalayan belt above 5000 m is covered with permanent snow layer. The Trans Himalayan Bhot valleys have extremely cold and dry climates.

Physiographic zone

Tarai belt: Above the alluvial plain lies the Tarai strip, a seasonally marshy zone of sand and clay soils. The Tarai has higher rainfall than the plains, and the downward-rushing rivers of the Himalaya slow down and spread out in the flatter Tarai zone, depositing fertile silt during the monsoon season and receding in the dry season. The Tarai has a high water table due to groundwater percolating down from the adjacent

zone. The central part of the Tarai belt is occupied by the Tarai-duar savanna and grasslands, a mosaic of grasslands, savannas, deciduous and evergreen forests that includes some of the world's tallest grasslands.

Siwalik Hills: Also called Churia or Margalla Hills, Siwalik Hills is intermittent outermost range of Foot-hills. Permeable conglomerates and other rocks allow rainwater to percolate down slope into the bhabhar and Tarai, supporting only scrubby forests upslope. The Churia have been over-shadowed in Nepal both in terms of biodiversity conservation and sustainable development. The Churiya, also known as the siwalik, make the southernmost chain of the Himalayas and bridge the Gangetic subtropics in the south and the temperate forests in the north.

Bhabhar belts: Above the Tarai belt is an upland zone known as the bhabhar, a zone of porous and rocky soils, made up of debris washed down from the higher ranges. The bhabhar and the lower siwalik ranges have a subtropical climate. The Himalayan subtropical pine forests occupy the western end of the subtropical belt, with forests dominated by Chir Pine *(Pinus roxburghii)*. The central part of the range is home to the Himalayan subtropical broadleaf forest, dominated by *Shorea robusta*.

Inner Tarai or Dun valleys: The inner Tarai valleys are open valleys north of Siwalik Hills or nestled between siwalik subranges. Examples: Chitwan and Dang.

Mid-hills: This zone prominent range 2,000 to 3,000 metres high forming along the main boundary thrust fault zone with a steep southern face and gentler northern slopes, nearly continuous except for river gorges. Rivers gather in candelabra form to the north to break through this range in relatively few places. ‘Hilly’ region averaging about 1,000 m immediately north of the Mahabharat range, rising over about 1000 to about 4,000 m at the main frontal Trust fault zone where the Greater Himalaya begin.

2.3.2 Sampling process

Although collecting adequate, representative samples of communities or populations of organism is difficult. Sophisticated techniques for the treatment of survey data cannot compensate for inadequacies in the original sampling (Hellawell, 1978). Some useful guidance for planning of biological sampling programs is discussed by Elliott (1971). For the calculation of abundance, frequency, density, and species diversity,

the quadrat sizes were taken 25 m × 25 m. In each of the forest 10 quadrat of size 25 m × 25 m were located by stratified random sampling method Elliott (1971). In each quadrat, all species of macrofungi were collected and photographed (Sony DSC S980 camera, China). The collection of macrofungi in these studies was entirely based on their sexual reproductive structures, visible to the neked eye above ground. Along with its vascular plant species have also been recorded from the same plot area.

The habit and habitat (forest type, soil type, etc) including ecological parameters viz. altitude were taken (by altimeter, Model No. 7030, Japan), latitude and longitude were recorded by means of GPS compass (Garmin, Eterex, Germany), of each plots were obtained by means of GPS, slope and aspect of the landscape was measured by means of clinometers (SILVA MOD 15, Sweden) Temperature, humidity and time obtained by Thermo-Hygrometer-Clock (Model: TempTec, Hongkong), soil pH by means of digital pH meter (Model No. 335, Systronics, Ahmedabad, India, Serial No. 2719) & Nail pH meter Model: Takemura, Japan) and Soil moisture was taken by the same Nail pH meter. Litter cover (%) and canopy coverage (%) were estimated visually from the centre of each quadrat.

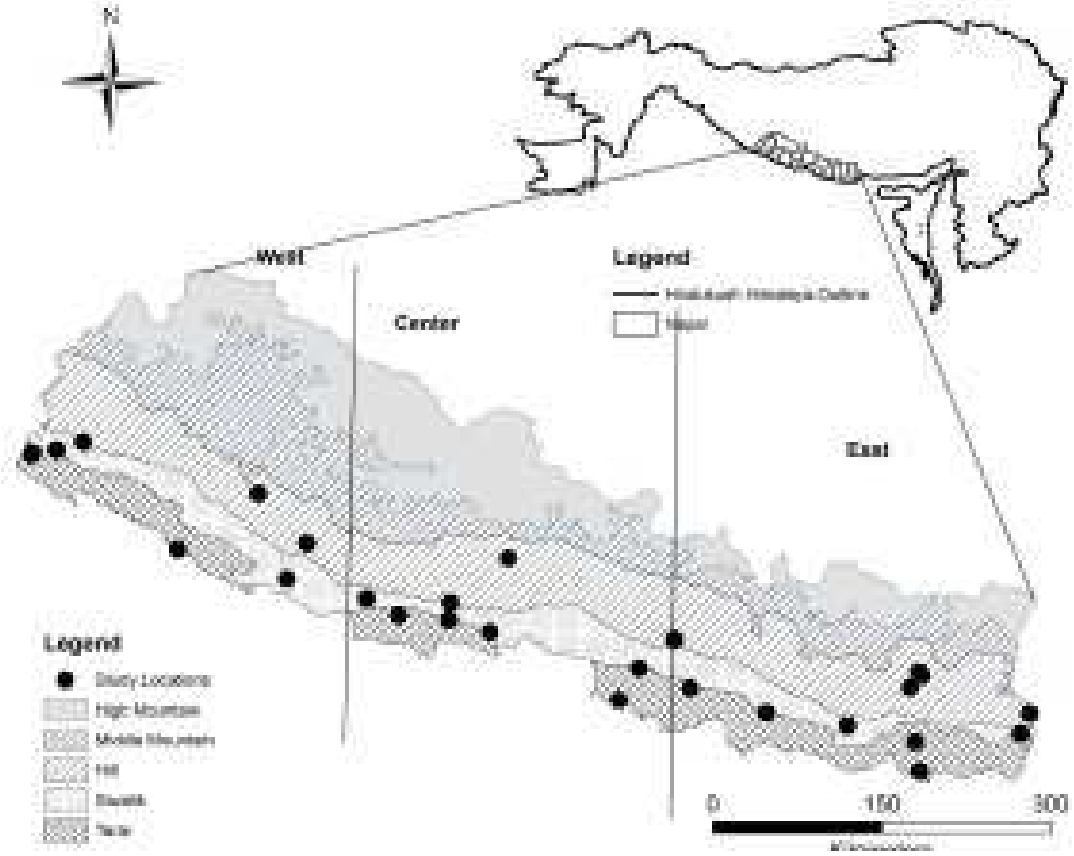


Figure 3: Sample collection sites.

Collection of Mushrooms

Exhaustive inventory (Table 1 and 2) of all probable localities and habitats of *Termitomyces* in Nepal were done. Samplings were made in different habitats representing different ecological belts (Tarai, Siwalik and Mid-hills corresponding to tropical, subtropical and temperate bioclimatic zones) within three phytogeographic divisions (East, Centre and West; Stearn, 1960; Dobremez, 1972) of Nepal. A reconnaissance field visit was made in different types of forest of 36 districts including Dun valley of Nepal, (between latitude of 26°44′08″ N in Morang and 29°06′32″ N Dadeldhura District and between the longitude of 80°18′02″ E in Kanchapur and 88°08′27″ E in Jhapa District) for the sample collection and selection of sampling site for in-depth study (Figure 3). Subsequent field visits were made covering pre-monsoon (April-May), monsoon (June-September) and post-monsoon (October-November) seasons in the years 2010, 2011 and 2012. Local people in each study site were consulted to identify the localities of *Termitomyces* as well as for their support in research activities. Local people were also consulted for the local name of individual specimen along with its food and medicinal use (if any).

During sample collection, key morphological characters, *viz*. shape, size and colour of the fresh samples were noted. The specimens were photographed in their natural habitat and well dried in mushroom drier. Some specimens were preserved in liquid medium (Figure 5). Spore prints of representative specimens were taken in the field. The local name of individual specimens along with their medicinal used by native people was also noted on the spot. Specimens were packed in wax-paper bags wrapped with aluminum foil to prevent external infection and intermixing of the spores, and labeled. The collected samples and spore print were brought into the Lab for the macro- and micro-morphological studies.

2.3.3 Laboratory Analysis

The specimens were brought to the Lab, CDB, TU, for the microscopic characters studied under an Olympus microscope (Model No. CX 22, Japan) and identified. For determining the groups, chemical reaction (Tulloss, 1994) were carried out by placing a very fine section passing through the epicutis to hymenial surface (spore bearing layer) were taken with the help of razor blade. The section of dried materials were then mounted in water with a drop of 10% KOH solution (this solution revives the section to its original size for the measurement), followed by NH_3OH, $FeSO_4$ (crystal

and solution), I_2 and Phelopthlene (its changed in colour were compared with the commercial colour chart leaf-let www.asianpaints.com. Then the section was covered with microscopic coverslip and gently tapped with a blunt pencil and observed under 15 × 10 and 15 × 40 powers used and when necessary with emersion oil in 15 × 100 power were used for further studies. The cyanophility was observed by placing the parts of species in cotton blue and mounting in lactophenol reagent and amyloidity of spores was observed by using Melzer's reagent Photographs were taken with a digital camera (Sony DSC S980 camera, China).

According to objective of the research, I had confined into the *Termitomyces* R. Heim preparation of their account with details, containing information about edibility, ecology, seasonal availability and nutraceutical relevance along with workable key for identification (Key to the species) of various *Termitomyces* species, which has not study still now were investigated. So that out of (241) taxa (Appendix 17), (14) species, and (3) forma, belongs to the genus *Termitomyces* are identified by standard literature and (2) species are newly proposed to the science. All the species of this genus are new to Nepal (except *T. clypeatus* & *T. eurrhizus*). They were subjected to the systematic treatment (viz. chemical reaction, key to the identification of the species, description of the species), data analysis in context of macro and micro morphological characters and cluster matrix analysis of *Termitomyces* in responses to species relationshop of the Nepalese *Termitomyces.*

Spore Print

During the sample collection period, spore print was taken, the stipe of the fruit body was cut and the cap was set on a piece of white paper (for white spores fungi, black paper were used or half white and half black paper) turning the gills downwards. A drop of water was mounted on the cap in order to minimize drying out of tissues. The materials were then placed on a container and incubated some time depending on the nature of the fruit body. Finally, caps were removed carefully and the characteristics of the spore prints were noted (Kuo, 2006).

Preservation of Mushrooms

The specimens were preserved in dry condition and some were in liquid condition.

Dry Preservation

The specimens gathered were dried immediately in order to prevent from rotting. Sun drying is the suitable for drying mushrooms. However, due to poor weather conditions and collections were made in rainy season, all the specimens were not dried only in sunlight. Wire trays with mushrooms specimens were placed over the hearth in about 40°C to dry mushrooms. After well dried, the specimens were packed in paper bags with some Naphthalene bulbs (or crystal of 1, 4 dichlorobenzene) and shield in auto lock plastic bags to prevent from decay and insects (Saini & Atri, 2000). Preserved specimens were thoroughly checked and were sun dried in the interval of 1 to 2 months (Figure 4). The mushrooms were carried (Fresh or dry preserved) to Lab for microscopic examination and identification (with the help of standard literature) in Central Department of Botany, Tribhuvan University, Kathmandu, Nepal, The dried specimens are housed in NHM, TU., Nepal.

Figure 4: Dry preserved samples.

Liquid Preservation

The fleshy specimens for museum were preserved in a mixture of distilled water, alcohol and formalin (Figure 5) or distilled water and formalin with 70:25:5 or 95:5 concentrations followed by Ainsworth (1971).

Figure 5: Liquid preserved samples.

2.3.4 Identification

Identification was done based on diagnostic morphological characteristics and microscopic examinations. For this basidiospores (length and width) of each species were measured in the lab based on a range of 25 spores, and its mean values were provided; using a high-power microscope (Olympus CX22, magnification $100_{\times}$). For determining the interrelationship between the species of *Termitomyces* using hierarchical cluster analysis of dendrogram using average linkage (nearest neighbor) rescalede distance cluster combine method by applying spss 20 version. The concerning literatures for identification of the samples were used as follow: (Heim, 1941, 1942c, 1951, 1958, 1977); (Natarajan, 1979); (Bels & Pateregetvit 1982); (Rawla, *et al.*, 1983); (Leelavathy, *et al.*, 1985); (Morris, 1986); (Singer,1986); (Piearce, 1987); (Vander & Eicker, 1990); (Bother & Eicker, 1991); (Zang, 1992); (Atri & Kaur, 2003); (Harkonen & Mwassumbi, 2003); (Tang, *et al*., 2006); (Tie *et al*., 2006); and on line data base such as: Biodiversity Library.org, Index fungorum, Jstor.org, Mycobank.org, tropicos.org) Voucher specimens are deposited in NHM, Swayambhu, Tribhuvan University, Kathmandu, Nepal.

Processing of the specimens

Experimental lab works were conducted for the anatomical study of the gills, its measurements including spores and its slides were prepared. Collected materials for their microscopic detail were examined and internal features of systematic importance of its microphotographs also taken. Measurement of spores and anatomical study of gills and stipes of the *Termitomyces* were done. Collected samples were documented and identified. Here, I adopted the classification of (Kuo, 2006).

2.4 Results

2.4.1 Basidioma macro-morphology

Nineteen *Termitomyces* taxa recorded in the study were identified based on diagnostic morphological characteristics and microscopic examinations (Pegler & Vanhaecke, 1994; Tibuhwa, *et al*., 2010; De, 2011; Sawhasan, *et al*., 2011; Tibuhwa, 2012b; Frøslev on-line). Measurements of pileus (diameter) and stipe (length and diameter) of each species were based on the average and range of 10 independent samples collected on the spot (Table 3).

2.4.1.1 Cap

Colour: This study showed that colour is a useful criterion for confining the species within the genus *Termitomyces*. Colour of the cap varies from whitish to greyish (*T. albuminosus, T. mammiformis, T. microcarpus f. santalensis*), greyish-brown (*T. clypeatus, T. eurrhizus, T. umkowaan, T.* new I and *T.* new II, ochraceous (*T. fuliginosus and T. straitus f. ochraceous*), creamy-white (*T. heimii* and *T. schimperi*), brown to grey (T. *straitus f. griseus, T. aurantiacus*) and tawny-brown to pale cream (*T. globulus, T. le-testui, T. microcarpus, T. robustus* & *T. badius*) between the species.

Surface: Cap surface differ between species. On the basis of cap surface taxa are divided into two groups. In first group are the species with wrinkled, smooth, glabrous, silky, viscid, rugulose, radially striate, whitish to greyish brown scale scattered over the pileus and included 17 taxa (*T. albuminosus, T. clypeatus, T. eurrhizus, T. fuliginosus, T. globulus, T. heimii, T. le-testui, T. mammiformis, T. microcarpus, T. microcarpus f. santalensis, T. stratus f. griseus, T. stratus f. ochraceous, T. umkowaan, T.* new I, *T.* new II, *T. aurantiacus* & *T. badius*); and in second group are the species with concentrically scrobiculate or radially ridged, glabrous, smooth, viscid, brown scales with squamules and included two species (*T. robustus* and *T. schimperi*).

Margin: Margin of the cap varies between the species. On the basis of its cap margin they can be divided into 5 groups. In first group are the species with incurved margin, which includes (*T. albuminosus, T. fuliginosus, T. globulus, T. heimii, T. mammiformis, T. microcarpus, T. microcarpus f. santalensis, T. schimperi, T. straitus f. ochraceous, T.* new I, *T.* new II, *T. aurantiacus* & *T. badius*). In second group are the species with appendiculate, irregular lobed margin, which includes (*T. clypeatus* and *T. le-testui*). In third group is the only one species (*T. robustus*) have lacerate margin. In fourth group are the species with incurved margin when young and slightly up lifted when mature, which includes (*T. straitus f. griseus* & *T. umkowaan*) and in the fifth group is the only one species (*T. eurrhizus*) have margin regular but not incurved.

Shape: Similarly its shape also varies between the species. According to the shape of the pileus, they can be divided into 8 groups. In the first groups is the only one species (*T. albuminosus*) have applano-umbonate in shape. In the second groups are the

species with plano-umbonate shape which includes (*T. clypeatus* & *T. heimii*). In third group are the species with umbo shape, which includes (*T. eurrhizus, T. mammiformis, T. microcarpus, T.* new I, *T. aurantiacus* & *T. badius*). In fourth group are the species with bullate-umbonate shape, which includes (*T. fuliginosus* and *T.* new II). In fifth group is the only one species (*T. globulus*) with gibbous shape cap. In sixth group is the also only one species (*T. le-testui*) with companulate-umbo shape. In seventh group are the species with conical to convexo-applanate shape of pileus, which includes (*T. microcarpus f. santalensis, T. robustus, T. schimperi, T. stratus f. griseus* & *T. stratus f. ochraceous*) and in eighth group is the only one species (*T. umkowaan*) have obtus-umbo shape of pileus.

Sizes: Caps size varies between the species, ranging from less than 4 cm (small) to 26 cm (large). For easy to understand on the basis of cap size *Termitomyces* are divided into 3 groups. In first group are the species with small size cap (< 4 cm), which includes *Termitomyces microcarpus* and *T. microcarpus f. santalensis.* In second group are the species with medium size cap (between 4-20 cm), they include *T. albuminosus, T. aurantiacus, T. badius, T. clypeatus, T. fuliginosus, T. globulus, T. heimii, T. mammiformis, T. stratus f. griseus, T. stratus f. ochraceous, T.* new I and *T.* new II., and in the third group are the species with a larger size cap (≥20 to 26 cm), which includes *T. eurrhizus, T. le-testui, T. robustus, T. schimperi* and *T. umkowaan.*

2.4.1.2 Perforatorium

Colour: Colour is also a useful for confining the species of this genus. Colour of the perforatorium varies from olivaceous to ochraceous (*T. albuminosus, T. straitus f. ochraceous* & *T. badius*), Creamy to dark-grey (*T. clypeatus, T. eurrhizus, T. fuliginosus T. microcarpus, T. microcarpus f. santalensis, T. schimperi* & *T. aurantiacus*) and dark-brown (*T. globulus, T. heimii, T. le-testui, T. mammiformis, T. robustus, T. straitus f. griseus, T. umkowaan, T.* new I & *T.* new II) between the species.

Shape: Perforatorium shape varies according to variation between the species. According to the shape of the perforatorium the *Termitomyces* can be divided into 6 groups. In the first groups are the species with slightly raised convex with prominent conical-umbonate to bullate-umbonate, which includes (*T. albuminosus, T. eurrhizus,*

T. fuliginosus, T. globulus, T. heimii, T. schimperi, T. straitus f. griseus, T. umkowaan, T. new I, *T.* new II, *T. aurantiacus* & *T. badius*). In the second group is the only one species (*T. clypeatus*) with strongly spiniform perforatorium. In the third group is also an only one species (*T. le-testui*) with cylindrical umbo "teat". In the fourth group is also an only one species (*T. mammiformis*), with mammiform and scrobiculate cone like pointed projection. In the fifth groups are the species with small conic, spiniform papilla, which includes (*T. microcarpus, T. microcarpus f. santalensis* & *T. ochraceous*). In sixth groups is also an only one species (*T. robustus*) with conical, submamilate continuing of cap.

2.4.1.3 Gills

Colour: Colour is also a useful for confining the species of this genus. Colour of the gills (lamellae) varies from white (*T. albuminosus, T. clyapeatus, T. eurrhizus, T. globulus, T. heimii, T. le-testui, T. mammiformis, T. microcarpus, T. microcarpus f. santalensis, T. robustus, T. schimperi, T. straitus f. griseus, T. umkowaan, T.* new I, *T.* new II, *T. aurantiacus* & *T. badius*), pink in (*T. fuliginosus*) and orange in colour (*T. straitus f. ochraceous*) between the species.

Ornamentation: Ornamentation varies according to variation between the species. The gills of the *Termitomyces* can be divided into 5 groups. In the first groups are the species with dense, free adnexed, crowded, thick, which includes (*T. albuminosus, T. fuliginosus, T. microcarpus* & *T. robustus*). In the second groups are the species with free, narrow, sometimes unequal, crowded, thick, which includes (*T. aurantiacus, T. badius, T. eurrhizus, T. globulus, T. le-testui, T. mammiformis, T. schimperi, T. straitus f. ochraceous, T. umkowaan, T.* new I, & *T.* new II.). In the third groups are the species with free, crowded and entire margin, which includes (*T. clypeatus* & *T. straitus f. griseus*). In the fourth group is the only one species (*T. microcarpus f. santalensis*) with sinuate; crowded with lamellulae of three unequal lengths and in the fifth group is also an only one species (*T. heimii*) with free, crowded and serrulate margin.

2.4.1.4 Stipe

Colour: Colour is also a useful for confining the species of this genus. Colour of the stipe varies from white with greyish tint (*T. albuminosus, T. badius, T. globulus, T.*

heimii, T. le-testui, T. mammiformis, T. microcarpus, T. microcarpus f. santalensis, T. schimperi, T. umkowaan, T. new I, & *T.* new II), Buffy-brown and below the pellicle grey in colour (*T. fuliginosus* & *T. straitus f. ochraceous*), pale brown (*T. robustus*), grey in colour (*T. microcarpus f. santalensis*) and concolorous (T. *straitus f. griseus*) between the species.

Surface: Similarly *Termitomyces* surfaces differ between the species. On the basis of its surface they are divided into 3 groups. In first group are the species with smooth and glabrous surface, which includes (*T. albuminosus, T. badius, T. mammiformis, T. microcarpus, T. microcarpus f. santalensis, T. robustus, T. schimperi, T. straitus f. griseus, T. straitus f. ochraceous, T.* new II). In the second group are the species with fibrous, fibrillose, smooth, glabrous, having thick pellicle, pubescent surface, which includes *T. clypeatus, T. eurrhizus, T. fuliginosus, T. globulus, T. le-testui, T. umkowaan, T.* new I & *T. aurantiacus*) and in the third group is the only one species (*T.* heimii) with stuffed surface.

Shape: Stipe shape varies according to variation between the species. According to the shape of the stipe the *Termitomyces* can be divided into four groups. In the first groups are the species with long, cylindrical, solid, slender, which includes (*T. albuminosus, T. badius, T. clypeatus, T. eurrhizus, T. fuliginosus, T. globulus, T. heimii, T. le-testui, T. mammiformis, T. schimperi* & *T. straitus f. ochraceous*). In the second groups are the species with small slender, cylindrical, uniformly thick, slightly elongated below soli which includes (*T. microcarpus* & *T. microcarpus f. santalansis*). In the third group is the only one species (*T.* robustus) with conical, tapering toward down ward and in the fourth group are the species with central, cylindrical, solid, slightly swollen at the middle, which includes (*T. aurantiacus, T. straitus f. griseus, T. umkowaan, T.* new I & *T.* new II).

Size: Its size varies between the species, ranging from ≤ 4 (small) to ≥ 10 cm (large). For easy to understand on the basis of its size *Termitomyces* are divided into 3 groups. In first group are the species with small size stipe (< 5 cm), which includes (*T. albuminosus, T. badius, T. fuliginosus* & *T. microcarpus f. santalensis*). In second group are the species with medium size stipe (≥ 4 − ≤ 10 cm), which includes (*T. clypeatus, T. globulus, T. heimii, T. mammiformis* & *T. straitus f. ochraceous*) and in third group are the species with larger size stipe (≥ 10 cm), which includes (*T. aurantiacus, T. eurrhizus, T. le-testui, T. microcarpus, T. robustus, T. schimperi, T. straitus f. griseus, T. umkowaan, T.* new I & *T.* new II).

2.4.1.5 Annulus

Annulus varies from completely absent (*T. aurantiacus, T. badius, T. clypeatus, T. fuliginosus, T. globulus, T. microcarpus, T. microcarpus f. santalensis, T. robustus, T. straitus f. griseus, T. straitus f. ochraceous, T. umkowaan* & *T.* new I,), to microscopic squamules, (*T. eurrhizus* & *T.* new II.) to fugacious appendiculate (*T. le-testui* & *T. mammiformis*) and to very thick ring like structure encircling the stipe (*T. albuminosus, T. heimii* & *T. schimperi*).

Table 3: Summary of macro-morphological characters of the Nepalese *Termitomyces.*

SN	Taxa	Cap				
		Colour	**Surface**	**Margin**	**Shape**	**Size (l×w) cm**
1	*T. alb*	White to greyish	Smooth, wrinkles, scales	Incurved	Applanato-umbonate	9.0-14.0 (Medium)
2	*T. aur.*	Brown to grey	Smooth	Incurved	Umbonate	10-15 (Medium)
3	*T. badi*	Pale-grey to dark brown	Smooth, glabrous	Incurved	Umbonate	10-14 (Medium)
4	*T. cly*	Greyish brown	Silkt, smooth, glabrous	Irregular lobed	Plano-umbonate	5.5-7.5 (Medium)
5	*T. eur*	Dark greyish brown	Viscid, rugulose, scaly	Regular	Umbonate	20.5-22.0 (Large)
6	*T. ful*	Ochraceus	Smooth	Incurved, entire	Bullate-umbonate	4.75-5.50 (Medium)
7	*T. glo*	Tawny brown	Smooth, glabrous, radially striate	Incurved, lobed, splitting	Gibbous	6.0-9.0 (Medium)
8	*T.hei*	Creamy-white	Smooth, silky	Incurved	Plano-umbonate	7.5-9.0 (Medium)
9	*T. let*	Cream to light brown	Smooth, tomentose	Apendiculate	Companulate-umbo	21.0-22.5 (Large)
10	*T. mam*	Whitish grey	Smooth, covered by firm creamy partial veil	Entire	Umbo	4.5-7.5 (Medium)
11	*T. mic (small)*	Creamy-white	Smooth, glabrous	Entire, splits	Umbo	1-3 (Small)
12	*T. mic (large)*	Whitish to greyish	Smooth	Incurved, entire	Conical to umbonate	1-4 (Small)

13	*T. rob*	Tawny brown to pale cream	Smooth, glabrous, viscid, concentrically scrobiculate or radially ridged	Lacerate	Conical to convexo-applanate	20.5×21.5 (Large)
14	*T. sch*	Cream-white	Smooth, concentric ring of brown scales with squamule	Incurved	Conical	20.5-21.0 (Large)
15	*T. st. f. gri*	Grey to greyish brown	Radially striate	Incurved	Conical to convexo-applanate	4.5-8.5 (Medium)
16	*T. st. f. och*	Ochraceus-brown	Smooth	Incurved	Conical to convexo-applanate	4.0-9.0 (Medium)
17	*T. umk*	Grey to brown buff	Smooth, viscid, radially wrinkled	Incurved	Obtuse-umbo	21-26 (Large)
18	*T.* new I	Greyish brown	Smooth, radially striate	Incurved	Umbonate	9-15 (Medium)
19	*T.* new II.	Dark- grey	Wrinkles, smooth	Entire	Bullate-umbonate	8.0-16.0 (Medium)

Table 3. Continued...

SN	Perforatorium		Gills	
	Colour	**Shape**	**Colour**	**Ornamentation**
1	Olivaceous	Umbonate	White	Dense, free, adnex
2	Dark-grey	Umbonate	White to pale-grey	Dense, crowded, free
3	Olive	Umbo	Grey	Dense, free
4	Dark-grey	Spiny	Creamy-white to pink	Crowded, free, edge entire with lamellulae
5	Dark-grey	Rounded-umbo	White	Crowded, free, with lamellulae
6	Grey	Bullate-umbonate	Pink	Crowdwd, free, adnexed
7	Dark-grey	Umbonate	White to pale pinkish	Densely crowded, free, narrow with lamellulae
8	Dark-brown	Umbo	White to pinkish	Crowded, free, serrulate margin
9	Dark-brown	Cylindrical. Umbo "teat"	White	Free, crowded unequal
10	Blackish-brown	Mammiformis & scrobiculate, cone like pointed projection	White to pinkish	Crowded, free, with lamellulae
11	Cream	Small spiniform	White	Crowded, free, thick

12	Creamy-grey	Conic	Concolourous	Sinuate, crowded, with lamellulae, smooth, entire edge
13	Partly blackish	Conical, sub-mammillae,	Whitish	Densely crowded, free
14	Dark-grey	Conical to bullate umbo	Whitish	Dense, crowded, free
15	Brown	Convex with prominent umbo	Whitish	Crowded, free, equal, smooth edge
16	Olivaceous	Small spinifirm	Pale-yellow	Crowded, free
17	Yellowish-brown	Umbo	White	Dense, crowded, free
18	Brown	Umbo	White	Dense, free
19	Brown	Bullate-umbonate	White to pale grey	Dense, crowded, free

Table 3. Continued...

SN	Stipe				Annulus
	Colour	**Surface**	**Shape**	**Size (l×w) cm**	
1	White with greyish tint	Smooth, fibrillose	Long, cylindrical, solid	30×1	Thick, white, persistance
2	Silvery-white	Smooth	Solid, swollen at base & tapering toward distal end	7.0-9.0× 2.0-2.5	Absent
3	White	Smooth, glabrous	Solid, straight	5.2×0.75	Absent
4	White to dirty brown	Smooth, fibrous, glabrous	Long, cylindrical, slender, solid, bulbous at middle & below it hollow	7-9×1.2	Absent
5	Creamy-white	Fibrillose	Tubular, cylindrical, bulbous base, above ground hollow below ground solid	20×1.5	Apical annulus, persistance
6	Buffy-brown and below pellicle grey	Smooth, fibrous, glabrous, pellicle thick	Cylindrical, solid, slightly tapering down wards	5×0.9	Absent
7	Creamy-white	Smooth, fibrous, glabrous	Cylindrical, solid	7×1.5	Absent
8	Creamy-white	Above annulus stuff & below its fibrous, smooth	Cylindrical, solid, bulge near the pileus	5×1.5	Thick, white, persistance

9	Cream	Pubescent	Solid, fusiform	12-18× 2-3	Present
10	White	Smooth, glabrous	Solid, cylindrical, slightly enlarged at ground level	8×1.2	Persistent
11	White	Smooth, glabrous	Slender, hollow,	3.5×0.5	Absent
12	Grey	Smooth, glabrous	Cylindrical, slender, slightly elongated below soil	3-8× 0.4-0.6	Absent
13	Pale-brown	Smooth, glabrous	Conical, tapering towards down ward	18×3	Absent
14	Creamy	Smooth, glabrous	Solid	15×2.5	Present
15	Concolorous	Smooth, glabrous	Cylindrical, slightly swollen at the middle solid	7×1.7	Absent
16	Buffy-brown	Smooth, glabrous	Solid	4×1.8	Absent
17	White	Smooth	Solid, cylindrical, swollen at base	20×0.7	Absent
18	White to pale yellow	Smooth	Umbonate	10×2	Absent
19	White	Smooth	Solid, swollen at base	7×2	Present

Table 3. Continued...

SN	**Pseudorrhizea**			
	Colour	**Surface**	**Shape**	**Size (l×w) cm**
1	Dark-brown	Cartileginous	Hollow, tapering towards the distal end	30×1
2	Dark-brown	Cartileginous	Solid & tapering towards distal end	5×0.5
3	Brown	Smooth, glabrous	Solid, cylindrical, & widening at certain depth, then tapering towards distal end	5×1
4	Pale-grey	Smooth, glabrous, fibrous	Solid, slender, bulbous, tapering towards distal ends	3-5×1
5	Dark-brown	Cartileginous	Solid, cylindrical, tapering towards the distal end	60×1.5
6	Grey to brown	Smooth, fibrous	Solid, cylindrical, widening at certain depth then tapering towards distal ends	3.5×0.7
7	Tawny to rusty brown	Smooth, fibrous	Solid, slender	6×1
8	Cream to pale-grey	Leathery	Hollow, fleshy, cylindrical, widening at certain depth then tapering towards distal end	60×1.7

9	Dark-brown	Smooth, glabrous, fibrous	Hollow, cylindrical,tapering towards the distal end	18×1.5
10	Pale-grey to dark brown	Leathery-smooth	Hollow, cylindrical, tapering towards the distal end	19×1.2
11	Absent	Absent	Absent	Absent
12	Greyish brown	Smooth, glabrous	Solid, cylindrical, tapering towards distal end	5×0.3
13	Dark-brown	Cartileginous	Hollow, cylindrical, tapering towards the distal end	30×1.5
14	Dark-brown	Smooth	Solid, cylindrical, widening at certain depth then tapering towards distal ends	17×1.6
15	Pale to dark brown	Smooth, glabrous	Cylindrical, slender, bulbous	3.5×1
16	Pale to dark brown	Smooth, glabrous	Cylindrical, slender, bulbous	3.5×1
17	Rusty-brown	Stuffed	Solid, cylindrical, tapering towards distal end	12×1
18	Buffy-brown	Smooth, glabrous	Solid, cylindrical, widening at certain depth then tapering towards distal ends	5×0.5
19	Brown	Smooth, glabrous	Solid, cylindrical, slender, widening at certain depth then tapering towards distal end	7×0.5

Table 3. Continued...

SN	**Flesh**			**Spore print**
	Colour	**Hyphae**	**Clamp connection**	**Colour**
1	Whitish	Inamyloid,inflatened,thin walled	Absent	Brown
2	Whitish	Inamyloid, thin walled, inflatened, soft	Absent	Pink
3	Whitish	Inamyloid, thin walled, inflatened, soft	Absent	Pinkish
4	Whitish	Inamyloid,inflatened,thin walled, interwoven, hyaline	Absent	Pink
5	White	Inamyloids, inflated, thin walled	Absent	Pink
6	Buffy-brown	Hyaline, thin walled, soft	Absent	Purplish
7	Whitish	Firm to spongy, inflated	Absent	Pinkish
8	White	Inamyloids, inflated, thin walled, septed,parallel, hyaline, soft, fleshy	Present	Brown to pinkish grey
9	Whitish	Inamyloids, thin walled, interwoven, soft, hyaline	Absent	Pinkish
10	White	Inamyloids, thin walled, cylindrical, fleshy, soft, hyaline	Absent	Pinkish
11	Whitish	Inamyloids, thin walled, interwoven, soft, fleshy, hyaline,	Present	Pale-pinkish

12	Whitish	Inamyloid, thin walled, hyaline, spongy, inflatened,interoven	Present	Brown
13	Grey	Inamyloid, thin walled, easily separable from the cuticle, soft, fleshy, hyaline,	Absent	Pinkish
14	Whitish	Inamyloid, thin walled, filamentous, hyaline,	Absent	Pale-cinnamon
15	Pale-grey	Inamyloid, thin walled, filamentous, hyaline,	Absent	Pinkish-cream
16	Whitish	Inamyloid, thin walled, cylindrical, hyaline	Absent	Cool-pink
17	Whitish-grey	Thick, hyaline	Absent	Brownish
18	Grey	Inamyloid, inflatened, soft	Absent	Pinkish
19	Grey	Inamyloid, inflatened, soft	Absent	Light-brown

2.4.1.6 Pseudorrhizea

Colour: Colour is also a useful for confining the species of this genus. Its colour also varies between the species. On the basis of its colour they are divided into 4 groups. In first group are the species with dark-brown pseudorrhizea, which includes (*T. albuminosus, T. aurantiacus, T. clypeatus, T. le-testui, T. robustus, T. schimperi* & *T. umkowaan*), In second group are the species with pale-grey colour of pseudorrhizea, which includes (*T. clypeatus, T. fuliginosus, T. globulus, T. heimii, T. mammiformis* & *T. microcarpus f. santalensis*), In third group is the species with none of pseudorrhizea (*T. microcarpus*), In fourth group are the species with brown colour of pseudorrhizea, which includes (*T. badius, T. straitus f. griseus, T. straitus f. ochraceous, T.* new I & *T.* new II).

Surface: Similarly their surfaces differ between the species. On the basis of its surface they are divided into 5 groups. In first group are the species with cartilaginous surface, which includes (*T. albuminosus, T. aurantiacus, T. eurrhizus* & *T. robustus*). In second group are the species with smooth and glabrous surface, which includes (*T. badius, T. clypeatus, T. fuliginosus, T. globulus, T. le-testui, T. microcarpus f. santalensis, T. schimperi, T. straitus f. griseus, T. straitus f. ochraceous, T.* new I & *T.* new II). In third group are the species with smooth and leathery surface, which includes (*T. heimii* & *T. mammiformis*). In fourth group is the only one species (*T. umkowaan*) with fibrillose and stuffed surface. In fifth group is the species with none of pseudorrhizea (*T. microcarpus f. santalensis*).

Shape: Similarly its shape also varies between the species. According to the shape of the pseudorrhizea, they can be divided into three groups. In the first groups are the

species with hollow, cylindrical and widening at certain depth, than narrowing down to the point of attachment to the termite nest, its length determined by the depth of the termite comb, of pseudorrhizea, which includes (*T. albuminosus, T. heimii, T. le-testui, T. mammiformis, T. mammiformis f. santalensis* & *T. robustus*). In the second groups are the species with solid, cylindrical and widening at certain depth, than narrowing down to the point of attachment to the termite nest, its length determined by the depth of the termite comb, of pseudorrhizea, which includes (*T. aurantiacus, T. badius, T.* clypeatus, *T. eurrhizus, T. fuliginosus, T. globulus, T. schimperi, T. straitus f. griseus, T. straitus f. ochraceous, T. umkowaan, T.* new I & *T.* new II,). In third group is the species with none of pseudorrhizea (*T. microcarpus*).

Size: Its size varies between the species, ranging from ≤ 5 (small) to ≥ 12 cm (large). For easy to understand on the basis of its size they are divided into 4 groups. In first group are the species with small size pseudorrhizea (< 5 cm), which includes (*T. aurantiacus, T. badius, T. clypeatus, T. fuliginosus, T. microcarpus f. santalensis, T. straitus f. griseus, T. straitus f. ochraceous, T.* new I & *T.* new II), In second group are the species with medium size pseudorrhizea (5 to 12 cm), it include only one species (*T. globulus*), in the third group are the species with a large size pseudorrhizea (≥ 12 cm), they are (*T. albuminosus, T. eurrhizus, T. heimii, T. le-testui, T. mammiformis, T. robustus, T. schimperi* & *T. umkowaan*) and none of them (*T. microcarpus*).

2.4.1.7 Flesh

Colour: Colour is also a useful for confining the species of this genus. Colour of the flesh varies from white (*T. albuminosus, T. aurantiacus, T. badius, T. clyapeatus, T. eurrhizus, T. globulus, T. heimii, T. le-testui, T. mammiformis, T. microcarpus, T. microcarpus f. santalensis,* T. robustus, *T. schimperi, T. straitus f. ochraceous, T. umkowaan, T.* new I & *T.* new II) and buffy-brown (*T. fuliginosus* & *T. straitus f. griseus*).

Hyphae: Hyphal nature of the flesh, are also a useful for confining the species of this genus. It became varies between the species from nonamyloid, hyaline, thin walled interwoven, cylindrical, easily separable from the cuticle, which includes (*T. albuminosus, T. aurantiacus, T. badius, T. clyapeatus, T. eurrhizus, T. fuliginosus, T. globulus, T. le-testui, T. mammiformis, T. microcarpus, T. microcarpus f. santalensis, T. robustus, T. straitus f. ochraceous, T. umkowaan, T.* new I & *T.* new II) and septed, parallel, filamentous, which includes *T. heimii, T. schimperi* and *T. straitus f. griseus.*

Clamp connection: Presence or absences of clamp connection are also a useful for confining the species of this genus. Clamp connection of the hyphae varies due to the absence (*T. albuminosus, T. aurantiacus, T. badius, T. clyapeatus, T. eurrhizus, T. fuliginosus, T. globulus, T. le-testui, T. mammiformis, T. robustus, T. schimperi, T. straitus f. griseus, T. straitus f. ochraceous, T. umkowaan, T.* new I & *T.* new II) and presence (*T. heimii, T. microcarpus* & *T. microcarpus f. santalensis*).

2.4.1.8 Spore prints

On my studied and investigation samples, of the colours of spore prints are pink (in *T. aurantiacus, T. clypeatus, T. eurrhizus* & *T. mammiformis,*), brown (in *T. albuminosus, T. heimii, T. umkowaan* & *T.* new II) and pinkish (in *T. fuliginosus, T. globulus, T. le-testui, T. microcarpus, T. microcarpus f. santalensis, T. robustus, T. schimperi, T. straitus f. griseus, T. straitus f. ochraceous* & *T.* new I).

2.4.2 Dendrogram analysis

Among the nineteen taxa of Nepalese *Termitomyces* in dendrogram for a hierarchical clustering analysis show that Tm of 11 and Tsg of 15 numbers i.e. *T. microcarpus* and *T. straitus f. ochraceus* are distinct to other *Termitomyces* (Figure 6). On the basis of hierarchical cluster analysis between the species they can be categorized into seven groups. In group-A (*T.* n1, *T.* n2, *T. badius, T. globulus, T. schimperi, T. fuliginosus,* & *T. le-testui*) are present. In group-B (*T. albuminosus, T. clypeatus, T. mammiformis* & *T. eurrhizus*) are closely interrelated. In group-C, (*T. robustus* & *T. umkowaan*) are showed the similar characters in many respects). In groups-D (*T. aurantiacus* & *T. heimii*) are similar. In group-E (*T. microcarpus*) its characters are unique due to smallest in size. In group-F (*T. microcarpus f. santalensis* & *T. straitus f. ochraceous*) are similar to one another. In groups-G (*T. straitus f. griseus*) is another unique character, due to perforatorium with continuation of cap, not so long spiniform papilla, though all the species are interlinked within the studied taxa.

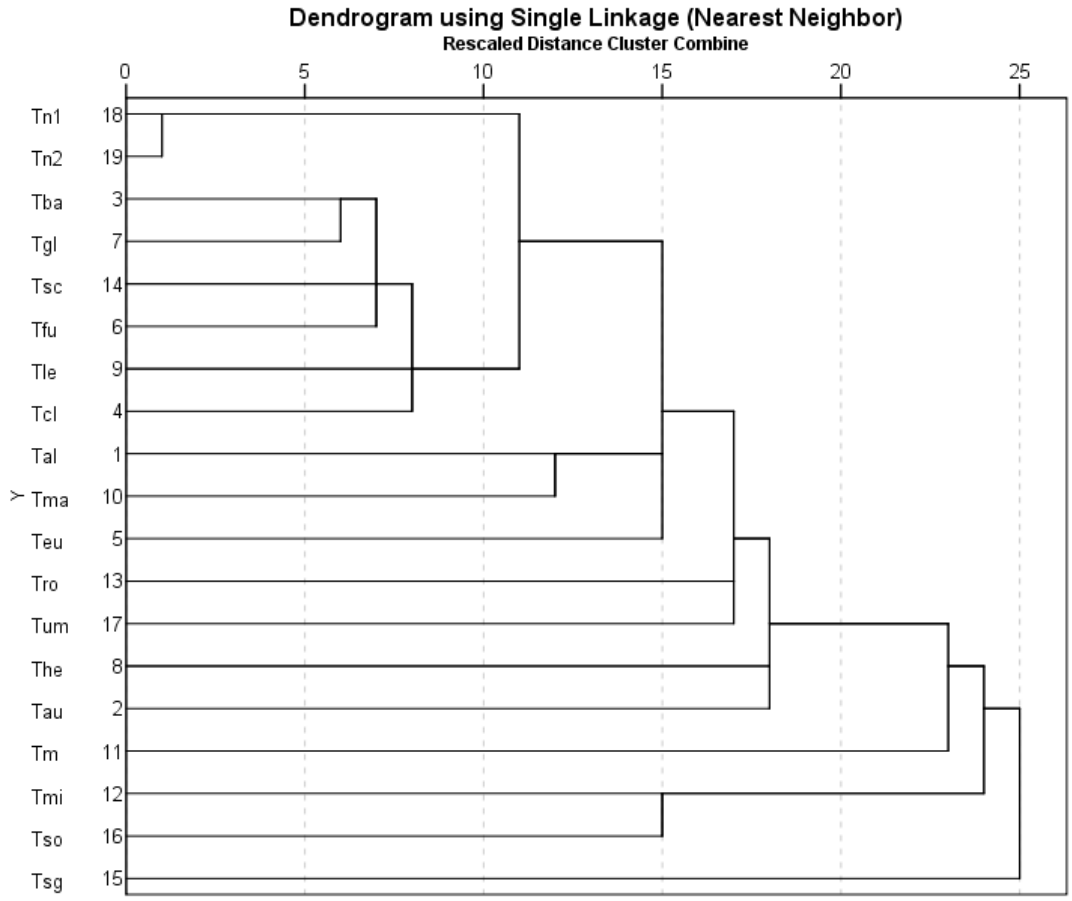


Figure 6: Results of cluster analysis.

Note: Among the species based on macro-morphological characters of the Nepalese *Termitomyces* (Tal= *T. albuminosus*, Tau= *T. aurantiacus*, Tba= *T. badius*, Tgl=*T. globulus* Tcl= *T. clypeatus*, Teu= *T. eurrhizus*, Tfu= *T. fuliginosus*, The= *T. heimii*, Tle= *T. le-testui*, Tm= *T. microcarpus*, Tmi= *T. microcarpus f. santalensis*, Tma= *T. mammiformis*, Tro= *T. robustus*, Tsc= *T. schimperi*, Tsg= *T. straitus f. griseus*, Tso= *T. straitus f. ochraceous*, Tum= *T. umkowaan*, Tn1= *T.* new I, Tn2= *T.* new II).

2.4.3 Basidioma micro-morphology

Micro-morphological characters mainly study in basidia, cystidia and basidiospores (Table 4). It also provides ample information for species identification.

2.4.3.1 Basidia

Its size is useful for identification usually for more closely related species. From the results, the basidia size within the genus little differ (10-12 × 4-6 of µm of *T. microcarpus* to 30.0µ × 0.9µ of *T. fuliginosus*).

Table 4: Summary of micro-morphological characters of the Nepalese *Termitomyces.*

SN	Species	Basidia	Cystidia	Basidiospores
1	*T. albuminosus* (Berk.) R. Heim	(22.5-24.5 × 6.8-8.4) µm	(29-31 × 20-22) µm	(6.5-8.5 × 4-6) µm -ellipsoid
2	*T. aurantiacus* (R. Heim) R. Heim	(21.5-23.5 × 4.9-6.9) µm	(24-26 × 10.5-12.5) µm	(5.5-7.5 × 3.5-5.5) µm - ellipsoid
3	*T. badius* Otieno	(23-25 × 6-8) µm	(18-20 × 9.5-11.5) µm	(5.5-7.5 × 2.5-4.5) µm - ellipsoid
4	*T. clypeatus* R. Heim	(15.5-17.5 × 4.5-6.5) µm	(19-21 × 9-11) µm	(5.5-7.5 × 3.5-5.5) µm - ellipsoid
5	*T. eurrhizus* (Berk.) R. Heim	(10.66-12.66 × 5.9-7.9) µm	(34-36 × 10.5-12.5) µm	(8.3-10.3 × 5.8-7.8) µm - ellipsoid
6	*T. fuliginosus* R. Heim	(29-31 × 0.8-1.0) µm	(24-26 × 18-20) µm	(7-9 × 3-5) µm -ovoid
7	*T. globulus* R. Heim & Gooss.-Font.	(20-25 × 5-7) µm	(27-29 × 14-16) µm	(6.5-8.5 × 3.5-5.5) µm - ellipsoid
8	*T. heimii* Natarajan	(16.5-18.5 × 5.5-7.5) µm	(34-36 × 19-21) µm	(5.5-7.5 × 3.5-5.5) µm - ovoid
9	*T. le-testui* (Pat.) R. Heim	(23.1-25.1 × 6.3-8.3) µm	(35-37 × 14-16) µm	(6.25-8.25 × 2.5-4.5) µm - oblong
10	*T. mammiformis* R. Heim	(21.1-23.1 × 5.5-7.5) µm	(44-46 × 14-16) µm	(4.5-6.5 × 2.5-4.5) µm - ellipsoid
11	*T. microcarpus* (Berk. & Broome) R. Heim	(10-12 × 4-6) µm	(21-23 × 10-12) µm	(4-6 × 2-4) µm - ellipsoid
12	*T.microcarpus* (Berk. & Broome) R. Heim *f. santalensis*	(15-20 × 5-7) µm	(23-25 × 11-13) µm	(5-7 × 3-5) µm - ellipsoid
13	*T.robustus* (Beeli) R. Heim	(19.5-21.5 × 5.5-7.5) µm	(24-26 × 11-13) µm	(4.5-6.5 × 3.5-5.5) µm - ellipsoid
14	*T.schimperi* (Pat.) R. Heim	(26.5-28.5 × 7-9) µm	(31.5-33.5 × 13.5-15.5) µm	(5.5-7.5 × 3.5-5.5) µm - ellipsoid
15	*T. straitus f. griseus* **(**Beeli) R. Heim	(19.5-21.5 × 4.5-6.5) µm	(36-38 × 9-11) µm	(4.5-6.5 × 3.5-5.5) µm - ellipsoid
16	*T.straitus f.* ochraceus *R.* Heim.	(21-23 × 5.5-7.5) µm	(39.5-41.5 × 11-13) µm	(5.5-7.5 × 4.5-6.5) µm - ellipsoid
17	*T. umkowaani* (Cooke & Massee) D.A. Reid	(21-23 × 6.5-8.5) µm	(44-46 × 9-11) µm	(5.5-7.5 × 3.5-5.5) µm - ellipsoid
18	*T. palpensis* sp. nov. (new I)	(16.5-18.5 × 5.5-7.5) µm	(24-26 × 14-16) µm	(3.5-5.5 × 2.5-4.5) µm - ellipsoid
19	*T. arghakhanchensis* sp. Nov. (new II)	(18-20 × 5-7) µm	(29-31 × 16-18) µm	(3.5-5.5 × 2.5-4.5) µm - ovoid

2.4.3.2 Cystidia

Its size varies among the species 19.0 × 10.5 of *T. badius* to 45.0 × 10.5 of *T. mammiformis*. Its shape also varies from clavate to obovoid pyriform.

2.4.3.3 Basidiospores

They have little variation in their morphometry thus provides little taxonomic information for designating the species. All are ellipsoid except *T. le-testui,* which has oblong in shape. *T. eurrhizus* have somewhat large basidiospores and broadly clavate to pyriform cystidia. Untetra basidiosores is the distinguishing characters found in *T. umkowaan.* Size of the basidia and basidiospores are diverged.

2.4.4 Systematic treatment

For the exact identification of the species, there should be required chemical reaction test of xanthochoric and cynophilic reaction for determining the groups of the species and another is the identification key which helps to reach the specific species of the taxon.

2.4.4.1 Chemical reaction test

Determining the groups, of macro fungi, chemical reactions of mushroom tissue (spore bearing layer) with 10% KOH followed by NH_3OH, $FeSO_4$ (crystal and solution), I_2 and Phelopthlene were conducted (Tulloss, 1994). This helps to identify the agaricoid fungi. Its cynophility was observed with the lactophenol whereas amyloidity of spores was observed by using Melzer's reagent (Table 5).

2.4.4.2 Key to the species of Termitomyces

A diagnostic key is presented for 19 taxa of *Termitomyces* species based on the present collections, literature and descriptions by Pegler and Vanhaecke (1994), Tibuhwa et al. (2010), De (2011), Tibuhwa (2012b), and Frøslev (on-line). So far, keys to *Termitomyces* were constructed based on colour of the cap, shape of the umbo (papilla), characteristics of an annulus and nature of stipe and pseudorrhizea (Table 3). In addition to the above-mentioned features (description of 19 taxa), (Table 4 & 5); I also have considered, in developing the following key for almost all the

Termitomyces occurring in the field during sample collection. Although some taxa are not distinctly classified as separate groups (Figure 6, Appendix 2). Based on the current observations, they can be delimited by specific features. In such cases, the key provides to identify the specific name.

Table 5: Chemical test.

Mushrooms tissus reacts with following chemicals, and after the reactions its changed colour compared with the commercial colour chart leaf-let (www.asianpaints.com).

SN	Collection No.	Accession No.	Taxa	Phenolphthalein	NH_3OH	KOH
1	VN-1010525-CDBP	NHM TU 2-2-1666	*T. albuminosus*	Cool Cream 3272	No Reaction	Cool Cream 3272
2	VN-1007232-CDBP	NHM TU 2-2-1682	*T. aurantiacus*	Beacon 3109	Spice 0477, melted	Cool Cream 3272
3	VN-1008296-CDBP	NHM TU 2-2-1683	*T. badius*	Cool Cream 3272	Sugarcane 0332	Promise 0P09
4	VN-1010530-CDBP	NHM TU 2-2-1667	*T. clypeatus*	Beacon 3109	No Reaction	Sugarcane 0332
5	VN-1007119-CDBP	NHM TU 2-2-1668	*T. eurrhizus*	Cool Cream 3272	White	White
6	VN-1008279-CDBP	NHM TU 2-2-1676	*T. fuliginosus*	Raw silk 0351	Sugarcane 0332	Yellow
7	VN-1007482-CDBP	NHM TU 2-2-77	*T. globulus*	Beacon 3109	No Reaction	Sugarcane 0332
8	VN-1010524-CDBP	NHM TU 2-2-1669	*T. heimii*	Ivory 0315	Barley 0572, soft	White
9	VN-1007481-CDBP	NHM TU 2-2-1670	*T. le-testui*	Volcano 0578	Barley 0572, soft	Beacon 3109
10	VN-1007154-CDBP	NHM TU 2-2-1678	*T. mammiformis*	Ivory 0315	White	White
11	VN-1008133-CDBP	NHM TU 2-2-1671	*T. microcarpus*	Cool Cream 3272	No Reaction	Sugarcane 0332
12	VN-1007429-CDBP	NHM TU 2-2-1679	*T. microcarpus f. san*	Cool Cream 3272	Sugarcane 0332	Sugarcane 0332

13	VN-1007158-TUCH	NHM TU 2-2-1672	*T. robustus*	Sugarcane 0332	Spice 0477, melted	Cool Cream 3272
14	VN-1007155-CDBP	NHM TU 2-2-1673	*T. schimperi*	Promise 0P09	Barley 0572, soft	Casablanca 7927
15	VN-1007251-CDBP	NHM TU 2-2-1675	*T. straitus. f. griseus*	Cool Cream 3272	No Reaction	Cool Cream 3272
16	VN-1007179-CDBP	NHM TU 2-2-1674	*T. striatus. f. ochraceus*	Raw silk 0351	White	Volcano 0578
17	VN-1107590-CDBP	NHM TU 2-2-1665	*T. umkowaan*	Ivory 0315	Cherry Brandy X126	Milk Toffee 7968
18	VN-1008184-CDBP	NHM TU 2-2-1680	*T. palpensis* sp. nov. (new I)	Sugarcane 0332	No Reaction	White
19	VN-1008227-CDBP	NHM TU 2-2-1681	*T. arghakhanchensis* sp. Nov. (new II)	Sporty Yellow X104	White	Rust 0569

Table 5. Continued....

SN	$FeSO_4$ solution	$FeSO_4$ crystal	Iodine
1	Stipe: outer white, Inner: rosy coral 8031, Cap: white, Gills: strawberry 0481	Stipe: outer white, Inner: Sunrise 0526, Cap: white, Gills: sugarcane 0332	Dorsal: white, Ventral: Beacon 3109
2	Stipe: outer white, Inner: rosy coral 8031, Cap: white, Gills: Raw Silk 0351	Stipe: outer negative and inner: strawberry 0481, Cap: negative. Gills: Raw Silk 0351	Dorsal: light Coffee 0470, Ventral: Ivory 0315
3	Stipe: outer Ting of rose 8084, Inner: Pale Rose 0421, Cap: Ting of Rose 8084, Gills: Macrame 0495	Stipe: outer Lavender Dew 0941, Inner: In Vogue 8105, Cap: Lavender Dew 0941, Gills: Magnolia 0387	Dorsal: white, Ventral: Bathstone 0301
4	Stipe: outer white, Inner: rosy coral 8031, Cap: white, Gills: Raw Silk 0351	Stipe: outer negative and inner: strawberry 0481, Cap: negative. Gills: Raw Silk 0351	Dorsal: light Coffee 0470, Ventral: Ivory 0315
5	Stipe: outer white, Inner: rosy coral 8031, Cap: white, Gills: Raw Silk 0351	Stipe: outer negative and inner: strawberry 0481, Cap: negative. Gills: Raw Silk 0351	Dorsal: light Coffee 0470, Ventral: Ivory 0315
6	No reaction	Stipe: outer white, Inner: Sunrise 0526, Cap: white, Gills: sugarcane 0332	Dorsal: white, Ventral: Beacon 3109

7	Stipe: outer white, Inner: Rosy Coral 8031, Cap: white, Gills: Raw Silk 0351	Stipe: outer negative and inner: strawberry 0481, Cap: negative. Gills: Raw Silk 0351	Dorsal: light Coffee 0470, Ventral: Ivory 0315
8	Stipe: outer white, Inner: Pale Rosy 0421, Cap: white, Gills: Bliss 0170	Stipe: outer cream, Inner: volcano 0578, Cap: cream, Gills: Baby Blush 8138	Dorsal: white, Ventral: Barley 0572
9	Stipe: outer white, Inner: Red Earth 8029, Cap: white, Gills: Sandalwood 0485	Stipe: outer cream, Inner: Marco Polo 8607, Cap: cream, Gills: Pale Cream 0328	Dorsal: white, Ventral: Dawn 0469
10	Stipe: outer white, Inner: Red Earth 8029, Cap: white, Gills: Sandalwood 0485	Stipe: outer cream, Inner: Marco Polo 8607, Cap: cream, Gills: Pale Cream 0328	Dorsal: white, Ventral: Dawn 0469
11	No reaction	Stipe: outer white, Inner: Sunrise 0526, Cap: white, Gills: sugarcane 0332	Dorsal: white, Ventral: Beacon 3109
12	No reaction	Stipe: outer white, Inner: Sunrise 0526, Cap: white, Gills: sugarcane 0332	Dorsal: white, Ventral: Beacon 3109
13	Stipe: outer Ting of rose 8084, Inner: Pale Rose 0421, Cap: Ting of Rose 8084, Gills: Macrame 0495	Stipe: outer Lavender Dew 0941, Inner: In Vogue 8105, Cap: Lavender Dew 0941, Gills: Magnolia 0387	Dorsal: white, Ventral: Beacon 3109
14	Stipe: outer white, Inner: rosy coral 8031, Cap: white, Gills: strawberry 0481	Stipe: outer white, Inner: Sunrise 0526, Cap: white, Gills: sugarcane 0332	Dorsal: white, Ventral: Beacon 3109
15	Stipe: outer white, Inner: Pale Rosy 0421, Cap: white, Gills: Bliss 0170	Stipe: outer cream, Inner: volcano 0578, Cap: cream, Gills: Baby Blush 8138	Dorsal: white, Ventral: Barley 0572
16	Stipe: outer Ting of rose 8084, Inner: Pale Rose 0421, Cap: Ting of Rose 8084, Gills: Macrame 0495	Stipe: outer Lavender Dew 0941, Inner: In Vogue 8105, Cap: Lavender Dew 0941, Gills: Magnolia 0387	Dorsal: white, Ventral: Bathstone 0301
17	Stipe: outer white, Inner: Pale Rosy 0421, Cap: white, Gills: Bliss 0170	Stipe: outer cream, Inner: volcano 0578, Cap: cream, Gills: Baby Blush 8138	Dorsal: white, Ventral: Barley 0572
18	No reaction	Stipe: outer white, Inner: Sunrise 0526, Cap: white, Gills: sugarcane 0332	Dorsal: white, Ventral: Beacon 3109
19	Stipe: outer Ting of rose 8084, Inner: Pale Rose 0421, Cap: Ting of Rose 8084, Gills: Macrame 0495	Stipe: outer Lavender Dew 0941, Inner: In Vogue 8105, Cap: Lavender Dew 0941, Gills: Magnolia 0387	Dorsal: white, Ventral: Bathstone 0301

Key to Identification of species of Nepalese *Termitomyces* R. Heim

1a Fruit-body differentiated into prominent head and tail on mound 7

1b Fruit-body differentiated with short or long stipe, on soil or mound 2

2a Lower side of the cap with gills, smooth, dense, free 5

2b Lower side of the cap either decurrent or adnexed gilled 3

3a Stipe always longitudinally fibrous (when broken), fruit-bodies not sobrittle ..... *T. aurantiacus*

3b Stipe central, slender, hollow, white, fleshy-fibrous, smooth, papilla umbonate 4

4a Lamellae (mature) white, cream, pale yellow or very pale pink, free, crowded, dense, olivaceous perforatorium, pileus umbonate, incurved margin,stipe not rooting, not bulbous, without annulus, lamellae sub free, adnexed or deeply decurrent ..... *T. badius*

4b Lamellae (mature) white and spores dark brown or black; stipe moderately to deeply rooting (pseudorrhiza) or originating from a hypogeous sclerotium with bulbous base, pseudorrhiza originating from termite nest; nosclerotium..... *T. palpensis* sp. Nov. (new I)

5a Cap very small (< 2 cm diam.), pointed; fruit-bodies gregarious in large numbers, without pseudorrhizea and papilla, clamp connection present..... *T. microcarpus*

5b Cap larger, usually at least 4 cm diameter..... 6

6a Fruit body with pseudorrhizea (up to 5 cm long), & cap 1-4 cm diam., papilla conic..... *T. microcarpus f. santalensis*

6b Fruit-body small with pseudorrhiza; cap medium, approx 10 cm diameter, dark brown, perforatorium, bullate-umbonate annulus present..... *T. arghakhanchensis* sp. Nov. (new II)

7a Fruit bodies large and short pseudorrhizea, pileus yellowish brown or grayish yellow, smooth to radially wrinkled, umbo spiniform to sharply pointed, stipe bulbous near the soil surface, with long rust-brown pseudorrhiza ..... *T. umkowaan*

7b Fruit bodies in groups, growing on the sandy soil or on the shaded ground in association with termites nests 8

8a Pileus medium, 5-10 cm diameters, brownish orange, concentrically scrobiculate, umbo obtuse ..... *T. fuliginosus*

8b Pileus expanding to applanato-umbonate, perforatorium prominent and pointed, spiniform papilla, veil absent, stipe solid, pleurocystidia smooth 9

9a Perforatorium not so long spiniform, continuous, or demarcated, or absent 10

9b Perforatorium slightly raised, black ..... *T. albuminosus*

10a Perforatorium smooth, brown to blackish brown; cap silky greyish brown to ash grey ..... *T. clypeatus*

10b Perforatorium demarcated and prominent, either submammillate, mamiform or cylindric 11

11a Perforatorium absent or not demarcated, small, broadly conical, a continuation of the cap 12

11b Perforatorium mamiform and scrobiculate, blackish brown; cap white, 3−7 cm ..... *T. mammiformis*

12a Cap brownish or dark brown, 9-25 cm, perforatorium cylindric, smooth 13

12b Cap brownish, tomentose; perforatorium cylindric, dark brown; annulus persistent ..... *T. le-testui*

13a Cap scrobiculate or radially ridged, glabrous; perforatorium sub mamilate; no annulus ..... *T. robustus*

13b Cap large with distinct velar remnants, annulus present 14

14a Cap without velar remnants or with few minute whitish evanescent membranous squamules.....15

14b Cap white, with large dark squamules; perforatorium absent.....*T. schimperi*

15a Pseudorrhiza blackish (cortex); cap greyish brown, viscid, rugulose; annulus inconstant.....*T. eurrhizus*

15b Pseudorrhiza pale, not entirely blackish.....16

16a Cap medium, without papilla and annulus, ochraceous brown.....17

16b Cap smaller, globose, perforatorium low, small and poorly developed, stipe usually without annulus, greyish brown.....*T. globules*

17a Cap 10-20 cm, concentrically scrobiculate; pseudorrhiza partly blackish.....*T. robustus*

17b Cap first conical, then convexo-applanate (large size); perforatorium small, broadly conical, perforatorium 18

18a Cap whitish, smooth, with greyish brown, broad umbo.....*T. heimii*

18b Cap smaller (4-12 cm), not concentrically scrobiculate, at most radially striate, pileus ochraceous brown to grey brown, stipe without annulus ..... (*T. striatus*) 19

19a Stipe without annulus; cap ochraceus brown, with remnant, middle, radially striate.....*f. ochraceus*

19b Stipe without annulus; cap grey to greyish brown, without remnant, smaller, radially striate .....*f. griseus*

2.4.4.3 Taxonomic description of Nepalese *Termitomyces*

i) ***Termitomyces albuminosus*** (Berk.) R. Heim, *Mem. Acad., Sci., Paris* **44**, 72 (1941)

Local name: Vend Chhanii, Bagale, Vemtee.

Taxonomic position: Basidiomycota, Agarocomycetes, Agaricomycetidae, Agaricales, Tricholomataceae.

Identifying characters: The most distinguishing characters of this species are prominent, cone-like pointed, dark coloured projection at the centre of the cap i.e. spiniform papilla. Pileus expands to applanato-umbonate; veil absent, stipe solid, Pleurocystidia smooth. Sporophores are found in groups, growing on the sandy soil or on the shaded ground in association with termite's nests.

Description

Sporophores: Usually growing solitary in termite hills, characterized by its obligate symbiont with termites; usually centrally stipitate, white but olivaceous near the umbonal region.

Pileus: 9-14 cm in diameter, white, fleshy, surface covered with wrinkles, medium in size, sub conical to expanded, obtusely umbonate, whitish with grayish or brownish scale scattered over the pileus with smooth, incurved margin, perforatorium (Papilla) is slightly raised, black. Pelicles are thick, separable, below it whiter in colour. Lamellae: adnexed, white, dense, free. Stipe: cylindrical, 30 cm × 1 cm, smooth, fibrous, white, solid, with grayish tint, a created upwards cartilaginous outside, Annulus thick, white, persistent. Pseudorrhiza: Significantly long and hollow root like extension, size up to 30 × 1 cm below the ground level. Colour Dark brown, surface cartilaginous, shape hollow, cylindrical tapering toward distal end. It became wide at certain depth, than narrowing down to the point of attachment to the termite nest, its length determined by the depth of the termite comb. Flesh: Whitish in colour, soft and inflated, hyphae nonamyloid, without clamp-connection. Basidiospores: 7.5 × 5.0 µm, hyaline, smooth walled, ellipsoid. Basidia: 23.5 × 7.6 µm, tetra sterigmata bearing tetra basidiospore. Spores: Pinkish in colour. Spore: 22.5 × 7.5 µm, nonamyloid, thin walled elliptical, hyaline, smooth. Spore print: Brown in colour. Cystidia: 30.0 × 21.0 µm. Hymenophoral trama: regular (Figure 7).

Habitat: On termite nests in tropical forest. Season: June-August.

Chemical test with

Phenolphthalein : Cool cream

NH_3OH : No change (i.e. dorsal (outer) milky white and inner cool cream, soapy.

KOH : Dorsal (outer) milky white and ventral (inner) cool cream.

$FeSo_4$ solution : Stipe: outer white & inner royal coral, cap white. Gills: strawberry.

$FeSo_4$ crystal : Stipe outer white, inner sunrise, cap: white, gills: Sugarcane.

Iodine : Dorsal (outer): white, inner: Beacon.

Specimen examined: On termites nest, common in *Shorea robusta* dominated subtropical deciduous hill forest of Thada-Dohonthe, Arghakhnchi district. Elevation 500 m asl, longitude: 83.09029° E, latitude: 27.86208° N, Aspect: 270° W, Slop: 20° W, Temp. 21.9-26.7° C; Humidity: 75-87%, Rain fall: 16 mm, moisture: 6 mm, Climate: subtropical monsoon climate, Soil pH: 5.7; Time: 11:46 am; Date: 19.07.2011. VN-1010525-CDBP. Accession No. NHM TU 2-2-1666. Report for new to Nepal.

Distribution: Equatorial, southern Africa, Madagascar, South East Asia including Nepal.

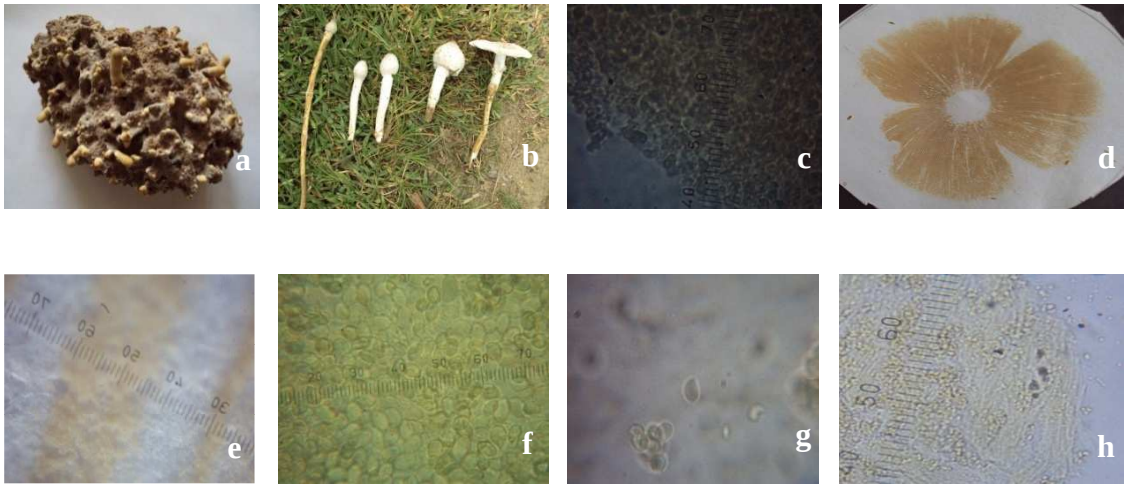


Figure 7: *Termitomyces albuminosus*, a.Termite's nest with fruiting Basidiocarp, b. Basidiocarp in ascending order of fruiting body, c. Basidiumes (1div=3.75 µm), d. Spore print, e. Lamellae difference of albuminosus (1div=3.75 µm) and f, g and h. spores (1div=3.75 µm)

ii) ***Termitomyces aurantiacus*** (R. Heim) R. Heim, *Termites et Champignons* (Paris), 56(1977)

Local name: Chotki Vend (Awadhi)

Taxonomic position: Division: Basidiomycota class: Agarocomycetes sub-class: Agaricomycetidae order: Agaricales family: Tricholomataceae.

Identifying characters: The most distinguishing characters of this species are medium size cap, (10-15 cm in diameter), brown to grey, smooth, glabrous. Perforatorium small, olivaceous broadly conical, a continuation of cap, demarcated and prominent, partly blackish, dark coloured projection at the centre of the cap, not so long spiniform papilla, demarcated as umbonate, dark grey in colour. Cap umbonate, without velar remnant, margin incurved, stipe with pseudorrhiza pale, not entirely blackish, originating from termite nest; no sclerotium, no annulus.

Description

Sporophores: Usually growing solitary in termite hills, characterized by its obligate symbiont with termites; usually centrally stipitate white but olivaceous near the umbonal region.

Pileus: Size 10-15 cm, (Medium size) brown to grey towards margin, olivaceous at the apex, umbonate, surface smooth, incurved margin. Lamellae: Free, dense, crowded, white to pale grey. Stipe: Solid, swollen at base, size 7-9 × 2.0-2.5 cm, silvery white, surface smooth, fibrous, annulus absent. Pseudorrhiza: Size 5 × 0.5 cm long, solid, dark brown in colour, surface cartilaginous, solid and widening at certain depth, then narrowing down to the point of attachment to the termite nest, its length determined by the depth of the termite comb. Flesh: Soft, thin walled, white, inflated, clamp connection absent, nonamyloid. Spore print: Pink. Basidia: 22.5 × 5.9 µm. Cystidia: 25 × 11.5 µm. Basidiospores: 6.5 × 4.5 µm, ellipsoid. Hymenophoral trama: regular (Figure 8).

Habitat: On termite nests in tropical forest. Fruiting season: July-September.

Chemical test with

Phenolphthalein	:	Beacon 3109
NH_3OH	:	Spice 0477, melted
KOH	:	Cool Cream 3272
$FeSo_4$ solution	:	Stipe: outer: white, and inner: rosy coral 8031, Cap: white, Gills: Silk 0351.
$FeSo_4$ crystal	:	Stipe: outer: negative, and inner: strawberry 0481, Cap: negative.
Iodine	:	Dorsal: light Coffee 0470, Ventral: Ivory 0315.

Specimen examined: Growing on surface land on soil, common in Sal dominant forest, Rupandehi (Kumhargaddi), Alt.: 195m, long. 83° 26.236'E, lat, 27° 29.245'N, Aspect: 140° S/E; Slop: 20° S/W; Temp.: 21.9-32.3° C; Humidity: 69-87 %; Soil pH: 6; Time: 9:06 am; Date: 04.08.2011. VN-1007232-CDBP. Accession No. NHM TU 2-2-1682. Report for new to Nepal.

Distribution: Equatorial, southern Africa, Madagascar, S. E. Asia including Nepal.

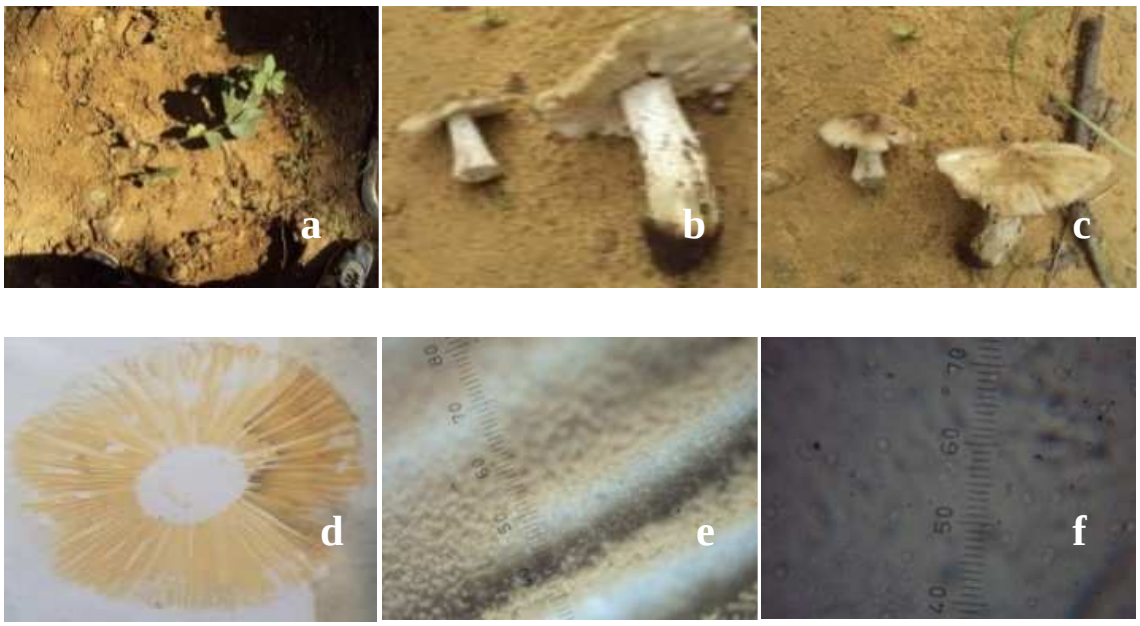


Figure 8: *Termitomyces aurantiacus*, a.Termatotium after digging, b. Basidiocarp ventral view, c. Basidiocarp dorsal view, d. Spore print, e. Lamellae difference (1div=11.66 µm), f. Spores (1div=11.66µm)

iii) ***Termitomyces badius*** Otieno, *Sydowia* **22**(1-4), 161 (1969)

Local name: Kanayo (Kumhal)

Taxonomic position: Division: Basidiomycota class: Agarocomycetes sub-class: Agaricomycetidae order: Agaricales family: Tricholomataceae.

Identifying characters: The most distinguishing characters of this species are the silky, smooth, glabrous, white stipe with dark brown to blackish pseudorrhiza originating from termite nest; no sclerotium. Cap medium in size (up to 10 cm in diameter). Colour ochraceous-grey, without velar remnant, umbonate. Perforatorium are umbo, olive, margin radially straight, incurved. Pseudorrhiza small (up to 5 cm), absence of clamp connection. Sporophores are, growing on sandy soil in groups or on the shaded ground in association with termite's nests.

Description

Usually growing solitary in termite hills, characterized by its obligate symbiont with termites; usually centrally stipitate white but olivaceous near the umbonal region. Sporophores: Spread of this fungus is quite striking; they are abundantly found solitary in the humus rich grass land or termite disused nests, characterized by its obligate symbiont with termites; usually centrally stipitate white but olivaceous near the umbonal region.

Pileus: Size 10-14 cm (Medium size) in diameter; umbonate in shape, surface smooth, glabrous, pale grey in colour, radially straight, incurved margin, dark brown; olive in the centre, papillate, umbo. Stipe: Size 5.2 × 0.75 cm long, more or less straight, solid, white, surface smooth, glabrous, absent of annulus, a part of it is inside the soil, nearly 3.2 cm, Lamellae: free dense, grey in colour. Pseudorrhidea: Size 5 × 1 cm long, solid, brown in colour, cylindrical, glabrous, smooth, and widening at certain depth, then narrowing down to the point of attachment to the termite nest, its length determined by the depth of the termite comb. Flesh: whitish, soft, inflated, clamp connection absent, thin walled, inamyloid. Basidia: 24 × 7 µm, clavate, Basidiospores: 4 in number, smooth, hyaline, 6.5 × 3.5 µm, ellipsoidal. Spore print: Pinkish. Cystidia: 19 × 10.5 µm. Hymenophoral trama: regular (Figure 9).

Habitat: On termite nests in tropical forest. Fruiting season: July-September.

Chemical test with

Phenolphthalein	:	Cool Cream 3272
NH_3OH	:	Sugarcane 0332
KOH	:	Promise 0P09
$FeSo_4$ solution	:	Stipe: outer: Ting of rose 8084, and inner: Pale Rose 0421, Cap: Ting of rose 8084, Gills: Macrame 0495.
$FeSo_4$ crystal	:	Stipe: outer: Lavender Dew 0941, inner: In Vogue 8105, Cap: Lavender Dew 0941, Gills: Magnolia 0387
Iodine	:	Dorsal: white, Ventral: Bathstone 0301.

Specimen examined: Growing on termite disused nests, common. Nawalparasi (Arunkhola), Alt.: 202m, long. 84.05827° E; lat. 27.62584° N; Aspect: 50° N/E; Slope: 12° S/W; Temp.: 21.9-30.1°C; Humidity: 64-87%; Soil pH: 5.62; Time: 2:00

pm., Date: 24.07.2011. VN-1008296-CDBP. Accession No. NHM TU 2-2-1683., Report for new to Nepal.

Distribution: Equatorial, southern Africa, Madagascar, South East Asia including Nepal.

Figure 9: *Termitomyces badius,* a. Termite nest, b.Basidiocarp in dorsal view, c. Basidiocarp in ventral view, d. Umbo papili, e. Spore print, f. Lamellae difference (1div= 11.66 µm) and g. Basidiumes with spores (1div=3.75 µm)

iv) *Termitomyces clypeatus* R. Heim, *Bull. Jard. Bot. Etat.,* **21**, 207 (1951)

Local name: Dewale, Eklemama (Magar)

Taxonomic position: Basidiomycota, Agarocomycetes, Agaricomycetidae, Agaricales, Tricholomataceae.

Identifying characters: The most distinguishing characters of this species of perforatorium is the prominent, smooth, dark grey to brown in colour, very long and cone-like pointed, dark coloured projection at the centre of the cap i.e. spiniform papilla. Cap up to 7.5 cm, in diameter, silky grayish brown to ash in colour. Stipe with long pseudorrhiza, originating from termite nest; no sclerotium. Clamp connection absent. Cystidia present. Sporophores are growing on sandy soil in groups or on the shaded ground in association with termite's nests.

Description

Sporophores: in groups, growing on the sandy soil or on the shaded ground in association with termite's nests. perforatorium is the prominent, smooth, brown to blackish brown, very long and cone-like pointed, dark coloured projection at the centre of the cap i.e. spiniform papilla. Cap medium in size, silky pale brown to ash, medium, (usually at least 5 cm diameter), umbo. Gills are free. Stipe with long, white

coloured pseudorrhiza, originating from termite nest; no sclerotium. Pileus: 5.5-7.5 cm in diameter (medium size), plano-umbonate, with a high spiniform dark-grey perforatorium; surface dark brown at centre, generally grey to grayish brown and paling towards margin, smooth and glabrous, striate and radially rimose; margin straight and splitting fissile, irregularly lobed, pileal surface silky; epicutis consisting of slender, repent hyphae. Lamellae: with lamellulae, free, creamy-white, pink with maturity, crowded, with lamellulae, edge entire. Stipe: 7.0-9.0 × 1.2 cm, central, cylindrical, slender; surface at first white to dirty brown, fleshy and fibrous, smooth and glabrous, longitudinally striate, lacking both an annulus and other velar remnants, solid, fibrous, sometimes bulbous besides soil, hollow. Pseudorhiza: size 3.0-5.0 × 1 cm, pale grey in colour, surface smooth, glabrous and fibrous, shape solid, cylindrical, slender bulbous in shape, tapering towards the distal ends and widening at certain depth, than narrowing down to the point of attachment to the termite nest, its length determined by the depth of the termite comb. Flesh: context of pileus white, soft, fleshy, of inflated, hyaline thin walled, interwoven hyphae, inamyloid, without clamp connections. Basidiospores: 8.5 × 3-3.5 µm, sub hyaline, thin-walled, obovoid to broadly ellipsoidal, smooth, thin walled. Basidia: 15-16.5 × 5.5-6.5 µm, clavate, sub hyaline and thin-walled, tetrasporic (tetra sterigmata bearing tetra basidiospores). Spore: hyaline, ellipsoid, non-amyloid, thin-walled, smooth, 10.5-11.5 × 4-5 µm. Spore print**:** Pink. Cystidia: 20.0 × 10.0 µm. Cheilocystidia: Clavate to pyriform, hyaline, thin-walled. Pleurocystidia: Pyriform, hyaline and thin-walled, rare. Hymenophoral trama: regular, hyaline, thin-walled. Subhymenial layer: narrow consists of branched, repent hyphae (Figure 10).

Habitat: Grow from subterranean termite nests in tropical forest. Fruiting Season: June-August.

Chemical test with

Phenolphthalein	:	Beacon 3109
NH_3OH	:	Negative on all the parts
KOH	:	Yellows grey on cap surface
$FeSo_4$ solution	:	Stipe: outer: negative & inner: Strawberry 0481, Cap: negative. Gills: Raw Silk 0351.
$FeSo_4$ crystal	:	Stipe: outer: negative, inner: Rosy Coral 8031 Cap: negative, Gills: Raw Silk 0351.

Iodine : Dorsal: light Coffee 0470, Ventral: Ivory 0315.

Specimen examined: Growing commonly in termite's hive of *Shorea robusta* dominant forest. Parroha VDC, Rupandehi, Alt.: 233m, long. 27. 68854° N, lat. 083.33732° E, Aspect: 210° S/E, Slope: 20° N/E, Temp.: 20.3-31.4° C, Humidity: 67-87%, Time: 8:13 am, Date: 09.08.2011. VN-1010530-CDBP. Accession No.NHM TU 2-2-1667. New to area of collection.

Previous report: From Kathmandu valley at 1350 masl (Adhikari *et. al*., 2005) and 1500 masl (Pandey 2008) and from Dolpa hill at 2954 m asl (Devkota, 2008).

Distribution: Equatorial, southern Africa, Madagascar, South East Asia including Nepal.

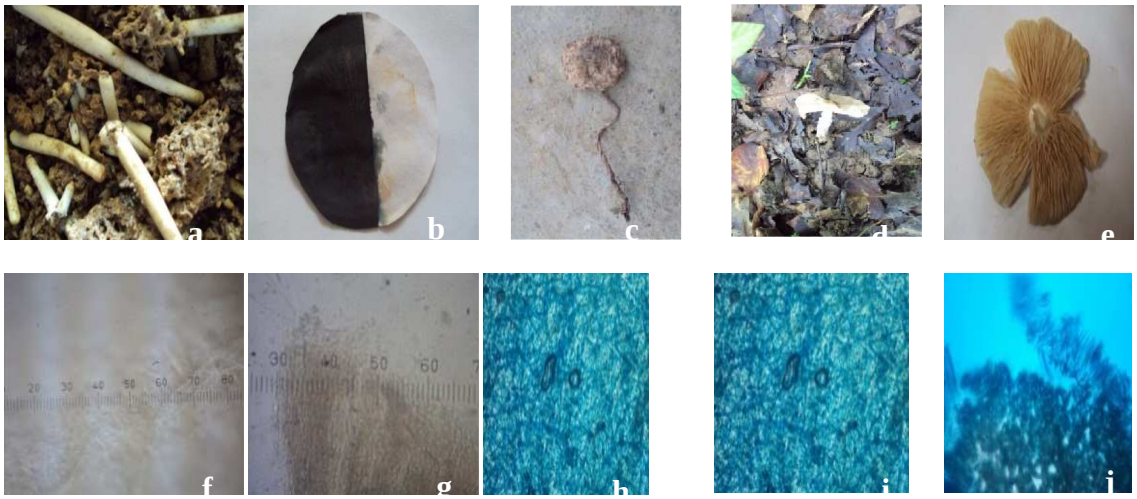


Figure 10: *Termitomyces clypeatus,* a. Fruiting body on termite's nest, b. Spore prints, c. Dry Basidiocarp, d. Basidiocarp on habitat, e. Gills, f. Lamellae difference (1div=11.66 µm), g. Anatomy basidium with spores (1div=11.66 µm), and h, I and j. Anatomy of gills and stipe

v) ***Termitomyces eurrhizus*** (Berk.) R. Heim, *Arch. Mus. Hist. Nat. Paris*, ser. 6, **18,** 140(1942).

Local name: Sadaaka, (Awadhi), Puchima, Jhyarbo, Chyarmo, Kalunge, Kalunge chyarmo.

Taxonomic position: Division: Basidiomycota class: Agarocomycetes sub-class: Agaricomycetidae order: Agaricales family: Tricholomataceae.

Identifying characters: The most distinguishing characters of this species are the stipe with long dark brown to blackish pseudorrhiza originating from termite nest; no

Sclerotium. Cap large, (up to 22 cm in diameter), colour grayish brown, viscid; rugulose, without velar remnant or with few minute whitish evanescent membranous squamules are present. Perforatorium absent or a continuation of cap. Pseudorrhizea long (60 cm). Veil present, persistency variable. Cheilocystidia and pleurocystidia present. Sporophores are, growing on sandy soil in groups or on the shaded ground in association with termite's nests.

Description

Sporophores: usually growing solitary in termite hills, characterized by its obligate symbiont with termites; usually centrally stipitate, white but olivaceous near the umbonal region.

Pileus: Conic to flat, fleshy, dark-grayish brown pale towards the margin, without papilla; 20.5-22.0 cm in diameter, (large size), at first convex, later expanded with prominent umbo, obtusely rounded perforatorium. Universal veil, are white glabrous, viscid, rugulose adherent to pileal surface in the form of a circular patch with free margins. Scales present on the surface, firm, margin regular, not incurved Lamellae: crowded, with lamellulae, distinctly formed, free to sub adnate, pliable, white, entire, Stipe: Size 20.0 × 1.5 cm long, cylindrical**,** central, tubular, fibrillose), creamy-white, somewhat tough, bulbous base, solid above ground, hollow below the soil, penetrating the soil to some distance, with a persistent apical annulus Pseudorrhiza: Size 60.0-11.0 × 1.5 cm or more**,** dark brown in colour, solid, cylindrical with a cartilaginous and widening at certain depth, than narrowing down to the point of attachment to the termite nest, its length determined by the depth of the termite comb. Flesh: White, inflated, thin walled hyphae, nonamyloid, without clamp connection. Basidia: clavate, 4-spored, 11.66 × 6.9 µm (tetra sterigmata bearing tetra basidiospore). Basidiospores: Somewhat large, hyaline, ellipsoid, thin walled 6.8-9.3 × 5.1-6.8 µm; Spore deposit: salmon pink. Spore: hyaline, ellipsoid, smooth, non-amyloid, thin-walled, 6.0-8.0 × 4.0-5.0 µm. Spore print: pink. Cystidia: 35.0 × 11.5 µm, obovoid to pyriform. Heilocystidia: present, numerous; pleurocystidia: clavate, sometimes ventricose-rostrate above the general level of the hymenium. Cheilocystidia: usually napiform, crowded. Hymenophoral trama: bilateral when young, regular at maturity, with hyaline hyphae. Subhymenial layer: thick, narrow; consist of branch repent hyphae (Figure 11).

Habitat: Growth from subterranean termite nests. Fruiting season: July-September.

Chemical test with

Phenolphthalein	:	Cool Cream 3272
NH_3OH	:	White
KOH	:	White
$FeSo_4$ solution	:	Stipe: outer white, Inner: rosy coral 8031, Cap: white, Gills: Raw Silk 0351.
$FeSo_4$ crystal	:	Stipe: outer negative and inner: strawberry 0481, Cap: negative. Gills: Raw Silk 0351.
Iodine	:	Dorsal: light Coffee 0470, Ventral: Ivory 0315.

Specimen examined: Growing on termites nest soil, common in Sal forest, Rupandehi (Saljhandi), 195m, long. 083° 26.236′E, lat, 27° 29.245′ N, Aspect: 140° S/E; Slop: 20° S/W; Temp.: 21.9-32.3° C; Humidity: 69-87 %; Soil pH: 6; Time: 9:06 am; Date: 31.07.2010. VN-1007119-CDBP. Accession No. NHM TU 2-2-1668. New to area of collection.

Previous report: From Banepa valley at 1200 m asl (Singh & Shrestha, 1986); Kathmandu valley at 1350 m asl (Joshi & Joshi, 2008), Shaktikhor-Chitwan at 300 m asl (Rijal, 2011) and Twanrakhola-Makawanpur at 500 m asl (Uprety *at. al*., 2012).They also growing on soil in mixed forest, Nagarkot (1620m); Pokhara (980m); in pine forest, kakani (1780m) and sold in Kathmandu market (Adhikari, 1996).

Distribution: Equatorial, southern Africa, Madagascar, South East Asia including Nepal.

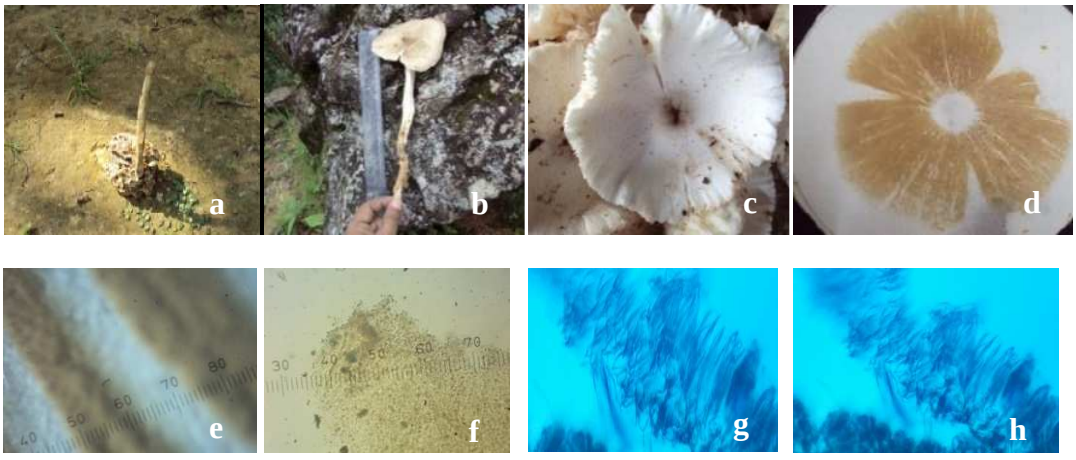


Figure 11: *Termitomyces eurrhizeus, a*. Fruiting body on termite's nest, b. and c. Basidiocarp, d. Spore print, e. Lamellae difference (1div= 11.66 µm), f. Spore, Cystidia and Basidium (1div=11.66 µm), and g. and h. Basidium (1div=11.66 µm)

vi) ***Termitomyces fuliginosus*** R. Heim, *Arch. Mus. Hist. Nat. Paris*, ser. 6, **18,** 147 (1942)

Local name: Chuchanari

Taxonomic position: Division: Basidiomycota class: Agarocomycetes sub-class: Agaricomycetidae order: Agaricales family: Tricholomataceae.

Identifying characters: The most distinguishing characters of this species are the prominent, cone-like pointed, dark coloured projection at the centre of the cap i.e. spiniform papilla. Caps are ochraceous-orange, perforatorium bullate-umbonate, grey, medium size.

Description

Sporophores: Growing on the sandy soil in groups, or on the shaded ground in association with termite's nests, characterized by its obligate symbiont with termites; usually centrally stipitate white but olivaceous near the umbonal region.

Pileus: 4.75-5.50 cm in diameter (medium size), convexoapplanate, smooth, surface grayish brown to yellow brown, perforatorium spiniform, radially fibrillose, sometimes radially striate, entire and incurved margin. Stipe: Buffy-brown, medium cylindrical, 5.0 × 0.9 cm, solid, cylindrical or slightly tapering downward; surface white above and pale brown on the bulb, fibrous, pellicle thick, and below it grey in colour, annulus absent. Pseudozhiza: Present, solid, fibrous, grey to brown in colour and size up to 3.5 × 0.7 cm, cylindrical and widening at certain depth, than narrowing down to the point of attachment to the termite nest, its length determined by the depth of the termite comb Lamellae: free, crowed, white to pink, adnexed Flesh: soft, buffy-brown, hyaline, thin walled, clamp connection absent. Spore print: purplish. Spore deposite: Ivory. Basidia: 30.0 × 0.9 µm, tetra sterigmata bearing tetra basidiospore, clavate. Basidiospores: 8.0 × 4.0 µm ovoid to sub cylindrical or ellipsoid, hyaline, smooth. Cystidia: 25.0 × 19.0 µm. Hymenophoral trama: regular (Figure 12).

Habitat: On termite nests in tropical forest. Fruiting season: July-September.

Chemical test with

Phenolphthalein	:	Raw silk 0351
NH_3OH	:	Sugarcane 0332
KOH	:	Yellow
$FeSo_4$ solution	:	No reaction.
$FeSo_4$ crystal	:	Stipe: outer white and inner: sunrise 0526, Cap: white. Gills: sugarcane 0332
Iodine	:	Dorsal: white, Ventral: Beacon 3109.

Specimen examined: Growing on termites nest soil, common in Sal forest, Nawalparasi (Bisashya, Sunwal-6), 130m, long. 83.65415° E, lat. 27.62274° N, Aspect: 198° S/W; Slop: 10° S/W; Temp.: 21.9-29.1°C; Humidity: 79-87 %; Soil pH: 6.5; Time: 9:10 am. Date: 06.10.2011. VN-1008279-CDBP. Accession No. NHM TU 2-2-1676. Report for new to Nepal.

Distribution: Equatorial, southern Africa, Madagascar, South East Asia including Nepal.

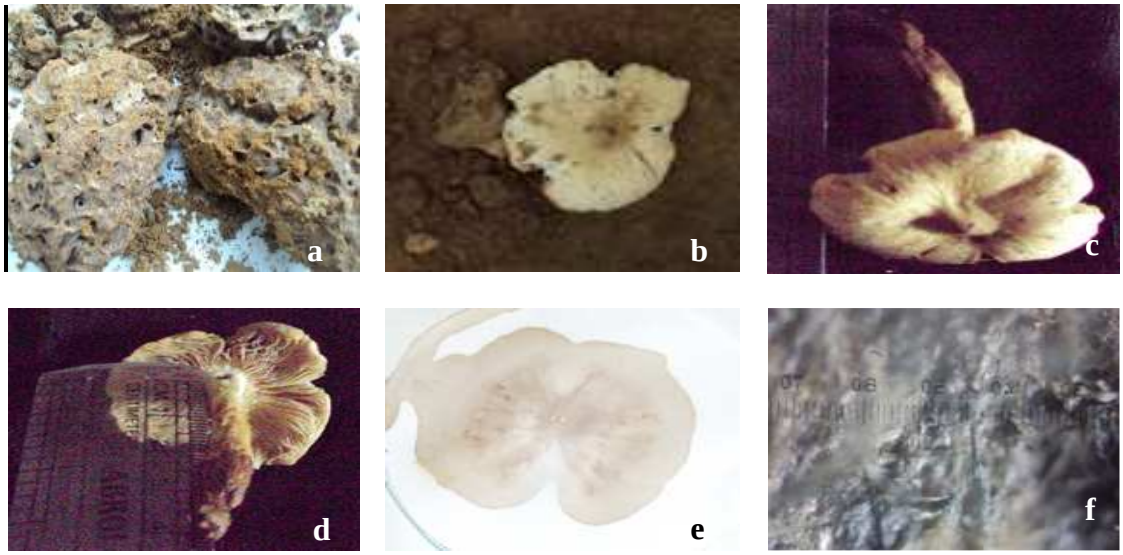


Figure 12: *Termitomyces fuliginosus,* a. Nest, b. and c. Basidiocarp (dorsal view), d. Basidiocarp (ventral view), e. Spore print and f. Lamellae difference (1div=11.66 µm)

vii) ***Termitomyces globulus*** R. Heim & Gooss. -Font. in *Bull. Jard. Bot. État.*, **21,** 216 (1951).

Local name: Dallu vend

Taxonomic position: Division: Basidiomycota class: Agarocomycetes sub-class: Agaricomycetidae order: Agaricales family: Tricholomataceae.

Identifying characters: The most distinguishing characters of this species are the prominent, gibbous shape with umbo, dark brown perforatorium at the centre of the cap. Sporophores are found in groups, growing on the sandy soil or on the shaded ground in association with termite's nests.

This is one of the largest species in the genus, and basidiomata are often collected in both Africa and Asia. It has a more robust stipe than *T. striatus* (Beeli) R. Heim, together with a less developed perforatorium.

Description

Sporophores: Usually growing solitary in termite hills, characterized by its obligate symbiont with termites; usually centrally stipitate, white but olivaceous near the umbonal region.

Pileus: 6.0-9.0 cm diam., subglobose, finally expanding but margin usually remaining incurved, with the perforatorium scarcely developed or nil; surface pale ochraceous brown to tawny brown, paler at margin, glabrous and smooth, radially striate; margin lobed, often splitting. Lamellae: free, narrow, up to 8 mm broad, white to pale pinkish, densely crowded, with lamellulae of three lengths. Stipe: size 6.0 × 1.5 cm, cylindrical, solid; surface cream coloured, fibrous and smooth. Pseudorrhiza: elongate, size up to 6 × 1 cm or more, slender, tawny to rusty brown. Flesh: whitish in colour, firm to spongy, consisting of thin-walled, inflated hyphae, clamp connection absent. Spore deposit: pinkish cream in colour. Spores: 6.0-8.5 × 3.5-4.5 µm, obovoid to ellipsoid, thin-walled. Spore print: Pinkish. Basidia: 20.0-25.0 × 5.0-7.0 µm, clavate, bears four sterigmata. Cheilocystidia: crowded, polymorphic ranges from globose or pyriform to cylindric and subcapitate, often septate wall. Cystidia: 28 × 15 µm. Basidiospores: 7.5 × 4.5 µm, ellipsoidal. Pleurocystidia: similar to cheilocystidia. Hymenophoral trama: subregular, of thin walled, inflated hyphae. Subhymenial layer: interwoven (Figure 13)**.**

Habitat: On termite nests in tropical forest. Fruiting season: July-September.

Chemical test with

Phenolphthalein : Beacon 3109

NH_3OH : No reaction

KOH : Sugarecane 0332

$FeSo_4$ solution : Stipe: Outer: white, Inner: Rosy Coral 8031 Cap: white, Gills: Raw Silk 0351.

$FeSo_4$ crystal : Stipe: outer: negative and inner: strawberry 0481, Cap: negative. Gills: Raw silk 0351.

Iodine : Dorsal: light Coffee 0470, Ventral: Ivory 0315.

Specimen examined: Growing on termites nest soil, common in mixed forest, Kanchanpur (Shukla-Phanta WR) 320m, long. 80.18024°E, lat. 28.97291° N, Temp.: 20.3-37.9° C, Humidity: 70-87 %; Soil pH: 5.5; Time: 16:54 pm; Date: 31.08.2011. VN-1007482-CDBP. Accession No. NHM TU 2-2-1677. Report for new to Nepal.

Distribution: Equatorial, southern Africa, Madagascar, South East Asia including Nepal.

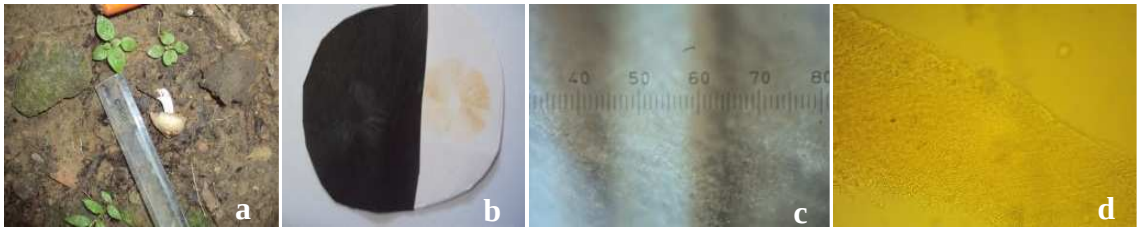


Figure 13: *Termitomyces globulus*, a. Basidiocarp in habitat, b. Spore print, c. Lamellae difference (1div= 11.66 µm) and d. Spores with basidium (1div=11.66 µm)

viii) ***Termitomyces heimii*** Natarajan, *Mycologia*, 71(4), 853-855 (1979).

Local name: Vavnethi, Bemtee 'Vemtee' (Tharu)

Taxonomic position: division: Basidiomycota class: Agarocomycetes sub-class: Agaricomycetidae order: Agaricales family: Tricholomataceae.

Identifying characters: The skin of the cap peels off. Partial veil membranous, at first covering whole surface of the pileus and the stipe, then broken into tough squamules and forming a persistent double annulus on the upper part of the stipe; of narrow hyphae, parallel and perpendicular to the surface of the pileus and the stipe. The stalk is long (up to 25 cm) and swollen near the attachment with the cap. Sporophores are growing on sandy soil in groups or on the shaded ground in association with termite's nests.

Description

Sporophores: Growing in groups in symbiont with termite nests; centrally stipitate; pluteoid; Pileus: Silky white, turns into the grey to brown-white-creamy in colour, Smooth**,** size, 7.5-9.0 cm in diameter, (medium size), convex or campanulate at first, then expanding to plano-umbonate with a patch of veil at the apex, obtuse (umbo) perforatorium; surface pale brown to dark brown at centre. Whitish grey at the umbonal, margin incurved and cracking (the cap splits at a few places from margin); covered at first by a firm partial veil, which disrupts to form persistent velar squamules at maturity; margin radially striate, often splitting; pileal surface an epicutis consisting of thin walled, repent hyphae. Lamellae: Free, surface white to pinkish cream with age, margin serrulate, crowded, with lamellale. Stipe: size 5.0 × 1.5 cm, central, cylindrical, bulges near the pileus and possess a thick ring, surface creamish white stuffed, and smooth above thick and white persistent annulus, with pale brownish velar squamules below; solid, fibrous. Pseudorrhiza: Significantly long, size 60 × 1.7 cm**,** up to 15 cm below ground level, fleshy, pale-grey, cylindrical, hollow, terminated by a disk connected to the termite comb; leathery, hollow, surface cream, and widening at certain depth, than narrowing down to the point of attachment to the termite nest, its length determined by the depth of the termite comb. Flesh: white, fleshy, soft; of inflated, hyaline, thin-walled, septed with clamp connection hyphae. Basidiospore deposite: pinkish cream. Basidiospores: 6.5 × 4.5 µm, ovoid to ellipsoid, hyphae, non-amyloid, smooth, sub hyaline and thin-walled. Basidia: 17.5 × 6.5 µm, sub hyaline and thin-walled, clavate, with four sterigmata, (tetra sterigmata bearing tetra basidiospore) Spore print: Brown to pinkish grey. Cystidia: 35.0 × 20.0 µm. Cheilocystidia: Occasionally, clavate to pyriform. Pleurocystidia: clavate, similar to cheilocystidia, rare. Hymenophoral trama: regular, hyaline, thin walled, parallel hyphae (Figure 14)**.**

Habitat: On termite nests in tropical forest. Fruiting Season: July to August.

Chemical test with

Phenolphthalein : Ivory 0315

NH_3OH : Barley 0572

KOH : White

$FeSo_4$ solution : Stipe: Outer: white, Inner: Pale Rosy 0421, Cap: white, Gills: Bliss 0170.

$FeSo_4$ crystal : Stipe: outer: Cream and inner: volcano 0578, Cap: cream. Gills: Baby Blush 8138.

Iodine : Dorsal: white, Ventral: Barley 0572.

Specimens examined: Growing on termites nest soil, common in Sal forest, Kapilwastu (BCF), 247m; long. 83.10818° E, lat. 27.68348° N, Aspect: 22° N/E; Slop: 20° S/W; Temp.: 21.9-33.5° C; Humidity: 76-87 %; pH: 5.5; Time: 9:17 am; Date: 18.09.2010; VN-1010524-CDBP. Accession No. NHM TU 2-2-1669. This species is reported first time in Nepal.

Distribution: Equatorial, southern Africa, Madagascar, South East Asia including Nepal.

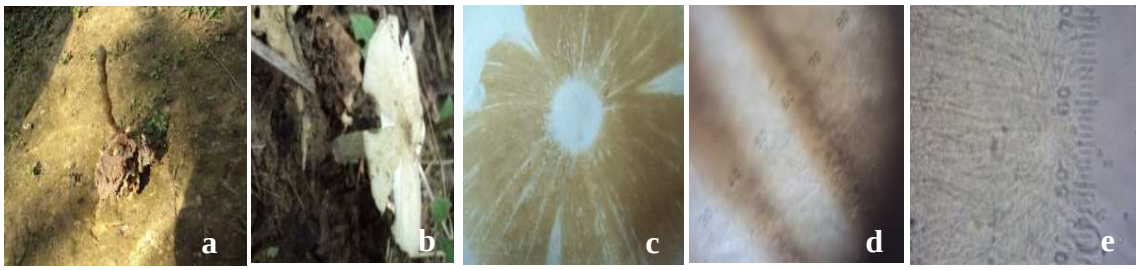


Figure 14: *Termitomyces heimii*, a. Fruiting body on termite's nest, b. Basidiocarp, c. Spore print, d. Lamellae difference (1div= 11.66 µm) and e. Spores with basidium (1div=3.75 µm)

ix) ***Termitomycs le-testui*** (Pat.) R. Heim, *Arch. Mus. Hist. Nat. Paris*, ser. 6, **18,** 109 (1942).

Local name: Dudhamunte.

Taxonomic position: Division: Basidiomycota class: Agarocomycetes sub-class: Agaricomycetidae order: Agaricales family: Tricholomataceae.

Identifying Characters: The most distinguishing characters of this species are, cap brownish or black brown, larger, 21.0-22.5 cm in diameter, tomentose; perforatorium prominent, cylindric, smooth, dark brown, demarcated and prominent, either subnammillate, mammiform or cylindric, long, continuous projection at the centre of the cap as a 'teat of mammals' like structure of papilla, stipe with long pseudirrhiza originating from termite nest; no sclerotium, annulus persistent. Sporophores are found in groups, growing on the sandy soil or on the shaded ground in association with termite's nests.

Description

Sporophores: Growing on termite nests in early to during monsoon period. The fruit-bodies from inside the tunnels and bore through the very hard layer of insert matter, forming their way through it with a special umbo, sometimes quite pointed, called a "driller." A 'teat' likes structure.

Pileus: size 21.0-22.5 cm, (large), cream to light brown, fleshy, campanulate then convex, eventually fairly expanded, with a conspicuous cylindrical umbo of perforatorium, which is dark brown from the small brown scales about disc. Cutical dry and whitish fairly pale pinkish-gray, squamulose and cracked except toward margin, which is usually appendiculate. Lamellae: white, almost free, crowded, unequal. Stipe: size 12.0-18.0 × 2.0-3.0 cm or more, solid, fusiform, deeply rooting, cream to whitish, pubescent beneath membranous ring, which is large, double, variable but complete and persistent, pendant or sheath like. Pseudorhiza: 18 × 1.5 cm long, dark in colour, hollow, cylindrical and widening at certain depth, than narrowing down to the point of attachment to the termite nest, its length determined by the depth of the termite comb. Flesh: whitish, soft, thin walled, interwoven, nonamyloid, hyaline hyphae, clamp connection absent. Spore print: Pinkish. Spores: white, elliptical, smooth, 6.0-9.0 × 3.0-5.0 µm. Basidia: 24.1 × 7.3 µm, tetra sterigmata bearing tetra basidiospore Basidiospores: 7.25 × 3.5 µm, oblong. Cystidia: 36.0 × 15.0 µm (Figure 15).

Habitat: On termite nests in tropical forest. Fruiting season: July-September.

Chemical test with

Phenolphthalein	:	Volcano 0578
NH_3OH	:	Barley 0572, soft
KOH	:	Beacon 3109
$FeSo_4$ solution	:	Stipe: Outer: white, Inner: Red Earth 8029, Cap: white, Gills: Sandalwood 0485.
$FeSo_4$ crystal	:	Stipe: outer: Cream and inner: Marco Polo 8607, Cap: cream. Gills: Pale Cream 0328.
Iodine	:	Dorsal: white, Ventral: Dawn 0469.

Specimen examined: Growing on termite nest, common in Sal forest, Nawalparasi (Basabasahi), 130m, long. 83.65415° E, lat. 27.62274° N, Aspect: 198° S/W; Slope: 10° S/W; Temp.: 21.9-29.1° C; Humidity: 79-87 %; Time: 9:10 am. Date: 18.09.2011. VN-1007481-CDBP. Accession No. NHM TU 2-2-1670. Report for new to Nepal.

Distribution: Equatorial, southern Africa, Madagascar, South East Asia including Nepal.

Figure 15: *Termitomyces le-testui,* a. Termite nest, b. Basidiocarp, c. Spore print, d. Lamellae Difference (1div= 11.66 µm) and e. Spores (1div= 11.66 µm)

x) ***Termitomyces mammiformis*** R. Heim, *Arch. Mus. Hist. Nat. Paris,* ser. 6, **18,** 147 (1942).

Local name: Thuli Mugan (Magar)

Taxonomic position: Division: Basidiomycota class: Agarocomycetes sub-class: Agaricomycetidae order: Agaricales family: Tricholomataceae.

Identifying features: Perforatorium mammiform and scrobiculate, blackish brown demarcated. Prominent, cone like pointed, projection at the centre of the cap subnammillate, mammiform or cylindric, not so long spiniform papilla, continuous, or demarcated. Partial veil**,** membranous, at first covering the whole surface of the pileus and the stipe, then broken into membranous squamules (or occasionally granules) and forming the persistent double annulus on the upper surface; of narrow hyphae 2.0-2.5 µm diameter, with parallel palisadic arrangement and perpendicular to the surface of the pileus and the stipe. Cap medium in size (4.5-7.5 cm in diameter). Stipe with long pseudorrhiza, originating from termite nest; no sclerotium. Sporophores are found in groups, growing on the sandy soil or on the shaded ground in association with termite's nests.

Description

Sporophores: solitary, growing on the ground in association with termite nests; fleshy, centrally stipitate, 7.5 cm long.

Pileus: whitish grey to silvery shining, size 4.5-7.5 cm in diameter; medium in size, smooth, campanulate at first, and then applanate with a protruding mammiform, sharply differentiated scrobiculate perforatorium when mature; with a sharply differentiated umbo, margin inflexed, entire, surface pale brown to dark brown at centre, white to creamy elsewhere, covered by firm creamy partial veil when young, attached with some persistent pale grey or pale brown velar squamules when mature; surface or margin with radial fine stripes. Lamellae: free, white to pinkish, crowded with lamellulae. Stipe: size 8.0 × 1.2 cm, central, cylindrical or occasionally slightly enlarged at ground level; Surface white and glabrous above annulus, cream-coloured and with pale brown squamules below; solid, with a pseudorhiza, fibrous, persistent annulus present, persistent. Pseudorrhiza: Size up to 19 × 1.2 cm, cylindrical, pale grey, smooth, terminal with a disk connected to the surface of the termite comb, with blunt end, lathery, hollow; surface creamy. Dark in colour, cylindrical and widening at certain depth, than narrowing down to the point of attachment to the termite nest, its length determined by the depth of the termite comb. Flesh: white, fleshy and soft, of inflated, hyaline, cylindric, nonamyloid, thin-walled hyphae, clamp connection absent. Spore print: Pink. Basidiospores deposit: cream. Basidiophores: 5.5 × 3.5 µm, ovoid to ellipsoid, sub hyaline and thin-walld, smooth, inamyloid. Basidia: 22.1 × 6.5 µm, sub hyaline, thin-walled, clavte, with four sterigmata, club shaped (tetra sterigmata bearing tetra basidiospore). Cystidia: 45.0 × 15.0 µm**.** Cheilocystidia: clavate to pyriform, hyaline, thin-walled occasionally with small protuberances. Pleurocystidia: Similar to cheilocystidia, rare. Hymenophorae trama: bilateral in the primordium, regular at maturity, and hyaline, thin-walled, cylindric hyphae. Subhymenial layer: narrow radial hyphae (Figure 16)**.**

Habitat: On termite nests in tropical forest. Fruiting season: July-September.

Chemical test with

Phenolphthalein	:	Ivory 0315
NH_3OH	:	White
KOH	:	White
$FeSo_4$ solution	:	Stipe: Outer: white, Inner: Red Earth 8029, Cap: white, Gills: Sandalwood 0485.
$FeSo_4$ crystal	:	Stipe: outer: Cream and inner: Marco Polo 8607, Cap: cream. Gills: Pale Cream 0328.
Iodine	:	Dorsal: white, Ventral: Dawn 0469.

Specimen examined: Growing on termites nest soil, common in Sal forest, Nawalparasi (Bisashya, Sunwal-6), 130m, long. 83.65415° E, lat. 27.62274° N, Aspect: 198° S/W; Slope: 10° S/W; Temp.: 21.9-29.1° C; Humidity: 79-87 %; Time: 9:10 am. Date: 06.10.2011. VN-1007154-CDBP. Accession No. NHM TU 2-2-1678. Report for new to Nepal.

Distribution: Equatorial, southern Africa, Madagascar, South East Asia including Nepal.

Figure 16: *Termitomyces mammiformis*, a. Trematorium, b. Basidiocarp, c. Spores print, d. Lamellae difference (11.66 µm) and e. Spores with Basidium (1 div=3.75 µm)

xi) ***Termitomyces microcarpus*** (Berk. & Broome) R. Heim, *Mem. Acad. Sci. Inst. France* **64**, 72 (1942). [Small form]

Local name: Jhari (Magar), Rai (Tharu), Nakaphunani (Awadhi).

Taxonomic position: Division: Basidiomycota Class: Agaricomycetes Sub class: Agaricomycetidae Order: Agaricales Family: Tricholomataceae.

Identifying characters: Basidium small, pileus less than 3 cm diameter. Pseudorhiza absent, basidiomata growing from ejected termite nest remmants at soil surface. Inflated pleurocystidia present.

Description

Sporophores: Growing in termite hills, characterized by its obligate symbiont with termites; centrally stipitate, white but olivaceous near the umbonal region.

Pileus: **c**ream white to light grey in colour, with cream dark in centre, smooth with glabrous margin, small spiniform perforatorium, and umbo, without papilla, size 1.0 - 3.0 cm in diameter, the cap splits from its margins and resembled the petals of flower. Lamellae: free, white and crowded, thick, turning creamy at maturity. Stipe: central, slender, hollow, white, fleshy-fibrous, smooth, size 3.5 × 0.5 cm, uniformly thick, glabrous, slightly enlarged below soil, absent of annulus and other velar remnants.

Pseudorhiza: absent. Flesh: soft, fleshy, whitish, hyaline, thin-walled hyphae, clamp connection present. Basidiospores: 5.0 × 3.0 µm, ellipsoid. Basidia: 10.0-12.0 × 4.0-6.0 µm in size, tetra sterigmata bearing one basidiospore on each sterigma. Spores: sub-globose, smooth, thin-walled, inamyloid, hyaline, 10.0-12.0 × 4.0-6.0 µm. Spore print: pale pinkish. Cystidia: 22.0 × 11.0 µm. Hymenophoral trama: regular, lamellate (Figure 17).

Habitat: On termite nests in tropical forest. Fruiting season: July-September.

Chemical test with

Phenolphthalein	:	Cool Cream
NH_3OH	:	No change in colour in dorsal surface i.e. Milky white and ventral cool cream, soapy
KOH	:	Soapy, melted
$FeSo_4$ solution	:	no reaction, stiff brown in colour.
$FeSo_4$ crystal	:	Stipe: outer white, inner: sunrise, cap: white, gills: Sugarcane.
Iodine	:	Dorsal: white, Ventral: Beacon.

Specimen examined: Growing on termite's hive, common in *Shorea robusta*, 1016 m, Lumbini zone, Palpa district, Mashyam, V.D.C. W.N. 9. long. 83.50969°E, lati. 27.76451° N, Aspect: 270° W, Slope: 50° S/W, Temp.: 21.9-26.8° C, Humidity: 79-87 %, Soil pH: 6.2, Time: 9:43 am, Date: 17. 08. 2011. VN-1008133-CDBP. Accession No. NHM TU 2-2-1671. Report for new to Nepal.

Distribution: Equatorial, southern Africa, Madagascar, South East Asia including Nepal.

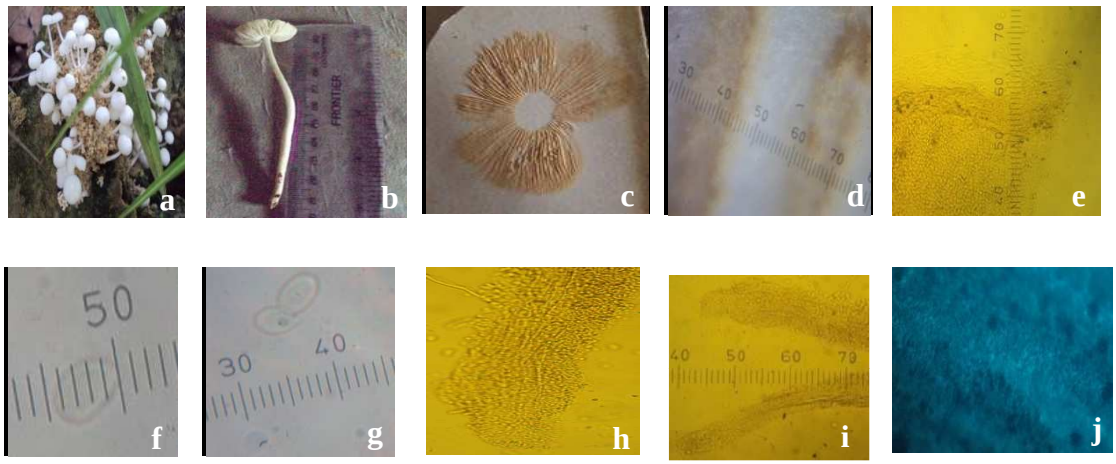


Figure 17: *Termitomyces microcarpus*, a and b. Basidiocarp, c. Spore print, d. Lamellae difference (1div=11.66 µm), e. Spores (1div=3.75 µm), f. and g. Spores (1div=1.66 µm, in emersion oil), h. Mycelium: 1div=11.66 µm; i and j basidium (1div=11.66 µm)

xii) ***Termitomyces microcarpus*** (Berk. & Br.) Heim *forma Santalensis* Heim, *Mem. Acad. Sci*. *Inst.* France **64**, 73 (1941)

Local name: Jhari (Magar), Rai (Tharu)

Identifying characters: This fungus grows abundantly in open grass land. It is marked by presence of pseudorrhiza. Pileus up to 4 cm diameter, Presence of conic papilla.

Description

Sporophores: Usually growing solitary in termite hills, characterized by its obligate symbiont with termites; usually centrally stipitate white but olivaceous near the umbonal region.

Pileus: 1-4 cm, whitish to grey in colour, papillate, conic, becoming olivaceous to brown at the papilla non-viscid, smooth, margin thin, incurved, entire, or incised. Lamellae: sinuate, concolourous, arcuate, crowded, with lamellulae of three unequal length; edge entire, smooth, concolourous. Stipe: 3-8 × 0.4-0.6 cm, central, straight, cylindrical, grey in colour, surface smooth, annulus absent. Pseudorrhiza: attenuated at the base to form a long, solid, greyish-brown in colour, size : 5 × 0.3 cm, hollow, slender, cylindrical, surface glabrous, smooth, and widening at certain depth, then narrowing down to the point of attachment to the termite nest, its length determined by the depth of the termite comb. Flesh: Spongy, whitish, consisting of interwoven hyphae, which are nonnamyloid, thin-walled, inflated hyphae, with clamp-connection. Spores: 5.6-7.5 × 4-5 µm, elliptical, hyaline, inamyloid, thin-walled. Spore print: brown. Basidia: 15-20 × 5-7 µm, clavate, bears 4-sterigmata. Cystidia: 24 × 12 µm. Cheilocystidia: abundant, and forming a sterile gill edge, claviform, hyaline, thin-walled. Pleurocystidia: absent. Basidiospores: 6 × 4 µm, ellipsoidal. Hymenophoral trama: sub regular, hyaline, inamyloid, consisting of thin walled hyphae. Subhymenial-layer: well developed, interwoven (Figure 18).

Habitat: On termite nests in tropical forest. Fruiting season: July-September.

Chemical test with

Phenolphthalein : Cool Cream 3272

NH_3OH : Sugarcane 0332

KOH : Sugarcane 0332

$FeSo_4$ solution : No reaction

$FeSo_4$ crystal : Stipe: outer: white, and inner: Sunrise 0526, Cap: white, Gills: Sugarcane 0332.

Iodine : Dorsal: white, Ventral: Beacon 3109

Specimen examined: Growing on open surface on pine forest, Salyan (Aringalependi-Dhanwag VDC-1), 1110m, long. 82.37703° E, lat. 28.25742° N; Aspect: 124° S/E; Slop: 40° S/W; Temp.: 21.9-28.5°C; Humidity: 76-87 %; Soil pH: 5.62; Time: 13:35 pm. Date: 24.08.2011. VN-1007429-CDBP. Accession No. NHM TU 2-2-1679. Report for new to Nepal.

Distribution: Equatorial, southern Africa, Madagascar, South East Asia including Nepal.

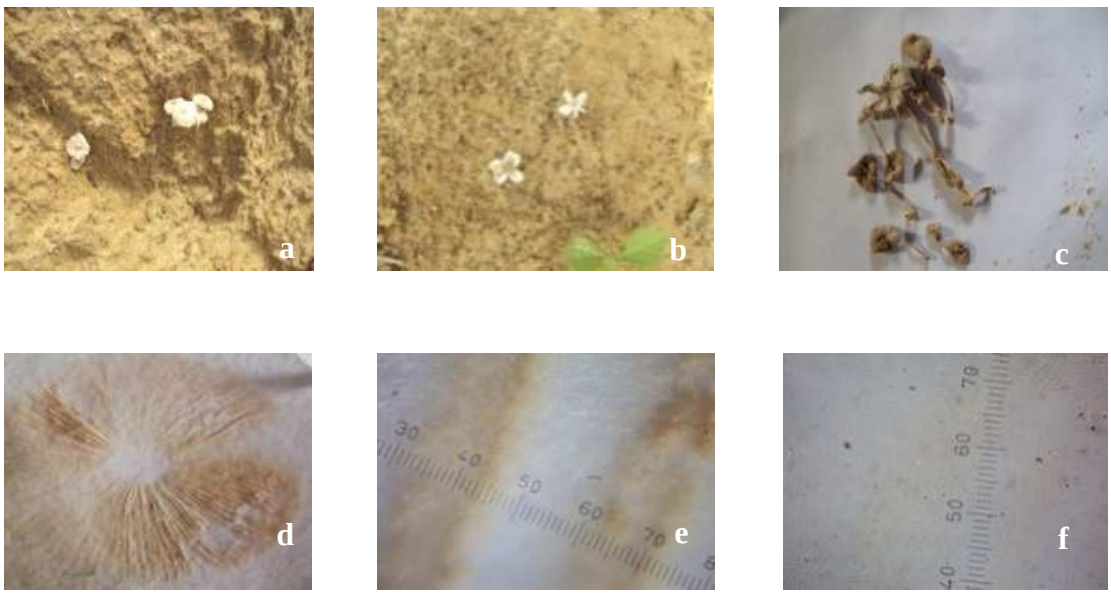


Figure 18: *Termitomyces microcarpus f. Santalensis*, Fig a and b. Basidiocarp on habitat, c. Dry basidiocarp, e. Spore print, f. Lamellae difference (1div=11.66 µm), g. Spores (1div=11.66 µm)

xiii) ***Termitomyces robustus*** (Beeli) Heim, *Bull. Jard. Bot. État* **21,** 210 (1951)

Local name: Bagale

Taxonomic position: Division: Basidiomycota class: Agarocomycetes sub-class: Agaricomycetidae order: Agaricales family: Tricholomataceae.

Identifying characters: The most distinguishing characters of this species are larger cap, (20.5-21.5 cm in diameter) concentrically scrobiculate or radially ridged, brownish or black brown, glabrous. Perforatorium small, broadly conical, a continuation of cap, submamilate, cylindric, smooth, demarcated and prominent, partly blackish, dark coloured projection at the centre of the cap, either subnammillate, mammiform or cylindric, not so long spiniform papilla, continuous, or demarcated, or absent. Cap first conical, then convexo-applanate, without velar remnant or with few minute whitish evanescent membranous squamules stipe with long pseudirrhiza pale, not entirely blackish, originating from termite nest; no sclerotium, no annulus.

Description

Sporophores: Growing on the sandy soil or on shaded ground in association with termite's nests, characterized by its robust size, mammiform papilla, and dark brownish perforatorium present in the pileus. Pseudorrhiza originating from the termite nest, they are fleshy, black, long and tough.

Pileus: Size 20.5-21.5 cm in diameter, (large size), brownish cream, margin inflexed when young, but flat reflexed or lacerate margin with age, surface smooth, viscid, concentrically scrobiculate or radially ridged, conical to convexo-applanate in shape. Perforatorium partly blackish and sub-mamilate, shape in conical continuation with the cap. Lamellae: densely crowded, whitish, free or sub adnate. Stipe: 18.0 × 3.0 cm thick (above ground), conical, tapering towards downward, pale brown, surface smooth, glabrous, annulus absent. Pseudorrhiza: size 30.0 × 1.5 cm, gradually attenuating to a narrow distal end, dark- brown in colour, cylindrical and widening at certain depth, than narrowing down to the point of attachment to the termite nest, its length determined by the depth of the termite comb. Flesh: grey, soft, fleshy, easily separable from the cutical, thin walled, nonamyloid, clamp connection absent. Hymenophoral trama: regular. Basidia: 4-spored, club shaped 20.5 × 6.5 µm, tetra sterigmata bearing tetra basidiospores, clavate. Basidiospores: hyaline, non-amyloid, ovoid to broadly ellipsoidal, smooth walled, 5.5-8.0 × 4.0-4.5 µm. Spore print:

pinkish, Cystidia: 25.0 × 12.0 µm. Cheilocystidia: abundant, thin walled, pyriform. Pleurocystidia: present (Figure 19).

Habitat: On termite nests in tropical forest. Fruiting season: July-September.

Chemical test with

Phenolphthalein	:	Sugarcane 0332
NH_3OH	:	Spice 0477, and melted
KOH	:	Cool Cream 3272
$FeSo_4$ solution	:	Stipe: outer: Ting of Rose 8084 and Inner: Pale Rose 0421, Cap: Ting of Rose 8084, Gills: Macramé 0495.
$FeSo_4$ crystal	:	Stipe: outer: Lavender Dew 0941, and inner: In Vogue 8105, Cap: Lavender Dew 0941, Gills: Magnolia 0387
Iodine	:	Dorsal: white, Ventral: Beacon 3109

Specimen examined: Growing on termite nest, common in sal forest, Chitwan (Jugadi), 228m, long. 84.47458° E, lat. 27.76132° N; Aspect: 158° S/E; Slop: 52° S/W; Temp.: 21.9-31.2°C; Humidity: 670−87 %; Soil pH: 5.62; Time: 9:17 am. Date: 10.08.2011. VN-1007158-CDBP. Accession No. NHM TU 2-2-1672. Report for new to Nepal.

Distribution: Equatorial, southern Africa, Madagascar, South East Asia including Nepal.

Figure 19: *Termitomyces robustus*, a. Premordium on its combs, b, c and d. Basidiocarps, e. Spore print f. Lamellae dfference (1 div= 11.66 µm), g. Spores (1 div= 3.75 µm) and h. Basidium (1div= 11.66 µm)

xiv) ***Termitomyces schimperi*** (Pat.) R. Heim, *Arch. Mus. Hist. Nat. Paris*, ser. 6, **18**, 114 (1942).

Local name: Dhamire.

Taxonomic position: Division: Basidiomycota class: Agarocomycetes sub-class: Agaricomycetidae order: Agaricales family: Tricholomataceae.

Identifying characters: The most distinguishing characters of this species is larger cap (usually at least 4 cm diameter), white, with distinct velar remnants, and large squamules; perforatorium not so long spiniform, prominent, demarcated, broadly conical, a continuation of cap, dark coloured projection at the centre of the cap. Stipe with long pseudorrhiza, originating from termite nest; no sclerotium. Sporophores are found in groups, growing on the sandy soil or on the shaded ground in association with termite's nests.

Description

Sporophores: Usually growing solitary in termite hills, characterized by its obligate symbiont with termites; usually centrally stipitate white but olivaceous near the umbonal region.

Pileus: 20.5-21.0 cm, (large size), cream white with concentric rings of brown scales, margin incurved. Perforatorium are conical to bullate umbo, in shape, dark grey in colour. Lamella: whitish, dense, crowded and free. Stipe: Size 15 × 2.5 cm, creamy-white in colour, solid, surface smooth, glabrous. Annulus covered by thick membranous of velar remains. Pseudozhiza: Size 17 × 1.6 cm, dark-brown in colour, solid, cylindrical, slender, surface smooth and widening at certain depth, than narrowing down to the point of attachment to the termite nest, its length determined by the depth of the termite comb**.** Flesh: whitish, thin walled, filamentous, nonamyloid, hyaline, clamp connection absent. Spore print: pale cinnamon. Basidia: 27.5 × 8.0 µm, tetra sterigmata bearing tetra basidiospore Cystidia: 32.5 × 14.5 µm. Basidiospores: 6.5 × 4.5 µm, ellipsoid (Figure 20)**.**

Habitat: On termite nests in tropical forest. Fruiting season: July-September.

Chemical test with

Phenolphthalein	:	Promise 0P09
NH_3OH	:	Barley 0572, and soft
KOH	:	Casablanca 7927
$FeSo_4$ solution	:	Stipe: outer: White, and Inner: Rosy coral 8031, Cap: White, Gills: Strawberry 0481.
$FeSo_4$ crystal	:	Stipe: outer: White, and inner: Sunrise 0526, Cap: White, Gills: Sugarcane 0332.
Iodine	:	Dorsal: white, Ventral: Beacon 3109

Specimen examined: Growing on termite's hive, common in *Shorea robusta*, 1016 m, Lumbini zone, Palpa district, Mashyam, V.D.C. W.N. 9. Longitude: 83.50969°E, latitude: 27.76451° N, Aspect: 270° W, Slope: 50° S/W, Temp.: 21.9-26.8° C, Humidity: 79-87%, Soil pH: 6.2, Time: 9:43 am, Date of collection: 17. 08. 2011. VN-1007155-CDBP. Accession No. NHM TU 2-2-1673. Report for new to Nepal.

Distribution: Equatorial, southern Africa, Madagascar, South East Asia including Nepal.

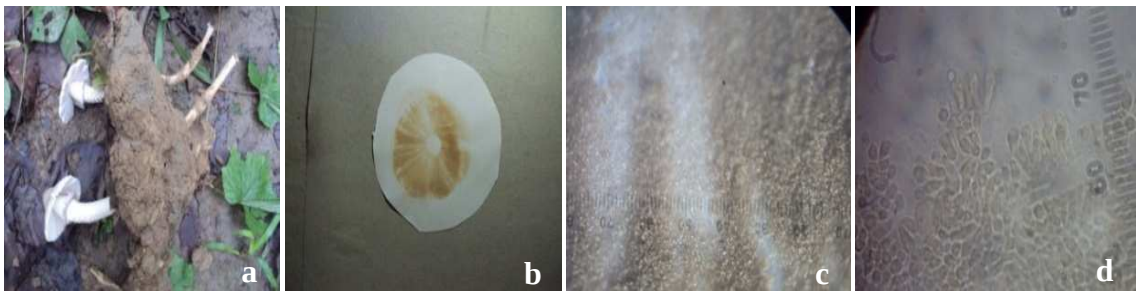


Figure 20: *Termitomyces schimperi,* a. Basidiospores on habitat, b. Spore print, c. Lamellae difference and d. Spores with Basidium (1div=3.75 µm)

xv) ***Termitomyces straitus*** **(Beeli) Heim** ***f. griseus*** **Heim,** *Mém. Acad., Sci., Paris* **44,** 72 (1942); *Fl. Icon. champ. Congo* **7,** 144 (1958).

Local name: Kanayo (Kumal), Dhamire chyau (Nepali)

Taxonomic position: Division: Basidiomycota class: Agarocomycetes sub-class: Agaricomycetidae order: Agaricales family: Tricholomataceae

Identifying characters: The most distinguishing characters of this species are medium size cap, (usually at least 4.5 cm in diameter), grey to grayish brown in colour, not concentrically scrobiculate, at most radially striate, first conical, then convexo-applanate, without velar remnant or with few minute whitish evanescent membranous squamules, prominent, cone-like pointed, dark coloured projection at the centre of the cap i.e. spiniform papilla. Ochraceous brown to grey brown in colour. Perforatorium not so long spiniform, a continuation of cap, or demarcated, or not demarcated or absent, small, broadly conical. Stipes are usually without annulus with pseudorrhiza originating from termite nest; no sclerotium. Pseudorrhiza are pale, not entirely blackish.

Description

Sporophores: Growing on the sandy soil in groups or on the shaded ground in association with termite's nests.

Pileus: **s**ize 4.5-8.5 cm, (medium size), grey to greyish-brown in colour, conical to convexo-applanate, surface radially striate, margin incurved when young but slightly uplifted when mature. Convex with prominent umbo papilla of brown in colour. Lamella: whitish, crowded, free, smooth, equal edge. Stipe: **s**ize 7 × 1.7 cm, fleshy, central, cylindrical, slightly swollen at the middle, solid, concolorous, surface smooth, glabrous, annulus absent. Pseudorrhiza: Size 3-5 × 1 cm, dark-brown in colour, cylindrical slender, smooth, glabrous, fibrous, bulbous and widening at certain depth, than narrowing down to the point of attachment to the termite nest, its length determined by the depth of the termite comb. Flesh: Pale-grey in colour, thin walled, filamentous, nonamyloid, hyaline, clamp connection absent. Basidia: 20.5 × 5.5 µm, tetra sterigmata bearing tetra basidiospore. Spore print: pinkish cream Cystidia: 37 × 10 µm. Basidiospores: 5.5 × 4.5 µm, ellipsoid (Figu**r**e 21)**.**

Habitat: On termite nests in tropical forest. Fruiting season: July-September.

Chemical test with

Phenolphthalein : Cool Cream 3272

NH_3OH : No reaction

KOH : Cool Cream 3272

$FeSo_4$ solution : Stipe: outer: White, and Inner: Pale Rosy 0421, Cap: White, Gills: Bliss 0170. 8031, Cap: White, Gills: Strawberry 0481.

$FeSo_4$ crystal : Stipe: outer: cream, and inner: Volcano 0578, Cap: Cream, Gills: Blush 8138.

Iodine : Dorsal: white, Ventral: Barley 0572.

Specimen examined: The species found around the Elephant dung, common, near Dipterocarpus, in Bardiya (BNP Forest) 132m, long. 81.34325°E, lat. 28.20712° N, Aspect: 64° N/E, Slop: 5° S/W, Temp.: 21.9-30.3° C, Humidity: 75-87%; Soil pH: 5.5; Time: 7:30 am; Date: 29.08.2011; VN-1007251-CDBP.Accession No. NHM TU 2-2-1675. Report for new to Nepal.

Distribution: Equatorial, southern Africa, Madagascar, S. E. Asia including Nepal.

Figure 21: *Termitomyces straitus f. griseus,* a. Premordium on its combs, b. and c. Basidiocarp, d. Spore print, e. Lamellae difference (1div= 11.66 µm) and f. Basidium and spore (1div= 11.66 µm)

xvi) Termitomyces *straitus f. ochraceus* *Mém. Acad., Sci., Paris* **44,** 72 (1942); *Fl. Icon. champ. Congo* **7,** 144 (1958).

Local name: Kalunge

Taxonomic position: division: Basidiomycota class: Agarocomycetes sub-class: Agaricomycetidae order: Agaricales family: Tricholomataceae

Identifying characters: The most distinguishing characters of this species are medium size cap, (usually at least ≥4 cm in diameter), ochraceus brown in colour, not

concentrically scrobiculate, at most radially striate, first conical, then convexo-applanate, without velar remnant or with few minute whitish evanescent membranous squamules, prominent, cone-like pointed, dark coloured projection at the centre of the cap i.e. spiniform papilla. Perforatorium not so long spiniform, a continuation of cap, either demarcated, or not or absent, small, broadly conical. Stipe, are absence of annulus with pseudorrhiza originating from termite nest; no sclerotium. Pseudorrhiza are pale, not entirely blackish.

Description

Sporophores: Growing on the sandy soil in groups or on the shaded ground in association with termite's nests.

Pileus: Size 4.0-9.0 cm, (medium size), without or small spiniform papilla of olivaceous in colour, conical to convexo-applanate in shape, surface smooth, incurved margin when young, slightly uplifted when mature, ochraceous in colour. Lamella: Dense, crowded, free, pale yellow in colour. Stipe: size 4 × 1.8 cm, small, fleshy, solid, surface smooth, glabrous, buffy-brown in colour, annulus absent. Pseudorrhiza: pale to dark-brown in colour, cylindrical slender, smooth, glabrous, fibrous, bulbous and widening at certain depth, than narrowing down to the point of attachment to the termite nest, its length determined by the depth of the termite comb. Flesh: whitish, thin walled, cylindrical, nonamyloid, and hyaline. Clamp connection: absent. Spore print: cool pink. Basidia: 22 × 6.5 µm, tetra sterigmata bearing tetra basidiospores. Cystidia: 40.5 × 12 µm. Basidiospores: 6.5 × 5.5 µm ellipsoidal (Figure 22)**.**

Habitat: On termite nests in tropical forest. Fruiting season: July-September.

Chemical test with

Phenolphthalein	:	Raw silk 0351
NH_3OH	:	White
KOH	:	Volcano 0578
$FeSo_4$ solution	:	Stipe: outer: Ting of rose 8084, and Inner: Pale Rose 0421, Cap: Ting of Rose 8084, Gills: Macrame 0495.
$FeSo_4$ crystal	:	Stipe: outer: Lavender Dew 0941, and inner: In Vogue 8105, Cap: Lavender Dew 0941, Gills: Magnolia 0387.
Iodine	:	Dorsal: white, Ventral: Bathstone 0301.

Specimen examined: Growing on termite nest, common in Humicolous, Scattered, on *Shorea robusta* dominated TDRF forest, Nawalparasi (Chalishkilo), 202m, long. 84.05827° E, lat. 27.62584° N; Aspect: 50° N/E; Slop: 12° S/W; Temp.: 21.9-31.2°C; Humidity: 67-87 %; Soil pH: 5.62; Time: 4:41 pm.; Date: 24.07.2011. VN-1007179-CDBP. Accession No. NHM TU 2-2-1674. Report for new to Nepal.

Distribution: Equatorial, southern Africa, Madagascar, South East Asia including Nepal.

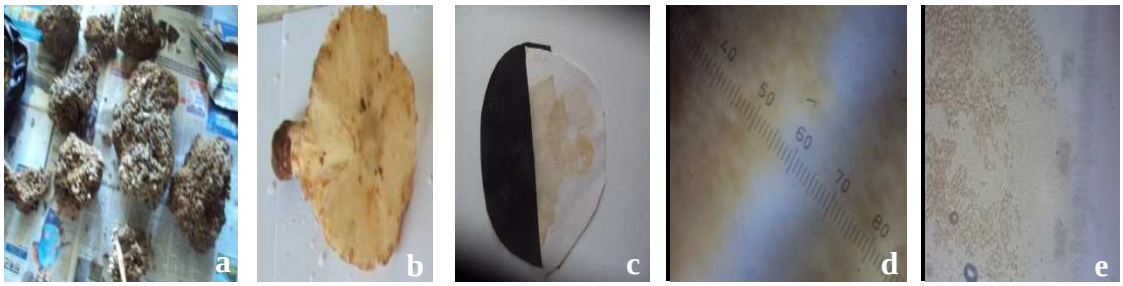


Figure 22: *Termitomyces straitus f. ochraceus*, a. Premordium on its combs, b. Basidiocarp, c. Spore print d. Lamellae difference (1div= 11.66 µm), and e. Spores with Basidium (1div= 11.66 µm)

xvii) ***Termitomyces umkowaan*** (Cooke & Massee) D. A. Reid, *Contr. Bolus Herb.* **7,** 118 (1975)

Local name: Sangraino.

Taxonomic position: Division: Basidiomycota class: Agarocomycetes sub-class: Agaricomycetidae order: Agaricales family: Tricholomataceae.

Identifying characters: The most distinguishing characters of this species are large fleshy agaric with yellowish brown cap, obtuse umbo, whitish gills and long stipe with pseudorrhiza, scattered to gregarious on termite mounds or on soil, rare, odour pleasant, taste excellent, edible, untetra basidiospores.

Description

Sporophores: Usually growing solitary in termite hills, characterized by its obligate symbiont with termites; usually centrally stipitate white but olivaceous near the umbonal region.

Pileus: Size 21.0-26.0 cm diameter, (large size), at first campanulate becoming expanded to shallowly convex and later up turned with spiniform to broadly conical

umbo perforatorium and straight to incised or split margin when young and uplifted when mature; at first greyish yellow, fading to yellowish brown, radially wrinkled, smooth and viscid or slimy when wet, otherwise dry. Lamellae: white, free, broad, crowded, regular; short gills of three unequal lengths. Stipe: white, surface smooth, fibrillose swollen at the base, extending to rust brown, size 20 × 0.7 cm (epigeal), central, cylindrical, without annulus, long; slightly swollen or bulbous base near the soil surface, Pseudozhiza: Size 12 × 1 cm, and rust-brown in colour, cylindrical and widening at certain depth, than narrowing down to the point of attachment to the termite nest, its length determined by the depth of the termite comb, surface fibrillose and stuffed. Flesh: Whitish-grey to creamy in colour, moderately thick and fleshy, hyaline, clamp connection absent. Basidia: 22.0 × 7.5 µm, divergence. Spore print: Brownish. Cystidia: 45.0 × 10 µm broadly clavate to pyriform Basidiospores: 6.5 × 4.5 µm, untetra basidiospores, broadly ellipsoidal, divergence, hyaline and smooth (Figure 23)**.**

Habitat: On termite nests in tropical forest. Fruiting season: July-September.

Chemical test with

Phenolphthalein	:	Ivory 0315
NH_3OH	:	Cherry Brandy X126
KOH	:	Milk Toffee 7968
$FeSo_4$ solution	:	Stipe: outer: white, and Inner: Pale Rose 0421, Cap: White, Gills: Bliss 0170.
$FeSo_4$ crystal	:	Stipe: outer: Cream, and inner: Volcano 0578, Cap: Cream, Gills: Baby Blush 8138.
Iodine	:	Dorsal: white, Ventral: Barley 0572.

Specimen examined: Growing on termite nest common in *Shorea robusta* dominated STDHF forest, Palpa (Charchare), Alt.: 905m, long. 84.50969° E, lat. 27.76451° N; Aspect: 158° S/E; Slop: 52° S/W; Temp.: 21.9-31.2°C; Humidity: 67-87%; Soil pH: 5.62; Time: 9:17 am. Date: 11.07.2011. VN-1107590-CDBP. Accession No. NHM TU 2-2-1665. Report for new to Nepal.

Distribution: Equatorial, southern Africa, Madagascar, South East Asia including Nepal.

Figure 23: *Termitomyces umkowaan,* a. Premordium on its combs, b. Basidiocarp dorsal view, c. Basidiocarp ventral view, d. Spore print e. Lamellae difference, and f. Basidium with spores (1div= 3.75 µm)

xviii) *Termitomyces* new I (A)

Local name: Bhalu mugan (Magar)

Taxonomic position: Division: Basidiomycota class: Agarocomycetes sub-class: Agaricomycetidae order: Agaricales family: Tricholomataceae.

Identifying characters: The most distinguishing characters of this species are fleshy agaric with greyish brown cap, umbonate, white gills and bulbous base of stipe with pseudorrhiza. The basidiocarps scattered to gregarious on termite mounds or on soil, rare, odour pleasant, taste excellent, edible.

Description

Sporophores: Usually growing solitary in termite hills, characterized by its obligate symbiont with termites; usually centrally stipitate white but olivaceous near the umbonal region.

Pileus: size 9-15 cm, (Medium size), greyish brown pale toward the margin and olivaceous near the umbonal region, umbonate, incurved margin, surface smooth, radially striate, Perforatorium umbo and brown in colour. Lamellae: Free, white, dense. Stipe: size 10 × 2 cm, solid, swollen at base i.e. bulbous base, whitish to pale yellow in colour, surface smooth, fibrillose, absence of annulus. Pseudorrhiza: Size 5 × 0.5 cm, buffy brown in colour, solid, cylindrical. Pseudorrhiza: 5 × 0.5 cm long, solid, brown in colour, cylindrical, surface glabrous, smooth, widening at certain depth, then narrowing down to the point of attachment to the termite nest, its length determined by the depth of the termite comb. Flesh: Grey, soft, inflated, clamp connection absent, nonamyloid. Spore Print: pinkish. Basidia: 17.5 × 6.5 µm. Basidiospores: 4.5 × 3.5 µm, oblong**.** Cystidia: 25 × 15 µm**.** Hymenophoral trama: regular (Figure 24)**.**

Habitat: On termite nests in tropical forest. Fruiting season: July-September.

Chemical test with

Phenolphthalein	:	Sugarcane 0332
NH_3OH	:	No reaction
KOH	:	White
$FeSo_4$ solution	:	No reaction
$FeSo_4$ crystal	:	Stipe: outer: White, and inner: Sunrise 0526, Cap: White, Gills: Sugarcane 0332.
Iodine	:	Dorsal: white, Ventral: Beacon 3109.

Specimen examined: Growing on termite nest, common. Palpa (Simaldanda), 816m, long. 83.50969°E, lat. 27.76451° N, Aspect: 270° W, Slop: 50° S/W, Temp.: 21.9-26.8°C, Humidity: 79-87 %, Soil pH: 6.2 Time: 9:43 am; Date: 04.08.2010; VN-1008184-CDBP. Accession No. NHM TU 2-2-1680. Propose for new to Science.

Distribution: Equatorial, southern Africa, Madagascar, South East Asia including Nepal.

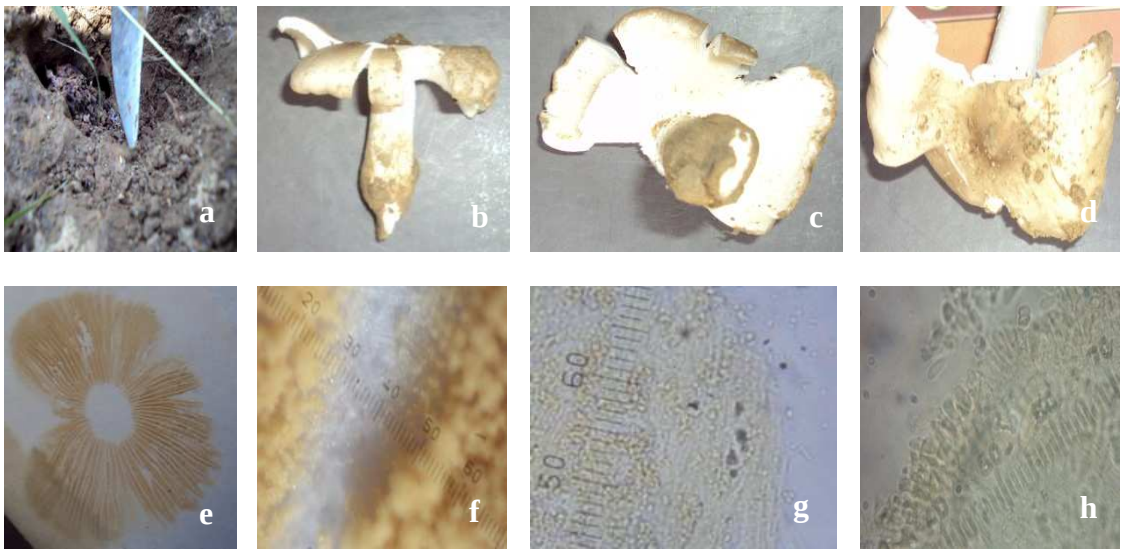


Figure 24: *Termitomyces* new I, a. Termite in termire nest, b. Basidiocarp dorsal view, c. Basidiocarp ventral view, d. Umbonate papilla, e. Spore print, f. Lamellae difference (1div=11.66 µm), g. Spores (1div=11.66 µm) and h. Basidium with spores (1div=3.75 µm)

xix) *Termitomyces* new II (B)

Local name: Tuse cyau (Khash)

Taxonomic position: Division: Basidiomycota class: Agaricomycetes sub-class: Agaricomycetidae order: Agaricales family: Tricholomataceae.

Identifying characters: The most distinguishing characters of this species are medium size cap, (usually at least ≥ 8 cm in diameter), dark-grey to rusty brown in colour, cap bullate-umbonate in shape, entire in margin, surface wrinkles, smooth, without velar remnant or with few minute whitish evanescent membranous squamules, prominent, cone-like pointed, dark-brown coloured projection at the centre of the cap. Perforatorium are broadly conical. Stipe, are presence of annulus with pseudorrhiza originating from termite nest; no sclerotium. Pseudorrhiza are pale, not entirely blackish.

Description

Sporophores: Usually growing solitary in termite hills, characterized by its obligate symbiont with termites; usually centrally stipitate white but olivaceous near the umbonal region.

Pileus: Size 8-16 cm, (Medium size), dark grey to black in colour, bullate-umbonate, surface wrinkled, smooth, entire margin, papilla brown in colour. Lamellae: Free, dense, crowded, white to pale grey. Stipe: Size, 7 × 2 cm, solid, swollen at base, whitish, surface smooth, annulus thin demarcation and persistent. Pseudorrhiza: Size 7 × 0.5 cm long, solid, brown in colour cylindrical, slender, surface glabrous, smooth, and widening at certain depth, then narrowing down to the point of attachment to the termite nest, its length determined by the depth of the termite comb. Flesh: Soft, grey, inflated, clamp connection absent, inamyloid. Spore print: light brown. Basidia: 19 × 6 µm. Basidiospores: 4.5 × 3.5 µm, ovoid**.** Cystidia: 30 × 17 µm. Hymenophoral trama: regular (Figure 25).

Habitat: On termite nests in tropical forest. Fruiting season: July-September.

Chemical test with

Phenolphthalein	:	Sporty Yellow X104
NH_3OH	:	White
KOH	:	Rust 0569
$FeSo_4$ solution	:	Stipe: outer: Ting of rose 8084, and inner: Pale Rose 0421, Cap: Ting of Rose 8084, Gills: Macrame 0495.
$FeSo_4$ crystal	:	Stipe: outer: Lavender Dew 0941, and inner: In Vogue 8105, Cap: Lavender Dew 0941, Gills: Magnolia 0387.
Iodine	:	Dorsal: white, Ventral: Bathstone 0301.

Specimen examined: Growing on termite nest, common. Arghakhanchi (Thada), 1261m, Long. 83.09029° E, lati.27.86208° N, Aspect: 270° W, Slop: 20° W, Temp.: 21.9-26.7° C; Humidity: 75-87%, Soil pH: 6.2, Time: 11:46 am.; Date: 19.07.2011. VN-1008227-CDBP. Accession No. NHM TU 2-2-1681; Proposed for new to Science.

Distribution: Equatorial, southern Africa, Madagascar, South East Asia including Nepal.

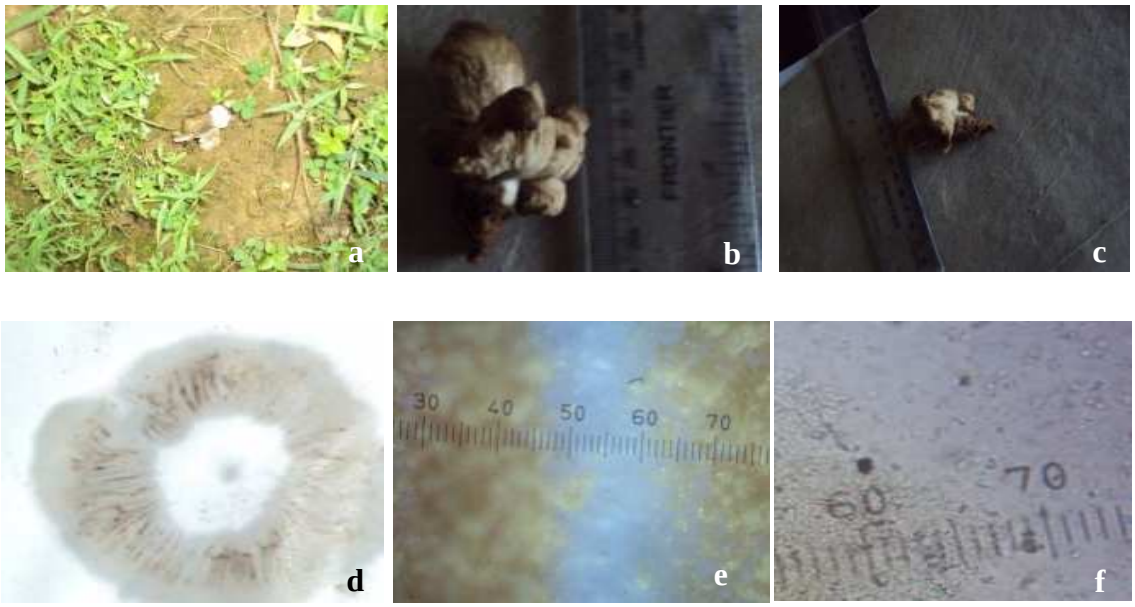


Figure 25: *Termitomyces* new II, a. Basidiocarp on habitat, b. and c. Basidiocarp dorsal and ventral view, d. Spore print, e. Lamellae difference (1div=11.66 µm), and f. Spores (1div=11.66 µm)

2.5 Discussion

2.5.1 Macro-morphology

The cap size, shape, margin, surface and colour vary according to age and environmental conditions. This character is very useful for narrowing the identification of the taxon (Table 3). This also help in narrowing the wide-range of comparisons in the field guide and more systematically important, for making identification work easier (Heim, 1977; Harkonen & Mwasumbi, 2003; Harkonen, *et al*., 1995).

Cap as a morphological feature being environmentally influenced (Alexopoulos & Mims, 1993). Piearce (1987), reported on the effects of the architecture, depth, size and nutritional status of the parent comb and resistance of the soil to the cap, but he

did not explain the relationship of the effects. Kunugawa and Furukawa (1965) found out that temperature affects the development of fruit bodies since mushrooms have their minimal, optimal and maximal temperatures for their healthy growth while Webester (1970) noted the effect of light intensity on mushroom fruit body morphology, often with much elongated stipe and poor cap development depending on the light intensity. Thus it can be said that on the basis of cap size, it is very useful for comparisons between the taxa for identification. A distinct umbo (or papilla) is one of the important diagnostic features of *Termitomyces.*

Colour of cap, perforatorium, gills, stipe, pseudorrhizea and flesh also help for identification of the taxon during present study. Colour is also a useful for confining the species of the genus (Heim, 1977). Colour of basidioma cap, stipe and lamellar are the most useful macro morphological characters but difficulty is that pigments responsible for these, are unknown in many groups of agarics including *Termitomyces* (Singer, 1986). So that it is necessary for more study to determine pigments responsible for particular type of colour in order to make distinguishing the characters. However variation of colour was found in this genus from whitish to greyish, greyish to brown and Tawny-brown to pale cream.

A distinct umbo (or papilla) is one of the important diagnostic features of *Termitomyces*. Shape of the perforatorium implies that some species are close to one another. For example *T. eurrhizus, T. heimii, T. umkowaan, T.* new I and *T. badius* have an umbo; similarly *T. microcarpous* (small & large form) both have small spiniform papilla, whereas they differ to one another due to varied form of perforatorium. For example *T. clypeatus, T. le-testui,* and *T. mammiformis* due to the presence of spiniform, cylindrical umbo “teat” perforatorium, and mammiform scrobiculate cone like pointed projection respectively.

Ornamentation of gills also helps for narrowing the taxon during identification but it is less significant for reaching the species level, because all the *Termitomyces* having free, crowded and dense lamellae.

Shape, size and surface of the stipe also help for narrowing the species but it is less significant for determining the species level, because usually all the *Termitomyces* having solid, cylindrical, and long.

Presence of an annulus in termitomycetes also serves as an important character. It varies from completely absent to microscopic squamules, to fugacious appendiculate

and to very thick ring like structure encircling the stipe. In some condition of (*T. clypeatus, T.* new II, *T. aurantiacus,* and *T. badius*) preliminary stage it appears but at matures it become completely disappears. Similarly *T. straitus f. griseus, T. straitus f. ochraceus,* and *T.* new II, it may be present or absent, so that practically these characters are very difficult to use identification, because due to its wide range of variation and unsTable taxonomic information. In the present study, out of nineteen, seven species (*T. albuminosus, T. eurrhizus, T. heimii, T. le-testui, T. mammiformis, T. Schimperi,* and *T. mallus f. arghakhanchensis*), of Nepalese *Termitomyces* possess, presence of annulus, of which, two (*T. albuminosus* and *T. heimii*) have thick and white, four (*T. eurrhizus, T. heimii, T. le-testui* and *T. mammiformis*) was persistent annulus and left of the twelve species had absence of annulus.

Similarly, the presence (*T. clypeatus, T. eurrhizus, T. heimii* and *T. umkowaan*) or absence (*T. microcarpus,* small form) of pseudorhizae in termitomycetes also constitutes a distinguishing feature. The length of the hidden pseudorhizae (if present) varies from 3-60 cm and varies in shape (cylindrical, unevenly widening and narrowing in certain sections). The size of the pseudorrhizea varies between the species, ranging from $\leq$ 5 (small) to $\geq$ 12 cm (large) and completely absent in *T. microcarpus* (small form). Its presence or absence is helpful for identifying between the large and small form of *T. microcarpus* among the termitophilous mushroom, similarly its length depends on the depth of the termite comb. So, ranging the length we can categorize whether they are belongs. Similarly its shape (morphometry) also varies between the species. They are either solid (or stuffed) or hollow. Presence or absence, solid or hollow, and wideness also help to grading of the species. Surface also varies between the species. Whether they are cartilaginous or leathery or glabrous-smooth or fibrillose-stuffed, these characters also helps for narrowing the species among the genus of termitophilous mushrooms. The colour of the pseudorhizae contrasts sharply with the stipe (white to light brown vs. buffy brown to dark brown). They are either solid (or stuffed) or hollow. Its colour also varies between brown to dark-brown, to pale-grey in colour among the species. According to Van-der and Eicker (1990), the aspect of colour and morphometry are not much used as distinguishing characters between species. But, during present investigation its colour and morphometry help for distinguishing between the species of *T. microcarpus* (small and large form) and other species except *T. eurrhizus, T. globulus* and *T. umkowaan.* In some respect some species are close resembles to one another,

while in some respect they are far apart, eg. *T. eurrhizus, T. globulus* and *T. clypeatus* their colour and shape of pseudorrhizea, are dark brown, solid, cylindrical, widening at certain depth, then narrowing down to the point of attachment to the termite nest, (depends on the depth of combs). Whereas other studied species they are contrasting to one another. The stipe either has a bulbous base (*T. clypeatus, T. eurrhizus* and *T. umkowaan*) or is devoid of it (*T. microcarpus*). In *T. heimii*, the base was broader than the stipe as well as pseudorrhiza. Presence of an annulus in termitomycetes also serves as an important character, which ranges from none through squamulose and appendiculate to a thick ring encircling the stipe. In the present study, out of nineteen, seven species (*T. albuminosus, T. eurrhizus, T. heimii, T. le-testui, T. mammiformis, T. Schimperi,* and *T.* new II), of Nepalese *Termitomyces* possess, presence of annulus, of which, two (*T. albuminosus* and *T. heimii*) have thick and white, four (*T. eurrhizus, T. heimii, T. le-testui* and *T. mammiformis*) was persistent annulus and left of the twelve species had absence of annulus.

Presence or absence of clamp connection also helps for narrowing the species. For example *T. heimii* and *T. microcarpus* (small & large) have presence of clamp connection rest all studied species have absent. During present investigation it is difficult by only study the hyphal structure for narrowing the taxon, because generally all the species have nonamyloid, hyaline, interwoven, thin walled (except *T. umkowaan,* it has thick wall). Though *T. heimii, T. schimperi* and *T. straitus f, griseus* are close to one another on the basis of septed, parallel and filamentous hyphae.

According to Heim (1977) and Singer (1986), all the species have pinkish spore in mass and colour of the spore print occasionally turn pale brown with long storage. During present investigation, all the species had pink to brown to pinkish in colour hence justify the statement on this experiment.

2.5.2 Micro-morphology

Micro-morphological characters also provide crucial information for species descriptions (Table 4). This statement also supported by Harkonen *et al*. (1995) and Bother and Eicker (1991).

Basidia size varies among the species (10.0 × 4.0 µm of *T. microcarpus* (small form) to 30.0 × 0.9 µm of *T. fuliginosus*). All species, of basidia, they contain tetra

sterigmata bearing one basidiospore on each sterigma, but in case of *T. umkowaan* it contain untetra basidiospore on (1-2) sterigmata. During present investigation *T. umkowaan* had larger basidia and basidiospores with comparisons to other species. Vander and Eicker (1990) also reported the same in support of earlier statement. But they further reported that no any other basidia character appeared to have any systematic significance. On my present investigation on the basis of size of basidia they are narrowing down on 4 groups. In the first group its size varies 10-12 × 4-6 to 15-20 × 5-7 µm [(*T. microcarpus*) (small and large form)]. Second groups its size varies 16.5 × 5.5, 17.5 × 6.5, 11.66 × 6.9, 17.5 × 6.5, 19.0 × 6.0 and 20.5 × 6.6 µm (*T. clypeatus, T.* new I, *T. eurrhizus, T. heimii,* T. new II., and *T. robustus*). Third groups, its size varies 23.5 × 7.6, 22.5 × 5.9, 20-25 × 5-7, 24.0 × 7.0, 24.1 × 7.3, 22.1 × 6.5, 20.5 × 5.5, 22.0 × 6.5, and 22.0 × 7.5 µm (*T. albuminosus, T. aurantiacus. T. globulus, T. badius, T. le-testui, T. mammiformis, T. straitus f. griseus, T. straitus f. ochraceous,* and *T. umkowaan*), and fourth groups, its size varies 30.0 × 0.9 and 27.5 × 8.0 µm (*T. fuliginosus,* and *T. schimperi*).

Cystidia size varies among the species (19.0 × 10.5 of *T. badius* to 45.0 × 15.0 µm of *T. mammiformis*). Its shape was found to be useful to more similar species like *T. umkowaan* and *T. eurrhizus*. Cystidia shapes stand as a difference between the two, *T. umkowaani* being broadly clavate to pyriform (Vander and Eicker, 1990) while the *T. eurrhizus* being obovoid to pyriform (Pegler & Vanhaecke, 1994).

They have little variation of morphometry in basidiospores, thus they provides little taxonomic information for designating the species. All are ellipsoid except *T. le-testui,* which has oblong in shape. *T. eurrhizus* have somewhat large basidiospores and broadly clavate to pyriform cystidia. Untetra basidiospore is the distinguishing characters found in *T. umkowaan.* Size of the basidia and basidiospores are diverged. The divergence of one species from other members of the genus suggests that it may be of a different genus (Singer, 1986; Bougher & Katrina, 1998).

Vander and Eicker (1990), distinguished *T. umkowaan* from *T. eurrhizus* using the basidiospores size and shape, by describing *T. eurrhizus* having somewhat large basidiospores and broadly clavate to pyriform cystidia. This study found inconsistencies in the basidiospore size such that they overlap between the two species. For example, the basidiospores of *T. eurrhizus* as reported by Pegler &

Vanhaecke (1994), is 6.5-8.5 × 4-5 µm, Harkonen *et al.* (1995), 6.8-10.0 × 4.1-6.0 µm, Heim (1977) 6.5-8.5 × 4.0-5.0 µm and present study is in larger size 9.3 × 6.8 µm while that of *T. umkowaan* is 6.1-11.3 × 4.3-5.6 µm as observed by Vander and Eicker (1990), and 6.8-8.0 × 4.3-5.6 µm observed by Tibuhwa *et al*. (2010) and smaller size 6.5 × 4.5 µm were observed in this study. This inconsistency and overlap of the basidiospore size limits its use in demarcating the two species.

Micro morphological characters are generally overlapping to one another. For example, cystidia (obovoid to pyriform in *T. eurrhizus* and *T. globulus*, and clavate to pytiform in *T. umkowaan*), basidia (clavate in all three) and basidiospores (elliptical in *T. eurrhizus* & *T. globulus* and broadly elliptical in *T. umkowaan*) are the little bit contrasting characters to one another. Harkonen *et al*. (1995), and Bother and Eicker (1991) reported that micro-morphological characters of *Termitomyces* provide little information for species determination. But present study, ample help for narrowing the species. Generally all the species having basidia of tetra sterigmata bearing tetra basidiospores except *T. umkowaan,* such results also reported by Heim, (1942c, 1977); Bels and Pataragetvit, (1982); singer, (1986) and Tibuhwa *et al*. (2010).

2.6 Conclusion

This morphological classification helps to solve the taxonomic problem of *Termitomyces* species. Although for determining the species, macro and micro-morphological characters provides ample information. But it is not sufficient at all, so that it is necessary to ascertaining the molecular level of study for determining the more similar species in the systematics. During investigation (19) taxa were identified of the genus *Termitomyces.* The identified samples belong to class: Basidiomycotina; order: Agaricales; family: Tricholomataceae. Survey of literature shows that out of (19) taxa, (15) are new addition to the macrofungal flora of Nepal, and (2) species are newly proposed to the macrofungal flora of the world, whereas remaining (2) species of *Termitomyces clypeatus* and *T. eurrhizeus* constitute the first authentic report from the study area. This research work provides in depth information about the genus *Termitomyces* for further studies on taxonomic field. Hence the recent investigation creates an enthusiasm towards intensive exploration on the works.

CHAPTER 3

3. ETHNOMYCOLOGY OF TERMITOMYCES R. HEIM IN NEPAL

3.1 Introduction

Before the invention of ethno mycological study, wild mushrooms were simply collected and reported whether they are applicable or not, for the human beings (Singh, 1966). An ethno mycological study includes, with regards to those who consumed the edible fungi, by the selected ethnic tribes or group in specific geographical regions. Ethnomycology is especially dedicated to mushrooms. The investigation and study about the utilization of mushrooms by different ethnic groups is not studied in detail in Nepal (Adhikari, 2000). “Dictionary of fungi” defined Ethnomycology as a branch of Ethnology in which edible mushrooms and religion is closely inter-related. Wasson (1969) is also credited with coining the term “Ethnomycology”. Wild edible mushrooms are one of the important minor forest products, which are locally traded in local markets of different part of Nepal (Kharel & Rajbhandary, 2005). In this regards *Termitomyces* species has culinary purposes as well as medicinal utility for treatment of different types of disease and hygienic disorders have been documented (Table 6).

Hypothesis

Termitomyces are one of the underappreciated groups of Non timber forest products (NTFPs) in Nepal.

Objective

To documents the *Termitomyces* spp. on ethnomycological aspects.

3.2 Literature review

3.2.1 Ethnomycology: An overview

The world treasure of fungi probably comprises of 1.5 million species, of which 69,000 species have yet been described (Hawksworth, 1974) and others are awaiting their discovery. Among the described species include about 10,000 species of fleshy macro fungi (Kendrick, 1985), very few of which are deadly poisonous. About 2000

species belonging to more than 30 genera are edible. Among these edible forms, 80 species have been grown experimentally, 40 species cultivated economically, about 20 species cultivated commercially and 5-6 species are produced on an industrial scale (Chang & Miles, 1993). Some of the significant contributions on termitophilous mushrooms from ethno-biological aspects, are found by Sacherer, 1979; Akpaja, *et al*., 2003; Atri, *et al*., 2005. Other significant contribution on fungi from ethno-biological perspectives are by Adhikari, 2000; Joshi & Joshi, 1999; Rana & Giri, 2008 etc.

Some ethnic groups covered in ethno mycological studies include Chepang (Tullous & Bhandary, 1992; Pandey, 2008), Sherpas (Sacherer, 1979), Tamangs (Kharel, 1999; Pandey, 2008), Thakali (Bill & Cotter 1989). The geographical area covered by ethno mycological studies in Nepal includes Kathmandu (Singh, 1966; Singh & Nisha, 1974), Kathmandu valley and adjoining area (Adhikari, 2000; Pandey, 2008), Dumre, Pokhara, Mustang, Manang (Bhandary, 1991), Pokhara and Kathmandu valley (Joshi & Joshi 1999), Rolwaling (Sacherer, 1979), Western Central Region of Nepal (Adhikari, *et. al*., 2005), and Sukhaura Hariyali Community forest, Rupandehi (Bhattarai, 2013).

However, till 2000 A.D., the total number of mushrooms of ethno mycological significance recorded in Nepal included more than 110 edible species, of which 13 were medicinal, 45 were toxic and 6 species were of other uses (Adhikari, 2000). Singh (1966) reported 18 wild edible species of mushrooms sold at Kathmandu market in bamboo package. Sacherer (1979), in an ethno mycological study of Rolwaling Sherpas, listed about 14 specimens of mushrooms in their local names without proper taxonomic identification. Bhandary (1980) collected 13 species of macro fungi related to seven different families, among which eight species were new to Nepal. Bhandary (1984) prepared a checklist of edible and poisonous mushrooms along with their local names. Adhikari (2000) threw light on different ethnic groups associated with the collection and consumption of wild edible mushrooms in different phytogeographical belts of Nepal. Bills and Cotter (1989) studied taxonomy and ethnomycology of *Lactarius* Sec. Dapetes (Russulaceae) in Nepal. Bhandary (1991) reported 25 species of edible and medicinal fungi from Dumre to Manang, Mustang and Pokhara area. In this regard, edibility of *Coltricia perennis* and *Daldinia*

concentrica and medicinal application of *Daldinia concentrica* and *Pycnoporus cinnabarinus* were not reported earlier. Tullous and Bhandary (1992) reported *Amanita chepangiana* from Jugedi, Chitwan, used as food by Chepang ethnic groups.

Adhikari (1996) reported that 821 species of Basidiomycetes including the list of Hymenomycetes (520) with taxonomic studies on the genera such as *Amanita*, *Russula*, *Lactarius* and Gasteromycetes species. It included 40 species new to Nepalese mycoflora with seven species new to science. Adhikari (2000) studied the ethnomycological approaches with the Ayurvedic concepts about the mushrooms. Joshi and Joshi (1999), in their ethno mycological study, presented the ethno botany of 36 species of wild mushrooms collected from different parts of Kathmandu and Pokhara. Kharel (1999) reported *Lentinellus ursinus*, an edible mushroom from Bhardeo VDC, Lalitpur. Pandey (2008) highlighted the relation of mushrooms with the different ethnic groups of Kathmandu valley. Kharel and Rajbhandary (2005) studied upon the ethnomycology of some wild mushrooms in Bhardeo, Lalitpur. Devkota *et al*. (2005b) reported 31 species of mushrooms from Lumle, Kaski, from western Nepal, in which they have documented brief ethno mycological knowledge among the local people. Many writers have made studies on *Cordyceps sinensis*. In some parts of Nepal, *C. sinensis* is powdered and combined with the rhizome of *Dactylorhiza hataginea* for consumption (Adhikari, 2000). It is also used for tonic for Yak and Sheep. Local people believe that it gives good strength and hence they used to give it as gift to relatives and friends (Devkota, 2006). *C. sinensis* is consumed mixed with rice flour in boiled milk. Traditionally, it has been consumed with a variety of meats of chicken, duck and pork (depending upon the type of ailments) in the form of a medicinal soup (Zhu, *et al.*, 1998; Winkler, 2004).

Christensen, *et al*., (2008b) studied upon the uses of wild mushrooms in the Annapurna Conservation Area. They have reported 38 species with their different values among local peoples. Pandey (2008) gave the account of mushrooms in Langtang area with their use and local names where listed 94 Tamangs name of mushroom and their use among the Tamang of Nepal. The thesis focused on the diversity of species used and the distribution of use pattern of various wild mushrooms among the Tamangs of Nepal and she also described the ethnic view of Chepang on mushrooms and their local names.

Basically ethnomycological knowledge can be studied into two parts: One which is gained in school and universities and the nest is acquired from local knowledge, which is usually communicated verbally. In Nepal it is traditionally knowledge acquired by experience and observation, which is communicated vocally, and which has formed the basis for Nepalese ethno botany. The impetus for collection and research on mushrooms and other fungi of Nepal gained momentum after the founding of post-graduate teaching in botany in TU in November 1965. Specimen collection became a part of the academic activity since they were needed for Lab works (Singh, 2007). For the first time, Singh and Nisha (1973ab) initiated a mini-project on "Inventions on wild Mushrooms and other fungi of Kathmandu valley", thus research and cultivation of mushroom can be said to be started in 1973 in Nepal (with experiments). Adhikari (2000) has presented a very impressive account of mycological investigations and species of mushrooms reported from Nepal in book "Mushroom of Nepal".

3.2.2 Mycological studies in Nepal

Nepal possesses diverse phytogeographical zones related to altitude, variation of topography, climate and latitudinal change within a short distance. Thus the vegetation varies greatly from east to west and from north to south. These varied elements have enriched Nepal with economically important mycoflora (Pandey, 2008). The noteworthy contributions in mycological field are those of Singh and Nisha (1974), Adhikari (1996, 2000), Devkota (2005a). So far, 812 mushroom species have been identified (Adhikari, 2009). Among them, (228) species are edible (Christensen, *et al.*, 2008a), (66) species are poisonous (Pandey, 2008; Adhikari 2009), (73) species are medicinal (Adhikari, 2009). The present work was concentrated in the tropical to temperate (up to 3000m) and east to west of Nepal.

Nepalese ethnicity

The population of Nepal embraces different kinds of ethnic caste with diverse traditional cultures. The relationship of ethnic groups with macrofungi on one hand on the castes those are aware of the religious sacredness of the Hinduism and on the other hand on traditional knowledge. The origin and distribution of some ethnic castes are found to localize in particular zone or area (Figure 26). The ethnic groups are the traditional collectors whose knowledge on mycoflora is quite different. However, due

to urbanization, social factors, the displacement and migration, it is becoming more and more difficult to point out or localize the exact origin of these groups (Adhikari, 2000).

Nepal is a cultural heritage for diverse ethnic groups of Mongolian, Australoid and Aryan stocks differing in language, race, religion, custom and cultures. The different ethnic groups, with their own distinct culture and language handed down gradually by ancestors, are traditionally linked in different ways to resources available in forest (Siwakoti & Siwakoti, 1998); and the forest biodiversity has supported the livelihood of many indigenous tribal people who live in inaccessible remote area of Nepal.

Total 102 ethnic groups in Nepal are distributed in the entire topographical zone except Nival belt (CBS, 2001). They have 93 different Native languages. Among them, they are still found to depend heavily on collection and consumption of wild mushrooms for food and medicine include (63) different ethnic groups (Figure 26) where mainly Ahir, Barai, Bantar, Bing, Damai, Dhanuk, Dhimal, Dhobi, Dom, Dusad (Pasi), Gangai, Harijan, Jhagar, Kahar, Kalwar, Kamar, Kami, Kanu, Koeri, Kurmi, Lodha, Lohar, Mallah, Musahar, Muslim, Pathrkot, Rajbangsi, Santhal, Sundi, Tajpuriya, Teli, and Tharu in the Tarai plain, Bhujel (Khawas), Badi, Bote, Chhanthal, Churaute, Danuwar, Darai, Gaine, Jirel, Khash-chhetri, Khash-Bramhan, Khuna, Kumal, Magar, Majhi, Newar, Praja (Chepang) and Sanyasi, Sarki, in the siwalik and Gurung, Lama, Limbu, Rai, Raute, Sherpa, Tamang Thakali, Thakuri, Thami and Yakkha at mid hills region of Nepal.

3.2.3 *Termitomyces* Mushrooms

Termitophilous mushrooms are very important for their edibility and nutraceutical utility (See pp 7, pt no 1.4 Termitophilous Mushrooms in Nepal). Several workers have estimated the nutritional values and secondary metabolites of these and reported to be superior to all other edible mushrooms and common vegetables (Mukiibi, 1973; Purkayastha & Chandra, 1975 and Botha & Eicker, 1992). Their fresh fruit bodies are sold in the different markets of Thailand, at roadsides in Old World Tropics (Turnbull & Watling, 1999) and many parts of India (Atri, *et al.*, 2005).

In the trade and manufacture of valuable compounds from *Termitomyces* mushrooms, scientists from African countries and South-East Asian countries like South Africa,

Thailand, Old Tropics, Tanzania, China and North America have done a lot (Ferreira, *et al*., 2009, 2010).

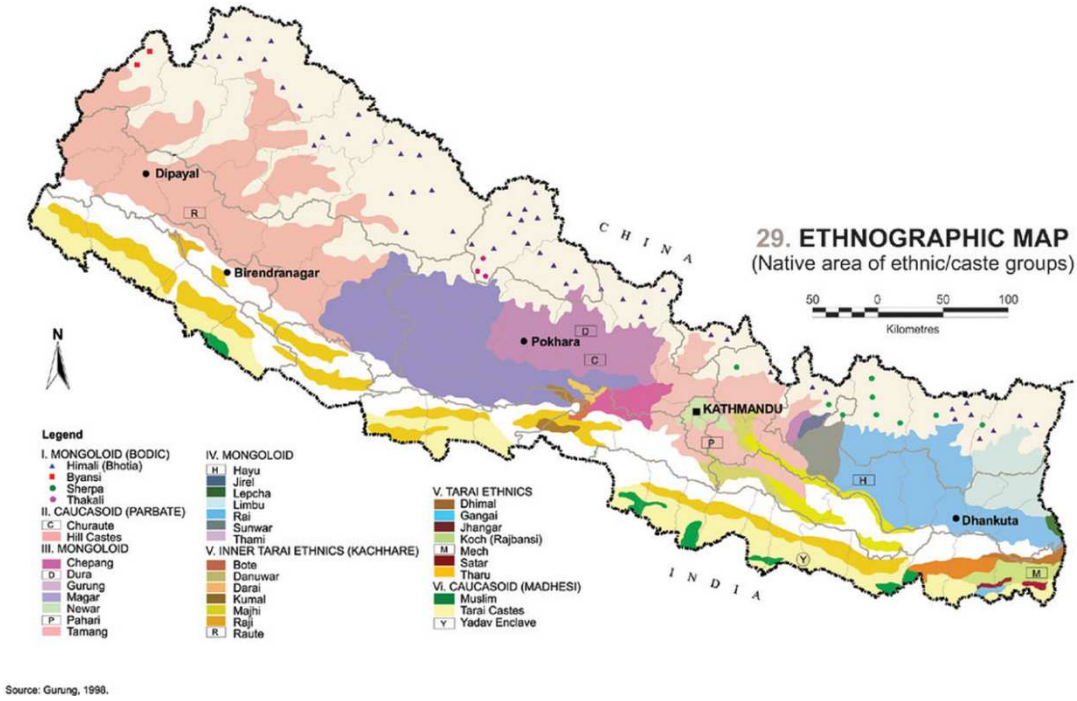


Figure 26: Map of Nepal showing main ethnic groups

3.3 Materials and Methods

Exhaustive surveys of termitomycetes on various localities were carried out in Tarai, Siwalik and Midhills of Nepal during southwest monsoon and post-monsoon seasons (June-November, 2010-2012). *Termitomyces* spp. were recorded in different habitats like mixed plantations, *Acacia*, *Dalbergia*, *Tectona* monoculture forests, AGP, Community managed forest (CMF), GNF, PF, GPF, Sacred groves (SG) and natural (evergreen, moist deciduous and Sal) forests. These were conducted on the basis of information found from the local informant (the information of the sites was obtained from the interaction with 300 members of the individuals of 63 ethnic groups and interviewed had also been taken regarding indigenous knowledge), on the basis of availability of the species (Appendix 1). The PRA technique was adopted with the local people to get information on nutritional as well as medicinal aspects and exploring the utility of investigated taxa, to examine its nutritional and sociobiological aspect. Rests of the methodology are detailly provideds in chapter-2, pp 15-25, pt. no. 2.3.1-2.3.4.

3.4 Results

During present investigation it was found that all these mycoelement are used for vegetable, also as food including local medicines by different methods of preparation and administration by local people in different parts of tropical and subtropical regions (Table 6), because of their unique texture, flavour and taste. Their market price fluctuates in between 5 and 6 US Dollar/Kg (1 US Dollar = 100 Rs/- NC).

During the survey, it was found that respondents in the urbanized areas hardly possessed any information about their edibility. However, in the rural areas some respondents above 60 years possessed substantial knowhow about their edibility, recipes and medicinal utility particularly their use in the form of soup for the persons suffering with indigestion, malnutrition and anemia, fever, physical weakness, muscular pain etc (Table 6). Because of the importance attached to these due to their culinary relevance different mushrooms are popular by variety of their indigenous names in the areas of their occurrence. These are regularly collected during the monsoon season for personal consumption and some of them like *T. albuminosus, T. eurrhzus, T. heimii, T. mammiformis, T. robustus, T. schimperi,* and *T. straitus* for earning revenue by selling these in bulk to local vendors in different parts of the country. Because of their delicious nature wide variety of these mushrooms are being regularly hunted from their natural habitats for consumption and earning revenue.

The present research would bring out the picture of mushrooms and their inter-relationship with the human beings. Hence, the research helps to emphasize the importance of wild mushroom in rural livelihoods and the information obtained from the present research also helps for the management of natural resources and towards uplifting the peoples' livelihood.

Table 6: Traditional use of *Termitomyces* spp. by different ethnic groups

SN	Taxa	Pathological Conditions	Mode of Preparation and administration	Region	Local Name	Ethnic Group
1	*T. albuminosus*, *T. striatus*, *T. mammiformis*	Measles	Pouring water in dry mushroom in pot overnight and its filtrate consumed orally, one cup thrice a day for 2/3 days. This is repeated till the recovery is evident in Magar tribe (Gulmi District).	CM WM	Kaltauke-Mugan Chikish	Magar Magar
2	*T. albuminosus*, *T. heimii*, *T. eurrhizus*, *T. mammiformis*	Yellow fever	Pouring water in dry mushroom in pot overnight and its filtrate consumed orally, one cup thrice a day for 2/3 days. This is repeated till the recovery is evident in Tharu tribe (Kapilwastu District), Kumal tribe in (Nawalparasi).	CT	Vavnethi Puchina, Bammhukan	Tharu, Barai Newar
3	*T. umkowaan*, *T. clypeatus*, *T. mammiformis*	Jaundice	Pouring water in dry mushroom in pot overnight and its filtrate consumed orally, one cup thrice a day for 2/3 days. This is repeated till the recovery is evident in Magar tribe (Palpa District).	CM ET	Kaltauke-Mugan Kavak	Magar, Kami Kanu,Sundi, Teli
4	*T. albuminosus*, *T. schimperi*	Inappetence / Abdominal disorder	Used as powdered 5-10 gm. with hot water, by mixing a pinch of salt, twice a day up to recovery in Harijan tribe (Kailali District),	WT CT	Sadaaka Kukurmutta	Harijan, Bing Muslim, Ahir

			and Muslim tribe (Banke, Kapilwastu, Routahat).			
5	*T. microcarpus*, *T. albuminosus*	Diarrhoea	Pouring water in dry mushroom in pot overnight and its filtrate consumed orally, one cup thrice a day for 2/3 days. This is repeated till the recovery is evident in Tharu, Badi (Rupandehi, Bardiya District).	CT WT	Vavnethi, Rai Bemtee	Tharu Badi, Bhujel
6	*T. robustus*, *T. fuliginosus*	Bleeding Constipation, Wounds, Itching, Eczema etc.	For curing, its powder is used with mustard oil (in Santhal tribe in Morang, Jhapa District)/water (in Tharu tribe in Nawalparasi District). Parts of fruiting bodies are mashed fresh with water for several times to form a uniform paste. The paste is consumed orally after dinner for 2/3 days.	ET CT	Chirko Vavnethi, Rai	Santhal Tharu
7	*T. microcarpus*, *T. mammiformis*, *T. albuminosus*, *T. eurrhizus*	Muscular pain	Boiled fruits bodies are used as soup to relieve muscle pains and to maintain protect or restore immune health. It is administered in two ways- Rajbangsi (Morang, Jhapa District) tribe uses it as curry, soup while its roasted forms are used in Chepang (Makawanpur-District) tribe.	ET CS CM	Chuchina Dewale Jhyamo Chyarmo	Rajbangsi, Tajpuriya Chepang Sherpa

8	*T. eurrhizus*, *T. umkowaan*.	Skin diseases	Mixture of mushroom and *Cynodon dactylon* is crushed in mortar/pestle and applied on skin infection twice a day for 3 to 5 as Paste form.(Magar tribe in Dang District)	WS	Bagale-Mugan Deule	Magar Jirel,Gaine
9	*T. robustus*, *T. aurantiacus*, *T. badius*	Feaver	Dried powder and Black salt (Birenun) are used with hot water, twice a day up to recovery (in Thami and Khuna tribe Banke district; Badi in Doti District; Tharu, Kumhal in Rupandehi, Nawalparasi District). Similarly it make soup and drink in Newar and Rai tribe (in Makawanpur District; Tajpuriya, Bantar and Santhal tribe (Morang, Jhapa, Saptari District) and Newar tribe (Ilam District)	WT WS ET EM CS CT	Sangraino Dhamire Chirko/Chuchina Puchina Dewale Chotki Vend/Kanayo	Thami, Khash-chhetri, Khuna, Bote, Badi, Route Tajpuriya, Santhal, Bantar Newar, Rai Tharu/ Kumal
10	*T. robustus*, *T. globulus*	Cut wound	Fresh extract powder/paste of fruiting bodies is used for wound healing. [(In Kumal, Badi, Bote, Route and Khuna tribe in Kusum (Banke District)].	WT ET	Vend Chhanii Negeriya.Chhani	Khuna, Kumal Chamar, Gangai
11	*T. microcarpus*	Delivery pain	Decoction of fruiting bodies as soup or curry is given to as tonic for stimulating power. Khuna (Banke District), Kumhal, Churaute (Palpa	WT CM ET WM	Jhari/Rai Jhari/MujurKhutte Chucina/Chirko Sangraino, Jhari	Khuna, Kumal Kumal, Churaute Santhal Magar, Damai

			District) and Santhal (Morang, Jhapa District), Magar (Salyan District).			
12	*T. schimperi*, *T. fuliginosus*, *T. le-testui*, *T. microcarpus*	Fever/ Indigestion/ Malnutrition/ Stomachache	Dried powder and Black salt (Birenun) are used with hot water, twice a day up to recovery (in Khuna, Badi tribe Banke district).	WT ET	Sadaaka	Khuna, Kumal, Badi, Lodh, Pasi
13	*T. microcarpus*, *T. albuminosus*, *T. mammiformis*	Laziness/ Indolence/ Inactiveness	It is used to strengthen body, for increase in body healing ability and is associated with longevity. Its powder is taken one table spoon with hot water after dinner till the recovery is evident in Bote tribe (Chitwan District).	CS CT	Dewale Mujukhutte	Bote, Sarki, Sanyasi, Lama
14	*T. microcarpus*, *T. mammiformis*, *T. clypeatus*	Stiffness of Joints	To relief from the arthritics pain, frequently used over the painful area as a paste. It is administered in two ways Santhal (Morang, Jhapa) tribe uses it as pest while Dhimal (Morang) tribe mixed with mustard oil and used as ointment for the treatment of infected parts.	ET WT ET	Chirko/Chuchina Patiyari Negeriakukurmutta	Dhimal, Santhal, Kamar Mallah Jhagar
15	*T. mammiformis*, *T. fuliginosus*	Buccal cavity infection	Its powder is used as tooth powder with mixing mustard oil and common salt in Sherpa, Tamang (Sindhuli, District).	ES	Dhamire, Shamu Shymo/ Shyamu	Sherpa Tamang

16	*T. microcarpus, T. mammiformis*	Cough/Cold	Decoction of fruiting bodies in hot water is given one cup in two times a day Rai, Limbu and Yakkha tribe, (in Ilam District).	EM	Kalunge, Pat, Dhudhmuntte	Rai, Limbu, Khash-Brahman
17	*T. heimii*	Nourishment	It is recommended for nourishing mothers Tharu, (Nawalparasi), Gurung (Palpa) used as soup.	CT CM	Chhani-Vavnethi Chyabo	Tharu Gurung
18	*T. microcarpus*	Venereal diseases	It is used as paste till recovery. Tharu in Nawalparasi, Rupandehi and Saptari District); (*It is used for treating Gonorrhoes in Nigeria, Akpaja et al. (2003); Ayodele et al. (2009).	WT CT ET	Nakaphunani/ Chhatrak Rai Chatrika	Tharu, Majhi, Patharkot Kurmi, Tharu Koiri, Kahar
19	*T. le-testui*	Fever/ Abdominal disorder/ Removal of impurities of Blood	Dry mushroom powder used in hot water. Kumhal in Nawalparasi District [* It was used in pre-colonial time to cure bilharziosis (Chronic disease of blood fluke) in Africa and south America]	CS	Chuchnari, Bhuenphor	Kumal Chhantel
20	*T. eurrhizus*	Rheumatism, Diarrhoea and Blood pressure	Its soup used in Rheumatism, diarrhoea, lowering high blood pressure in Santhal (in Morang District); Darai (in Chitwan District)	ET CS ET	Chirko/Chuchanar Bagale Negeriakukurmutta	Santhal, Musahar Danuwar, Darai Dhanuk, Dom,

Note: T = Tarai, S = Siwalik, M = Mid-hill, E = East, C = Centre and W = West

3.5 Discussion

The world: overview

The main work on various aspects of these mushrooms including diversity, sociobiology, edibility, ecology and conservation aspects has been done in Europe, North America and Africa. Its collection and consumption are an important source of healthy nutrition for the rural communities during the rainy season in several parts of Africa, Asia, Europe and South America (Nakalembe, *et al.,* 2009). He recorded mycological knowledge and uses of termitophilous mushrooms in Mid-Western Uganda. Ethno-mycological findings by Akapaja *et al*. (2003) showed that the *Termitomyces* species were used as traditional food and good source of nutrients including proteins, fat, fibers and minerals and alternative source of income and employment to women and old people. Harkönen (2002) reported the selling of edible species of *Termitomyces*, *Cantharellus*, *Lactarius*, *Russula* and *Amanita* in Tanzania and road side markets. Atri *et al*. (2005, 2010) elaborated the edibility and sociobiological aspects of these mushrooms in Punjab state. Rajak and Rai (2005) made a documentary file on edible tribal mushroom resources of Central India and their ethnological aspects. During investigation place of collection, pronounce and traditional use of *Termitomyces* speces for the treatment of different types of therapeutic use and ailments by different ethnic groups in the studies area has been explored (Table 6).

Wild edible mushrooms are traditionally used in many Asian countries as food and medicine (Manzi, *et al*., 1999; Akapaja, *et al*., 2003; Adhikari, *et al*., 2005, Joshi & Joshi, 2008). Various medicinal uses were reported by Nakalembe *et al*. (2009) for many species of termitophilous mushrooms viz., *T. microcarpus, T. aurantiacus, T. eurhizus, T. clypeatus* and *T. tyleranus.* Amongst various termitophilous mushrooms *T. microcarpus* has been reported to serve as a medicinal mushroom for the treatment of abdominal discomfort in newly borne babies, measles, cough, whooping cough, tuberculosis, delayed labour in women, postnatal bleeding in mothers and treatment of umbilical cords in newly borne babies and diseases linked to high blood lipids (Nakalembe, *et al*., 2009; Nabubuya, *et al.,* 2010).

Nepalese perspetives

The study sites are phytogeographically a diverse terrain and rich in mushroom diversity and offers immense scope for ethno mycological studies. In spite of the fast

modernization process, the local communities of this area still hold on to their traditional faith and depend on indigenous species for their various domestic needs and traditional medicine. However, at present, the useful wild mushrooms and their ethno-information are being eroded as a result of loss or degraded of appropriate habitat of the species, unsustainable land use activities and over-exploitation of natural resources. During this study, an attempt has been made to document the useful wild mushrooms with existing traditional practices which are being used by different ethnic groups on the study area. The gathering of mushrooms revealed that there were plenty of species, which were edible and of medicinal value. Many mushroom species are gathered by local people for their daily livelihood and trade. They pronounce the mushrooms as their own language. They are sacked in bags and carried to market for sell. The area embraces the mycophagous ethnic communities' viz. Baraei, Bhujel (Khawas), Bote, Chamar, Chhanthal, Darai, Damaei, Dhimal, Dom, Dusad, Gaaeene, Jhangar, Kami, Koeeti, Kumal, Kurmi, Lodh, Majhi, Musahar, Praja (Chepang), Santhali, Sarki Sherpa and Tamang people are also consuming many of these macro fungi, still they are less habituated in taking these mushrooms in comparison to the Gurung Limbu, Magar Newar, Rai, Rajbanshi Tharu, Thakali, Thakuri as they are more developed than those of the other ethnic groups living in this area during my study period. There are some events, which I had found during sample collection, viz. most of the *Termitomyces* are used by the rural people in the remedy of local infection, therapeutic uses as its syrup as used as tonic. Now a days the medicinal use of mushrooms among the ethnic groups of the study area going in decline day by day. Regarding the collection of mushrooms in most part of country, after picking from the ground a central black part of pileus should keep in the hole made by its stipe so that in next season same collector could collect mushroom. This is a gentle thought for mushroom conservation that leaving some pileus portion over there means leaving spores for further life. Such type of events also reported in Lumle by Adhikari *et al*. (2005). Adhikari *et al.* (2005) and Aryal (2009b) also documented many beliefs and ideas about edibility poisoning problem and its mitigation of wild mushrooms in Western and Central Nepal.

Sustainable utilization

This study also revealed that these wild mushrooms are widely used by the rural people to fulfill their basic needs and also used for therapeutic purpose. Now a day the local people have developed a traditional knowledge system related to utilization of (NTFPs) in a sustainable manner; especially ageing people have significant

knowledge regarding usefulness of the species. During PRA (Participatory rural appraisal) surveys with the local people, when questioned about the changing status of the existing species, my respondents listed some important species such as, *Termitomyces albuminosus, T. clypeatus, T. eurrhizus, T. fuliginosus, T. globulus, T. heimii, T. le-testui, T. mammiformis, T. microcarpus, T. robustus, schimperi, T. straitus* var. griseus, *T. straitus var. griseus, T. umkowaan* which have also declined in abundance during the last two decade. These *Termitomyces* spp. are one of the underappreciated termitomycetes, which have high medicinal importance and high nutraceutical potential (Moordian & Thurman, 1999; Kabasa, *et al*., 2006; Jagadish, *et al*., 2010). Hence, efforts should be directed to conserve the valuable species and their habitats with the implementation of locally appropriate sustainable management measures involving local participation.

Challenges

The increasing depletion of the forest vegetation is dominated by species of the *Dipterocarpaceae, Combretaceae* and *Leguminosae* families dominant forest is an alarming signal in the appearance, distribution and dominance of these species. The present investigation shows that the pure stand of *Dipterocarpaceae* favors the growth of numerous species of mushroom flora. The litter debris of *Shorea robusta* and other species of vascular flora favors the regulation and maintenance of temperature and moisture in the soil. The recent investigation on ethno mycological study on some wild mushrooms of this area has created an enthusiasm towards its intensive exploration. It is visualized from the investigation that these important species, need special attention to conserve against the threat to avoid their unmanaged and unscientific exploitation by the people. Therefore, methods of harvesting should be done more scientific rather than traditional pattern. For this the mycoelements prevailing in this area need sustainable utilization and conservation. It is therefore necessary to conserve natural habitat of mushroom diversity sustainably. Hence the government should take special attention on these aspects.

Present status and road-map

During the current study, the interactions with the local people, these termite mushrooms was the most favored for consumption. Thus, among the indigenous edible mushroom species of Nepal, *Termitomyces* spp. appears to be highly suitable

for commercialization purpose by the preservation of tropical evergreen reverine forest. These species are also an economically valuable natural resource serving as an alternative to plant and animal derived foods. Besides nutritional value, its many species possess medicinal properties and have industrial applications. For example, *Termitomyces clypeatus* possess a significant quantity of proteins (31%), carbohydrates (32%), ascorbic acid (10-14%) and antioxidants (Ogundana & Fagade, 1981; Tibuhwa, 2012a). It also produces a variety of enzymes in culture media useful as additives in food, in leavening of bread, in processing silage and in other industrial applications (e.g. clarification of non-citrus fruit juices) (Khowala, *et al*., 1992; Ghorai, *et al*., 2009).

Therefore documentation of information on edible, medicinal and poisonous mushrooms as well as the different social and cultural practices associated with their use in ethno medicinal practices in different parts of the country is very important to sensitize the communities about the value of these mushrooms. Several edible mushrooms, including *Termitomyces* species, contribute significantly to tribal food security in most of the village of the marginal people in the country. This knowledge provides enough background to appreciate the diversity and their relevance in ecosystem maintenance in general and human welfare in particular. So priorities were gives to documents the useful macro fungi and their uses along with local knowledge and practices before these species are eliminated from the areas. Hence it needs to take steps to conserve and manage this minor forest product by checking over exploitation through enforcement of regulations available under “Convention on International Trade in Endangered Species of Wild Flora and Fauna”.

3.6 Conclusion

Documentation of food and medicinal use of *Termitomyces* in different social and cultural practices associated with their use in ethno medicinal practices in different parts of Nepal is very important to sensitize the communities about the significance. It plays a vital role in enrichment of the socio- economic life of the rural marginalized people. Besides their consumption, the use in local medicines also paves the way for the upbringing new industries.

CHAPTER 4

4. DISTRIBUTION AND DIVERSITY OF TERMITOMYCES R. HEIM ALONG ECOLOGICAL GRADIENTS IN NEPAL

4.1 Introduction

Fungi play an important role for the conversion of insoluble inorganic phosphate to soluble organic phosphate in the soil (Aryal, 2009a). They are major components of biodiversity, essential for the survival of other organisms and are crucial in global ecological processes (Hawksworth, 1991). Contributing to the nutrient cycle and maintenance of ecosystem fungi plays an important role in soil formation, fertility, structure and improvement (Hao-qin *et al.*, 2008). Similarly macro fungi play a principle role in recycling nutrients and influence plant community composition through symbiotic relationship (Dighton, 2003). There have been a few studies on forest ecology (Packham *et al.*, 2002) and relationship of macro fungi with environmental variables (Zamora & Cecilia, 1995).

Fungal diversity is a crucial component of biodiversity (Dix & Webster, 1995). They are an exceptionally diverse group central to the functioning of ecosystem (Newbound, 2009). They are the vital contributors of terrestrial ecosystems because of involvement in nutrient cycling (Jordan, 1985; Lodge, 1992) and are principal decomposers of dead organic matter, such as dead wood and litter (Harmon *et al*., 1986). Macro fungi have been shown to have a significant ecological function in the establishment and dynamic succession of plant communities, nutrient cycling and the protection of forest ecosystem, and likely to be crucial to sustainable development, ecological construction and stability (Schmit 2005, Dighton *et. al*., 2005, Lee *et. al*., 2009). In these aspect Termitophilous fungi are high value forest resources.

Although factors driving macro fungal diversity is unclear; climatic conditions such as rainfall, air, soil, temperatures, evapo-transpiration, relative humidity, and water deficits or excesses are generally regarded as major factors affecting macrofungal fructification (Brunner *et al*., 1992). Geomorphologic features as slop, aspects and altitude as well seems to influence the macro fungal communities (Yang *et al*., 2006). Similarly Biological interaction which occurs below the ground, among plants roots, animals and micro-organisms are dynamic and substantially influence ecosystem

processes (Hoff *et al.*, 2004). Fungi play important role in maintaining soil nutrient like other underground biota (Rossman *et al*., 1998). The nature and availability of soil solutes are fundamentally important from the stands point of fungal nutrition. Normally, inorganic solutes are absorbed in the ionic forms for their normal development but amounts of their requirements may vary among the one species to another species

Termitophilic mushrooms are usually seen in 25-32°C during rainy season in tropical to temperate belts. Different species exhibit different fruiting phenologies varying from year to year at different altitude and latitude; maximum richness occurs only during short period and differs among years. Investgation show that within a geographical region fruiting is influenced by elevation, latitude and their effect on temperature and precipitation (Ohenoja, 1988).

Up to 30 species of the genus *Termitomyces* are known and were accepted in the 10th edition of the Dictionary of the Fungi (Frøslev, *et al.*, 2003, Kirk, *et al.*, 2008). Until Heim (1942c) enlighted the genus *Termitomyces*, early mycologists assigned this genus to widely different genera like *Armillaria* (Fr.) Staude 1857, *Entoloma* P. Kumm. 1871, *Lentinus* Fr. 1825, and *Pluteus* Fr. 1836, as there are some morphological similarities (Frøslev, *et al.*, 2003) with these genera. More than 50% of the species of the genus *Termitomyces* recorded worldwide occur in Nepal. They were collected from tropical to temperate region. All the species has a wide distribution throughout the Nation. Keeping in view the vast scope and importance of the problem to ecosystem maintenance in general and human nutrition in particular, the present investigation concerning "Diversity of *Termitomyces* in Nepal" was initiated with the objective of study, to documents the occurrence and distribution of *Termitomyces* in Nepal.

Hypothesis

Species composition of *Termitomyces* is varied according to altitude and location.

Objective

To assess patterns of species diversity and distribution along the east-west and south-north gradients of Nepal Himalaya.

4.2 Literature review

The population of fungi globally has in recent times being a challenge to mycologist all over the world (Wood, 1992). Only a small parts of total fungal wealth has been subjected to scientific research and mycologist continue to unravel the unexplored and hidden wealth (Swapna *et al*., 2008). The number of fungal species has been estimated up to 13.5 million (Kirk *et al*., 2008), however the currently accepted working data is 1.5 million species (Hawksworth, 2001). It is estimated that with the current rate of species description it will take 1170 years to complete the global fungi inventory. The total number of mushrooms forming species has been estimated in between 53,000 and 110, 000 which suggest that only 18 to 38% of the total mushrooms have been documented (Mueller *et al*., 2007).

Regarding the fungus-growing termites, there are about 2,600 species of termites that belongs to 7 families and 281 genera (Duur *et al*., 2007). They are cosmopolitan, specially, limited to warm region (tropical and subtropical). Those termites, which belonging to subfamily macrotermitinae grow fungi not in all. These fungi growing termites are only found in Africa and Asia and important greatly on the decay of plant biomass (Wood & Sands, 1978). These termites (Isoptera: Termitidae: Macrotermitinae) are taxonomically identified in 11 genera and 330 species (Aanen *et al*., 2002), with 10 genera occurred in Africa, 4 in Asia and 1 in Madagascar (Nobre, *et al*., 2010). They maintain their fungal symbiont (genus *Termitomyces*, Basidiomycotina) on special structures in gardens inside their colonies, the nest, and the fungus combs, which are housed in specially constructed chambers, either inside a mound or dispersed in soil. Those fungus gardens are continuously provided with plant substrates, whereas older parts that have been well decomposed by fungi are consumed by termites (Aanen & Boomsma, 2005; Batra & Batra, 1979). Termite consumed dry plant materials converted to fecal pellets (primary feces) when added resulted rapidly development of fungal mycelium in the newly added substrate. After a few weeks the fungi start to produce vegetative structure, *nodules* (mushroom) that are consumed by workers. At a later stage, the entire combs structure permeated with mycelium is consumed (Darlington, 1994). The symbiosis between termites and *Termitomyces* fungi is 'symmetric' since both partners are obligatorily interdependent, and this dependence has a single evolutionary origin with no known reversals to non-symbiotic states (Aanen, *et al*., 2002, Rouland, 2002, Frøslev, *et. al.*, 2003).

Termitomyces is a white-rot fungus (Bignell, 2000). These fungi are among the few organisms that can digest lignin and cellulose. The optimal conditions (high, buffered temperature and high humidity) for these fungi are predominantly found in hot and wet habitats (Rayner, *et al*., 1988), such as rain forests. This fungal white-rot decay requires temperature-buffered, high-humidity environments, which tropical rain forests provide. Optimal growth conditions for *Termitomyces* are a relative humidity that is near saturation and constant temperatures of about 30ºC (Thomas, 1987).

Fungus combs required a temperature of 27º-28º C [(*Macrotermes jeanneli;* (Darlington, *et. al*., 1997)] or 30º C [(*Macrotermes bellicosus;* (Korb, 2003)] but the relative humidity is independent. Outside the nests, daily air temperature was 20º-37ºC and surface soil temperature was 21º- 45ºC. Conditions within the mound are therefore more similar to rain-forest climates. Although climate conditions are also buffered to some degree in non-fungus-growing Termitidae, such complex, highly climatically buffered mounds are unique to fungus-growing termites.

4.3 Materials and Methods

Soil samples were collected from 9 locations, each represents the individual topography as representatives for these comparative sites of sampling habitats. These soil samples were analyzed in the soil science division NARC, Khumaltar and Department of Agriculture in SMD (Soil Management Directorial), Hariharbhawan, Lalitpur, Go'N. About 500 gm. of soil was collected from the four corners of each quadrat from the depth of 15 cm, and mixed together thoroughly and placed in a polyethylene bag. In case of termite nest and casing soil they were simply taken from the ground surface and mixed as the same process. The samples were air dried while in the field and the drying was continued for one week after returning back to the Lab. Its pH was measured by taking soil sample and D/W (Distilled water) in the ratio 1:1. Before it, the digital pH meter was calibrated by adjusting in different concentration of buffer (pH 4, 7 & 9) solution to observe the soil pH (ASTM, 1995). Nitrogen test were obtained in percentage by Kjeldahl digestion method, (Kjeldahl, 1883). Phosphorus by Olsen's Bicarbonate method (Olsen, *et al.,* 1954), and Potash obtained by the adjustment of flame photometer (Merwin & Peech, 1950), from the filtrated ammonium acetate. (Walkley & Black, 1934) method was used for determining the organic matter present in the soil, where oxidation of organic carbon in an acid dichromate solution followed by titration of the remaining dichromate with ferrous ammonium sulphate was done (Zobel, *et al.,* 1987). Similarly micronutrients (Zn, Cu,

Mn, & Fe) analyzed by DTPA methods **(**Lindsay & Norvell, 1978**)**, and Boron by Azometchneh colorimetric method (Gupta, 1979).

The yield data of the fruting bodies for three flushes in a period of 30 days from first flush were recorded in terms of average number, Av.weight and Av. weight per fruit body, within a period of three year (rest of the methodology given in Chapter 2, pp 15-27, pt. no.2.3-2.7).

4.4 Results

During the present investigations several wild edible species have been collected and identified. Among them termitomycetes were selected for this investigation. These are *T. albuminosus, T. aurantiacus, T. badius, T. clypeatus, T. eurhizus, T. fuliginosus, T. glubulus, T. heimii, T. le-testui, T. mammiformis, T. microcarpus, T. microcarpus f. santalensis, T. robustus, T. schimperi, T. striatus f. griseus* and *ochraceus, T. umkowaani, T.* new I (sp a) and *T.* new II (sp b). Of these, all the *Termitomyces* are mostly common in tropical to subtropical region of the study area. They were found growing on the top of termatorium in different localities. The number of individual mushroom ranged from one individual to 2823 in Bardiya National park (BNP) locality. West Tarai region was rich *Termitomyces* growth. Species richness declined along increasing elevation in different ecological belts in Nepal (Figure 27).

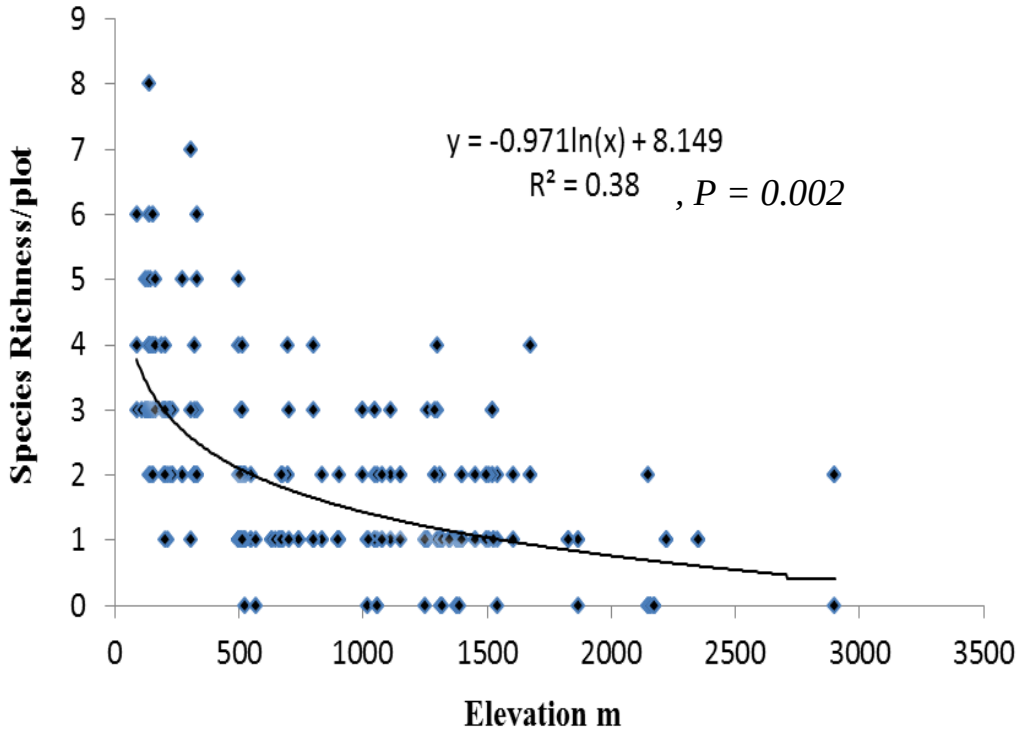


Figure 27: Relationship between species richness and elevation.

Linear regression testing the effects of different environmental parameters on species richness was significantly variable within physiographic regions, land use types, locality and altitude. The number of individuals were significantly influenced by physiography, land use, locality, altitude, air temperature and marginally by (P=0.08) liter cover (Table 7).

The effect of physiography on species composition, Canonical Correspondence Analysis (CCA) showed that the colon number composition of *Termitomyces* composition was significantly (p = 0.002) variable across different physiographic regions and altitude. In total, both physiographic and altitude explained 5.86 % of the total variation in the data set of species composition. *T.* new II (b) and *T.* new I (a), were strictly found in Siwalik, *T. umkowaan*, *T. microcarpus f. santalensis, T. robustus*, and *T. eurrhizus* in midhill, and *T. clypeatus, T. microcarpus, T. schimperi* and *T. auratiacus* were in Tarai region (Figure 28). Results of the CCA analysis showed that the effect of elevation on species composition of *T. eurrhizus, T. albuminosus* and *T. umkowaan* were found in higher elevation whereas *T. schimperi* and *T. heimii* were found in lower elevation. *T. robustus, T. fuliginosus* and *T. mammiformis* were found everywhere irrespective of any elevation (Figure 29).

Table 7: Linear regression on species richness of *Termitomyces.*

Predictors	**Df**	**Deviance**	**Resid. Df**	**Resid. Dev**	**F Value**	**P value**	**R^2**
Physiography	2	21.26051	272	67.43817	65.48979	<0.001	0.2397
Region	2	0.34043	270	67.09774	1.04864	0.3526	-
Land use	4	1.92509	266	65.17266	2.96497	0.0211	0.0217
Locality	80	26.99966	186	38.173	2.07921	<0.001	0.3044
Altitude	1	0.68845	185	37.48455	4.24135	0.0409	0.0078
Canopy cover	1	0.01043	184	37.47412	0.06425	0.8002	-
Litter cover	1	0.47766	183	36.99646	2.94272	0.0880	-
Soil moisture	1	0.01661	182	36.97985	0.10232	0.7494	-
Physiography	1	0.01939	181	36.96045	0.11948	0.7300	-
Nitrogen content	1	0.08466	180	36.87579	0.52159	0.4711	-
Phosphorus content	1	0.05183	179	36.82396	0.31932	0.5727	-
Potassium content	1	0.0941	178	36.72986	0.57973	0.4474	-
Organic matter	1	0.00792	177	36.72194	0.04879	0.8254	-
Air temperature	1	0.77839	176	35.94355	4.7954	0.0299	0.0088
Air humidity	1	0.00878	175	35.93478	0.05406	0.8164	-

Significant p-values ($p \leq 0.05$) are bold.

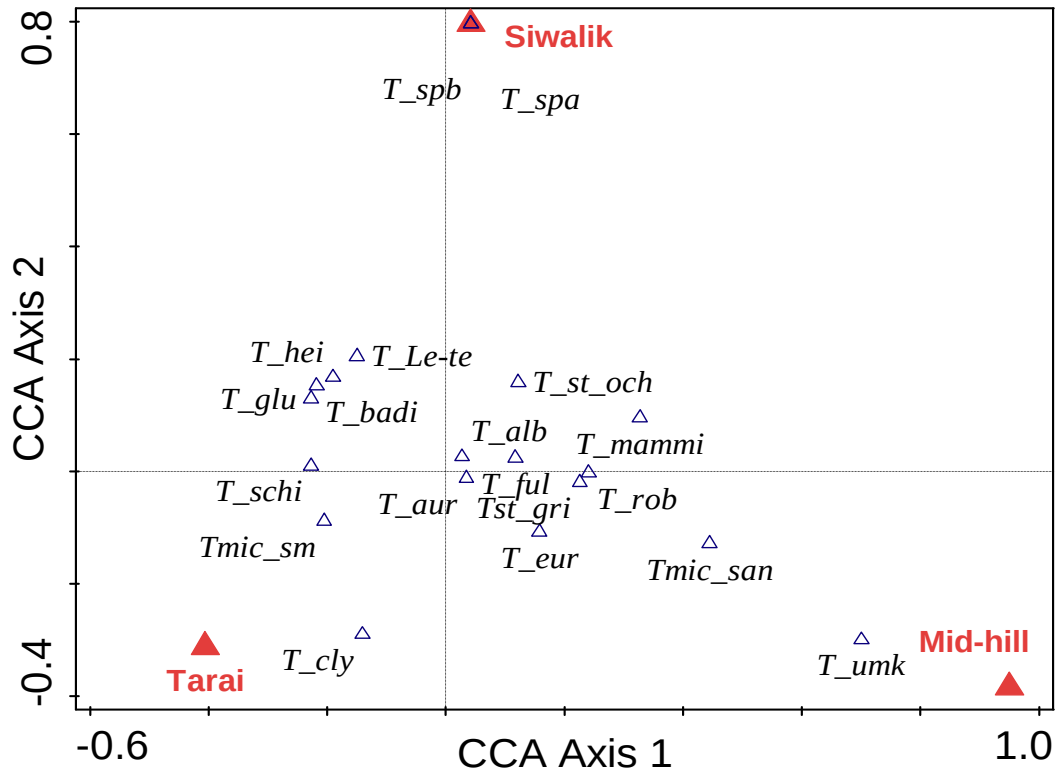


Figure 28: Effect of physiography on *Termitomyces* species composition (The 1st canonical axis explained 2.39 % and the 2nd explained 0.56 % of the total variation of the data set).

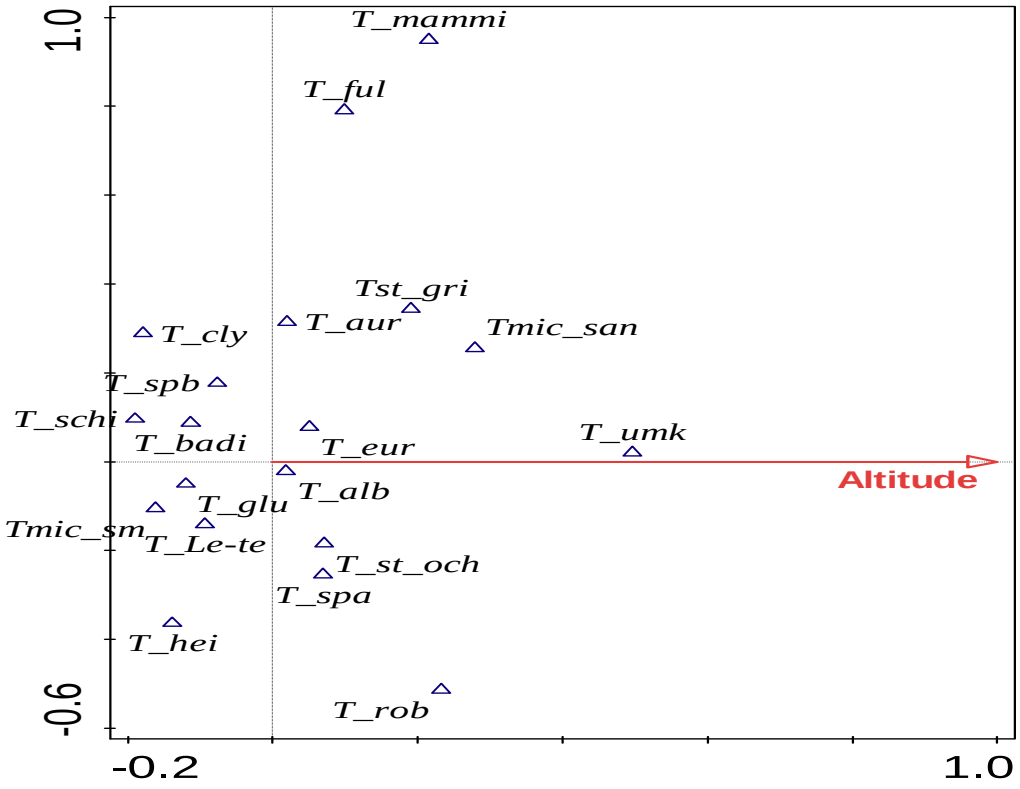


Figure 29: Effect of elevation on *Termitomyces* species composition (The 1st canonical axis explained 2.06 % and the 2nd explained 0.56 % of the total variation of the data set).

Results of the CCA analysis showed that the relationship between different environmental variable and different species of *Termitomyces* composition, looking at the total relationship between the number of colon of *Termitomyces*, it was found that colon of *T. straitus f. griseus*, *T. straitus f. ochraceus* and *T. albuminosus* were influenced by soil nutrients such as N, P, K and OM; *T. fuliginosus* and *T. clypeatus* by air temperature, *T. schimperi* and *T. globulus* by pH, *T. heimii* by litter content and *T. microcapus*, *T. robustus* and *T. auratiacus* by soil moisture (Figure 30).

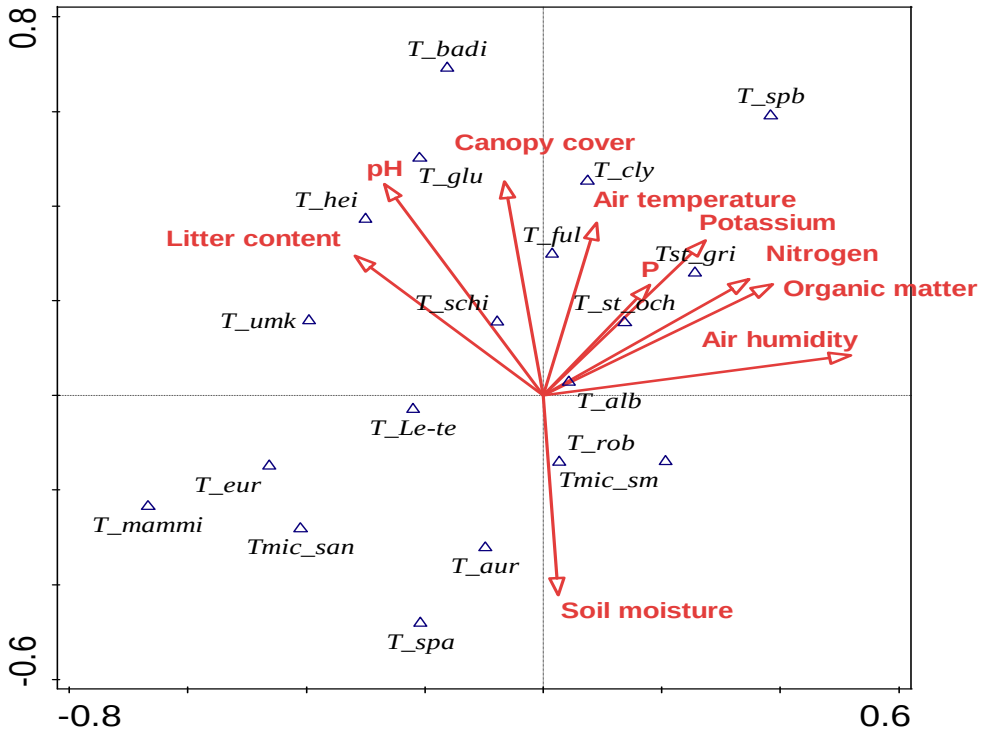


Figure 30: Relationship between environmental variable and species composition (The 1st canonical axis explained 0.88 % and 2nd canonical axis explained 0.64 % of the total variation of the data set).

The persent organic matter among the 9 spots of sampling habitat features were indicated highest as 40.3 % in Natural protected forest with the C: N ratio of 20, (Appendix 6) comparative to the least contained of percent organic matter as 0.8% in agro-pastoral land with the C: N ratio also as 20. However, the chemical properties of soil which indicated on the level of microelement content (ppm), such as Phosphorus (P), Potassium (K), Iron (Fe), Zinc (Zn), Copper (Cu) Manganese (Mn) and Boron (B) which were apparently higher in the most of natural protected forest with comparisons to the agro-pastoral land ecosystem and managed PF. These differences in obtained results (Table 8) were probably caused by the different in the topography of an areas, soil parent materials, erosion deposited and agricultural practice of the farmers in an area.

The higher biomass productions in the tropical riverine forest specially protected area were due to the favourable environment of the termite growth and longer growing season with comparision to the higher elevation. In midhill more humid climate and higher rates of soil erosion which may cause a greater loss of organic matter, leads lower the biomass yield (Table 9). The physical properties of soil among the 27 comparative sampling sites were recorded. All of sampling sites there were no significant difference of P-value of the pH with the soil on the (R) and (Ss) including (R×Ss). So that coefficient of variation is very less (13.51%).

Table 8: Interaction between different eco-zone & (Ss), against different nutrients.

Treatment	**%**		**pH**	**Kg/ha**		**ppm**				
Regions R	**OM**	**N**		**P_2O_5**	**K_2O**	**Fe**	**Cu**	**Mn**	**Zn**	**B**
Tarai	5.408	0.2733b	6.007	134.608	643.35	31.20b	3.922	162.2a	13.76a	3.939
Siwalik	2.158	0.1025b	6.193	72.7	457.475	33.76b	4.264	3.992c	14.57a	4.078
Mid-hill	2.475	0.6117a	6.164	101.758	651.867	40.14a	4.73	32.64b	8.767b	4.194
P-value	Ns	0.0056	Ns	Ns	Ns	0.001	Ns	0.001	0.001	Ns
LSD 0.05		0.2957				3.792		11.71	2.648	
SEM±		0.1008				1.293		3.992	0.903	
Soil source (Ss)										
Termite nest	7.9	0.6244^{a}	6.011	351.3^{a}	351.3^{a}	63.44^{a}	6.156^{a}	450.8^{a}	11.89^{c}	13.19^{a}
Casing soil	1.389	0.05000^{b}	6.153	5.678^{c}	5.678^{c}	12.56^{d}	3.502^{c}	32.64^{c}	3.578^{d}	0.8878^{c}
Vicinity area	2	0.3156^{ab}	5.783	5.478^{c}	5.478^{c}	46.60^{b}	4.720^{b}	65.82^{b}	15.00^{b}	1.727^{b}
Forest	2.1	0.3267^{ab}	6.538	49.64^{b}	49.64^{b}	17.53^{c}	2.844^{c}	36.11^{c}	19.00^{a}	0.4784^{c}
P-value	Ns	0.0192	Ns	0.001	0.001	0.001	0.001	0.001	0.001	0.001
LSD 0.05		0.3415		0.3415	0.3415	4.379	1.172	13.52	3.058	0.4995
SEM±		0.1164		0.1164	0.1164	1.493	0.3994	4.61	1.043	0.1703
(R) × (Ss)	Ns	Ns	Ns	Ns	0.0349	Ns	Ns	0.001	0.001	Ns
CV%	183.37	105.97	13.51	124.21	49.29	12.78	27.84	9.45	25.3	12.54

Note: Experimentation: Design: 2 factorial RCBD (Randomized Complete Block Deign). Note: (R) = Region, (Ss) = Soil source, CV= Coefficient of variation, (R×Ss) = interaction between region and soil sources.

Table 9: Biomass yield

Regions	**Av. No. of fruit**	**Av. Wt./fruit (gm)**	**Av. Biomass yield (gm)**
Tarai	28	16.66	590.55
Siwalik	22	12.98	337.92
Mid-hill	16.66	10.82	258.26

The present organic matter among the 27 sampling habitat features were indicated highest in Tarai (5.40%) in termite nest (7.90%) with the C: N ratio of 20, comparative to the low contained of organic matter as 2.47% in Midhill regions of cashing soil (1.38%) with the C: N ratio 20. There were no significant difference of P-value and interaction between (R) and (Ss) of the OM, but coefficient of variation is high (183.37%). Nitrogen contents of mound in 3 regions significantly different at 0.05% level of significant. It has maximum in mid-hill (0.61%) and the termite nest (0.62%), whereas lest in Siwalik and Tarai (0.1% & 0.27%) in the cashing soil respectively. There were significant differences on both of factors (R) (0.0056%) and (Ss) (0.0192%). Interactions between the (R) and (Ss) were not significantly difference at 0.05% level of significance. Its variation of coefficient is high (105.97%). However, the chemical properties of soil which indicated on the level of microelement content (ppm), such as Phosphorus (P), Potassium (K), Iron (Fe), Copper (Cu), Manganese (Mn), Zinc (Zn) and Boron (B), which were apparently higher in the most of places (Table 8). For instance, content of phosphorus in Taria (134.6) is highest in termite nest (351.3), content of potash in Midhills (651.86) is highest in termite nest (351.3), interaction between (R) and (Ss) of the potash is significantly different at 0.05% level of significant. Similarly P & K on the (Ss) are also significantly different at 0.05% level of significant. Likewise contents of Fe in Midhill (R) significantly different at 0.05% level of significant. It has maximum in Midhill (40.14ppm) and low in Tarai (31.20ppm), similarly Fe content in (Ss) is significantly different at 0.05% level of significant. It has maximum in termite nest (63.44 ppm) and low in cashing soil (12.56 ppm). Cu content is maximum in Midhill (4.730 ppm) but not significantly different at 0.05% level of significant and low in Tarai (3.922 ppm), whereas Cu content in (Ss) is significantly different at 0.05% level of significant. It has maximum in termite nest (6.15 ppm) and low in forest and cashing soil (2.84 & 3.50 ppm) respectively. Contents of Mn in (R) significantly different at 0.05% level of significant. It has maximum in Tarai (162.2 ppm) and low in Siwalik (3.99 ppm), similarly Mn content in (Ss) is significantly different at 0.05% level of significant. It has maximum in termite nest (450.8 ppm) and low in cashing soil (32.64 ppm), interaction between (R) and (Ss) of the Mn is significantly different at 0.05% level of significant. Contents of Zn in (R) significantly different at 0.05% level of significant. It has maximum in siwalik and Tarai (14.57 & 13.76 ppm) and low in Midhills (8.76 ppm), similarly Zn content in (Ss) is significantly different at

0.05% level of significant. It has maximum in forest soil (19.0 ppm) and low in cashing soil (3.57 ppm), interaction between (R) and (Ss) of the Zn is significantly different at 0.05% level of significant. B content in (R) is not significantly different at 0.05% level of significant. It has maximum in Midhill (4.19 ppm) and low in Tarai (3.93 ppm), but B content in (Ss) is significantly different at 0.05% level of significant. It has maximum in termite nest (13.19 ppm) and low in forest and cashing soil (0.47 ppm) and (0.88 ppm) interaction between (R) and (Ss) of the B is significantly different at 0.05% level of significant. So the biomass yield is highest in Tarai, then the Siwalik and Midhills. Hence the soil properties as an edaphic factors influence on *Termitomyces* distribution and abundance.

The present study based on a survey and the literature revealed that the occurrence of nineteen taxa of *Termitomyces* as described (in Chapter 2, pp 45. pt. no. 2.12), their details, including abundance in responses to land use and other environmental variable and vernacular names, given in Table 6. Most of the *Termitomyces* species were found in the forest where the vegetation is dominated by species of the Dipterocarpaceae Combretaceae and Leguminosae families in Tarai and Siwalik. Out of 19 (except *T. eurrhizus and T. clypeatus*) all are found for the first time in Nepal, among them two are new proposed to science. Among them, *T. albuminosus, T. mammiformis, T. microcarpus* and *T. robustus* were frequents; *T. clypeatus, T. eurrhizus, T. heimii, T. le-testui* and *T. schimperi* was common; *T, stratus* (var. griseus & ochraceus), *T. globulus, T. umkowaan* and two species of newly proposed *Termitomyces* (*T.*new I & *T.* new II) were found to be rare.

4.5 Discussion

Vascular flora affecting Mycoflora

Plant species composition also influence the number and species of macro fungi present because plants constitute the habitat and energy source for most fungi and all fungi show some degree of host or substratum specificity. Forest management tools (clearings, pruning, species selection, fire, fertilization) or any other factors (change in habitat and climate, urbanization, landslides, grazing, cutting down of trees, slash, or unscientific harvesting etc) can also play a crucial role in shaping macro fungal communities since they can modify vegetation parameters like tree density, canopy cover, primary productivity, basal area, understory plant communities, soil conditions

(Wiensczyk *et al*., 2002). Saprophytic fungi seem to be more associated to the substrate and shows preference for a specific tree or shrub litter (Roberts, *et al*., 2004).

Edaphic factor determine the abundance of fungi

During investigation the higher the OM and micronutrients that were found greater the abundance of termitomycetes. Because of physical structure of soil is improved by accumulation of mycelium within it. It binds fine soil particles to form water stable aggregetes. This is accompanied by the penetration of the mycelium through soil, forming a network whch traps small particles (Aryal, 2009a). Thus soil which hold different micelia should be preserved to maintain the growth of fungi. Conservation of biological diversity has been the subject of intense debate all over the world. Despite of rich mycodiversity, is lack of conservational practices carried out in Nepal (Adhikari, 2000). Not much has been done to conserve fungal diversity in general and mushrooms in particular. Conservation studies on fungal diversity are a recent development on the world scene. Some of the contributions in this regard are by Arnolds (1991), Kaul (1992), Adhikari (2000), Atri and Lakhanpal (2002).

Symbiotic Association between *Termitomyces* and Termites

The relationship of termites and fungi has been the subject of many investigations. Fungi in decaying wood provide nitrogen, vitamins, and other substances beneficial to termites, and may also destroyed toxic volatile materials or extractives in the wood. Fungi are also known to produce feeding stimulus. On the other hand, they can remove certain nutrients or produce toxic metabolites. Whether fungi are beneficial or not that depends on the species of termite and fungus and other variable (Ebeling, 1975) The higher termites (termitidae) in the fungus combs breakdown of lignin, and it also supplies nitrogenous materials and vitamins (Sands, 1970).

Many examples of mushroom cultivation by ants have been discovered (Weber, 1966; Abe, 1989). In contrast, there are few reports on the ecological aspects of the symbiotic relationship in *Termitomyces* and fungus growing termites. Termite eats dead and sometimes live plant tissues and piles their excrement in a porous structure

(Abe, 1989; Murrin, 1996). Then they make a fungus garden by cultivating *Termitomyces* hyphae on that structure. Finally they eat the mature hyphae or fungus combs. Termites cannot live without a fungus garden, and *Termitomyces* has been observed only in termite nest (Abe, 1989; Darligton, 1994). Fruiting bodies of *Termitomyces* have been reported to develop in fungus gardens after termites desert the nest (Dixon, 1983). Fruiting-body formation is assumed to also occur in garden being used by termites (Sagara, 2000). However, it is unknown whether some aspect of termite behavior prevents the formation of fruiting bodies of *Termitomyces* (Johnson, *et al.*, 1981).

Termite's mushroom is a rather expansive natural product. For domestication its explants were obtained from from the fruiting body. There has been till no reported about developing the fruiting body from mycelial of the symbiotic fungi in the lab. Its efforts to artificially cultivate and harvest it have not been very successful, though its domestication, were obtained from the explants of the fruiting bodies by tissue culture technique but mycelial give poor information about the taxonomy based on morphology. It is a rare and delicious natural product with high nutritional value. This investigation is aimed at factors, both physical and biological, affecting the distribution of fungus gardens in the study area of managed and natural forest including agro-pastoral land.

The termites build nest, which act as a greenhouse, and go out in search of plants materials with which to provision their fungus gardens. They offer their fungal symbionts finally broken down, usually dead; plants materials, such as wood and grass, and the fungi grow on this substrate. In turn, the fungi decompose carbon rich complex plant materials, such as cellulose, lignin and pectin which the termites do not have the enzymes to digest directly-forming nitrogen-rich fungal biomass that provides the termites with most of their food. Fungus is grown on a fungus combs maintained by termites. They constantly added new plant substrate. The termite eats the older fragments, for their food. The asexual fruiting bodies of the basidiospores contain spores of the fungus, which redistribute by the termites in the new substrate and speed up the cultivation process (Johnson *et al*., 1981; Darlington, 1994).

Occurrence of *Termitomyces*

Investigation revealed the occurrence of termitomycetes in association with termites either in permanent termite mounds or in temporary termite colonies. In their combs,

a specific microenvironment is created by the termites for cultivation of termitophilic fungi (Srivastava, *et al.* 2011). Understanding the precise conditions might help ex-situ simulation for production and exploitation of termitomycetes. Termitomycetes are gregarious in and around permanent termite mounds. They are also found as in solitary or scattered on ruminant-grazed land, AGP, and abandoned bunds of paddy fields. Some termitomycetes (both forms of *T. microcarpus*) prominently erupt wherever ruminant dung lies on the ground or at locations strongly influenced by ruminant activity (e.g. surroundings of cattle sheds, farmhouses and grasslands), therewith indicating the relationship between ruminants, termites and termitomycetes. Termite colonies take advantage of digested or partially digested lignocellulosic material in ruminant dung for cultivation of termitomycetes. In my study, *T. clypeatus* mainly preferred grassland in the protected forest (National Park), while it occurs in permanent termite mounds. Permanent termite mounds were preferred by *T. heimii* in protected as well as natural forest. Similarly, *T. umkowaan* also preferred the surroundings of permanent termite mounds in the natural forest. The other termitomycetes (*T. eurrhizus, T. microcarpus*) preferred mainly open places in forests and buffer zones (AGP, adjacent to forests) without prominent termite mounds. The activity of ruminants (e.g. cow, buffalo, sheep, horse and goat) is common in grassland and buffer zones of national park as well as natural forest. Physical disruption of lignocellulose by termite mastication supports further degradation by fungal enzymes (Nobre, *et al*. 2010). Such symbiosis enhances the degradation and turnover of lignocellulosic substrate in grasslands, on agricultural land and in forests. Based on the role of enzymes in tripartite association (bacteria- fungi-termites), Nobre and Aanen (2012) considered the termite mound as an external rumen. Besides, some termite workers (e.g. *Odontotermes formosanus*) show higher cellulase activity in their faeces than the symbiotic *Termitomyces* denoting the role of gut-derived and acquired enzymes by the bacteria and fungi. (Yang, *et al*., 2004; Deng, *et al*., 2008). There seems to be a specificity or selection between the termites and *Termitomyces*. For instance, colonies of the termite *Macrotermes natalensis* are associated with a single, sexually reproducing and horizontally transmitted lineage of a *Termitomyces* symbiont (Aanen, *et al*. 2007). Recent molecular research by Makonde *et al*. (2013) based on termite guts and termite combs also revealed that emergence of a particular *Termitomyces* species is due to monoculture by a specific termite population. In view of such specificity, it is necessary to follow the species of termites which exist in

forest and buffer zones of National park and the Community managed forest area of the study sites to exploit their beneficial association. Hence field observation implese that, mutualistic association of termite's comb and *Termitomyces* co-exist in a physiologically, ecologically and reproductively active state for long period of time in terrestrial ecosystem.

Role of Mycophagous Groups on Conservation Aspects

Knowledge on *Termitomyces* with proper mycophagous ethnic groups is very important for identifying the habitats and to develop strategies for habitat conservation. For instance, the ethnic groups of Rai, Limbu, Dhimal and Magar in Pathari-Morang, Rajbangsi, Santhali in Barhghare-Jhapa; Praja in the Churiyamai-Makawanpur and Juhedi-Chitwan; Khuna, Kumal in Kusum-Bairiya in Banke are dependent on the forest ecosystem for their nutritional security, which is partially met by *Termitomyces* mushrooms. Similarly, ethnic groups of marginal people like Tamang, Tharu, Chhanthal, Bote are experts in collecting mushrooms and aware of the specific localities and the seasonal availability of termitomycetes. Based on the survey and occurrence, it is concluded that the riverine sal forest of Tarai and Siwalik region to be the hotspots of *Termitomyces* as the ethnic groups extract illegally (as trading minor forest products is prohibited by the Government) up to tonnes in Figure (mixing of *T. albuminosus, T. eurrhizus, T. heimii, T. mammiformis, T. robustus*) annually for their livelihood and sold in near city even exported in border market in India. The study revealed that the harvesting of *Termitomyces* is exhaustive and points at the extent of anthropogenic pressure on their natural habitats. *Termitomyces* in the near city of the Tarai region very frequently sold by rural people is the mixture of *T. heimii, T. albuminosus, T. eurrhizus, T. mammiformis, T. robustus* which occurs gregariously in forest areas with termite mounds. It is collected immediately after thunderstorms during the early monsoon period (June-August). *Termitomyces microcarpus* (large and small forms) occurs in the mid rainy season (July-August), and is eaten by almost all concerning mycophagous ethnic groups or communities only privately, as they are laborious to collect and process. Thus, the local people should be trained to understand the importance of termitomycetes in human nutrition and the impact of anthropogenic pressure caused by overexploitation, and to initiate sustainable harvest and habitat restoration.

Extension of Knowledge for Propagation

Termitomyces is a paleo-tropical genus among the genera of gilled mushrooms, and are of major interest to the mycologists, entomologists and food technologists. Nearly 330 species of termites assigned to the subfamily *Macrotermitinae* distributed in Africa and Asia are involved in cultivation of termitomycetes (Aanen *et al*., 2002), which shows the widespread mutualistic symbiotic association. As termitomycetes are obligate symbionts with termites, ex-situ cultivation is a challenging task. However, suitable in-situ conditions could be maintained or restored in termite gardens based on the experience of expanding the natural cultivation of termitomycetes. Specific strategies to preserve small pockets rich in *Termitomyces* on cultivated lands (e.g. Tea, Coffee, *Areca*, Millet and Maize farms) are necessary. A recent report states that in Bangalore, India there were endowed with *T. clypeatus* and *T. microcarpus* (Pushpa & Purushothama 2010); this gives scope for in-situ maintenance and sustainable harvesting even in urban habitats. For this, there should be required the exploitation of termitomycetes in their natural habitats for commercial benefits purposes. Collectors should not destroy the location of termitomycetes during picking-up and should allow their further growth and dissemination as per traditional methods little bit of their back parts are remove, this helps for thire proper dissemination as well as method of conservation. Biodiversity conservation strategies will be beneficial if we include explicit and informal protection of traditions of mycophagous ethnic groups in a specific region for sustainability and food security. Concerning alternate protein energy nutritional sources especially in developing countries in Africa and Asia, habitat preservation, sustainable exploitation and research focused on termitomycetes will be highly rewarding.

Species Richness Influence by Environmental Variable

Data analysis were performed to see if the species richness was influenced by any environmental variable in Nepal, generalized linear model (GLM) with Poisson distribution and log function was used in S-Plus (S-PLUS, 2000). For the final analysis, species richness was transformed to square roots to obtain the normal distribution.

Multivariate analysis were performed to test the effect of locality, altitude, physiography and region, and characteristics of the local environment (canopy cover,

litter content of soil, soil moisture, soil pH, nitrogen, phosphorous, potassium and organic matter contents, air temperature and air humidity) *Termitomyces* fungi in Nepal. The differences between locality, altitude, physiography, region and landuse, and the other predictors on distribution of *Termitomyces* were found significant. The data were analyzed using unimodal technique (Lepš and Šmilauer, 2003) and gradient length was quite long (4.78 SD units)). Therefore, CCA was used to show the relationship between *Termitomyces* colon composition and different environmental variables. The significance was tested by performing Monte Carlo permutation test. In the analysis, rare species were down weighted to reduce the negative influence of rare species on the results. All tests were carried out using Canoco 5.01 (ter Braak & Smilauer, 2012).

Soil nutrients play a significant role on fruiting of the basidiome. It became depends on the effect of soil moisture, organic matter and soil litter on the natural habitat. Buol (2003) reported that soil parent materials and their weathering products had strongly influenced on the soil properties, including the depth of the regolith, texture, stoniness, clay type, nutrient contents and soil pH. Similarly Schuurman (2005), described that the influence of edaphic factors and plant diversity on detrivore community composition were showed that the dominant, mound building species, *Macrotermes michaelseni*, consumes both grass and wood litter and showed no clear preference. During studies higher biomass were found in tropical climatic region, this probably implied to the cases of the optimum in organic matter and major chemical microelements (Table 9) in protected forest of Tarai and siwalik belt which were allocated as the sites of this investigation. Distribution of species in Tarai to mid-hill land was observed on the effect of soil moisture, organic matter and soil litter surface thickness. Mostly the soil properties were varied tremendously in an area which greatly affected the land use potentials, vegetation pattern and hydrology. It was also noted that the differences in soil parent material, the natural erosion rates and vegetation coverall in difference comparative sampling sites. The soil properties, particularly clay content, were important as the determinant of Macrotermetine distribution collectively, this research finding also suggested that the edaphic factors have a major influence on termite distribution; meanwhile the local plant diversity may have some influence on abundance but is less an important determinant of distribution.

4.6 Conclusion

Edaphic factors on the termites distribution and abundance, showed that the soil texture, organic matter content of the soil surface (0-15 cm) and chemical micro or macro nutrient composition have played a major role in influencing termites nesting, fungus comb deposited under soil surface and termite's distribution. All of the sampling habitats in both arable and natural ecosystem were given as clump in termite dispersion pattern. The CL type of soil was observed as the preferable factor shown for predominated of termites nesting and termite's fungus combs distributed in Natural protected forest areas. It is difficult to conserve these mushrooms because of limited knowledge on their taxonomy, natural history, and ecology. Although present knowledge may indeed be meager on a species-by-species basis, however, it is broad in a general sense as has been observed during the present investigation.

CHAPTER 5

5. NUTRIENTS

5.1 Introduction

Several ethnic wild foods used by tribal or ethnic groups over the generations are known to possess medicinal properties apart from the nutritional ones. During the field survey several such examples were observed where the specific foods are prescribed during some specific health ailment (Chpter-3, pp 95, Table 6). Optimal nutrition is a key factor in influencing the physiological functions of an individual; therefore, in Ayurveda also, the importance of several plant species in supplementary diet for maintaining sound health has been very well emphasized. Kautilya's Arthasastra also discusses the food plants that can be used in emergency to give extraordinary strength (Sensarma, 1996).

Mushrooms are rich in proteins, fibers, unsaturated fatty acids; vitamins and minerals. They are poor in fat and carbohydrates. Its proteins contain all the essential amino acids required for the human health. Some of them, like tryptophan and lysine, which are absent in vegetables, are found in mushrooms. They are excellent sources of riboflavin, nicotinic acid and pantothenic acid. It also contains vitamins like A, B and D. Minerals, such as calcium, phosphorus and potassium, with fairly large amount of copper and iron so that mushrooms are said to be delight of diabetic (Suman & Sharma, 2007).

Mushrooms with their flavor, texture, nutritional value and high productivity per unit area have been identified as an excellent food source to alleviate malnutrition in developing countries (Eswaran & Ramabadran, 2000). They are used especially by the local people as food items and medicinal purpose (Akpaja, *et al*., 2003). It contains low amount of sodium and ample amount of potassium. Gbolagade, *et al*., 2006ab reported that, besides hematological, antiviral, antitumor, antibiotic and immunomodulating activities, their fungal biomass has various applications as far as fermentation is concerned, its biomass can be used as single cell protein (SCP), sources of lipids, flavors and other metabolites such as enzymes and polysaccharides. Okwulehie and Odunze (2004) reported that the increased demand for mushrooms could be contingent upon the phenomenal rise in the unit costs of the conventional

sources of animal proteins such as beef, pork, chicken and fish as opined by Aletor and Aladetimi (1995).

Similarly phytochemicals are the natural substance of vegetable origin, which provide a range of drugs for a number of diseases. Different hormones, vitamins, antibiotics etc. are obtained from range of phytochemicals such as saponins, tannin etc. present in mushrooms (Demain, 1999). Its composition data can provide useful information to promote sustainable use of biodiversity for food security and health and wellness (Evelyn, *et al*., 2006). These are the major bioactive compounds which provide health benefits (Klimczak, *et al.*, 2007). It makes food functional as many mushrooms are reported to produce a wide range of secondary metabolites with high therapeutic value (Wasser & Weis, 1999). These chemicals are also used for protection against chronic diseases (Rupasinghe, *et al.*, 2003). In this regards Ayurveda is not the science dealing only with disease, but is a holistic science of health care. Ayurveda was aware of nutraceutical. During exploratory phase and literature survey it was found that *Termitomyces* are not only source of food for the people but they also use them for medicinal purposes in rural area as remedy for certain illnesses and disorders (Chapter 3, pp 95, Table 6).

There are seventy three medicinal mushroom species which have been reported from Nepal (Adhikari, 2009). Medicinal plants are an integral part of the diverse traditional medical practices in Nepal and are codified in traditional medical systems such as Chinese, Ayurveda, Unani, Siddha, Homeopathy, Amchi, etc. (Manandhar, 2002). Crude-drugs are commonly given in the form of powder, decoctions, and infusions or in ointment forms.

Hence, preparation of monographs of four species of *Termitomyces* that would provide a systematic account on their phytochemical profiles is in urgent need for standardization of the traditional medicinal herbs, therapeutic benefits and their possible toxic effects. This study aimed to provide information on secondary metabolites of these *Termitomyces* of Nepal. This investigation explores on the qualitative phytochemical analysis of four species of Termite's mushrooms.

The purpose of the investigation is the food security remains issues in Nepal, 16% of the total Nepalese population are undernourished (FAO, 2012a). The chemical composition of the mushrooms determine their nutritional value and it differs

according to species as well as on the substratum, atmospheric condition, age, and the part of fructification (Manzi, *et al.*, 2001). Most of the analysis was done only on cultivated species and only few on wild mushrooms. Some species have been screened for chemical constituents (Adhikari, 2000).

The choice of *Termitomyces* as the termite mushrooms of interest in this investigation is based on its economic relevance and also medicinal importance among traditional medicine practitioners in the tropical regions of Asia and Africa. Therefore, there is need for a scientific study to ascertain the medicinal potentials of this mushroom. In view of their importance and meager amount of work on systematics, sociobiology, nutritional and nutraceutical aspects, ecology and conservation, present research problem have been undertaken for investigation. Therefore, this study focuses on the nutritional quality of this wild edible species.

Therefore on the basis of, most dominant, four (4) species of *Termitomyces* were subjected to the nutrients analysis in this research.

Hypothesis

Nutrient concentratation and avaivality is differ with ecological belt or altitude

Objective

To analyze and compare nutrient contents of four species of *Termitomyces* from different ecological zones.

5.2 Literature review

On ethnobiology study (Uprety, *et al*., 2012) has revealed that 81 species of wild plant species are used as food by tribals. Problems due to vitamin deficiency were unusual among the Magar tribe of Nepal, due to the high Vitamin A and B content of wild edible leaves (Dietz, 2005). Nutritional studies comprise of six classes of nutrients: carbohydrates, lipids, proteins, vitamins, minerals and water.

Nutritional and Nutraceutical value

Mushrooms have long been valued as nutritional foods and medicinal used by many scientists throughout the world. These are good sources of delicious food with

excellent flavor, aroma, exotic tasteful appeal and high nutritional traits because they contain good quality proteins, unsaturated fatty acids, minerals and vitamins (Manzi, *et al*., 1999; Chang & Miles, 2004). Some edible mushroom species are sources of bioactive compounds for medicinal applications, possessing antitumor, cardiovascular, antiviral, antibacterial and other activities (Ferreira, *et al*., 2009, 2010). Still there are several varieties of wild mushrooms whose nutritive profiles have not been described well. Termitophilous mushrooms are important group of fungi and are preferred for their edibility and nutraceutical utility (Munyanziza & Ngaga, 2006).

Gbolagade *et al.* (2007) investigated antagonistic effect of crude and purified extracts of Nigerian fungi against selected pathogenic microorganisms in which pure extracts of *Termitomyces microcarpus* and *T. robustus* fungi and reported best antibacterial activity in comparison to others. Mukherjee and Khowala (2002) and Mukherjee *et al.* (2006) evaluated cellobiase activity and secretion in the presence of glycosylation inhibitors or by coaggregation with sucrase in the extracellular, intracellular and cell bound fractions of *Termitomyces clypeatus*. Mukhopadhyay *et al.* (1997) reported the production of acetyl esterase in case of *T. clypeatus* when stimulated by xylan, cellulose, arabinose and arabinose-containing polysaccharides.

Food Value

It is use as a good source of food, probably as old as human civilization. It has been a delicacy since ancient times. Mushroom can be used for the food to solve the malnutrition problem (Manandhar, 2003).

As source of proteins

The protein contain of mushrooms has been reported to be twice that of vegetables and four times that of oranges and significantly higher than that of wheat (Bano, 1993). Bother and Eicker (1992) reported that the proteins of *T. umkowanii* has very high sulphur containing amino acids which exceeds the essential amino acid content of the reference protein by 150%. Aletor and Aladetimi (1995) found highest protein contents in *T. robustus* (33.8%) followed by other edible species. Adejumo and Awosanya (2005) reported highest protein content in *T. mammiformis* (36.8%) followed by *Lactarius triviralis* and other mushroom species. Oboh and Shodehinde

(2009) found higher protein in *T. mammiformis* than other edible mushrooms of Nigeria and more protein were recorded in pileus than stipe (28.6% & 24.8%, respectively). Masamba and Kazombo (2010) reported 3.9% protein in *T. le-testui* on fresh wt. basis). Proteins recorded by Ogundana and Fagade (1981), Tibuhwa (2012a) were 31% in *T. clypeatus*.

Carbohydrates and fiber

Carbohydrate constitutes one of the main components of macrofungi. It plays important health-promoting roles in gastrointestinal tract, supporting digestion and absorption and helping to eliminate toxins and waste products. Ogundana and Fagade (1981), Tibuhwa (2012a) recorded 32% in *T. clypeatus*. Oboh and Shodehinde (2009) reported that equal amount of carbohydrate found in stipe and pileus of *T. robustus.* Masamba and Kazombo (2010) reported 0.5% fiber in *T. le-testui* on fresh wt. basis. Fiber is one of the most important nutrients for health promotion and disease prevention. Fiber is commonly defined as "plant polysaccharides and lignin which are resistant to hydrolysis by the digestive enzymes of man" (Trowell, *et a1*., 1976). Adejumo and Awosanya (2005) documented (7.2%) fiber content in *T. mammiformis.* Nabubuya *et al*. (2010) while evaluating the nutritional properties of *T. microcarpus* reported (11.21%) crude fiber. In the same species Mukiibi (1973) reported (2.2%) fibers and (54.2%) carbohydrate.

Vitamins

Vitamin, it stimulates several immune system activities, possibly by promoting the growth, and preventing the stress-induced shrinkage, of the thymus gland. It also protects skin and gums (Groff, *et.al.,* 1995). Blindness also cause by vitamin deficiency (Smith & Steinemann, 2000). *Termitomyces clypeatus* possess a significant quantity of ascorbic acid (10-14%) (Ogundana & Fagade, 1981; Tibuhwa, 2012a). Besides, these mushrooms are also an excellent source of other valuable nutritive constituents like Vitamin C, Vitamin B_1, and Vitamin B_2 (Jiskani, 2001).

Fat

Crude fat are extremely important role for building block of the cell membranes. Masamba and Kazombo (2010) reported 0.7% fat in *T. le-testui* on fresh wt. basis). Nabubuya *et al*., (2010) while evaluating the nutritional properties of *T. microcarpus* reported (2.32%) lipid. In the same species Mukiibi (1973) reported 4.3% fat.

Minerals

The minerals present at levels more than 0.05% in the human body are defined as macro minerals. Calcium, Phosphorus, Magnesium, Potassium and Sodium belong to this category. These minerals work together closely. Calcium accounts for approximately 1.5% of total body weight. Calcium phosphate gives structure and strength to bones and it performs many other physiological activities (Groff, *et al*., 1995). Phosphorus is a key element in all known forms of life. Inorganic phosphorus [$(PO_4)_3$], is a major constituents in biological molecules such as DNA and RNA. An average adult human contains a little less than 1 kg of phosphorus. Similarly in our food must provide us with hundreds of milligrams of magnesium every day. Our nerves also depend upon magnesium to avoid becoming overexcited and all metabolic function and over 300 different enzymes in the body require magnesium in order to function (Abbott & Rude, 1993). Potassium is especially important in regulating the activity of muscles and nerves (Groff, *et al*., 1995). Counteract of it's the increased urinary calcium loss caused by the high-salt diets helps to prevent bones from thinning out at a fast rate (Sellmeyer, *et al.*, 2002). It also play a role in the prevention and/or treatment of atherosclerosis, cataracts, dehydration, diabetes, hepatitis, high blood pressure, inflammatory bowel disease and osteoporosis, potassium depletion due to excessive fluid loss from diarrhea, vomiting, or sweating (He & Mac, 2001). About 95% of the potassium in the body is stored within cells, while sodium and chloride are predominantly located outside the cell. Sodium is the principal cation in extracellular fluids. It regulates plasma volume and acid-base balance, involved in the maintenance of osmotic pressure of the body fluids, preserves normal irritability of muscles and cell permeability, activates nerve and muscle function and involved in Na^+/K^+-ATPase, maintenance of membrane potentials, transmission of nerve impulses and the absorptive processes of monosaccharaides, amino acids, pyrimidines, and bile salts. The changes in osmotic pressure are largely dependent on sodium concentration (Murray, *et al*., 2000).

The minerals present at levels less than 0.05 % in the human body are defined as micro minerals. The micro minerals are also known as the trace elements. Irons, Zinc, Copper, and Manganese are belonging to this group. Iron in the human body contains approximately 4 gm., function of the immune system is dependent on sufficient iron (Groff, *et al*., 1995). Zinc is needed in the diet on a daily basis, but only in very small

amounts (50 milligrams or less). Studies on zinc reflected some of the key functions served by this mineral, including regulation of genetic activity and balance of carbohydrate metabolism and blood sugar. Some nutrients interact with zinc. Copper is essential in all plants and animals. The human body normally contains copper at a level of about 1.4 to 2.1 mg for each kg of body weight (http://www.copper.org/consumers/health/papers/cu_health_uk/cu_health_uk.html).

Copper is found in a variety of enzymes. In addition to its enzymatic role, it is used for biological electron transport. Manganese in the human body contains a total of 15-20 mg; it serves as an enzyme activator and as a component of metalloenzymes. It activates the enzymes responsible for the utilization of several key nutrients including biotin, thiamin, ascorbic acid, and choline. It is a catalyst in the synthesis of fatty acids and cholesterol, facilitates protein and carbohydrate metabolism, and may also participate in the production of sex hormones and maintaining reproductive health (Groff, *et al*., 1995). Mukiibi, (1973) reported that ash (minerals) content 14.1% in *T. microcarpus*.

Bioactive Compounds

Termitomyces clypeatus possess a significant quantity of antioxidants (Ogundana & Fagade, 1981; Tibuhwa, 2012a).

Role in production of biofuel

Many products (e.g. enzymes, metabolites, therapeutic products, process technology) from this edible mushroom have been studied and commercialized around the world. *T. clypeatus* apart from having high nutrient and mineral content is known to produce a series of bio products, which find use in different bioprocess. The fungus also secretes a wide range of hydrolytic enzymes important for bioremediation and biofuel production. The enzymes like cellulases, hemicellulases, xylanases, amylases, proteases, pectinases, sucrase etc. have huge demand in food and feed industries and have considerable positive impact on environment through their use in biofuel production and bioremediation. Many of these enzymes have been purified and characterized in Lab and regulatory mechanism of their productions has been studied. Some of the enzymes have been novel and possess unique properties. The fungal biomass has important role in bioremediation of industrial effluents apart from its

nutrient value. The potential of *Termitomyces* sp. along with *Termitomyces clypeatus* strain will be presented with respect to nutritional components and important bio products and enzymes along with their range of important applications including their role in biofuel production and bioremediation. Hence it plays an important role for the process and product developments in biofuel and environmental biotechnology (Ghorai, *et al*., 2009). Therefore *Termitomyces* species are highly ranked mushrooms followed by *Pleurotus* species and *Polyporus tenuiculus* (Nakalembe, *et al.,* 2009).

Secondary metabolites

Various secondary metabolites synthesized by plants are biologically active for human beings, and thus, they impart medicinal value to the plant species. Mode of action of many such secondary metabolites is known (Evans, 1997). Volatile oils occur as droplets in the cell. They are used, for flavorings, perfumery and mainly, used for their therapeutic purpose as inhalations and mouth washes (www.functionofvolatileoil.com). Alkaloids are mostly poisonous but possess specific physiological action on human body when used in small quantities (Gurib, 2006). Steroids are used in treatment of various bone-degenerative diseases. It exhibit strong anti-inflammatory activity. Steroidal saponins act as hormones. Its least volume has appetite-suppressing properties (Enyiukwu, *et al.,* 2014).

Triterpenoids and Emodins possess remarkable anti-inflammatory, anti-bacterial, anti-fungal, anthelmintic, anti-malarial, and molluscicidal activities. They have vasorelaxant properties, whereas, in least volume has an appetite-suppressing properties (Inamdar, *et al.,* 1996). The function of carotenoids in human health is immune enhancement, reduction of risk of degenerative diseases like cancer, and slowdown ageing (www.coumarinwikipedia.org). Fatty acids are building blocks of phospholipids and glycolipids in human body and Flavonoids are the phenolic compounds, popularly known as antioxidant properties. Its least volume has appetite-suppressing properties (Evans, 1997). The **c**oumarine its least volume has appetite-suppressing properties (www.coumarinwikipedia.org). Anthracene glycoside**s** are important for, the pharmaco-chemical studies and used for the treatment of cardiac failure, and other several diseases associated with ageing (www.foodphytochemicals. com). Saponins are hemotoxic. They are important for, the pharmaco-chemical studies, mainly in sex hormones (Okwu, 2001). Tannins have great inhibitory power

of many hydrolytic enzymes used by plant pathogens (Cowan, 1999), it inhibit the growth of a number of microorganisms, resist microbial attack and are recalcitrant to biodegradation (Field & Lettinga, 1992). They act as antioxidants properties and are potentially important in biological systems (Scalbert, 1991). Glycosides are known for its antimicrobial properties, which inhibits on microbial pathogens (Cowan, 1999). Polyuronoides and Polyoses have demulcent and emollient properties for human health. It provides tightening effect to the skin, effective as anti-wrinkle agent (Scalbert 1991).

5.3 Materials and Methods

5.3.1 Nutritional constituents (Primary metabolites)

Selected *Termitomyces* species collected for the present study were first subjected to analysis of their content of nutrients, such as protein, carbohydrates, moisture, ash, fiber, fat, dry matter, Cu, Fe, Mg, Ca, K, P, Mn, Na, and Zn. The species (viz. *T. albuminosus, T. microcarpus, T. robustus,* and *T. schimperi*) was selected, on the basis of dominance among the termite's mushroom, having high nutritive potential with medicinal value together with indigenous knowledge and on the basis of the fact that the species has not been explored in Nepal till now. Their proximate analysis was performed in the Lab of Nepal Agriculture Research Council (NARC), Khumaltar Lalitpur and WETC, Dilli Bazar Kathmandu, Nepal, in accordance with Association of Official Analytical Chemists, 13^{th} edition.

The following methods were adopted during nutritional analysis:

5.3.1.1 Determination of moisture content and dry matter

For the determination of moisture content, the method described by (AOAC, 1995) was adopted. First of all a clean and dry silica crucible was heated at 135±2ºC. It was then cooled in dessicator and weighed. Five gram of prepared sample was kept in the crucible and dried in the oven at 135±2ºC for 4 hours and cooled in a dessicator. The process of heating and cooling was repeated till a constant weight was achieved. The moisture content was calculated using following equation:

$$\text{Moisture \%} = \frac{\text{loss of weight due to drying}}{\text{Weight of sample taken for analysis}} \times 100$$

The dry matter content was determined by deducting the moisture content from 100% as follow:

Dry matter % = 100 − Moisture%

5.3.1.2 Determination of total ash

The crucible used for the determination of moisture content was ignited in a muffle furnace at 600°C for 3 hours until more until it became free from all carbon containing materials and the ash changes to white or grayish white. The crucible was then transferred to hot oven air and heated for one hour. The crucible was then placed into a dessicator. Heating and weighing continued until a constant weight obtained. The ash content in the sample was determined by following formula:

$$\text{Total ash \%} = \frac{\text{wt. of ash after incineration}}{\text{wt. of sample taken for ashing}} \times 100$$

5.3.1.3 Determination of Protein

About 2 grams of moisture free sample was taken into a kjeldahl digestion flask. About 10 grams of digestion mixture (Copper Sulphate & Sodium Sulphate) was added followed by 10 ml. of Concentrated Sulphuric Acid. It was then heated at low temperature until the froth ceases and further heated with increased temperature until the solution becomes transparent blue and white fumes comes out. The mixture was then further heated for half an hour and cooled in room temperature. The whole content then quantitatively transferred to a 100 ml volumetric flask and the volume was made up by distilled water. Whole content of the digestion solution was then transferred to a distillation flask followed by distilled water 40% sodium hydroxide solution. The distillation set was arranged in such a way that the cold water continuously flow through the unit. The distillate was then collected in 4% boric acid solution that absorbs the liberated nitrogen content in a 250 ml beaker. Nearly 200 ml of the distillate was collected in the beaker and was titrated against the standard 0.05 N Hydrochloric acid. Using the following formula total nitrogen content was calculated and the protein content was determined by multiplying with 6.25.

$$\text{Total Nitrogen \%} = \frac{1.4\,(V - V1) \times N}{W \;\; X \; V3} = x$$

Protein % = X × 6.25

Where;

V = volume of standard acid used to neutralize the distillate

V_1 = volume of standard acid used to neutralize the blank

N = normality of standard acid

X = total Nitrogen percent

W = weight of sample taken for digestion

5.3.1.4 Determination of Fat

Five grams of moisture free sample was weighed in a glass fibre thimble. The mouth of thimble was plugged with white cotton. A clean and dry round bottom flask was weighed. The thimble along with sample placed into the Soxhlet apparatus. The Soxhlet was filled with petroleum ether by pouring it through the condenser at the top by means of a glass funnel. The apparatus was placed on a heating mantle at 60ºC followed by cold water circulation. The solvent vessel was continued to heat until all of the solvent has been evaporated and condensed in the Soxhlet extractor. Extraction was continued for 7 hrs. After the extraction was over, the thimble was taken out from the Soxhlet. The material was again heated to remove the remaining ether from the flask. The vessel containing the fat residue was dried in an oven at 103 ± 2ºC for 1 hour. The initial and final weight of the round bottom flask was used to determine the fat concentration in the sample.

$$\text{Fat \%} = \frac{M2 - M1}{E} \times 100$$

M_1= Initial weight (in gm.) of the dry empty round bottom flask

M_2= Final weight (in gm.) of the dry empty round bottom flask

E= weight of the sample in grams

5.3.1.5 Determination of crude fibre

The sample contents obtained from the fat determination was used for the measurement of crude fibre. The whole content was poured into a heating flask followed by boiled 200 ml 0.255 N H_2SO_4. The mixture was boiled for 30 minutes by means of keeping the volume constant by the addition of distilled water at frequent intervals. The mixture was then filtered through a muslin cloth and the residue washed with hot water till free from acid. The material was then transferred to the same flask followed by 200 ml of boiling 0.313 N NaOH. After boiling for 30 minutes (keeping the volume constant as before) the mixture was filtered through a muslin cloth and the residue kept in the crucible washed with hot water till free from alkali, followed by washing with some alcohol and ether. The residue was then placed into a silica crucible and dried at 110 ± 2ºC until constant weight was obtained. Further the whole content of the crucible was ignited for approximately 30 min in a furnace at 550°C until carbonaceous matter was destroyed. Again, the crucible was cooled in dessicator and weighed until constant weight was obtained. The loss in weight represented the crude fiber and calculated with following formula:

$$\text{Crude fibre \%} = \frac{\text{Loss in weight noted}}{\text{Weight of sample taken}} \times 100$$

5.3.1.6 Determination of Carbohydrate content

Carbohydrate estimation was done following Anthrone method of Thimmaiah (1999).

The anthrone reaction is the basis of rapid and convenient method for the determination of hexoses, aldopentose and hexauronic acids either free or present in polysaccharides. Carbohydrates are dehydrated by conc. H_2SO_4 to form furfural. Furfural condenses with anthrone (10-Keto-9, 10-dihydro anthracene) to form a blue-green coloured complex, which is measured colorimetrically at 630 nm.

Extraction and quantification: A 20 gm. small piece of mushrooms sample was taken in conical flask and was hydrolyzed with 250 ml of 2.5 N HCl for 3 hrs in boiling water bath and then it was allowed to cool at room temperature (28 ± 2ºC). The solution thus obtained was neutralized by solid sodium carbonate (until effervescence ceases) cooled and then it was centrifuged (8000 rpm, 10 min.), filtered

and supernatant was collected and used for analysis. One ml of this solution (diluted up to 10^{-3} times with distilled water) was mixed with 4 ml Anthrone reagent and was incubated in a water bath for 8 min. in a boiling tube. The tube was then cooled rapidly a green colour developed whose O. D. (optical density) was measured at 630 nm. Same procedure was applied to the standard glucose solution (range 0µg/ml-100µg/ml) for obtaining a standard graph with O.D. values on y-axis and carbohydrate conc. on the x-axis. Blank sets were prepared by taking 1ml of distilled water in place of solution. Quantity of carbohydrate in unknown sample was determined by comparing the value with standard curve of glucose (range 0-100µg/ml glucose). The amount of carbohydrate present in sample was calculated as following using standard graph of glucose.

$$\text{Amount of carbohydrate present in sample (\% mg)} = \frac{\text{Sugar valu e from graph (mg)} \times \text{Total volume of extract (ml)}}{\text{Aliquot sample used (ml)} \times \text{weight of sample (mg)}} \times 100$$

The data are presented in Table 12.

5.3.1.7 Quantitative Analysis of Metals in *Termitomyces* Samples

Five ml of conc. HCl was added to the 1 gm. Ash content obtained from the mushroom sample and heated to dryness in a beaker. Further 1ml of conc. Nitric acid was added and heated to dryness and cooled. 100 ml of distilled water was added in the beaker and heated to boil for 10 minutes and cooled in room temperature. Final volume was made up to 100 ml and filtered through Whatman No. 42 filter paper. The solution was then aspirated to the Atomic Absorption Spectrophotometer (Agilent Technologies-200 Series AA) for determination of metal content using respective hollow cathode lamps (Table 10 and 11). For each metal analysis a calibration curve of different concentration against absorbance was plotted. Standard concentrations, their absorbance, respective wavelengths along with calibration graph are presented below:

Table 10: Atomic Absorption: Working Conditions (Fixed)

Lamp current:	5 mA
Fuel:	Acetylene
Support:	Air
Flame Stoichiometry:	Oxidizing

Instrumental Parameters for Mn, Fe, K, Na Cu, and Zn

Table 11: Atomic Absorption Spectrophotometer: Working Conditions (Fixed)

Lamp current:	10 mA
Fuel:	Acetylene
Support:	Nitrous oxide
Flame stoichiometry:	Reducing; red cone 1-1.5 cm High

Instrumental Parameters for Ca and Mg

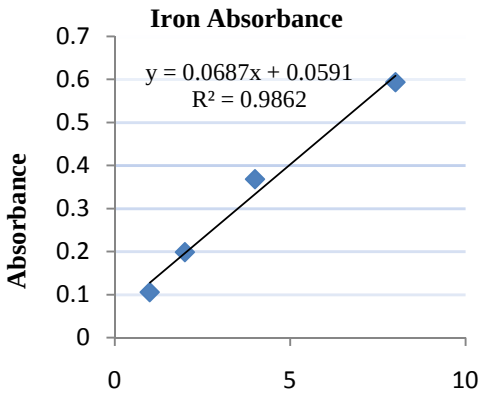
5.3.1.8 Quantitative Analysis of Non-Metal (Phosphorus)

Phosphorus reacts with molybdic acid to form a phosphomolybdate complex. It is then reduced with aminonaphtholsulphonic acid to the complex molybdenum blue which is measured colorimetrically.

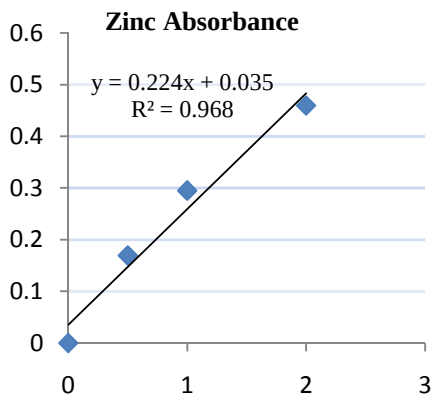
Procedure: To 5 ml of ash solution was obtained by dry ashing, added 5ml of molybdate reagent and mix. Addition of 2 ml of aminonaphtholsulphonic acid solution, mixed and made up the volume up to 50 ml. prepared similarly blank, using water in place of the sample. The solution was then aspirated to the UV-VIS Spectrophotometer for determination of Phosphorus content. Allowed to stand for 10 min and measured the colour at 650 nm setting the blank at 100% transmission.

Standard Curve: Potassium phosphate solution of 10 ml standard was diluted to 50 ml with water (1ml = 0.02 mg P). They were pipetted aliquots of that solution from 5 to 40 ml into 50 ml volumetric flasks. Added 5 ml of molybdate reagent and mixed. Then added 2 ml of aminonaphtholsulphonic acid reagent mixed and make up the volume up to 50 ml and measure the colour as in sample.

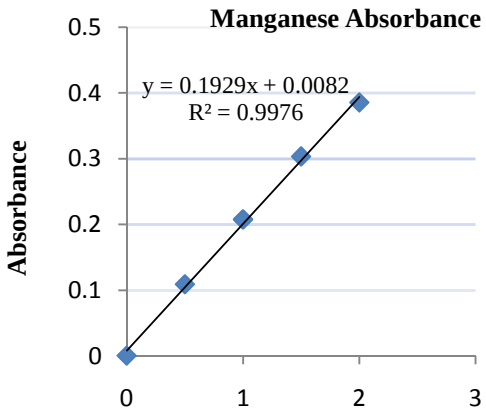
a)


Iron Absorbance
y = 0.0687x + 0.0591
R² = 0.9862
Absorbance
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
5
10

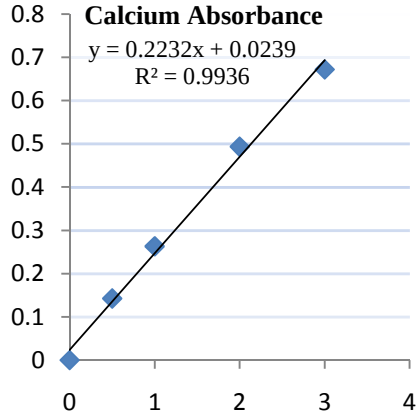
b)


Zinc Absorbance
y = 0.224x + 0.035
R² = 0.968
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3

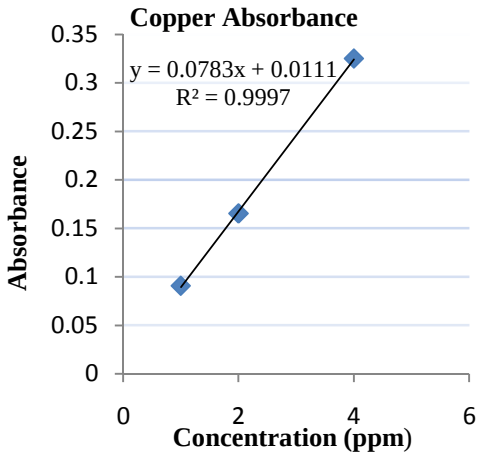
c)


Manganese Absorbance
y = 0.1929x + 0.0082
R² = 0.9976
Absorbance
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3

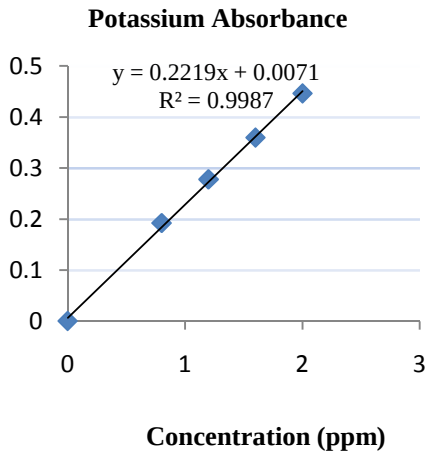
d)


Calcium Absorbance
y = 0.2232x + 0.0239
R² = 0.9936
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4

e)


Copper Absorbance
y = 0.0783x + 0.0111
R² = 0.9997
Absorbance
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
2
4
6
Concentration (ppm)

f)


Potassium Absorbance
y = 0.2219x + 0.0071
R² = 0.9987
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
Concentration (ppm)

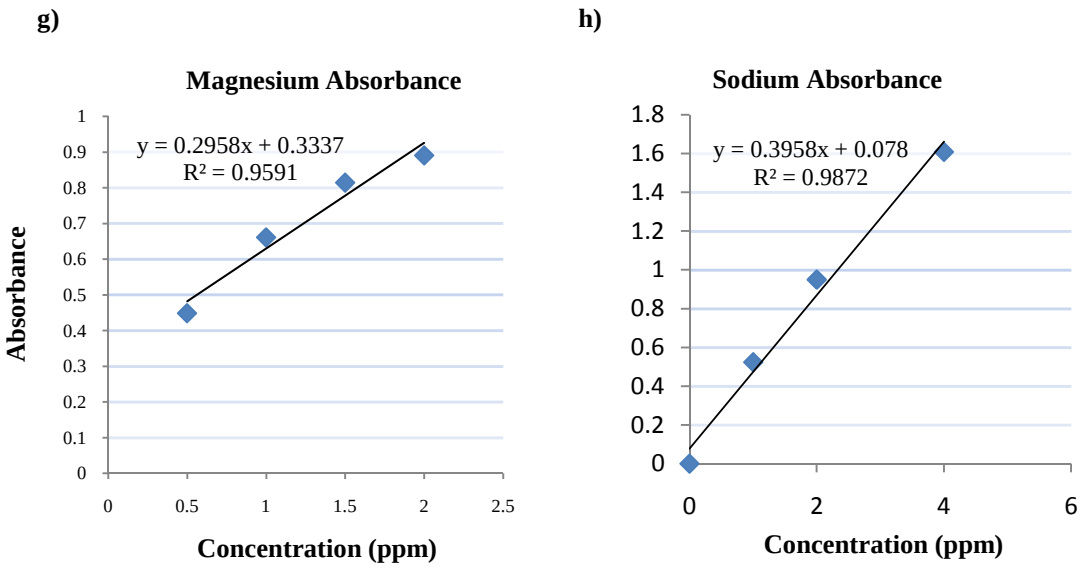


Figure 31: Calibration curve of different metals.

Analyzed a calibration curve plotted concentration (0.0, 0.2, 0.4, 0.8, 1.0, 1.2 ppm) against Absorbance (0.0, 0.115, 0.232, 0.442, 0.537, 0.63). Standard concentration its absorbance is, 880 nm wavelengths. The calibration graph and calculation presented below:

$$\text{Phosphorus mg/100 gm} = \frac{\text{mg of P in the aliquot of ash solution taken for estimation} \times \text{total volume of ash solution}}{\text{ml of ash solution taken for estimation} \times \text{Wt. of sample taken for ashing}} \times 100$$

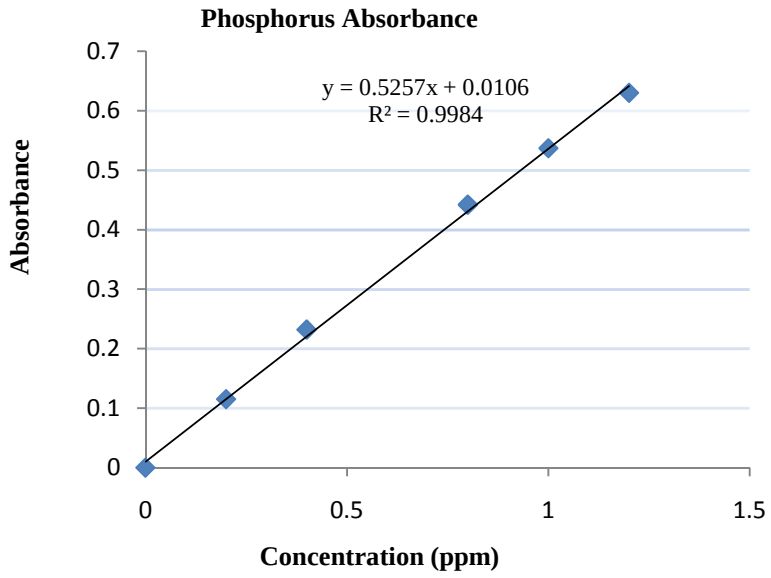


Figure 32: Calibration curve of Phosphorus (P)

5.3.2 Phytochemicals

Four species of *Termitomyces* were subjected to phytochemical analysis, nutritional as well as medicinal potential and they were assessed, on the basis of consumption as food and medicinal uses, which have been liked by the ethnic groups has been taken. To examine the secondary metabolites of *Termitomyces* species and selection of study area and layout the programmed. They were subjected to the qualitative phytochemical analysis (screening) had been done in Lab of DPR, Thapathali, Kathmandu, Go'N. Field sampling has been conducted three times (April-May, June-September and October-November) of the years 2010, 2011 and 2012, (Hellawell, 1978; Elliott, 1971).

5.3.2.1 Processing of the specimen

The mushroom was uprooted, washed and oven dried for 48 hours at 40°C, and they were turned repeatedly to avoid the microbial growth. The sample was pulverized using a manual blender and stored in a labeled air-tight container before analysis.

5.3.2.2 Phytochemical Screening

The experiment was carried out in Lab of DPR, Kathmandu. It was conducted according to standard methods described by Ciulei (1982). Briefly, 10gm. of powdered sample from each site was first extracted with petroleum ether using Soxhlet extractor until 6 hrs then with ethyl alcohol and finally with water. The obtained solutions in each extraction process were filtered through whatman filter paper No.1 and concentrated up to 20-25 ml using rotary evaporator at 40ºC.

5.3.2.3 Statistical Analysis

Variation of concentration of individual chemical compounds in east, centre and west Nepal was tested using chi square test. Similarly those variations were tested among the sample of Tarai, Siwalik and Midhill range, using same test, by Pearson's (1990), chi square test, differences were considered to be significant at $p<0.05$.

5.4 Results

5.4.1 Primary metabolites

The mean values were taken from the proximate analysed data of all studied eco-zones of Nepal. These showed ample amount of following essential nutrients (Table 12).

Table 12: Mean nutritive values of Termite's mushrooms (value in % by weight).

SN	Biochemical parameters	*Termitomyces. albuminosus*	*Termitomyces mammiformis*	*Termitomyces microcarpus*	*Termitomyces Robustus*
1	Protein	30 g/100g	21 g/100g	24 g/100g	19 g/100g
2	Carbohydrate	32	48	18	40
3	Moist	11	10	14	10
4	Ash	12	8	33	11
5	Fiber	9	14	8	16
6	Fat	2	1	1	1
7	Dry matter	90	90	87	91
8	Cu	4 mg/100g	4 mg/100g	3 mg/100g	3 mg/100g
9	Fe	20	62	38	17
10	Mg	208	42	72	35
11	Ca	47	170	528	105
12	K	2170	905	1927	905
13	P	11	9	15	9
14	Mn	8	6	5	3
15	Na	6	8	19	6
16	Zn	14	6	20	6

Comparison of macro-nutrients across Physiographic regions

Tarai

All the seven macro nutrients in four species of *Termitomyces*, are significantly different at 5% level of significance. Carbohydrate content was maximum in *T. mammiformis* and minimum in *T. microcarpus.* Similarly protein content was maximum in *T. albuminosus* and minimum in *T. robustus.* Moisture and fiber content was maximum in *T. robustus* and minimum in *T. albuminosus, T. microcarpus* and*. T. mammiformis.* Ash content was maximum in *T. microcarpus* and minimum in *T. albuminosus & T. mammiformis* Fat content was maximum in *T. albuminosus, T. microcarpus* & *T. robustus* and minimum in *T. mammiformis, T. microcarpus* and *T. robustus.* Dry Matter content was maximum in *T. mammiformis* and *T. microcarpus* and minimum in *T. robustus* (Appendix 3 of Table 1 & Figure 33a).

All together carbohydrate, protein, ash, fiber, fat and dry matter contain was maximum and moisture was low with comparison to the Siwalik and Midhill.

Siwalik

Except moisture all the seven macro nutrients in four species of *Termitomyces*, are significantly different at 5% level of significance. Carbohydrate & fiber content was maximum in *T. mammiformis* and *T. robustus* and minimum in *T. albuminosus* & *T. microcarpus.* Protein and fat content was maximum in *T. albuminosus* & *T. mammiformis* and minimum in *T. microcarpus* and *T. robustus.* Ash content was maximum in *T. microcarpus* and minimum in *T. mammiformis.* Dry Matter content was maximum in *T. robustus* and minimum in *T. microcarpus* (Appendix 3 of Table 2 and Figure 33b).

All together carbohydrate, protein, ash, fiber, fat and dry matter contain was less than Tarai but more than Midhill but moisture was less than Midhill.

Midhills

Except fat all the macronutrients of four species in Midhills are significantly different at 5% level of significance. Carbohydrate and fiber content was maximum in *T. mammiformis* & *T. robustus* and minimum in *T. microcarpus.* Protein and ash content was maximum in *T. albuminosus* & *T. mammiformis* and minimum in *T. microcarpus.* Moisture content was maximum in *T. microcarpus* and minimum in *T. robustus*. DM content was maximum in *T. robustus* and minimum in *T. microcarpus* (Appendix 3 of Table 3 and Figure 33c).

All together carbohydrate, protein, ash, fiber, fat and dry matter contain was low and moisture was highest with comperision to the Tarai and Siwaliks.

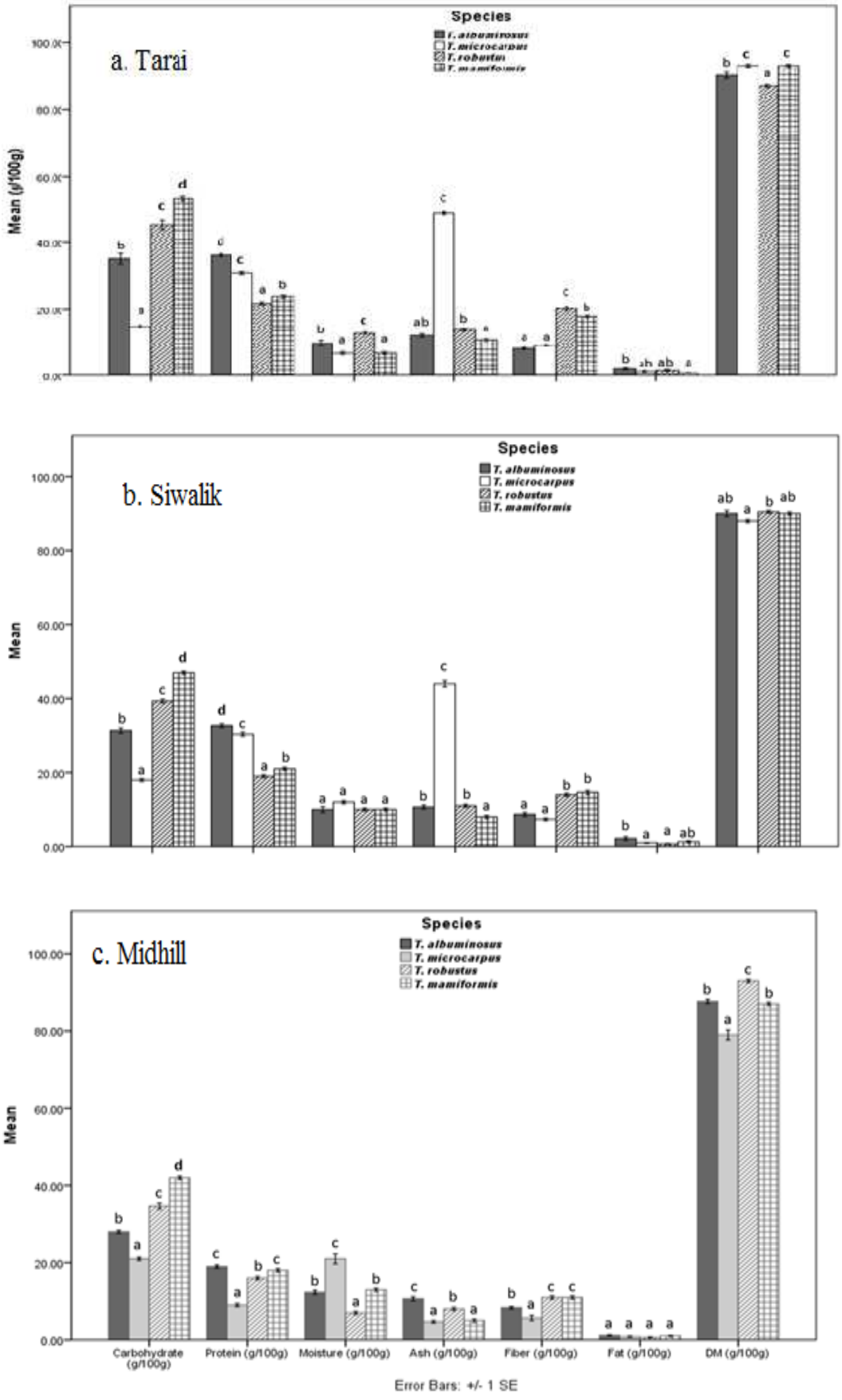


Figure 33: Comparision of macro-nutrients content of *Termitomyces* across physiographic region.

Comparison of macro-nutrient of each species along physiographic regions:

T. albuminosus

Investgated macro-nutrients contain of *T. albuminosus* in 9 regions significantly different at 5% level of significance. Carbohydrate content was maximum in central Tarai and minimum in entire Midhills. Protein was maximum in entire Tarai and minimum in entire Midhills. Moisture content was maximum in all eastern regions and central Midhills and minimum in central and western Siwaliks and western Tarai. Ash contents were maximum in Tarai and Siwaliks of eastern and central regions and minimum in all western regions and Midhills of eastern and central regions. Content of fiber was in uniform in almost all regions. Fat content were maximum in central Tarai and Siwaliks and eastern Tarai and its contents was low in almost all regions. Dry Matter content was maximum in central and western Siwaliks and western Tarai and minimum in all eastern regions and central Midhills (Appendix 3, Table 4 & Figure 34a).

T. mammiformis

Except fat all the macro-nutrients content of *T. mammiformis* in nine regions significantly different at 5% level of significance. Carbohydrate, protein, ash, fiber and dry matter contents were maximum in entire Tarai and minimum in entire Midhills. Moisture contents were maximum in entire Midhills and minimum in entire Tarai (Appendix 3 Table 5 & Figure 34b).

T. microcarpus

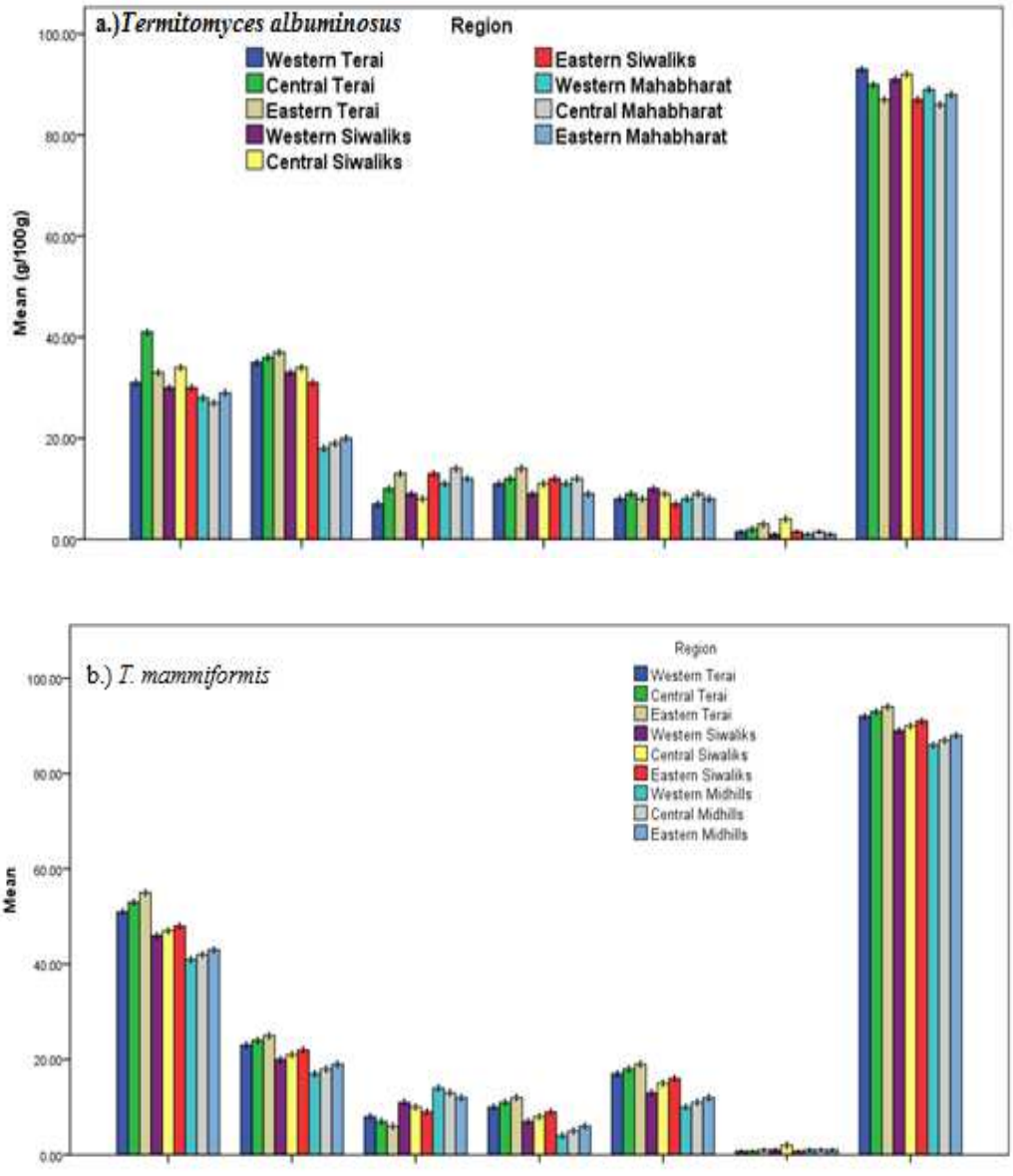
Except fat all the macro-nutrients content of *T. microcarpus* in nine regions significantly different at 5% level of significance. Carbohydrate, protein, ash, fiber and dry matter content was maximum in entire Tarai and minimum in entire Midhills. Moisture contents were maximum in entire Midhills and minimum in entire Tarai (Appendix 3, Table 6 & Figure 34c).

T. robustus

All the macro-nutrients content of *T. robustus* in nine regions significantly different at 5% level of significance. Carbohydrate content was maximum in centre Tarai and

minimum in west Midhills. Protein, Moisture and Ash contents were maximum in entire Tarai and minimum in entire Midhills. Fiber content was maximum in east Tarai and minimum in entire Midhills. Fat content was maximum in entire Tarai & centre Siwaliks and Midhills and minimum in east Siwaliks and Midhills, centre Siwaliks and Midhills and west Tarai, Siwaliks and Midhills. Dry Matter content was maximum in entire Midhills and minimum in entire Tarai (Appendix 3 of table 7 and Figure 34d).

Altogether carbohydrate, protein, ash, fiber and dry matter contant was higher in entire Tarai and gradually decreases with increasing an altitude together with east to west ecozone. But content of moisture were found higher in east midhill and gradually decreases towards west with the decrease elevation in all four investigated species.



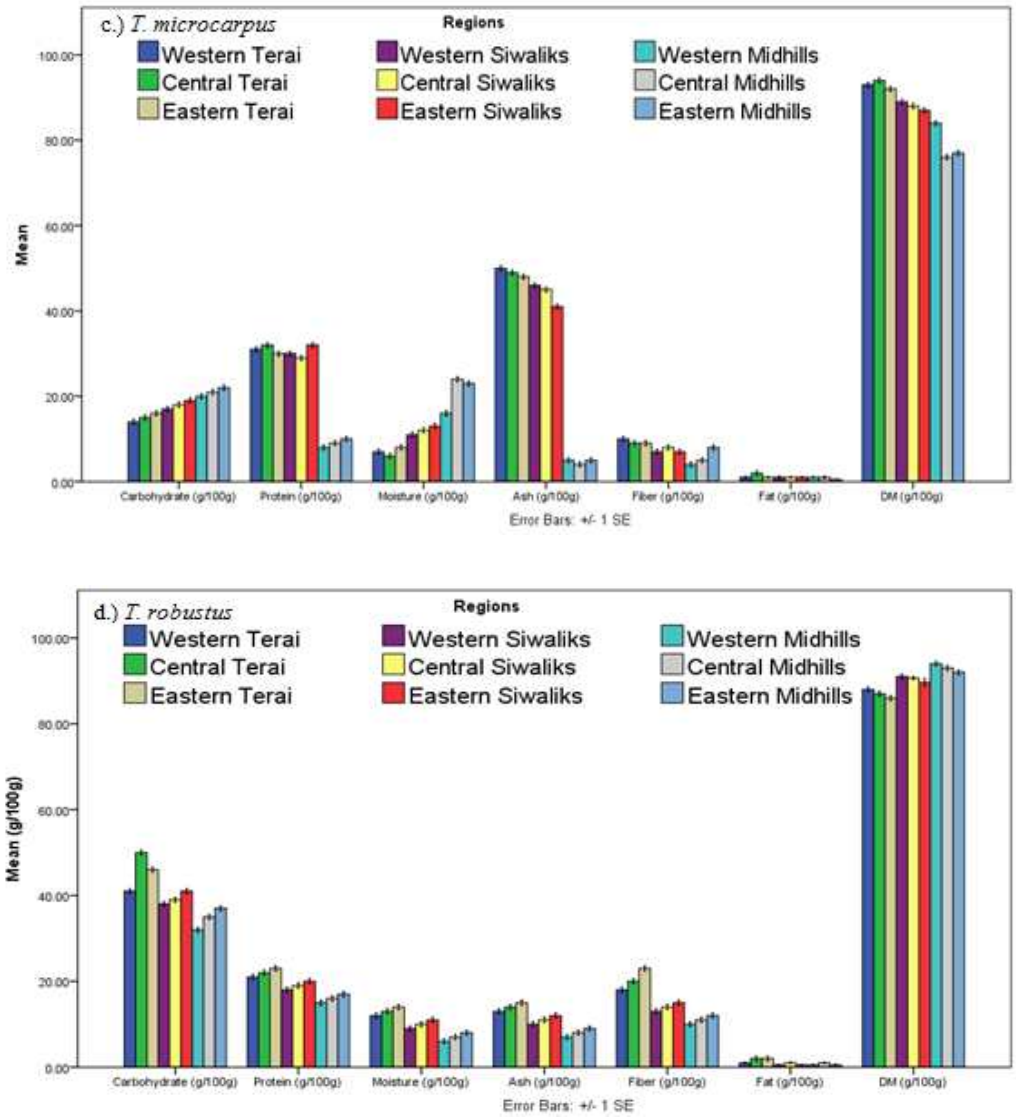


Figure 34: Comparision of macro-nutrients content of *Termitomyces* spp. along physiographic region.

Comparison of micro-nutrients, across Physiographic regions

Tarai

Except Copper all the micronutrients are significantly different at 5% level of significance. Iron and manganese content was maximum in *T. albuminosus* and *T. mammiformis* and minimum in *T. robustus.* Magnesium has maximum in *T. albuminosus* and minimum in *T. robustus.* Calcium and sodium content was maximum in *T. microcarpus* and minimum in *T. albuminosus.* Potassium content was maximum in *T. albuminosus* and minimum in *T. mammiformis.* Phosphorus content was maximum in *T. microcarpus* and minimum in *T. mammiformis.* Zinc content was maximum in *T. microcarpus* and minimum in *T. robustus* (Appendix 4 of Table 1 & Figure 35a).

Alltogether Cu, Mn, Na, Fe and Zn content was maximum and Ca, K, P and Mg content was low with comparison to the Siwalik and Midhill.

Shiwaliks

Except copper all the micronutrients are significantly different at 5% level of significance. Iron content was maximum in *T. mammiformis* and minimum in *T. albuminosus* and *T. robustus.* Magnesium, potassium, and manganese content were maximum in *T. albuminosus* and minimum in *T. robustus.* Calcium, phosphorus and sodium content were maximum in *T. microcarpus* and minimum in *T. albuminosus.* Zinc content was maximum in *T. microcarpus* and minimum in *T. mammiformis* & *T. robustus* (Appendix 4 of table 2 & Figure 35b).

All together Cu, Fe, K, P, Na & Zn content was maximum and Ca & Mg content was low with comparison to the Tarai and Midhill.

Midhills

All the micronutrients of four species in the Midhills are significantly different at 5% level of significance. Copper content was maximum in *T. mammiformis* and minimum in *T. microcarpus*. Iron content was maximum in *T. mammiformis* and minimum in *T. albuminosus.* Magnesium, potassium and manganese content were maximum in *T. albuminosus* and minimum in *T. robustus.* Calcium and sodium content was maximum in *T. microcarpus* and minimum in *T. albuminosus.* Phosphorus and zinc content was maximum in *T. microcarpus* and minimum in *T. robustus.* (Appendix 4 of Table 3 & Figure 35c).

All together Fe, Mg, Ca, K and P content was high and Cu, Mn, Na and Zn content was low with comparison to the Tarai and Siwaliks.

Comparison of micro-nutrient of each species along phytogeographic regions

T. albuminosus

All of the nine micronutrients content of *T. albuminosus* in 9 regions are significantly different at 5% level of significance. Copper contents were maximum in all the regions (except eastern Mid-hill) and minimum also in all regions (except eastern Tarai and central Siwaliks). Iron contents were maximum in all Tarai and minimum in central and western Siwaliks and eastern and western Midhills. Magnesium contents

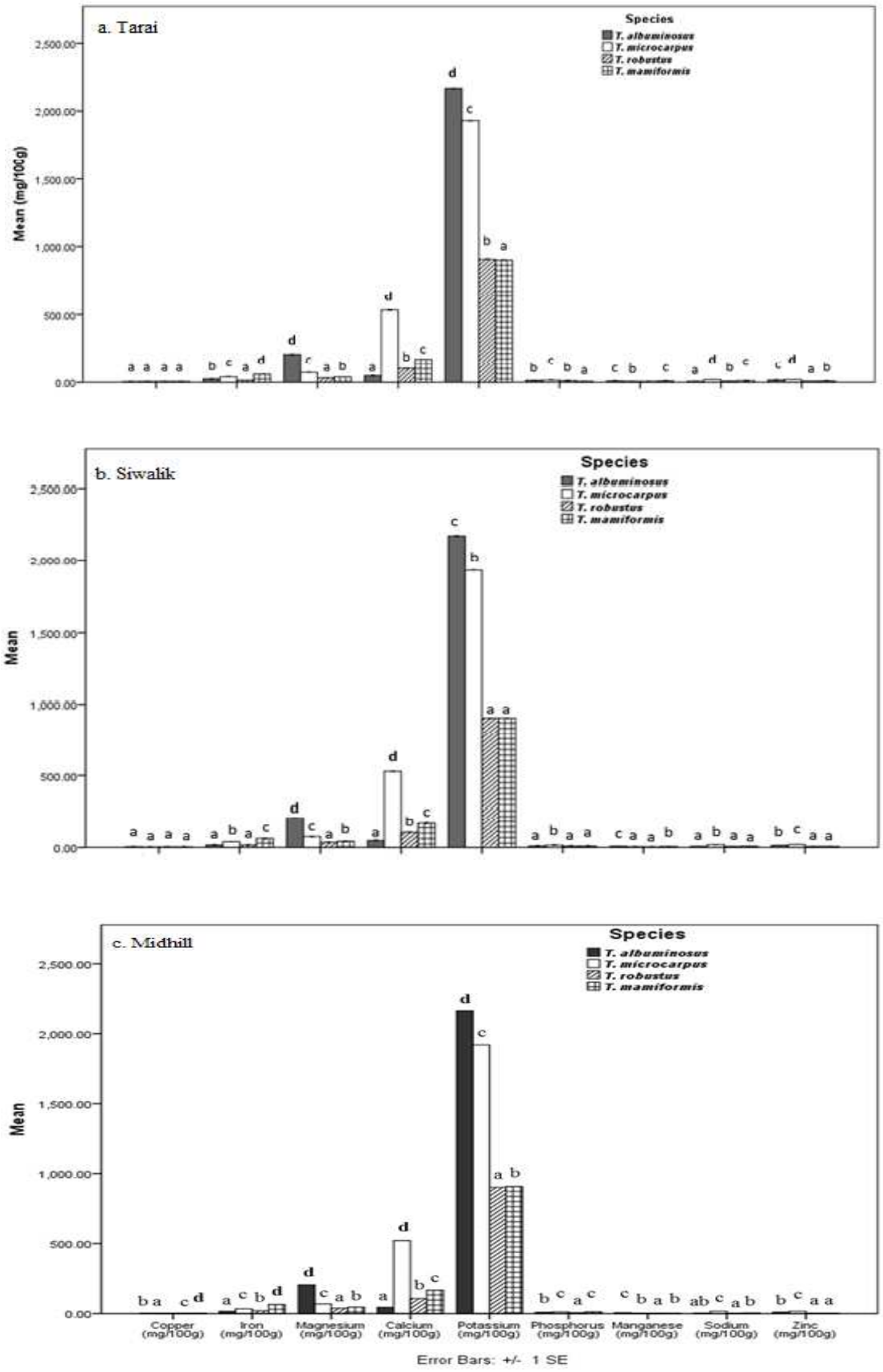


Figure 35: Comparision of micro-nutrients content (mg/100g) of *Termitomyces* across physiographic region.

were maximum in central and eastern Siwaliks and eastern Tarai and minimum in eastern Midhill and western Siwaliks. Calcium contents were maximum in central Tarai and minimum in eastern Midhill. Potassium contents were maximum in central Siwalik and Tarai and minimum in western Midhill. Phosphorus contents were maximum in east and centre Tarai and minimum in east, centre and west of Siwaliks and Midhills. Manganese contents were maximum in entire Tarai and east and centre Siwalik and minimum in entire Midhills and west Siwalik. Sodium contents were maximum in east and centre Siwaliks and minimum in east Tarai. Zinc contents were maximum in entire Tarai and east siwalik and minimum in entire Midhills (Appendix 4 of Table 4 and Figure 36a).

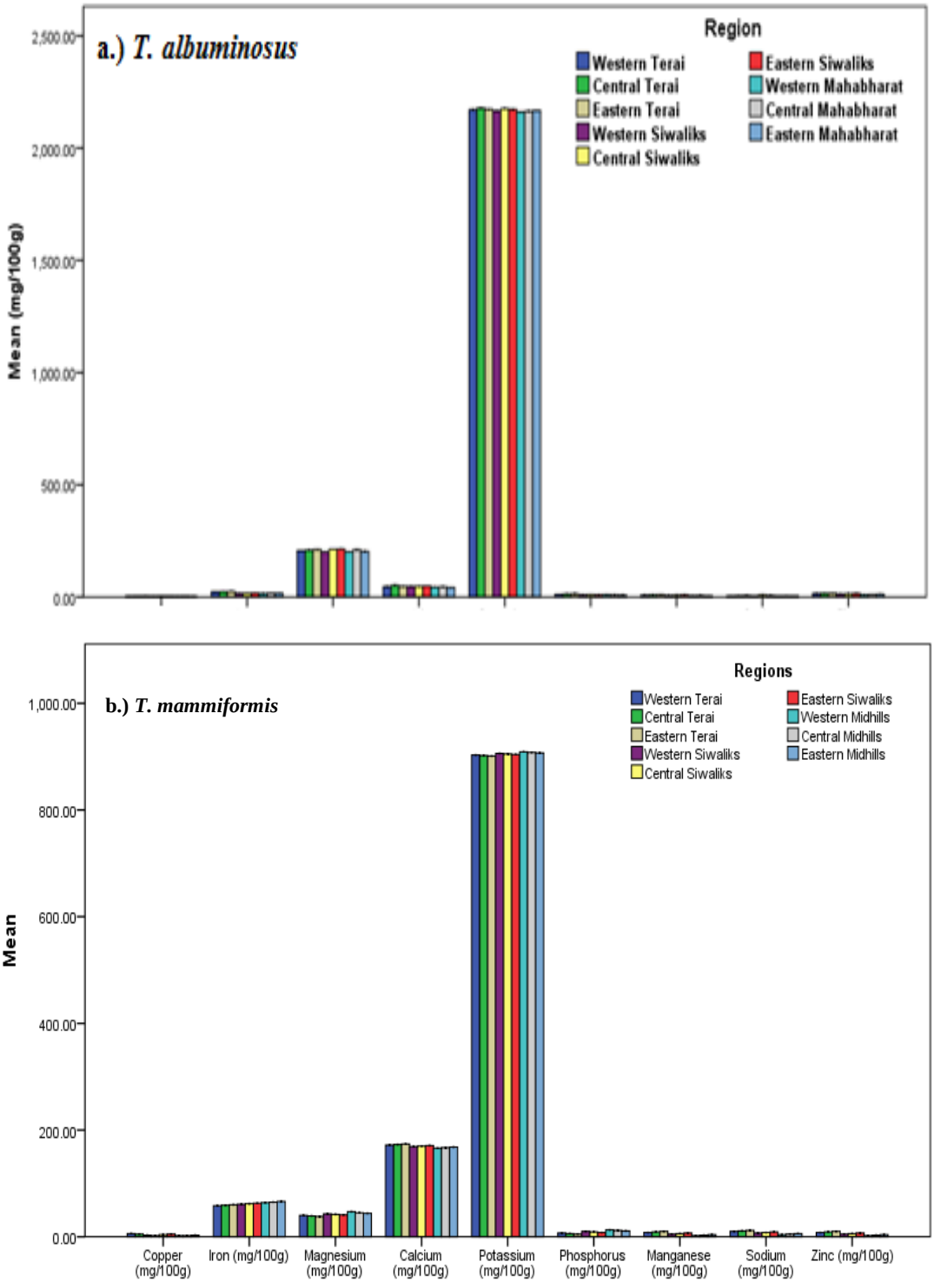
T. mammiformis

All the micro-nutrients content of *T. mammiformis* in nine regions are significantly different at 5% level of significance. Copper content was maximum in centre and west Tarai and east and centre Siwaliks and minimum in east Tarai & Midhills and centre and west Siwaliks and Midhills. Iron content was maximum in centre and west Midhills and east Siwaliks and minimum in entire Tarai. Magnesium content was maximum in west Siwaliks and east & centre Midhills and minimum in entire Tarai. Calcium content was maximum in centre and west Tarai and east Siwaliks and minimum in east, centre and west Midhills. Potassium content was maximum in west siwalik and east and centre Midhills and minimum in entire Tarai. Phosphorus content was maximum in entire Midhills and minimum in entire Tarai. Manganese content was maximum in entire Tarai and minimum in entire Midhills. Sodium content was maximum in entire Tarai and minimum in entire Midhills. Zinc content was maximum in entire Tarai and minimum in entire Midhills (Appendix 4 of Table 5 and Figure 36b).

T. microcarpus

All the micro-nutrients content of *T. microcarpus* in nine regions are significantly different at 5% level of significance. Copper content was maximum in entire Tarai and minimum in entire Midhills. Iron content was maximum in entire Midhills and minimum in entire Tarai. Magnesium content was maximum in entire Midhills and minimum in entire Tarai. Calcium content was maximum in entire Tarai and

minimum in entire Midhills. Potassium content was maximum in entire Midhills and minimum in entire Tarai. Phosphorus content was maximum in entire Midhills and minimum in entire Tarai. Manganese content was maximum in entire Tarai and minimum in entire Midhills. Sodium content was maximum in entire Tarai and minimum in entire Midhills. Zinc content was maximum in entire Tarai and minimum in entire Midhills (Appendix 4 of Table 6 and Figure 36c).



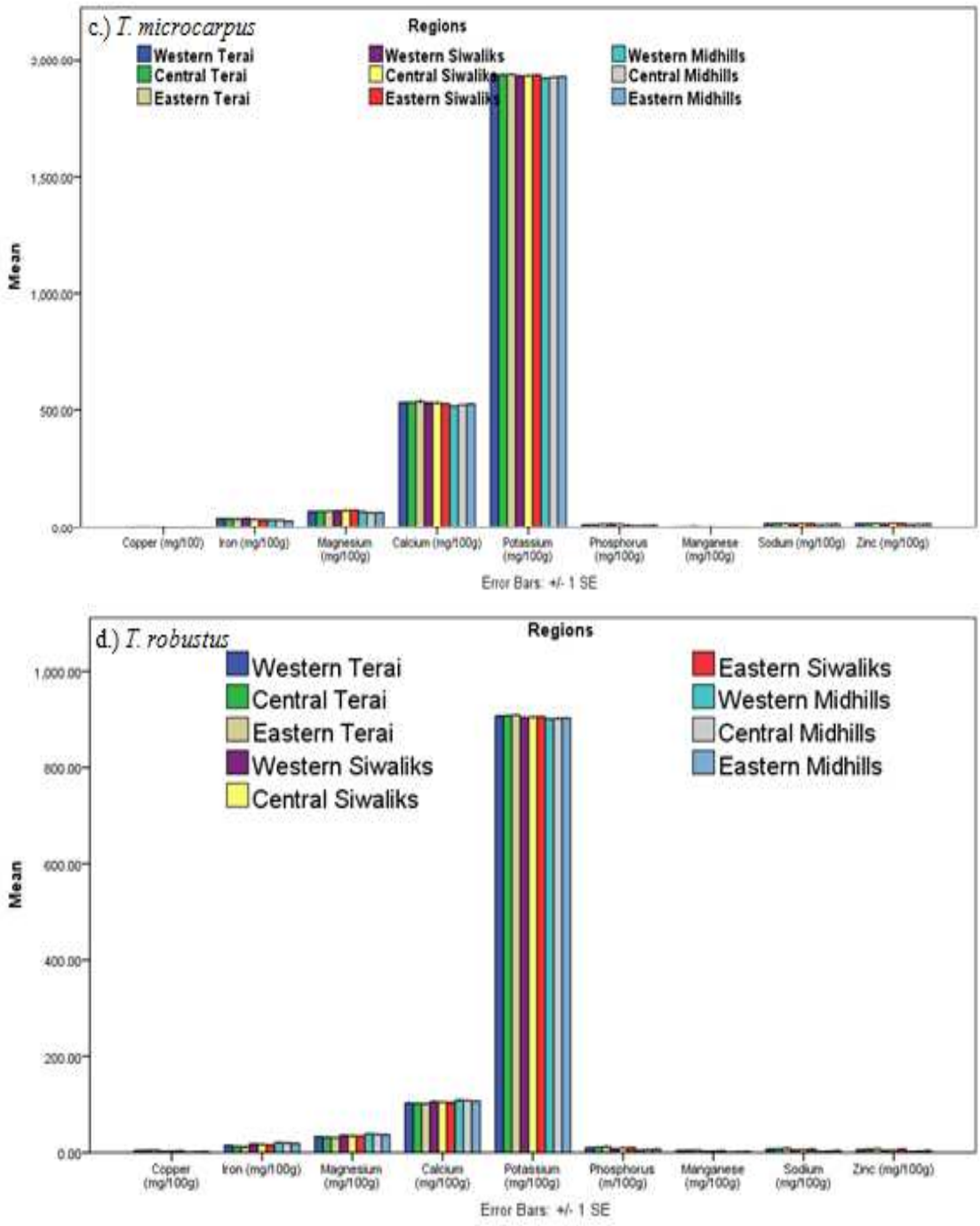


Figure 36: Comparision of Micro-nutrients contents of *Termitomyces* spp. along physiography regions.

T. robustus

All the micro-nutrients content of *T. robustus* in nine regions are significantly different at 5% level of significance. Copper, Potassium and Manganese content was maximum in entire Tarai and minimum in entire Midhills. Iron content was maximum in entire Midhills and minimum in east and centre Tarai. Magnesium and Calcium content was maximum in entire Midhills and minimum in entire Tarai. Phosphorus

and Sodium content was maximum in entire Tarai and east Siwaliks and minimum in entire Midhills. Zinc content was maximum in entire Tarai and east Siwaliks and minimum in entire Midhills and west Siwaliks (Appendix 4 of Table 7 and Figure 36d).

5.4.2 Secondary metabolites

In this investigation, four samples were analyzed from each region (Table 13), and sixteen (*T. mammiformis* & *T. microcarpus*), fifteen (*T. albuminosus*) and fourteen (*T. robustus*) of major chemical constituents have been found, where volatile oil is completely absent (*T. albuminosus* & *T. robustus*), among the tested samples of basidiome, during analysis, but in case of *T. robustus* volatile oil including steroids were absent. This chemical was also almost absent in *T. mammiformis* except a negligible concentration were found in east Tarai. Similarly presence of alkaloid in *T. mammiformis* was highest with comparisons to the other three species, in all topographic region, but absent in west Tarai in (*T.albuminosus* & *T. mammiformis*), and west Midhill & centre Midhill in (*T. albuminosus*). Its presence was very low in east and west Tarai in (*T. albuminosus & T. microcrpus*) respectively. Though its presence in almost all topographical region. Presence of steroid is highest in (*T. albuminosus* and *T. mammiformis*), moderate in (*T. microcarpus*) whereas completely absent in (*T.robustus*). Its presence was good in hilly region rather than Tarai, while in west mid hill was highest. Presence of triterpenoid and carotenoid were very good in almost all the topographical zone of all four tested samples (except in *T. albuminosus* of centre siwalik and *T. mammiformis* of west Tarai. Presence of fatty acid in Tarai was high, otherwise its presence was negligible or almost absent, in the entire topographical region in all four samples. Presence of emodine moderate in hilly region, otherwise its presence was low to negligible, in other topography in all four samples. Presence of flavonoid was moderate in all topography in all samples except in *T. albuminosus* of Tarai and *T. robustus* of hilly region. Presence of coumerine was almost absent in *T. microcarpus*, while in *T. robustus*, low in hill and rest in all topography there was absent, but in *T. mammiformis* it was little bit high then *T. albuminosus*; Whereas in *T. albuminosus* its presence was also very low. Presence of anthracene glycoside was very negligible in all topography and all samples but in *T. albuminosus* its presence only found in west Tarai and mid hill region. Presence of anthocinadine glycoside was very negligible to almost absent in all topography and all

samples but in *T. microcarpus* its presence was high in centre siwalik. Presence of saponin was very good in all topography and all samples but in *T. microcarpus* its presence was almost absent in mid hill region. Presence of tannins in *T. mammiformis* was highest with comparison to the other three samples in all topography similarly in *T. albuminosus* of east mid hills and *T. microcarpus* of centre siwalik was absent otherwise its presence was found in moderates. Presence of Glycoside in *T. albuminosus, T. mammiformis* (except WT) and *T. microcarpus* (except west Mid hillis) was very good in all topography, whereas in *T. robustus* its presence was high in hilly region and completely absent in other topography. Presence of polyruenoid and polyoses were moderate in all topography in all samples but there were absent in *T. robustus* of Tarai region.

In altogether presence of phytochemical in *T. mammiformis* is high, especially much more in hilly region and low in *T. robustus* of Tarai and Siwalik, whereas presence of chemicals in *T. albuminosus* and *T. microcarpus* were moderate mid hill and high in Tarai and Siwalik regions of the nation.

Table 13: Presence-absence of phytochemicals in different eco-zone of *Termitomyces* in Nepal.

Taxa	**Region**	**V**	**A**	**S**	**T**	**C**	**F**	**E**	**Fl**	**Co**	**An**	**Ant**	**Sa**	**Ta**	**G**	**P**	**Po**
T. albuminosus																	
Tarai	W	-	-	+++	+++	+++	+	+++	-	+	+++	+	+	+	+++	+++	+
	C	-	+++	+++	+++	+++	+++	+	+++	+++	+	-	+++	++	+++	-	++
	E	-	+	+	++	++	+++	+	+	-	-	-	+	++	+++	++	++
Siwalik	W	-	+	+	++	++	+++	+	+	-	-	-	+	++	+++	++	++
	C	-	+++	+	+	-	-	+++	-	-	-	-	+++	+	+	+	+
	E	-	+++	+	+++	+++	+	+	++	-	-	-	+++	++	++	+++	++
Midhill	W	-	-	+++	+++	+++	+	+++	-	+	+++	+	+	+	+++	+++	+
	C	-	-	+++	+++	+++	+	+	-	+	-	+	+++	+++	+	+	+
	E	-	+	+++	+++	+++	+	+	-	+	-	-	+++	-	++	+++	+
T. mammiformis																	
Tarai	W	-	-	-	+	-	+++	+	-	+	+	++	+	+++	-	++	++
	C	-	+++	++	+++	++	-	++	++	+	-	-	++	+++	++	-	-
	E	+	+++	-	+++	+++	+++	+	+	-	+	++	+++	++	+++	++	++
Siwalik	W	-	+++	++	++	++	+	+	++	++	+	+	+++	+++	+++	++	++
	C	-	++	+	+++	+++	+	+	+	+	-	+	+++	++	+++	+	+
	E	-	+++	++	+++	+++	++	+	++	+	+	+	+++	++	+++	++	++

Midhill	W	-	+++	++	+++	+++	++	+	+	+	+	++	+++	++	+++	++	++
	C	-	+++	++	+++	+++	-	-	+	++	-	-	+++	+++	+++	-	+
	E	-	+++	+++	+++	++	+	++	-	++	-	-	+++	++	++	+	+
T. microcarpus																	
Tarai	W	-	+	+++	+++	+	+++	-	-	-	-	-	+	++	+++	+++	+
	C	-	+++	+	+++	+++	-	++	+	-	-	-	+++	++	+++	++	++
	E	++	+++	-	+++	+++	++	-	+	-	+	++	+++	+	+++	-	-
Siwalik	W	-	+++	-	+++	+++	-	-	+	-	-	-	++	+	+++	+++	++
	C	-	++	+++	+++	+++	+	+	-	-	-	+++	+++	-	+++	+	-
	E	-	+	+	+++	+++	+++	++	++	-	-	-	+++	++	+++	+	++
Midhill	W	-	+	+	+	+	-	-	+	+	-	-	+	++	-	++	++
	C	-	+	+	+++	+++	+	+	+	-	+	-	-	++	+++	+	++
	E	-	+	+	++	++	+	+	+	-	++	-	-	++	++	+	++
T. robustus																	
Tarai	W	-	+	-	+++	+++	-	-	-	-	-	-	++	-	-	-	-
	C	-	+	-	+++	+++	-	-	-	-	-	-	++	+	-	-	-
	E	-	+	-	+++	+++	-	-	-	-	-	-	++	-	-	-	-
Siwalik	W	-	+	-	+++	+++	+	+	+	+	+	+	+++	++	+++	+	+
	C	-	+	-	+++	+++	+	+	+	+	+	+	+++	-	-	++	+
	E	-	++	-	+++	+++	+	+	+	+	+	+	++	++	++	+	+
Midhill	W	-	++	-	+++	+++	+	++	++	++	+	+	+++	+++	+++	++	++
	C	-	+++	-	+++	+++	+	++	++	+	+	+	+++	+++	+++	+	+
	E	-	+++	-	+++	+++	+	++	++	++	+	+	+++	+++	+++	++	++

Note: WT=Western Tarai; CT=Central Tarai; ET=Eastern Tarai; WS=Western Siwaliks; CS=Central Siwaliks; ES=Eastern Siwaliks; WM=Western Midhill; CM=Central Midhill and EM= Eastern Midhill.

Here, V= Volatile oil, A= Alkaloid, S= Steroid, T= Triterpenoid, C= Carotenoid, F=Fatty acid, E= Emodine, Fl= Flavonoid, Co= Coumarin, An= Anthracene, glycoside, Ant= Anthocyanadine glycoside Sa= Saponins, Ta= Tannins, G= Glycoside, P= Polyurenoids, Po= Polyoses.

Number of Phytochemicals along elevation and Physiographic zones

Number of phytochemicals (out of a total sixteen analyzed) present in four species taken along elevation and physiographic zones, the number of chemicals of *T. albuminosus* in west Nepal was highest along with south to north elevation and more or less gradually decreases from centre to east along with north to south gradients. In *T. mammiformis* and *T. microcarpus* in west Nepal was highest and gradually little bit decrease from west to east, where number was more in siwalik, then decreases from Tarai and Midhills respectively. In *T. robustus* west and centre of mid hills and siwalik was high then west, centre and east Tarai (Figure 37).

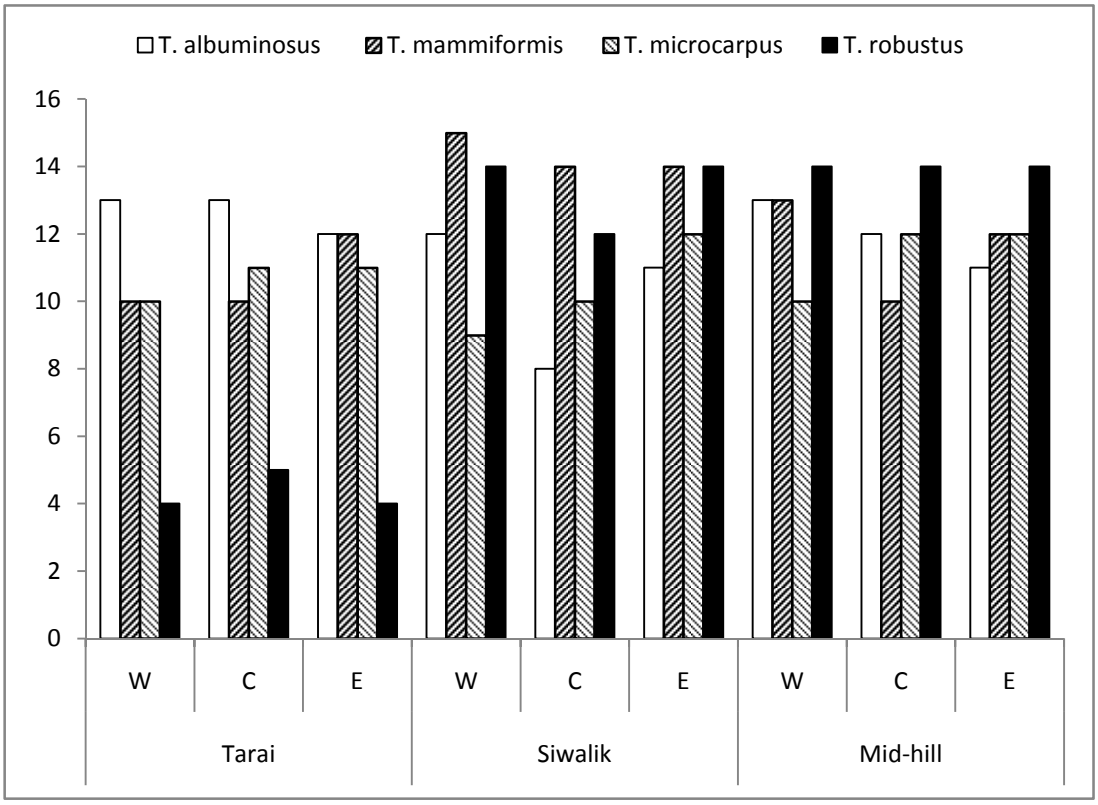


Figure 37: Number of phytochemicals present along elevation and physiographic zones.

Frequency of presence of phytochemicals

Frequency based on twenty-seven samples of each species (all together one hundred and eight) collected from different regions was analyzed (Appendix 5). Where its frequency of presence of volatile oil in *T. mammiformis* and *T. microcarpus* was lest; otherwise its frequency was zero in rest of the two samples. Similarly alkaloid in *T. microcarpus* and *T. robustus* was highest and followed by *T. mammiformus* and *T. albuminosus*. Steroid in *T. albuminosus, T. mammiformis* and *T. microcarpus* was in

moderate, while zero frequency in *T. robustus*. Triterpenoid in the entire tested sample was highest. Carotenoid in *T. microcarpus* and *T. robustus* was highest and in *T. albuminosus* and *T. mammiformis* was in higher. Fatty acid in *T. albuminosus* was higher and followed by *T. mammiformis* and *T. robustus***.** Emodine in *T. albuminosus* was highest and followed by *T. mammiformis, T. robustus* and *T. microcarpus.* Flavonoid in *T. mmmiformis* and *T. microcarpus* was higher and moderate in *T. robustus* and low in *T. albuminosus.* Coumerine in *T. mammiformis* was higher, and moderate in *T. robustus* and *T. albuminosus*, while least in *T. microcarpus.* Anthracene glycoside in *T. robustus* was moderate and followed by *T. mammiformis, T. albuminosus* and *T. microcarpus* among the tested samples. Anthocine glycoside in *T. robustus* was high and followed by *T. mammiformis*, *T. albuminosus* and least in *T. microcarpus*. Saponin in *T.* albuminosus, *T. mammiformis* and *T. robustus* were highest and higher in *T. microcarpus*. Tannin was highest in *T. mammiformis* and followed by *T. albuminosus* and *T. microcarpus* and moderate in robustus. Glycoside in *T. albuminosus* was highest, and higher in *T. mammiformis* and *T. microcarpus*, and moderate in *T. robustus*. Polyruenoids in *T. albuminosus* and *T. microcarpus* was higher and followed by *T. mammiformis* and *T. robustus* and frequency of presence of polyoses in *T. albuminosus* was highest and followed by *T. mammiformis T. microcarpus* and *T. robustus* (Figure 38) among the tested samples.

Over all frequency of presence of triterpenoid was highest and followed by alkaloid, carotenoid, emoidine, saponin, tannin, glycoside, polyruenoids, polyoses are in higher. Similarly steroid, fatty acid, flavonoids, coumerines are in moderate and volatile oil in least in the teasted samples.

In totality frequency of presence of phytochemicals in all four samples, triterpenoid was highest followed by carotenoids and saponins; then alkaloid, tannins, glycoside, polyreunoid & polyoses; then steroid, fatty acid, emoidine, flavonoid, coumerine; then anthracene glycoside, anthocine glycoside and least frequency of volatile oil were found, among the tested samples along the elevation and physiographic zones.

Likewise, there was significant difference in alkaloid, emodine, flavonoid, anthocyanadine, anthracene, saponins, tannins, glycoside, and marginal significance in triterpenoids, carotenoid and coumarin among the tested sample of *T. albuminosus* of three different eco-zones of east-west gradients of tropical to temperate region of Nepal. Similarly significant difference only found in saponins of three different eco-zones of north-south gradients (Table 14a). Likewise, there was significant difference

in alkaloid, steroid, triterpenoids, fatty acid, coumarin, anthracene glycoside and polyoses, contains of *T. mammiformis*, among the tested sample of three different eco-zones of north-south gradients of tropical to temperate region of Nepal. Similarly there was also significant difference in steroid, triterpenoids, fatty acid, emodine, anthracene glycoside, tannin, glycoside, polyurenoid and polyoses, contain among the tested sample of three different eco-zones of east-west gradients of tropical to temperate region of Nepal (Table 14b). Similarly there was significant difference in volatile oil, triterpenoids, carotenoid, fatty acid, emodine, flavonoid, coumarin, anthocyanadine glycoside, anthracene glycoside, saponins, glycoside, polyuren, polyoses, and marginal significance in tannins of *T. microcarpus*, among the tested sample of three different eco-zones of east-west gradients of tropical to temperate region of Nepal. Similarly there was also significant difference in all the chemicals contain among the tested sample of three different eco-zones of north-south gradients of tropical to temperate region of Nepal (Table 14c). Likewise, there was significant difference in fatty acid, emodine, flavonoid, anthocyanadine, anthracene and polyurenoid of *T. robustus* among the tested sample of three different eco-zones of east-west gradients of tropical to temperate region of Nepal. Similarly significant difference found in alkaloid, fatty acid, emodine, flavonoid, coumarin, anthocyanadine, anthracene, saponins, tannins, glycoside, polyurenoid and polyoses of three different eco-zones of north-south gradients (Table 14d).

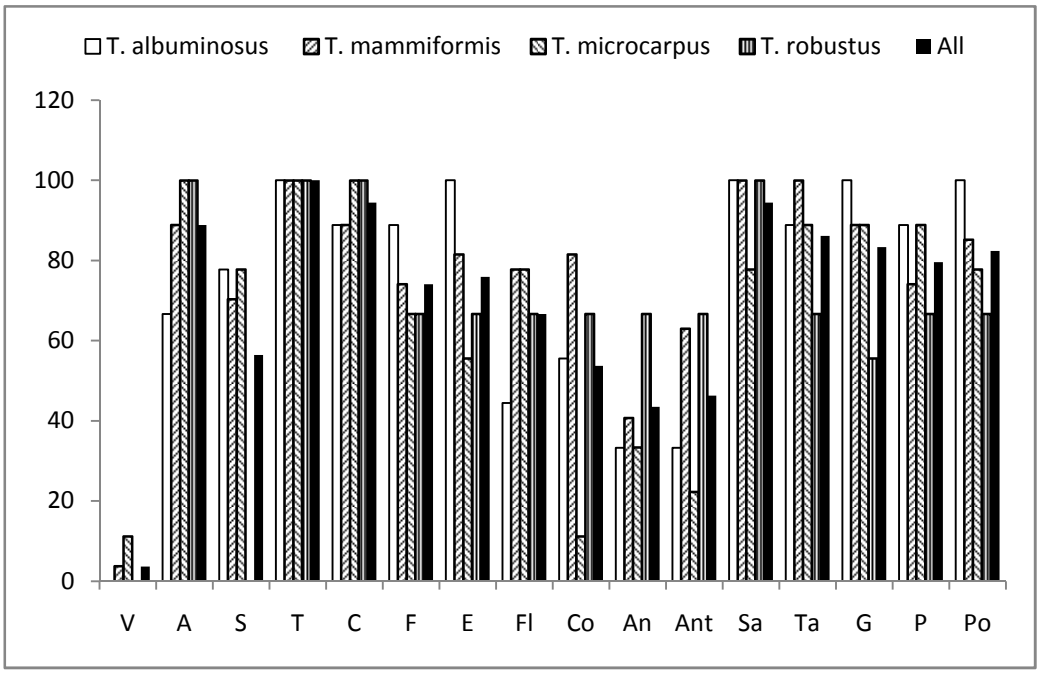


Figure 38: Frequency of presence of phytochemicals in *Termitomyces* species.

Table 14: Chi square test on variation of phytochemicals with different eco-zone.

Table 14a Results of P and X^2 of *T. albuminosus* on the variation of phytochemicals along with phytogeographic east-west (East, Central and West region) and north-south (Tarai, Siwaliks and Midhill belt) gradients with its frequency of each chemical at different eco zone.

East west gradient (East, Central and West regions)

Alkaloid	Carotenoid	Steroid	Triterpenoid	Fatty Acid	Emodine	Flavonoid	Coumarin
0.001	0.061	1.26	0.061	1.26	0.011	0.013	0.061
(18.0)	(9.0)	(7.2)	(9.0)	(7.2)	(9.0)	(16.2)	(9.0)
Anthocyanadine	**Anthracene**	**Tannins**	**Saponins**	**Glycoside**	**Polyurenoid**	**Polyoses**	**N**
0.0001	0.011	0.003	0.0001	0.0001	0.259	0.259	27
(21.0)	(9.0)	(19.5)	(18.9)	(28.0)	(2.7)	(2.7)	

North-South gradient (Tarai, Siwalik and Midhill belts)

Alkaloid	Carotenoid	Steroid	Triterpenoid	Fatty Acid	Emodine	Flavonoid	Coumarin
0.017	0.017	0.001	0.017	0.002	0.001	0.003	0.0001
(12.00)	(12.00)	(23.4)	(12.00)	(16.80)	(1.00)	(19.80)	(27.00)
Anthocyanadine	**Anthracene**	**Tannins**	**Saponins**	**Glycoside**	**Polyurenoid**	**Polyoses**	**N**
0.0017	0.011	0.006	0.259	0.029	0.036	0.005	27
(12.00)	(9.00)	(18.00)	(2.70)	(10.08)	(13.50)	(10.80)	

Table 14b Results of P and X^2 of *T. mammiformis* on the variation of phytochemicals along with phytogeographic north-south (Tarai, Siwaliks and Midhill belt) and east-west (East, Central and West region) gradients with its frequency of each chemical at different eco zone.

North-South gradient (Tarai, Siwalik and Midhill belts)

Volatile oil	Alkaloid	Carotenoid	Steroid	Triterpenoid	Fatty Acid	Emodine	Flavonoid	
0.354	0.001	0.126	0.019	0.012	0.003	0.137	0.134	
(2.077)	(18.00)	(1.20)	(15.15)	(12.857)	(19.442)	(6.975)	(9.791)	
Coumarin	**Anthocyanadine**	**Anthracene**	**Tannins**	**Saponins**	**Glycoside**	**Polyurenoid**	**Polyoses**	**N**
0.013	0.133	0.041	0.396	0.132	0.096	0.126	0.006	27
(12.646)	(9.80)	(9.975)	(4.077)	(7.067)	(7.875)	(9.964)	(18.25)	

East west gradient (East, Central and West regions)

Volatile oil	Alkaloid	Carotenoid	Steroid	Triterpenoid	Fatty Acid	Emodine	Flavonoid	
0.354	0.064	0.126	0.011	0.004	0.004	0.046	0.301	
(2.077)	(8.905)	(7.20)	(16.50)	(15.429)	(19.039)	(9.675)	(7.22)	
Coumarin	**Anthocyanadine**	**Anthracene**	**Tannins**	**Saponins**	**Glycoside**	**Polyurenoid**	**Polyoses**	**N**
0.423	0.133	0.016	0.007	0.132	0.024	0.0001	0.003	27
(3.877)	(9.8)	(12.225)	(14.115)	(7.067)	(11.25)	(24.857)	(19.75)	

Table 14c Results of P and X^2 of *T. microcarpus* on the variation of phytochemicals along with phytogeographic east-west (East, Central, and West region) and north-south (Tarai, Siwaliks and Midhill Region) gradients with its frequency of each chemical at different eco zone.

East west gradient (East, Central, West regions)								
Volatile oil	**Alkaloid**	**Carotenoid**	**Steroid**	**Triterpenoid**	**Fatty Acid**	**Emodine**	**Flavonoid**	
0.029	0.104	0.001	0.096	0.014	0.001	0.001	0.044	
(7.093)	(7.681)	(22.556)	(7.880)	(12.515)	(21.6)	(20.581)	(9.778)	
Coumarin	**Anthocyanadine**	**Anthracene**	**Tannins**	**Saponins**	**Glycoside**	**Polyurenoid**	**Polyoses**	**N**
0.049	0.012	0.009	0.056	0.001	0.014	0.001	0.032	28
(6.048)	(12.772)	(13.576)	(9.211)	(28)	(12.515)	(28.467)	(10.578)	
North-South gradient (Tarai, Siwalik and Midhill belts)								
Volatile oil	**Alkaloid**	**Carotenoid**	**Steroid**	**Triterpenoid**	**Fatty Acid**	**Emodine**	**Flavonoid**	
0.049	0.003	0.012	0.02	0.003	0.008	0.024	0.012	
(6.048)	(15.944)	(12.889)	(11.613)	(16.121)	(15.378)	(11.284)	(12.889)	
Coumarin	**Anthocyanadine**	**Anthracene**	**Tannins**	**Saponins**	**Glycoside**	**Polyurenoid**	**Polyoses**	**N**
0.029	0.016	0.014	0.016	0.001	0.003	0.005	0.005	28
(7.093)	(12.158)	(12.515)	(12.158)	(28.133)	(16.121)	(18.333)	(18.333)	

Table 14d Results of P and X^2 of *T. robustus* on the variation of phytochemicals along with phytogeographic east-west (East, Central and West region) and north-south (Tarai, Siwalik and Midhill belts) gradients with its frequency of each chemical at different eco zone.

East west gradient (East, Central and West regions)							
Alkaloid	**Carotenoid**	**Triterpenoid**	**Fatty Acid**	**Emodine**	**Flavonoid**	**Coumarin**	
0.126	3	3	0.0001	0.0001	0.0001	0.343	
(7.2)	(3)	(3)	(1)	(1)	(1)	(4.5)	
Anthocyanadine	**Anthracene**	**Tannins**	**Saponins**	**Glycoside**	**Polyurenoid**	**Polyoses**	**N**
0.001	0.001	0.174	0.259	0.174	0.001	0.343	27
(1)	(1)	(9)	(2.7)	(9)	(1)	(4.5)	
North-South gradient (Tarai, Siwalik and Midhill belt)							
Alkaloid	**Carotenoid**	**Triterpenoid**	**Fatty Acid**	**Emodine**	**Flavonoid**	**Coumarin**	
0.001	3	3	0.0001	0.0001	0.0001	0.0001	
(23.4)	(3)	(3)	(27)	(54)	(54)	(40.5)	
Anthocyanadine	**Anthracene**	**Tannins**	**Saponins**	**Glycoside**	**Polyurenoid**	**Polyoses**	**N**
0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	27
(27)	(27)	(42)	(18.9)	(27)	(30)	(40.5)	

5.5 Discussion

Utility of Termitophilous fungi

Collection and consumption of wild edible mushrooms is an important source of healthy nutrition for the rural communities during the rainy season in several parts of Africa, Asia, Europe and South America (Nakalembe, *et al.,* 2009). They are a good source of proteins, vitamins (C, B_1, and B_2) fats, carbohydrates, amino acids and minerals (Jiskani, 2001). The indigenous knowledge about their utility and consumption is transferred from one generation to other by the elders. There are number of reports of nutritional evaluation of mushrooms including termitophilous fungi from Africa, Asia (Mukiibi, 1973; Bother & Eicker, 1992; Adhikari, 2000; Pandey, 2008; Adejumo & Awosanya, 2005; Pushpa & Purushothama, 2010; Manjunathan & Kaviyarasan, 2011; Atri, *et al*., 2012), including Nepal. Nakalembe, *et al.,* (2009) reported that, among the *Termitomyces* species in Uganda, most favored species are *T. microcarpus, T. tyleranus, T. aurantiacus, T. eurhizus* and *T. clypeatus* (Nakalembe, *et al.,* 2009). During my investigation period all the species of *Termitomyces* throughout the country are highly appreciable by the local people. All the species of *Termitomyces* included in the thesis are being collected regularly by local inhabitants for personal consumption and some of them like *T. albuminosus, T. eurrhizus, T. heimii, T. robustus* and *T. striatus* for earning revenue as well. In India some of the *Termitomyces* species like *T. heimii* and *T. mammiformis* are collects by local people for earning revenue as well (Atri, *et al*., 2005). Present investigation was done four species of *Termitomyces,* were evaluated for their nutritional components followed by standard biochemical techniques (Table 10). The results obtained have been compared with similar such investigations by other workers (Aletor & Aladetimi, 1995; Adejumo & Awosanya, 2005; Oboh & Shodehinde, 2009; Nabubuya, *et al*., 2010; Ulziijargal & Mau, 2011).

Comparative study among the macronutrients

Adejumo and Awosanya (2005) reported highest protein content in *T. mammiformis* (36.8%) followed by *Lactarius triviralis* and other mushroom species. Higher amount of protein content has also been reported in *T. robustus* (33.8%) followed by other edible species (Aletor & Aladetimi, 1995). During the present investigation in

comparison, in *T. albuminosus* (30%) protein has been evaluated followed by *T. microcarpus* (24%), *T. mammiformis* (21%) and *T. robustus* (19%) (Table 10), which are less than documented by Adejumo and Awosanya (2005) in *T. mammiformis,* and Aletor and Aladetimi (1995) in *T. robustus*. Oboh and Shodehinde (2009) while working on *T. mammiformis* from Nigeria recorded (28.6%) protein in pileus and 24.6% protein in stipe, which is almost comparable with the present investigation. Masamba and Kazombo (2010) while working with *T. letestui* documented 3.9% protein, 0.7% fats and 0.5% fibers. In comparison the amount of carbohydrate, fat and fiber content is much more in all the presently worked out species of *Termitomyces of four species* from Nepal. The present results showed that *T. mammiformis* (48%) followed by *T. robustus* (40%) and *T. albuminosus* (32%) while lowest carbohydrate content was recorded in *T. microcarpus* (18%). The crude fiber content was highest in *T. robustus* (16% of dry weight) followed by *T. mammiformis* (14%), *T. albuminosus* (9%) while lowest crude fiber was recorded in *T. microcarpus* (8%) (Table 12). Adejumo and Awosanya (2005) documented (7.2%) fiber content in *T. mammiformis*, which is more than documented by Adejumo and Awosanya (2005) in *T. mammiformis* and Aletor and Aladetimi (1995) in almost comparable to the results arrived at with the Nepalese sample of *T. mammiformis* (14%) during the present investigation. Similarly the amount of moisture, Ash, fat, and dry matter contents were maximum in *T. microcarpus* (14%, 33%, 1%, 87%) followed by *T. albuminosus* 11%, 12%, 2%, 90%), *T. robustus* (10%, 11%, 1%, 91%) respectively whereas minimum quantities of moisture, Ash, fat, and dry matter has been recorded in *T. mammiformis* (10%, 8%, 1%, 90%) (Table 12).

Nabubuya, *et al*., (2010) while evaluating the nutritional properties of *T. microcarpus* reported (25.48%) protein, (2.32%) lipid and (11.21%) crude fiber. In the same species Mukiibi (1973) reported (27.4%) crude protein, (4.3%) fat, (2.2%) fibers, (14.1%) ash and (54.2%) carbohydrate in *T. microcarpus*. In comparison in the presently worked out Nepalese sample of *T. microcarpus* the amount of protein (24%), (Table 12), which is almost comparable with the results of Nabubuya *et al*. (2010) and Mukiibi (1973), while fat and lipid contents is low (1%) as compared with Nabubuya, *et al*., (2010) and Mukiibi (1973) and fiber (8%) content is low as compared with Nabubuya, *et al*., (2010) and high in compared with Mukiibi (1973) reports and carbohydrate content is much less (18%), and ash (33%) content is much

more as compared with Mukiibi (1973) reports in *T. microcarpus*. The crude fat percentage of 1% arrived at presently in *T. microcarpus* which is less than that of the values of 3.1-3.3 % obtained by Mendal (1898). As compared in all the species of *Termitomyces* evaluated presently, the percentage of proteins, fibers and ash is much more. The amount of protein present in the termitophilous mushrooms (19-30%) is much more than that of onion (1.4%), carrots (4%), peas (5.38 %), potatos (1.6%), orange (1.0%) and apple (0.3%) (FAO/WHO, 1989). Ulziijargal and Mau (2011) summarized the results of proximate studies of 38 fungi and 19 mycelial of medicinal mushrooms including *Termitomyces albuminosus*.

Micronutrients

Amongst the minerals investigated presently K content in *T. albuminosus* is highest (2170 mg/100g dry wt) followed by *T. microcarpus* (1927 mg/100g dry wt) and *T. mammiformis* & *T. robustus* (905 mg/100g dry wt). In addition *Termitomyces* species are quite rich in Ca which is 528 mg/100 g dry wt basis in *T. microcarpus* followed by *T. mammiformis* (170mg) *T. robustus* (105 mg) and minimum in *T. albuminosus* (47). Similarly *Termitomyces* species are rich in Mg, which is 208 mg/100 g on dry weight basis in *T. albuminosus* followed by *T. microcarpus* (72 mg), *T. mammiformis* (42) and minimum in *T. robustus* (35 mg/100 g). The amount of Fe, Zn and P contents were maximum in *T. mammiformis* (62, 6, and 9 mg/100 of dry wt) followed by *T. microcarpus* (38, 20 and 15 mg/100 g of dry wt), *T. albuminosus* (20, 14 and 11 mg/100 g of dry wt) respectively whereas minimum quantities of Fe, Zn and P has been recorded in *T. robustus* is minimum (17, 6 and 9 mg/100 g of dry wt). Na, contents were highest in *T. microcarpus* (19 mg/100 g of dry wt) it is followed by *T. mammiformis* (8mg/100g of dry wt), and *T. albuminosus,* and *T. robustus* (6 mg/100 g of dry wt). Mn contents were highest in *T. albuminosus* (8 mg/100 g of dry wt), followed by *T. mammiformis* (6 mg/100 g of dry wt), *T. microcarpus* (5 mg/100 g of dry wt) and minimum in *T. robustus* (3 mg/100 g of dry wt). Similarly Cu contents were highest in *T. albuminosus* and *T. mammiformis* (4 mg/100 g of dry wt) whereas minimum quantity of this element has been evaluated in *T. microcarpus* and *T. robustus* (3 mg/100 g of dry wt). While working with *T. microcarpus*, Nabubuya *et al.,* (2010) evaluated 1465 mg K, 221 mg Ca and 156 mg/100 gm P in this species. Presently it has been found that a termitophilous mushroom does absorb and accumulate toxic metals. Traces of some heavy metals viz. Fe (17-62 mg/100 g), Cu

(3-4 mg/100 g) Mn (3-8 mg/100 g) and Zn (6-20 mg/100 g) were found in the dry samples of termitophilous mushrooms (Table 12). The possible reason for this could be that the locations from where these were collected may be having some infestation of heavy metals. Mushrooms have been reported to possess very effective mechanisms that enable them to readily absorb heavy metals from their substrate (Isiloglu, *et al*., 2001). There are reports of high concentrations of heavy metals in the fruiting bodies of mushrooms collected adjacent to heavy metal smelters and oil polluted areas (Isiloglu, *et al*., 2001).

Termitomyces are an economically valuable natural resource serving as an alternative to plant and animal derived foods. Besides nutritional value, its many species possess medicinal properties and have industrial applications. For example, *Termitomyces clypeatus* possess a significant quantity of proteins (31%), carbohydrates (32%), ascorbic acid (10-14%) and antioxidants (Ogundana & Fagade, 1981; Tibuhwa, 2012a). It also produces a variety of enzymes in culture media useful as additives in food, in leavening of bread, in processing silage and in other industrial applications (e.g. clarification of non-citrus fruit juices) (Khowala, *et al*., 1992; Ghorai, *et al*., 2009).

Ample concentration of Secondary metabolites

Amongst the 16 phytochemicals evaluated (Table 13) the substantial amount of these compounds are found. Phytochemicals of alkaloid, steroid, triterpenoids, flavonoid, anthracene, saponins, tannins and glycosides are found in *T. reticulatus* by Loganathan, *et al.,* (2010). Fatty acids of *T. clypeatus* (Baraza, *et al.* 2007) and *T. letestui* play a significant role for the promotion of a variety of body biochemical function. These are potential nutritional food for individuals susceptible to diabetes (Arasmus, 1995).

‘Mushrooms contain low fat and oil’ (Okwulehie & Odunze, 2004). This statement also supports my experimental work. Because of it, they are recommended as good source of food supplement for patients with cardiac problems or at risk with lipid induced disorders. Mushrooms need antibacterial and antifungal compounds to survive in their natural environment (Lindequist, *et al*., 2005). Hence, they are rich sources of natural antibiotics, these wild mushrooms; possess inhibitory potential against bacteria associated with wastewater and leftover foods and also a potential

source of useful drugs. Many health promoting properties of mushrooms species are still unknown. This is because there is almost none information about these mushrooms and their medicinal potentials. *Termitomyces* species has ability to suppress postprandial hyperglycemia caused by prolonged high blood glucose level associated with diabetes (Moordian & Thurman, 1999). Phytochemicals are responsible for their nutritional and therapeutic uses. These results, therefore, not only make these wild edible mushrooms *T. albuminosus* (Berk.) R. Heim, *T. mammiformis* R. Heim *T. microcarpus* (Berk. & broome) R. Heim and *T. robustus* (Beeli) R. Heim, popular to consume as food sources but might also are valuable in drug development. Most important bioactive constituents of the plants are alkaloids, tannins, flavonoids and phelolic compounds (Cowan, 1999). These statements also support my investigation due to the presence of above mentioned secondary metabolite. Use of this mushroom is recommended for consumption. Due to the biological properties, it acts as a good dietary supplement as a functional food.

Bioactive compounds and their contained nutrients obtained may be responsible for their nutritional and therapeutic uses. Several ethnic wild foods used by ethnic groups over the generations are known to possess medicinal properties apart from the nutritional ones. During the field studies several such examples were observed where the specific foods are prescribed during some specific health ailment (Table 6). These results therefore not only make these wild edible mushrooms popular to consume as food sources but may also be valuable in drug development.

Kumari, *et al*., (2011ab) and Witkowska, *et al*., (2011) evaluated the ample concentration of carotenoids, alkaloids and flavonoids documented in *T. heimii, T. badius*, *T. striatus* and *T. radicatus* which emphasize the idea that for most of the antioxidant property of these mushrooms phenolic compounds may be the major contributors. Similar types of observations have been documented by Barros, *et al.,* (2007).

It is necessary to identify the biological and pharmacological potential of mushrooms especially of wild edible mushrooms, which are collected indigenously. It is also necessary to do research in identifying and isolating different species of mushrooms having nutraceutical and medicinal properties to commercialize. The production in large scale level would create a lot of employment opportunities especially in economically deprived rural area.

The plants which are rich in a wide variety of secondary metabolites belong to chemical classes such as alkaloid, triterpenoids, tannins; polyphenols are generally superior in medicinal property and exhibit physiological activity at a particular dose (Gurib, 2006). Most important bioactive constituents of the plants are alkaloids, tannins, flavonoids and phelolic compounds (Cowan, 1999). These statements support present investigation due to the presence of above mentions secondary metabolite. Due to the presence of phenolic as well as bioactive compounds it plays a significant role to develop the immunities strength in human health. Therefore, it is important to determine phytochemical content and to advocate their use as food. It was, therefore, felt necessary to evaluate the nutritional and even medicinal properties of wild edibles species of termitophilous mushrooms.

It is well known that several edible mushrooms, including *Termitomyces* species, contribute significantly to tribal food security in most of the village of the marginal people in the country. Documentation of information on edible, medicinal and different social and cultural practices associated with their use in ethnomedicinal practices in different parts of the country is very important to sensitize the communities about the value of these mushrooms.

5.6 Conclusion

All the investigated termitomycetes are edible and are being collected by local inhabitants for consumption. They have high nutritional and nutraceutical constituents. Documentation of these possess substantial amount of primary and secondary metabolites and negligible amount of heavy metals, which are well within the prescribed limits of edibility parameters as fixed by (FAO, 2012ab) is yet another property of these mushrooms which accounts for their nutritional credentials. All the *Termitomyces* are equally favored by the rural people. It contains different chemical concentration of bioactive compounds even in same ecological zone. Their concentration in order to affect by climatic variation. The co-relation between the traditional application and possession of secondary metabolites are useful to future workers to select a group of plants having similar chemical constituents to isolate biologically active principle or prepare remedies for particular case. Bioactive compounds with antibacterial properties can also be sourced from this underutilized termite mushrooms present in wild state.

CHAPTER 6

6. MYCELIALL GROWTH

6.1 Introduction

A growing organism is a changing entity, and attempt to estimate its size and composition at a particular time known as its growth rate. A fungus has wide range of structural variation so it is difficult to give some precise definition of its growth. Growth rate of fungi may be measured in terms of changes in number of cells, in linear dimensions, in cell mass, in cell volume or in amount of some cellular component (Bilgrami & Verma, 1981). Various factors such as humidity; temperature, pH, nutrients and other atmospheric condition, affects fungal growth, among them nutrients have significant influence on the mycelial growth (Hawker, 1957; Bilgrami & Verma, 1981; Kaul, 1999).

The conditions which permit spore formation are almost always of narrower range than those permitting mycelial growth. Thus, in general, spores growing are produced over narrower range of temperature, humidity, water content and pH of the substrate than those within which vegetative growth is possible. Most of the fungi which can show vegetative growth in the dark are unable to produce their spores in the absence of light. Conditions permitting the development of the 'perfect' spore stage usually differ far more from those sufficient for vegetative growth than those favoring asexual reproduction (Hawker, 1957).

Nutrition on fungal growth is the most important factor in the control of mycelial growth. Fungi received their food either by attacking living organisms host body, or by utilizing dead organic matter from the substrate. Kaul (1999) reported that the nutritional requirement for growth and reproduction of fungi are carbon, nitrogen, sulphur, phosphorus, vitamin and certain metallic or trace elements sources.

Because of scarcity of chlorophyll, fungi are not capable of photosynthesis nor do they include any forms comparable with the autotrophic bacteria, which are able to carry out this synthesis with the aid of chemical energy derived from the oxidation of inorganic compounds. Thus, they must all be supplied with suitable organic materials in the substrate (Hawker, 1957).

It has also been demonstrated that a number of metals, such as Fe, Zn, Cu, Mn etc., are required in such minute quantities that they are termed ‘trace elements’. Besides these essential nutrients, which are required by all fungi, traces of complex organic substances are necessary for growth of certain species, or strains, which are unable to synthesize them from simple carbohydrates and salts, Both trace elements and these organic ‘growth-substance’ have been shown to be, or are suspected to be, essential components of various enzyme systems (Hawker, 1957).

The nature, the concentration and the total quantity of the nutrient supply play a significant role in determining the time of initiation of mycelial growth, the quantity of mycelial produced and to some extent their form and size. The mycelium must obviously accumulate food material and synthesize sufficient complex substance (proteins, polysaccharides, fats, enzymes, etc.) from it (Hawker, 1957). Thus, to achieve maximum yield of fungal mycelium, optimum culture medium containing the nutrients in suitable amounts and combination should be formulated. Cultural characteristics optimization procedure aims at developing such a medium (Jackson, 1997).

The first step of using it in the best possible way strategy is the development of a defined or semi defined medium which supports good culture growth. Such medium is essential so that nutritional components of the medium can be varied and the impact of these changes assessed in terms of fungal growth. All of these factors must be considered during optimization. Initial studies are directed towards determining defined nutritional conditions which maximize growth. In general, significant biomass accumulation is necessary for optimal spore yield since spore production is usually dependent on the endogenous nutrients accumulated by the fungus during vegetative growth. Nutritional factors such as carbon, nitrogen, trace metals, vitamins and carbon-to-nitrogen ratio sources can all have an influence on growth. Once a defined medium has been developed which supports adequate growth, nutrients are varied in a directed way and their impact on spore yield and spore fitness can be assessed. The optimized defined medium serves as a nutritional framework from which a production medium can be formulated. In the production medium, the nutritional components of the defined medium are replaced with low-cost, complex substrates at less time. Use of this in the best possible way strategy aids in the development of production media for specific nutritional information which will be useful in developing production

media for commercial mycelial production which may be used as a source of nutrition and valuable products (Jackson, 1997). In this study, optimization of growth conditions for mycelial growth of *Termitomyces* species, an edible mushroom to Nepal was investigated.

Hypothesis

Artificial cultivation (especially myceliall growth) can be achieved in various supplements media (C, N, vitamins, amino acid and C: N ratio).

Objectives

- To identify the best nutritional source for myceliall growth
- To optimize the cultural media for the myceliall growth
- To raise the pure culture so as to domesticate and conserve the interesting taxa for further use.

6.2 Literature Review

Termitophilous mushrooms possess capability to produce lignocellulosic enzymes with potential to be efficient degrader of agro-wastes. Taprab, *et al*., (2005) postulated that symbiotic fungi viz. *Termitomyces* strain produces laccases which are potentially involved in fungus combs and facilitate mushroom growth. Subba (1975) observed that the effect of carbon and nitrogen sources on growth of *Choanephora infundibulifera* (Curry) D.D. Cunn. He was obtained the optimal myceliall growth at 26° C and in the pH 6.5. According to him among the carbon sources sucrose were the best and lactose supported poor growth. Similarly among the nitrogen sources ammonium nitrogen and aspartic acid were to be the best and nitrate nitrogen were moderate growth.

Chandra and Purukayastha (1977) studied and reported about growth requirements of *Agaricus campestris* L., *Lentinus subnudus* Berk., *Calocybe indica* P. & C., *Volvariella volvacea* (Bull.) Sing. and *T. eurrhizus* (Berk.) R. Heim. The optimal temperature and pH for growth varied from 25-30° C and from 4.0-6.5 respectively. Out of 19 carbon compounds, mannitol, dextrin, glucose, fructose and sorbitol were best utilized by *A. campestris, L. subnudus, C. indica, V. volvacea* and *T. eurrhizus*

respectively. Among 16 amino acids and amides tested glutamic acid was preferred by *A. campestris* and *V. volvacea*, while histidine was favored by *L. subnudus* and *C. indica. T. eurrhizus* showed best growth in a medium containing alanine. The ratio of carbon and nitrogen (C: N), and trace elements ($Cu2^{+}$, Fe 2^{+}, $Zn2^{+}$, and Mn 2^{+}) affected the growth of the test species significantly. Most of the test mushrooms utilized potassium and thiamine for their mycelial growth.

Shim, *et al*., (2003) observed and found out the fact that the optimal conditions for the myceliall growth of *Paecilomyces fumosoroseus* (Wize) A.H.S. Br. & G. Sm. were obtained at 25° C and in the range of pH 6-9 respectively. *P. fumosoroseus* showed the favorable growth on Hamada medium. The carbon and nitrogen source favorable for mycelial growth were dextrin and histidine, respectively. Optimum C: N ratio suitable for optimal growth of *P. fumosoroseus* was observed on the culture media adjusted to the ratio of 40:1.

Shim, *et al*., (2005a) studied cultural characteristics of *Macrolepiota procera* (Scop.) Sing., on several culture mediums, under different environmental conditions. They also compared mycelial growth on cultural media composed of various carbon and nitrogen source, and C: N ratio and found that the optimal conditions for the mycelial growth were 30°C and pH 7.0. M. procera showed rapid mycelial growth in the PDA media. The optimal carbon and nitrogen source were maltose and glycine respectively. The optimum C: N ratio was about 10:1 in case that 1% glucose was supplemented to the basal media as carbon source.

Shim, *et al*., (2005b) studied that *Cystoderma amianthinum*, one of the edible fungi belong to Agaricaceae of Basidiomycota, has a good taste and flavor. The optimal conditions for the mycelial growth were 25°C and pH 5 in PDA. *C. amianthinum* showed the favorable growth in the PDA and yeast maltextract agar (YMA). The favorable carbon and nitrogen sources promoting mycelial growth were fructose and histidine, respectively. The optimum C/N ratio was about 30: 1 in case that 1% glucose was supplemented to the basal medium as a carbon source.

Optimization of submerged culture conditions such as physical factors, chemical compounds and inoculums sizes for biomass production in *Pleurotus florida* Singer was investigated by Jonathan, *et al.,* (2006). This fungus produced the greatest

biomass at pH of 6.5 and 30°C. Among the monosaccharide, glucose stimulated the best biomass production. In the series of complex sugars and sugar alcohols, mannitol supported the highest biomass yield. The maximum biomass yield was obtained when 7.0 cm^3 of *P. florida* inoculums was inoculated into a submerged medium while the least biomass was obtained with 0.5 cm^3 of the inoculums.

Adebayo, et al., (2007) observed the effect of temperature, pH, vitamins and carbon source on the vegetative growth of *Schizophyllum commune* Fr. The result obtained revealed that this fungus had its optimum growth at 25°C. The best growth for the fungus was recorded at pH 5.5. Riboflavin and pyridoxine were the best growth promoting vitamins. Among the studied carbon, mannitol and sorbitol sources stimulated the best myceliall growth.

Imtiaj, *et al*., (2008) investigated and came to find that optimal growth conditions for the mycelial of 10 strains of *S. commune* Fr. The temperature range suitable for the myceliall growth and density was found to be 30~35° C. In case of pH, the most favorable growth was found at pH 5. Out of the tested carbon sources, dextrin and fructose were the most suitable and lactose, mannose and sorbitol were the most unsuitable for the fungus. Compact myceliall density was obtained from most of the carbon sources. Among the nitrogen sources, calcium nitrate, potassium nitrate and alanine were the most appropriate and the most incompatible were ammonium phosphate, histidine, urea and arginine for myceliall growth of *S. commune*.

Favorable myceliall growth conditions, such as pH, temperature, growth media, carbon sources and nitrogen sources were investigated by Jayasinghe, *et al*., (2008) for Korean strains in *Ganoderma lucidium* (Curtis) P. Karst. The most suitable temperature and pH for the myceliall growth was obtained at 30°C and 5 respectively. Dextrin, galactose and fructose were the best carbon sources for the growth of mycelial. The most suitable nitrogen sources were ammonium acetate, glycine, arginine and calcium nitrate.

Ayodele (2008) studied growth requirements of *Psathyrella atroumbonata* Pegler, a Nigerian edible mushroom. His study showed that glucose was the best carbon source for *P. atroumbonata*. The least growth was observed in lactose and cellulose. Yeast extract, an organic nitrogen source, proved to be the best nitrogen source as Ayodele

has studied in (2008). Thiamine was the best vitamin among the vitamins tested. This was followed by nicotinic acid and riboflavin. The least growth was observed in folic acid. Asparagine proved best among the amino acids tested followed by aspartic acid and least stimulatory amino acid was glycine. Carbon to nitrogen ratio of 4: 1 and 1: 4 stimulated good myceliall growth.

Pokhrel, *et al*., (2009b) investigated the effect of light, moisture, amino acids, vitamins and mineral nutrients on myceliall growth of the medicinal mushroom *Lyophyllum decastes* (Fr.) Singer on solid media. Glutamic acid was the best amino acid among the eight amino acids tested. Riboflavin was the best vitamin among the vitamins tested.

The growth requirements of *Lentinus tuber-regium* (Fr.) Fr., were studied by Manjunathan and Kaviyarasan (2010), an Indian edible mushroom for optimization using different carbon, nitrogen, vitamins and amino acids followed by different C: N ratios and showed that there was a significant increment on biomass of mycelium with the amendment of dextrose, yeast extract, thiamine and glycine. Different rational supplement of dextrose and yeast extract confirmed the effective myceliall formation with 1: 3 and 1:5 ratios.

Lai, *et al*., (2011) investigated the cultural characteristics of *Lignosus rhinoceros* (Cooke) Ryvarden, under different environmental conditions. They compared myceliall growth of this mushroom on culture media composed of various carbon and nitrogen sources in addition to C:N ratios and reported that the optimal conditions for myceliall growth were 30°C, at pH 6 and 7. Rapid myceliall growth of L. rhinoceros was observed on glucose-peptone and yeast extract-peptone-dextrose media. Carbon and nitrogen sources promoting myceliall growth of L. rhinoceros were glucose and potassium nitrate respectively. The optimum C: N ratio was approximately 10: 1 using 2% glucose supplemented as a carbon source in the basal media.

Adebayo, *et al*., (2011) investigated optimum culture condition for myceliall growth and exopolysaccharides (EPS) production by *Pleurotus ostreatus* (Jacq.) P. Kumm in submerged liquid culture. The optimum temperature for mycelial yield and EPS production was 25°C and 15° C in day 14 with yields of 1.40 $gm.l^{-1}$ and 2700 $mg.l^{-1}$ respectively. Optimum pH was found to be 8 with respective yields of 2.5 $gm.l^{-1}$ and 2665 $mg.l^{-1}$ in day 12 for mycelial and EPS. Mannitol (9.75$gm.l^{-1}$) and raffinose

stimulated the highest mycelium growth (3.0g.ml^{-1}) and EPS production (3000 mg.l^{-1}) at day 14 respectively. Mycelial yield (2.10gm.l^{-1}) and EPS production (2700mg.l^{-1}) was optimally supported by urea. Glycine (1.0g) and leucine (1.0g) supported optimum production of mycelial yield (2.5 g.ml^{-1}) and EPS concentration (2925 mg.l^{-1}) respectively.

Poudel (2012) repotted the optimization of cultural media for myceliall growth of *Volvariella taylorii* (Berk. & Broome) Singer, an edible mushroom of Nepal. He was obtained the optimal myceliall growth at 25 ± 2° C. According to him among the carbon sources maltose and sucrose, in nitrogen yeast extract and peptone, in vitamin serine, in amino acid nicotinic acid and in carbon to nitrogen ratio C_5:N_1, C_1: N_4, and C_1: N_5 were the best growth. Similarly Acharya (2012) in his experiment "A study on myceliall growth pattern of *Amanita chepangiana*, a wild edible Mushroom, on Different Nutrient Sources" repoted that among the carbon sources malt extract were the optimum growth at 22 ± 2° C. Similarly in nitrogen, sodium nitrate; in vitamins ascorbic acid; in amino acid, arginine and in carbon to nitrogen ratio C_4: N_1 were the optimum growth.

6.3 Materials and Methods

Exhaustive survey of various localities was conducted for the collection of materials in order to explore the domestication aspect of investigated taxa from the lab. For this 4 selected species were inoculated in culture tube on the field and brought in the lab for the process. Its details also provided in materials and methods of the capter-2.

6.3.1 Laboratory studies

The specimens (inoculated culture tubes) were brought to the Lab, CDB, TU, for the myceliall growth and were subjected to culture process. They were done experimentally using semi solid and liquid media and adopting the standard measurement and weighing procedures, to understand the effects of various carbon, nitrogen, vitamins, amino acids and its Carbon/Nitrogen ratio sources. Measurements of linear growth on semi-solid medium are done by means of a standard scale. Weighing of myceliall mass growth in liquid medium is done by a sophisticated digital chemical balance.

Domestication on pure culture of growth of fungal mycelium

Domestication of the selected mushroom species by pure culture technique were been done experimentally using semi solid and liquid media and adopting the standard measurement and weighing procedures, to understand the effects of various sources of carbon, nitrogen, vitamins, amino acids and C: N ratio on myceliall growth of four species of *Termitomyces*. Measurements of linear growth on semi-solid medium are done by means of a standard scale. Weighing of myceliall mass growth in liquid medium is done by a sophisticated digital chemical balance.

6.3.1.1 Sterilization of Instruments

The glass wares (petriplates, watch glass, glass rod, conical flask, test tube etc.), were sterilized in hot air oven. They were cleaned at first with the help of detergent, rinsed with the help of D/W, and then allowed to air dry. Then, they were wrapped in aluminum foil and sterilized in the hot air oven at 160-170°C for 2 hrs. The PDA media, basal media, cotton etc. were sterilized in an autoclave. For sterilization in the autoclave, the temperature was maintained at 121° C and 15 psi pressure for about 30 min.

6.3.1.2 Isolation on PDA media

All four species was selected, which had fresh and healthy. It was surface sterilized by submerging in 0.4 % sodium hypochlorite (NaOCl) for 1 min and was washed 4 times with sterilized water to remove residual NaOCl. Using a sterilized blade, its pileus and stipe were separated. The pileus portion was discarded. The stipe was split longitudinally into two equal halves and approximately 3 × 6 mm pieces of tissue were taken from depth of $1/4^{th}$ thickness of the upper end of the stipe so that they contained neither outermost portion nor the central tissues of the stipe. The tissue pieces were inoculated in the PDA plates. The plates were sealed with parafilm taped and were covered with the help of aluminum foil.

The inoculated petriplates were incubated at 25 ± 2°C in inverted position for seven days. After seven days, mycelium of the mushroom was observed to grow out of the inoculated tissue. Sub-cultures of the mycelium were done to obtained pure cultures. After obtaining pure cultures, it was transferred to PDA/PD slants for further study.

6.3.2 Media preparation

Vegetative growths of mycelium of the selected mushroom species were done experimentally by adopting the standard procedures, as described by Chandra and Purkayastha (1977); Fasidi and Olorunmaiye (1994). Study for the development mycelium of these mushrooms, cultural characteristics was investigated on different culture media on both semisolid and liquid condition, where composed of four important sources viz. Carbon (Dextrose, Lactose, Sucrose, Fructose, Mannitol, Maltose and Control), Nitrogen (Ammonium Nitrate, Peptone, Urea, Sodium Nitrate, Calcium Nitrate, Yeast extract and Control), Vitamins (Ascorbic acid, Nicotinic acid, Folic Acid, Thiamine, D-priotine and Control), Amino acids (Serine, Leusine, Valine, Glutamic acid, Arginine, Aspartic acid and Control), including C: N ratio source was applied under five treatments in each experiment, with their five replication. Mycelial growth was measured, after seven days by following Shim, *et al.*, (2005ab). The maximum and minimum diameters of the colony were also measured. The texture of the colony (compact, somewhat compact, thin, somewhat thin) was noted down.

6.3.2.1 Semisolid medium

Media preparation was done by following Chandra and Purkayastha (1977) and Shim *et al.* (2005a). The basal media and supplementary compounds were dissolved in 1 litre of distilled water. The media were sterilized by autoclaving at 15 psi and 121° C for half an hour. Then they were poured into petriplates, 20 ml per plate (approximately) and were allowed to solidify. The media, after cooling, were inoculated at the centre with 5 mm diameter agar blocks of seven-days-old mycelium colony of four species of *Termitomyces* and were incubated at 25 ± 2°C for 7 days. Each experiment was replicated five times. The medium were prepared as the composition of Potato 200 g, dextrose 15 g, Agar 20 g and distilled water 1 liter.

Potatoes were rinsed under running water and were added to 1 liter water in 2 liter conical flask. It was boiled for 1 hr. with constant stirring. The resulting extract was passed through fine sieve (muslin cloth) squeezing through as much pulp as possible. Agar was added to the extract and was boiled with constant stirring till agar dissolved. Dextrose was added and the extract was stirred till it dissolved. The total volume was made 1 liter by adding required amount of water. The resulting media was autoclaved at 15 psi for 20 min.

6.3.2.1a Carbon sources

Selected carbon sources were dextrose, maltose, fructose, sucrose, lactose and mannitol. The basal medium used consisted of 2.0 g of peptone, 0.5 g of KH_2PO_4, 0.5 g of $MgSO_4.7H_2O$ and 20 g of agar. Five gram of each carbon source was incorporated into the basal medium and distilled water was added to make 1liter. Basal medium alone without the carbon source was used as control. The media were heated with constant stirring till the agar was dissolved and then, pH was maintained at 6 by adding 1N HCl or NaOH solution. They were then sterilized by autoclaving at 15 psi pressure at 121°C for 30 minutes and were poured into 9 cm petriplates, 20 ml per plate (approximately). After the media plates cooled down, 5mm discs of inoculums were taken from 7-days-old colony of four species of *Termitomyces* and were placed at the centre of the media plates, one disc per plate. Then the inoculated petriplates were incubated at 25 ± 2°C for seven days.

6.3.2.1b Nitrogen sources

The nitrogen compounds selected were calcium nitrate, sodium nitrate, ammonium nitrate, peptone, yeast extract and urea. The basal medium used consisted of 10 g of fructose, 0.5 g of KH_2PO_4, 0.5 g of $MgSO_4.7H_2O$, 500 µg of Thiamine hydrochloride and 20 g of agar. Two gram of each nitrogen source was incorporated into the basal medium and distilled water was added to make 1 liter. Basal medium alone without the nitrogen source was used as control. The media were heated with constant stirring till the agar was dissolved and then, pH was maintained at 6 by adding 1 N HCl or NaOH solution. They were then sterilized by autoclaving at 15 psi pressure at 121°C, for 30 minutes and were poured into 9 cm petriplates, 20 ml per plate (approximately). After the media plates cooled down, 5 mm discs of inoculums were taken from 7-days-old colony of four species of *Termitomyces* and were placed at the centre of the media plates, one disc per a plate. Then the inoculated petriplates were incubated at 25 ± 2°C for seven days.

6.3.2.1c Vitamin sources

Vitamins selected were nicotinic acid, D-pirovic acid, ascorbic acid, folic acid and thiamine. The basal medium was the same as used for determination of nitrogen compounds. Each vitamin was added 500 µg to the basal medium and the final volume was made up to 1 liter. The set was treated the same way as for carbon and nitrogen. Basal medium alone was used as control.

6.3.2.1d Amino acids sources

The amino acids selected were arginine, aspartic acids, glutamic acid, valine, serine and leucine. The basal medium used was the same as that of nitrogen source. For each amino acid 500µg was added to the basal medium and made up to 1 liter. The setup was treated the same way as for carbon and vitamin sources described above. Basal medium alone without amino acid was used as control.

6.3.2.1e Carbon to nitrogen ratio (C: N) sources

The basal medium was similar to that used for nitrogen compounds. Glucose as a carbon source and yeast extract as a nitrogen source were added to the basal medium in varied ratios as per requirement. A concentration of 0.15 $g.L^{-1}$ of each of glucose and yeast extract in the basal medium served as 1: 1 ratio (Fasidi & Olorunmaiye, 1994). Other ratios (C: N=2:1, 3:1, 4:1, 5:1, 1:2, 1:3, 1:4 & 1:5) were prepared proportionately. The set up was treated the same way as for carbon, nitrogen, amino acids and vitamins sources. Basal medium alone was used as control.

6.3.2.2 Liquid medium

Here, submerge culture using of conical flask instead of petri-plate and the media were prepared without the addition of agar, rest all the setup was treated the same way as semisolid medium for carbon, nitrogen, vitamins, amino acid and C/N ratio sources as described above.

6.3.2.2a Carbon sources

In case of liquid medium there were no agar and the autoclaved (sterilized) media were poured on 250 ml sterilized conical flask, 20 ml per flask (approx.). After the media flask cooled down, 5mm discs of inoculums were taken from 7-days-old colony of four species of *Termitomyces* and were placed separately on tagged flask each, one disc per flask. Then the inoculated conical flasks were incubated at 25 ± 2°C for seven days. Each experiment was replicated five times.

6.3.2.2b Nitrogen sources

The setup was treated the same way as liquid medium for carbon, sources as described above.

6.3.2.2c Vitamin sources

The setup was treated the same way as liquid medium for carbon and nitrogen sources as described above.

6.3.2.2d Amino acids sources

In case of liquid medium, the setup was treated the same way as liquid medium for carbon, nitrogen and vitamin sources as described above.

6.3.2.2e Carbon to nitrogen ratio (C: N) sources

In case of liquid medium, the setup was treated the same way as liquid medium for carbon, nitrogen, vitamins and amino acid sources as described above.

6.3.3 Measurement of mycelial growth

Measurement of the mycelial growth, of liquid medium was done after seven days by following Shim *et al.* (2005a). Whereas in semisolid medium measurement were taken every 24 hrs of intervals of time till 168 hrs (seven days) or up to the coverage by the mycelium on 9 mm dia of petri-plate. The maximum and minimum diameters of the colony were measured. The texture of the colony (compact, somewhat compact, thin, somewhat thin) was also noted.

6.3.4 Statistical Analysis

To analyze the data and growth management the statistical tool were used standard error (SE) of means significance test etc. using SPSS 20.0 version. One Way Analysis of Variance (ANOVA) followed by multiple range test, post-hoc and Tukey HSD Test were performed to compare the results of different treatments.

6.4 Results

The different nutrient sources used in this research and their effects on mycelial growth of four species of *Termitomyces* are listed below:

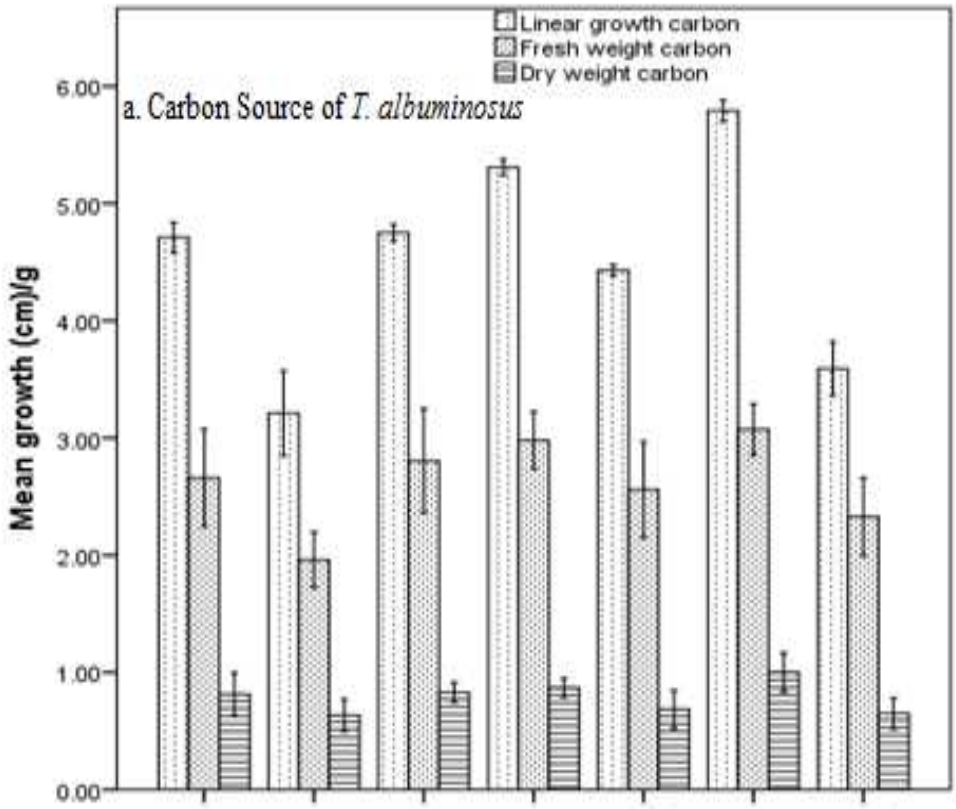
6.4.1 Effect of Carbon sources on growth of *Termitomyces* spp.

Termitomyces albuminosus

The effect of different carbon sources on linear growth of *T. albuminosus* was varied significantly with change in sources at 5% level of significance; it showed differential preferences for carbon sources for its metabolism. However, utilization varied from one carbon source to another. The maximum linear growth was observed in maltose with somewhat compact mycelial density while the least growth was observed on lactose with compact mycelial density ($p<0.05$). However, in control medium, mycelial growth was better than in lactose. Dextrose, fructose, mannitol and sucrose also showed optimum growth with somewhat compact myceliall density and better growth than control (Figure 39a, Photo plate 1). While both fresh and dry weight of mycelium in liquid medium did not vary significantly with change in carbon sources at 5% level of significance. It might be due to the agitation, lack of orbital incubation or continuation back up (Appendix 7a).

Termitomyces mammiformis

The effect of different carbon sources on linear growth of *T. mammiformis* was varied significantly with change in sources at 5% level of significance; it showed differential preferences for carbon sources for its metabolism. However, utilization varied from one carbon source to another. The best linear growth was observed in maltose with somewhat compact mycelial density while the least growth was observed on lactose with compact mycelial density ($p<0.05$). However, in control medium, mycelial growth was better than in lactose. Dextrose, mannitol and fructose also showed good growth with somewhat compact mycelial density and growth than control (Figure 39b). While both fresh and dry weight of mycelium in liquid medium did not vary significantly with change in carbon sources at 5% level of significance. It might be due to the agitation, lack of orbital incubation or continuation back up (Appendix 7b).


Linear growth carbon
Fresh weight carbon
Dry weight carbon
a. Carbon Source of T. albuminosus
Mean growth (cm)/g
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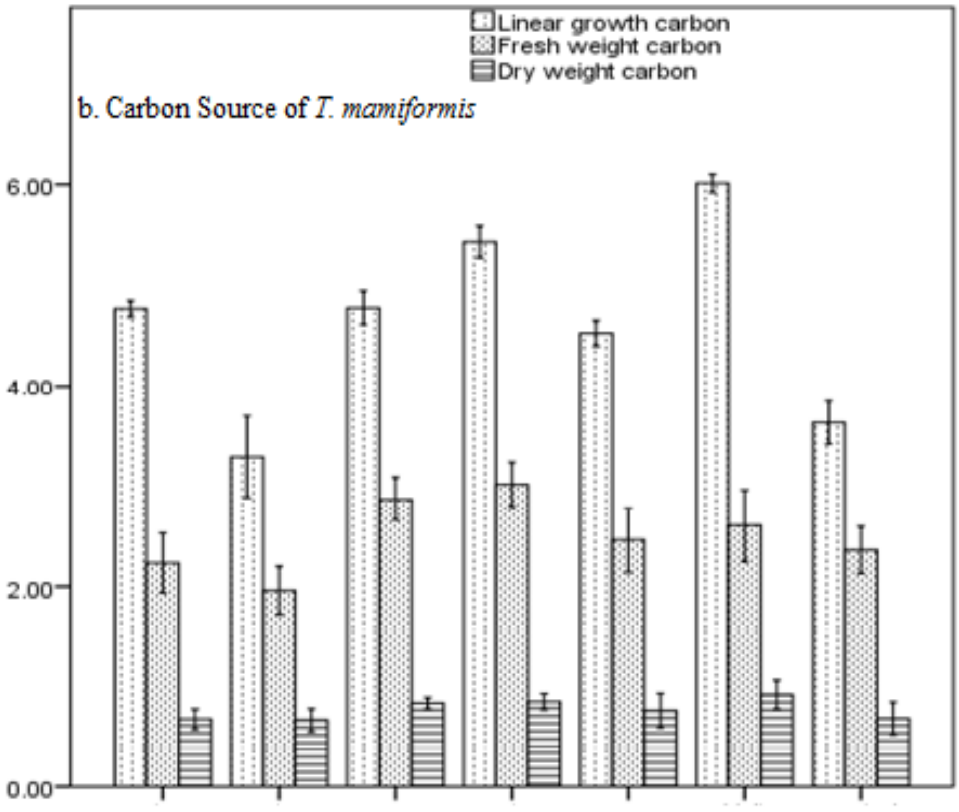
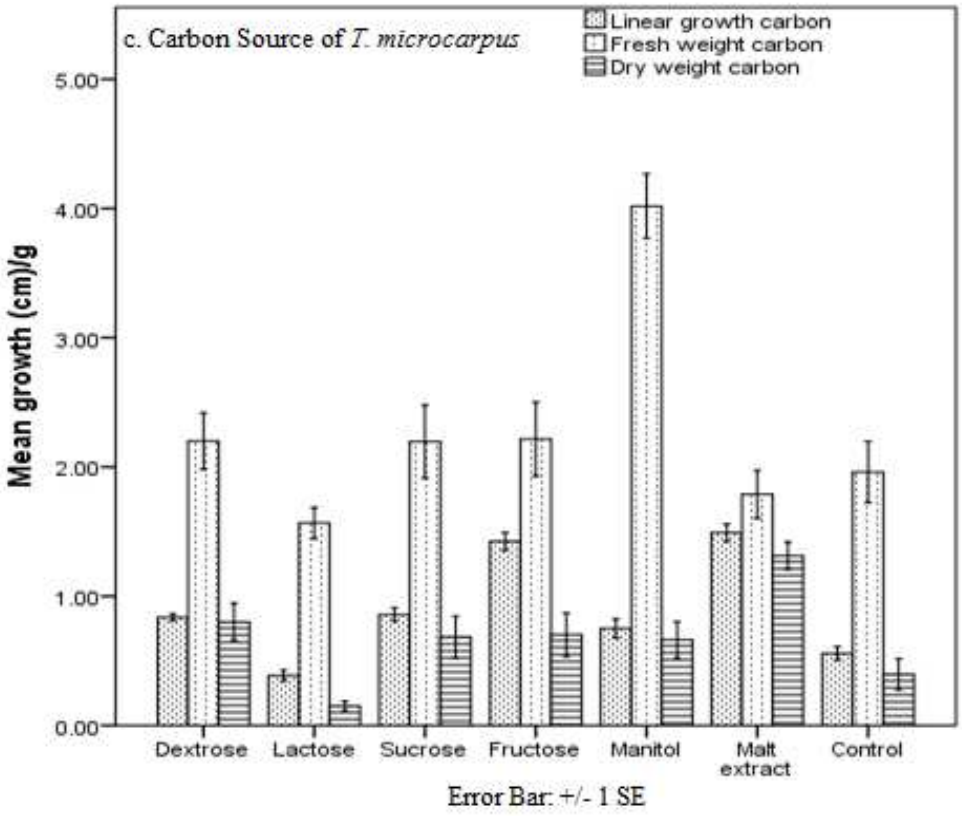
5.00
4.00
3.00
2.00
1.00
0.00


Linear growth carbon
Fresh weight carbon
Dry weight carbon
b. Carbon Source of T. mamiformis
6.00
4.00
2.00
0.00



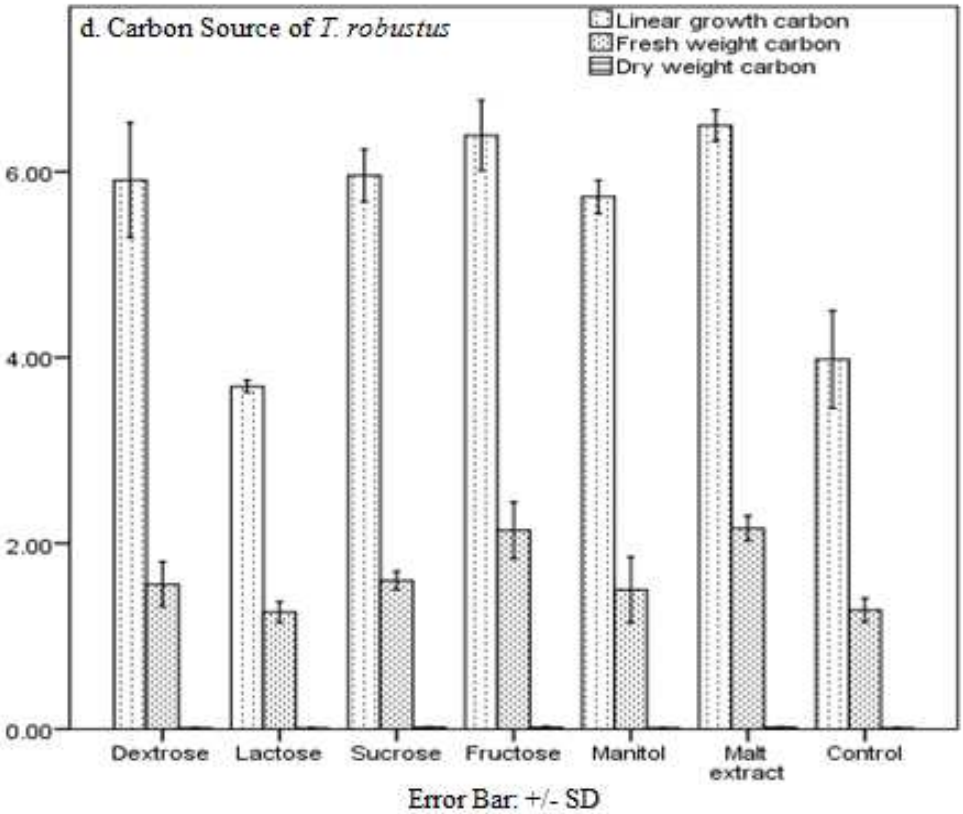


Figure 39: Mycelial growth of *Termitomyces* on different carbon sources; One-way ANOVA, Tukey HSD Test ($p<0.05$), multiple range and mean test.

Termitomyces microcarpus

The effect of different carbon sources on linear growth of *T. microcarpus* was varied significantly with change in sources at 5% level of significance; it showed differential preferences for carbon sources for its metabolism. However, utilization varied from one carbon source to another. The best linear growth was observed in maltose with somewhat compact mycelial density while the least growth was observed on lactose with compact mycelial density ($p<0.05$). However, in control medium, mycelial growth was better than in lactose and mannitol. Dextrose, fructose and sucrose also showed good growth with somewhat compact mycelial density and growth than control (Figure 39c). While both fresh and dry weight of mycelium in liquid medium did not vary significantly with change in carbon sources at 5% level of significance. It might be due to the agitation, lack of orbital incubation or continuation back up (Appendix 7c).

Termitomyces robustus

The effect of different carbon sources on linear growth of *T. robustus* was varied significantly with change in sources at 5% level of significance; it showed differential preferences for carbon sources for its metabolism. However, utilization varied from one carbon source to another. The best linear growth was observed in maltose with somewhat compact mycelial density while the least growth was observed on lactose with compact mycelial density ($p<0.05$). However, in control medium, mycelial growth was better than in lactose. Mannitol also showed optimum growth with somewhat compact mycelial density than lactose and control (Figure 39d). Similarly both fresh and dry weight of mycelium in liquid medium also varied significantly with change in carbon sources at 5% level of significance. The maximum fresh weight was also observed in maltose, while the minimum in lactose. However, in control medium, fresh weight of mycelial was better than in lactose. Sucrose also showed optimum growth with somewhat compact mycelial density than control. The maximum dry weight was observed in fructose, maltose and sucrose, and minimum was observed in lactose. However, in control medium, in dry weight of mycelial was better than in lactose (Appendix 7d).

6.4.2 Effect of Nitrogen sources on *Termitomyces* spp.

Termitomyces albuminosus

The effect of different nitrogen sources on linear growth of *T. albuminosus* was varied significantly with change in sources at 5% level of significance. The best linear growth was seen in yeast extract with compact mycelium density ($p<0.05$). However, in urea, least growth was observed with compact mycelial density (Figure 40a, Photo plate 2).

Similarly both fresh and dry weight of mycelium in liquid medium also varied significantly with change in carbon sources at 5% level of significance. The maximum fresh weight was observed in yeast extract while minimum in urea. However, in control of mycelial was better than in urea also showed optimum growth with somewhat compact mycelial density than control of ammonium nitrate, calcium nitrate and peptone. Likewise the maximum dry weight was observed in yeast extract while minimum in urea. However, in control dry weight of mycelial was better than in urea (Appendix 8a).

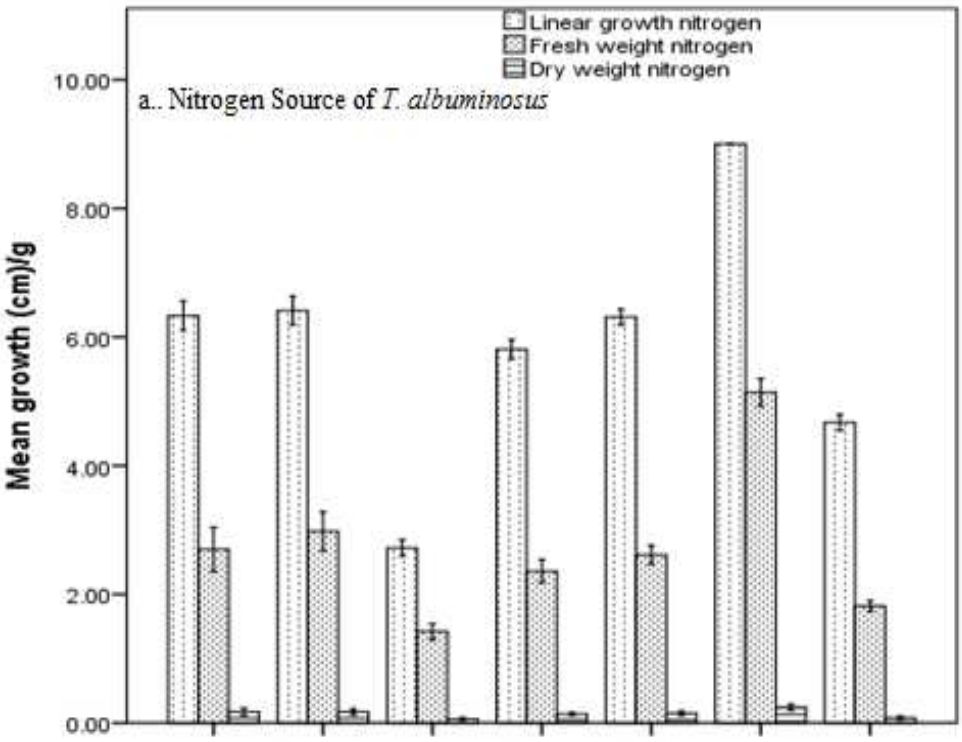
Termitomyces mammiformis

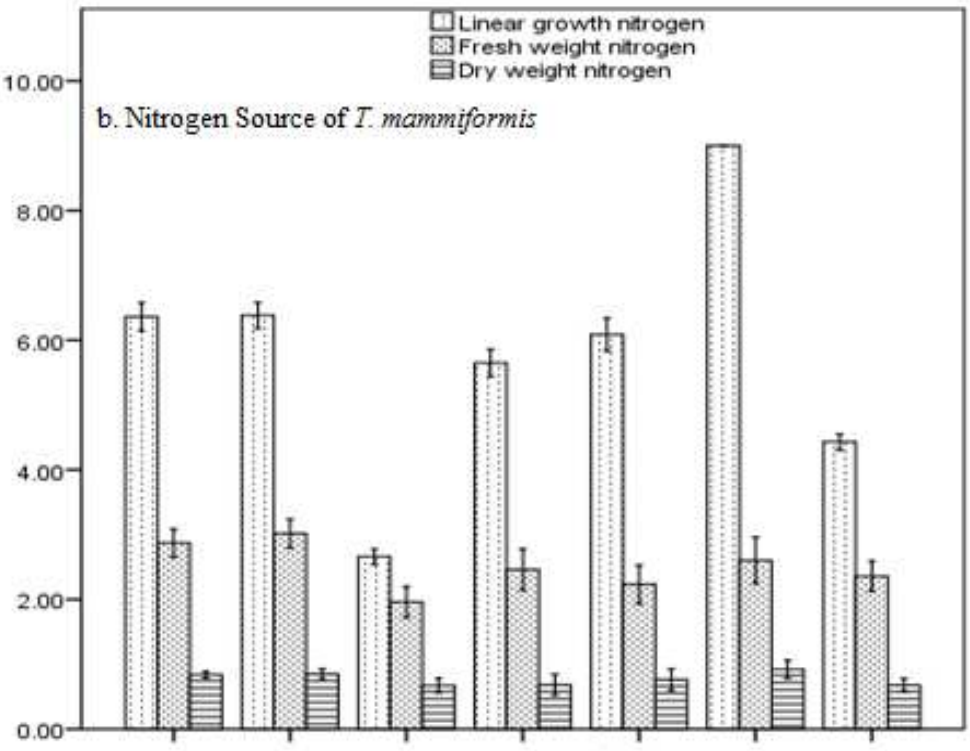
The effect of different nitrogen sources on linear growth of *T. mammiformis* was varied significantly with change in sources at 5% level of significance. The best linear growth was seen in yeast extract with compact mycelium density ($p<0.05$) while, in urea, least growth was observed with compact mycelial density (Figure 40b). However, in control of mycelial was better than in urea also showed optimum growth with somewhat compact mycelial density than control of ammonium nitrate, calcium nitrate and peptone and sodium nitrate. However, both fresh and dry weight of mycelium in liquid medium was did not varied significantly with change in nitrogen sources at 5% level of significance (Appendix 8b).

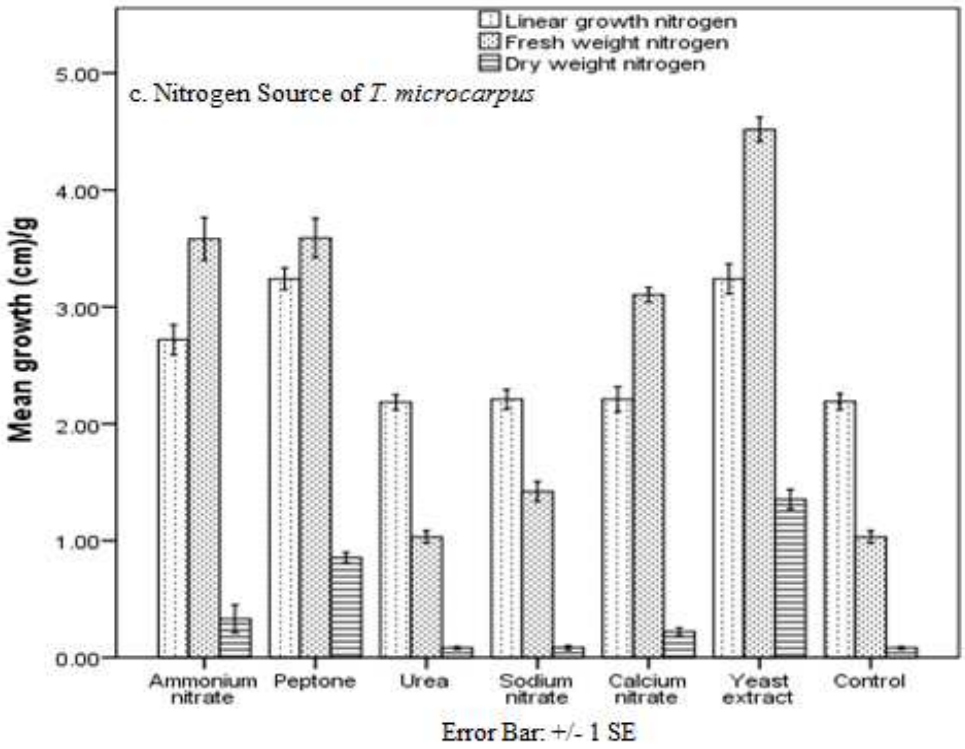
T. microcarpus

The effect of different nitrogen sources on linear growth of *T. microcarpus* was varied significantly with change in sources at 5% linear growth of *T. microcarpus* was varied significantly with change in sources at 5% level of significance. The best linear growth was seen in yeast extract with compact mycelium density ($p<0.05$) while, in

urea minimum growth was observed with compact mycelial density (Figure 40c). However, in control of mycelial was better than in urea also showed optimum growth with somewhat compact mycelial density than control of ammonium nitrate.







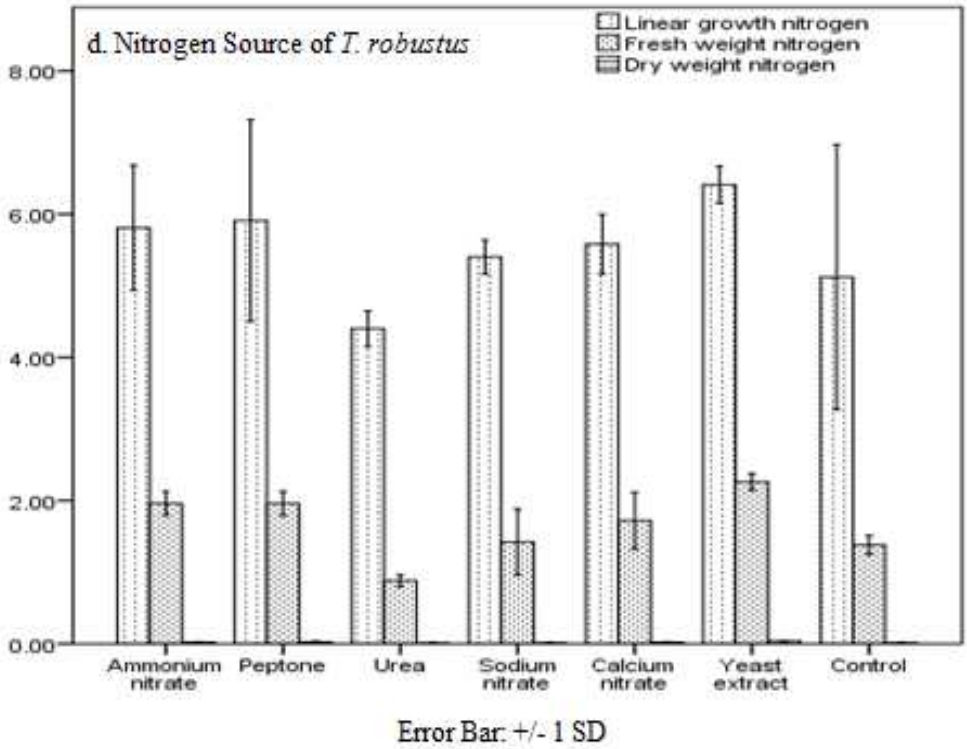


Figure 40: Mycelial growths of *Termitomyces* spp. on different nitrogen sources; One-way ANOVA, Tukey HSD Test ($p<0.05$), multiple range and mean test.

Similarly both fresh and dry weight of mycelium in liquid medium also varied significantly with change in nitrogen sources at 5% level of significance. The maximum fresh weight was observed in yeast extract while the minimum urea and control. However, in control of mycelial had equal to urea also showed optimum growth with somewhat compact mycelial density than control of ammonium nitrate, calcium nitrate and peptone. Likewise the maximum dry weight was observed in yeast extract while minimum in urea and control. However, in control, dry weight of mycelial had equal to the urea (Appendix 8c).

Termitomyces robustus

The effect of different nitrogen sources on linear growth of *T. robustus* was varied significantly with change in sources at 5% level of significance. The maximum linear growth was seen in yeast extract with compact mycelium density ($p<0.05$). However, in urea were observed minimum growth with compact mycelial density (Figure 40d). However, in control of mycelial was better than in urea.

Similarly both fresh and dry weight of mycelium in liquid medium also varied significantly with change in nitrogen sources at 5% level of significance. The maximum fresh weight was observed in yeast extract, while minimum in urea. However, in control media of mycelial was greater than urea also showed optimum growth with somewhat compact mycelial density than control of calcium nitrate and sodium nitrate. Likewise the maximum dry weight was observed in yeast extract while minimum in urea. However, in control, dry weight of mycelial was greater than the urea (Appendix 8d).

6.4.3 Effect of Vitamin sources on *Termitomyces* spp.

T. albuminosus

The effect of different vitamin sources on linear growth of *T. albuminosus* was varied significantly with change in sources at 5% level of significance. Out of five vitamins tested, thiamine was maximum linear growth for the growth of mycelium of *T. albuminosus* ($p<0.05$) while least growth was observed in folic acid. Control with somewhat compact mycelial density, ascorbic acid with somewhat thin mycelial

density and D-priotine with somewhat thin mycelial density showed the optimum growth (Figure 41a, Photo plate 3).

However, fresh weight of mycelium in liquid medium also varied significantly with change in vitamin sources at 5% level of significance. Maximum fresh weight was observed in D-priotine while minimum of ascorbic acid. But dry weight of mycelium in liquid medium did not vary significantly with change in vitamin sources at 5% level of significance (Appendix 9a).

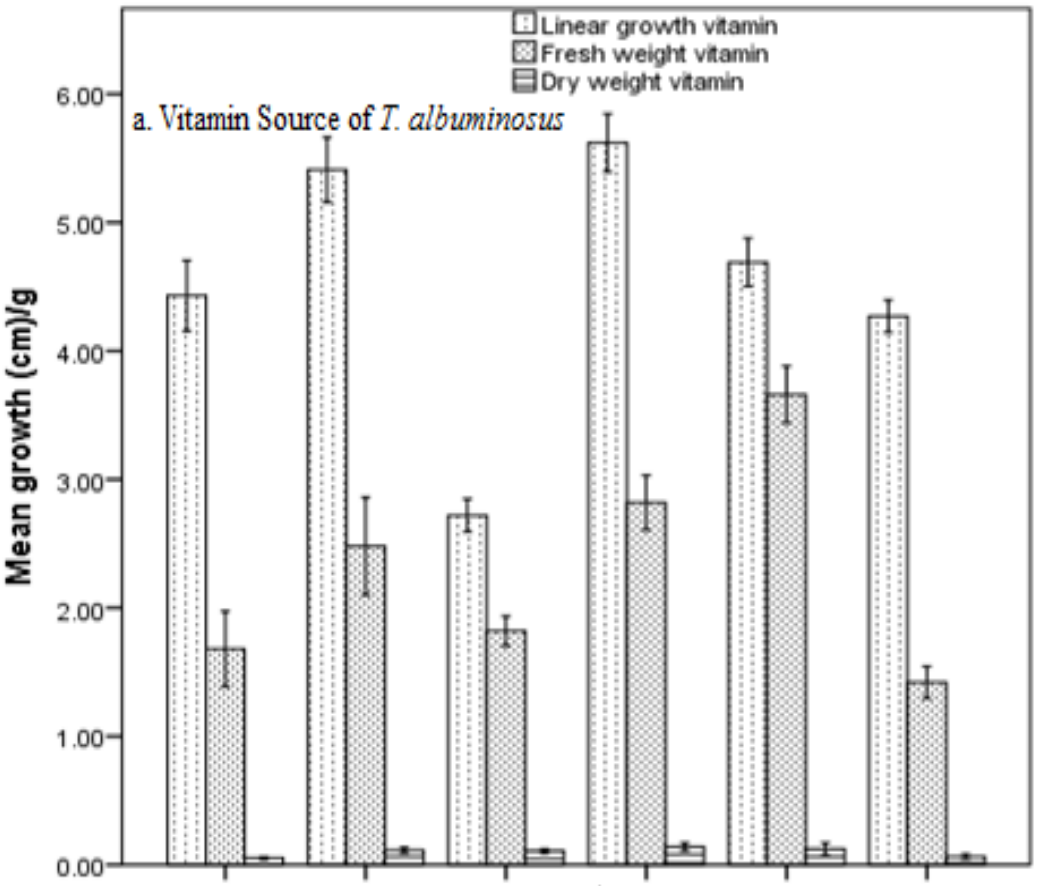
Termitomyces mammiformis

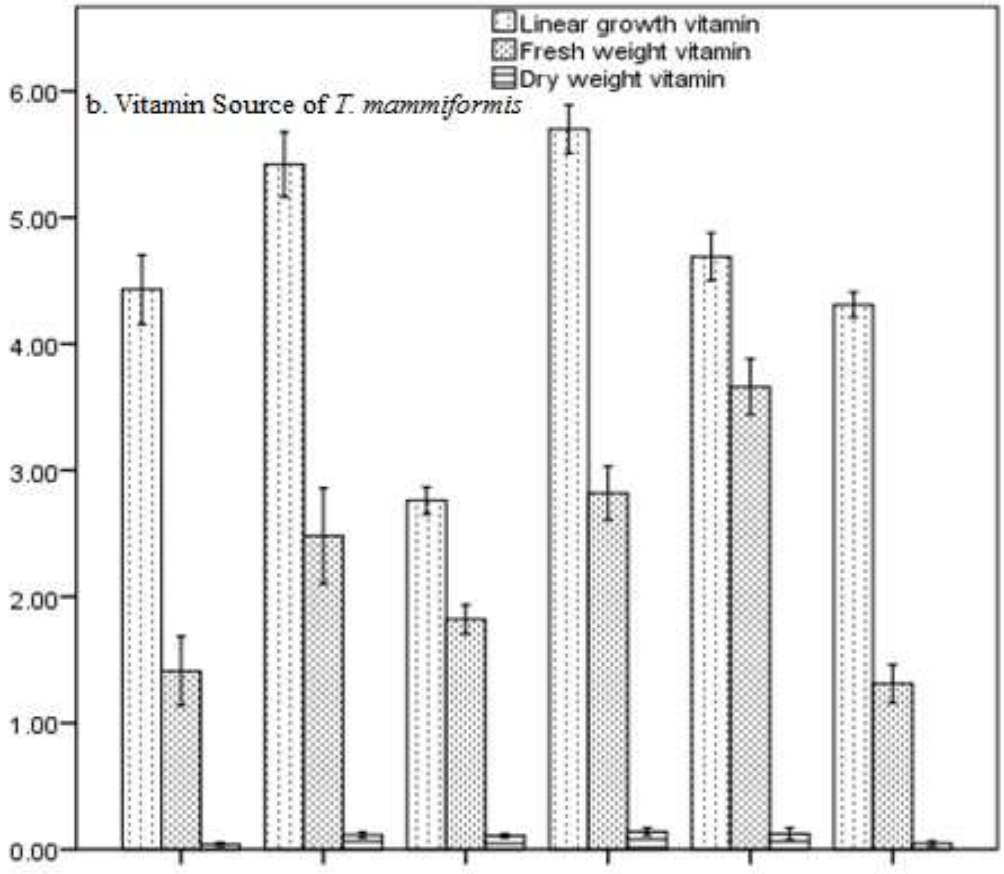
The effect of different vitamin sources on linear growth of *T. mammiformis* was varied significantly with change in sources at 5% level of significance. Out of five vitamins tested, thiamine was maximum linear growth and least in folic acid ($p<0.05$). Control with somewhat compact mycelial density, ascorbic acid and D-priotine with somewhat thin mycelial density showed the optimum growth (Figure 41b). However, fresh weight of mycelium in liquid medium also varied significantly with change in vitamin sources at 5% level of significance. Maximum weight was observed in D-priotine, while minimum of ascorbic acid and control. But dry weight of mycelium in liquid medium did not vary significantly with change in vitamin sources at 5% level of significance (Appendix 9b).

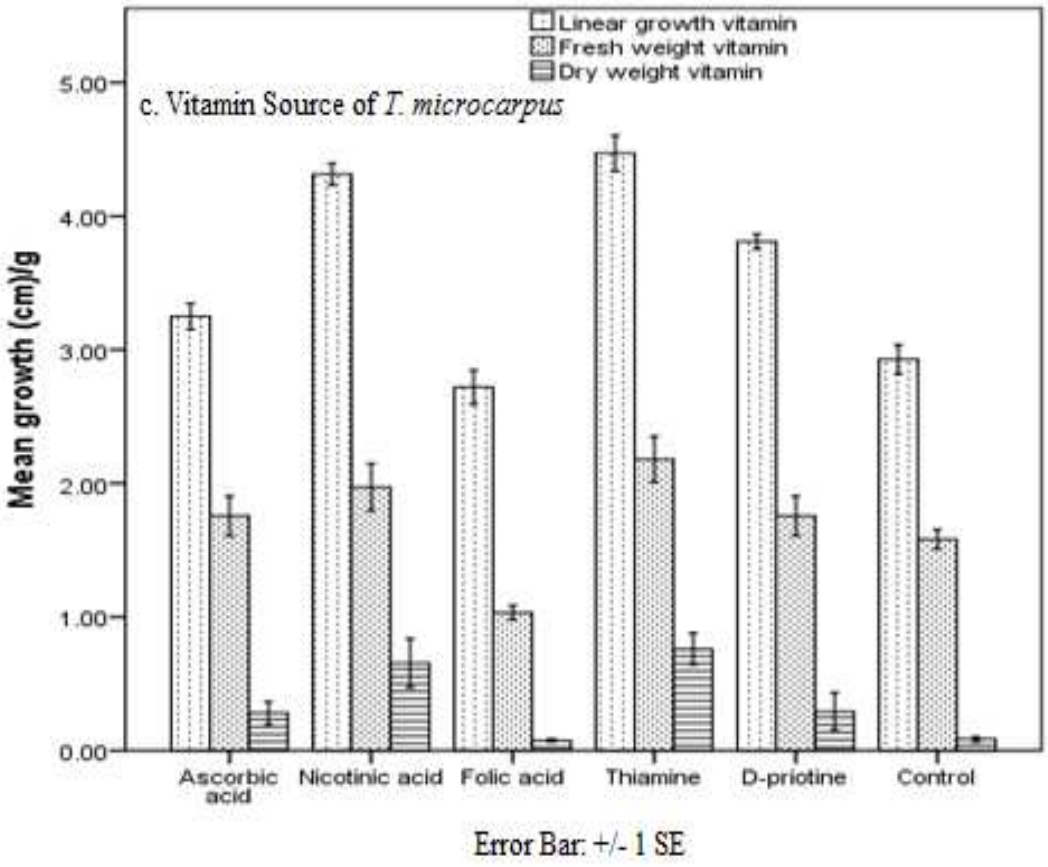
Termitomyces microcarpus

The effect of different vitamin sources on linear growth of *T. microcarpus* was varied significantly with change in sources at 5% level of significance. Out of five vitamins tested, thiamine was maximum and least in folic acid ($p<0.05$). D-priotine with somewhat compact mycelial density, ascorbic acid with somewhat thin mycelial density showed the optimum growth (Figure 41c). However, in control, was better than in folic acid.

Fresh and dry weight of mycelium in liquid medium also varied significantly with change in vitamin sources at 5% level of significance. Maximum weight was observed in thiamine while minimum of folic acid. However, in control was better than in folic acid.


Linear growth vitamin
Fresh weight vitamin
Dry weight vitamin
a. Vitamin Source of T. albuminosus
Mean growth (cm)/g
6.00
5.00
4.00
3.00
2.00
1.00
0.00


Linear growth vitamin
Fresh weight vitamin
Dry weight vitamin
b. Vitamin Source of T. mammiformis
6.00
5.00
4.00
3.00
2.00
1.00
0.00



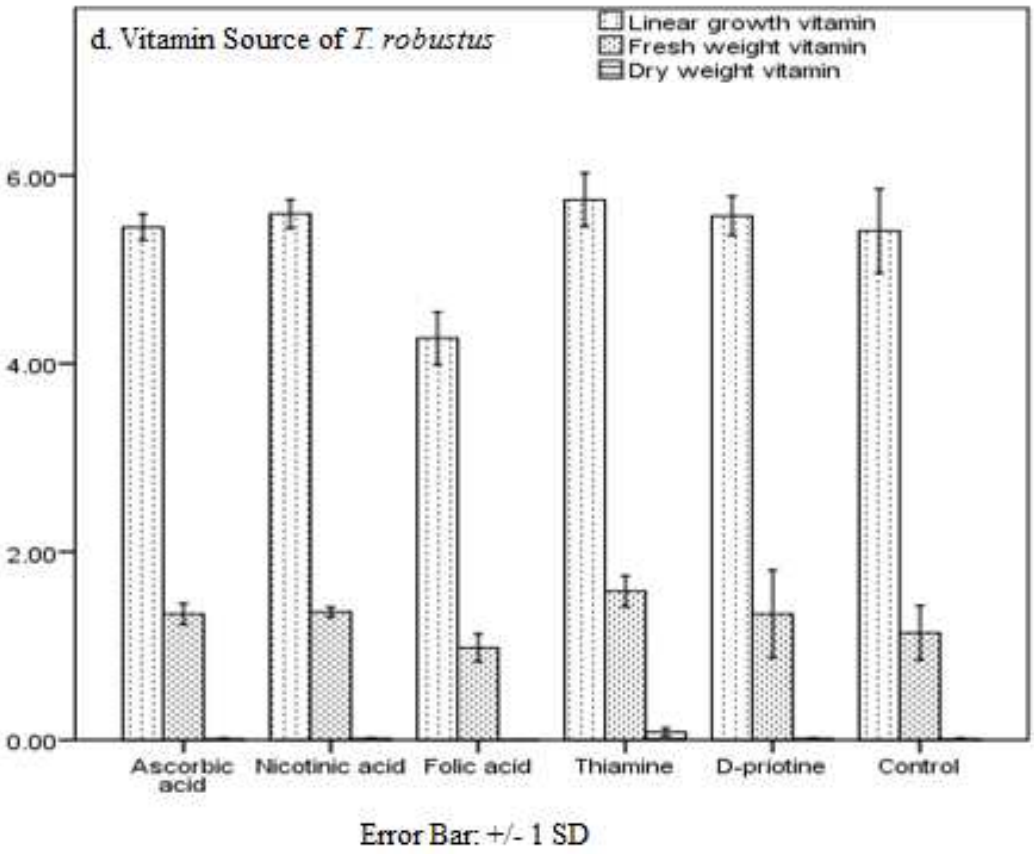


Figure 41: Myceliall growths of *Termitomyces* on different vitamins sources; One-way ANOVA, Tukey HSD Test ($p<0.05$), multiple range and mean test.

Similarly dry weight of mycelium in liquid medium was observed maximum in thiamine, while minimum in folic acid. However, in dry weight of mycelial of control was better than in folic acid (Appendix 9c).

Termitomyces robustus

The effect of different vitamin sources on linear growth of *T. robustus* was varied significantly with change in sources at 5% level of significance. Out of five vitamins tested, maximum linear growth was observed in thiamine while minimum in folic acid ($p<0.05$) (Figure 41d). However, control, was better than in folic acid.

Fresh and dry weight of mycelium in liquid medium also varied significantly with change in vitamin sources at 5% level of significance. Maximum fresh weight was observed in thiamine, while minimum of folic acid. However, in control was better than in folic acid. Similarly dry weight of mycelium in liquid medium was observed maximum in thiamine while minimum in folic acid. However of control was better than in folic acid (Appendix 9d).

6.4.4 Effect of Amino acid sources on *Termitomyces* spp.

Termitomyces albuminosus

The effect of different amino acid sources on linear growth of *T. albuminosus* was varied significantly with change in sources at 5% level of significance. Among them, glutamic acid proved to be the maximum linear growth with somewhat thin mycelial density while control showed the least growth with thin mycelial density ($p<0.05$) (Figure 42a, Photo plate 4). The control treatment showed intermediate growth with somewhat thick mycelial density. Leusine, valine, aspartic acid and serine was also observed with somewhat thin mycelial density showed the medium growth. However, both fresh and dry weight of mycelium in liquid medium did not vary significantly with change in amino acid sources at 5% level of significance (Appendix 10a).

Termitomyces mammiformis

The effect of different amino acid sources on linear growth of *T. mammiformis* was varied significantly with change in sources at 5% level of significance. Among them,

serine (4.69 ± 0.19 cm), proved to be the maximum linear growth with somewhat thin mycelial density while aspartic acid showed the minimum growth with thin mycelial density ($p<0.05$) (Figure 42b). The control treatment showed intermediate growth with somewhat thick mycelial density. However, both fresh and dry weight of mycelium in liquid medium did not vary significantly with change in amino acid sources at 5% level of significance (Appendix 10b).

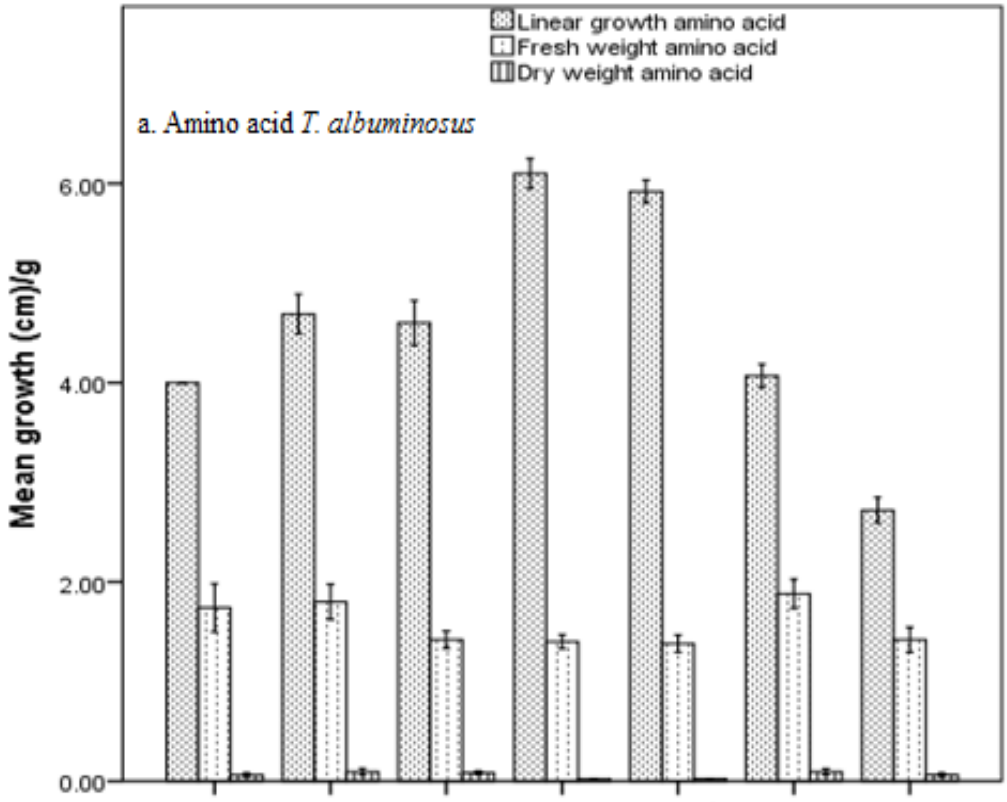
Termitomyces microcarpus

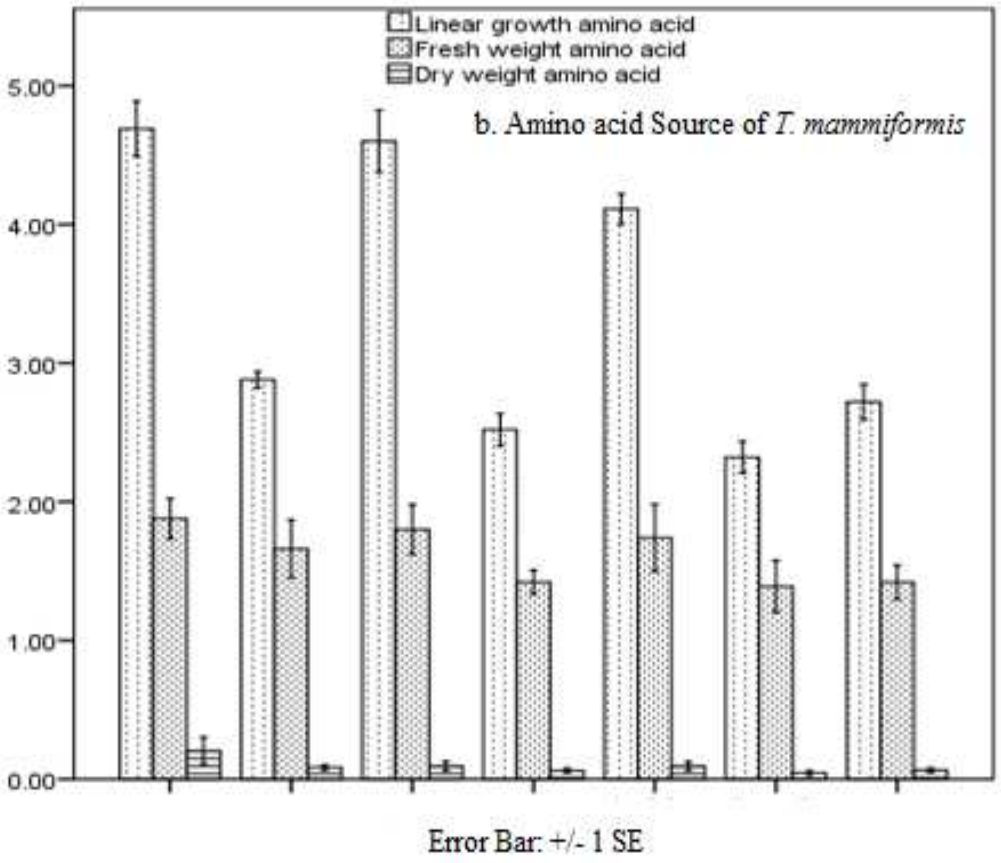
The effect of different amino acid sources on linear growth of *T. microcarpus* was varied significantly with change in sources at 5% level of significance. Among them, serine proved to be the maximum linear growth with somewhat thin mycelial density while glutamic acid showed the minimum growth with thin mycelial density ($p<0.05$) (Figure 42c). The control treatment showed intermediate growth with somewhat thick mycelial density. Leusine and valine was also observed with somewhat thin mycelial density showed the medium growth.

However, fresh and dry weight of mycelium in liquid medium also varied significantly with change in amino acid sources at 5% level of significance. Maximum fresh weight was observed in serine while minimum of aspartic acid. However, control and glutamic acid had equal fresh weight. But the control was observed higher growth than aspartic acid. Similarly dry weight of mycelium in liquid medium was observed maximum in serine while minimum in aspartic acid. However of control was better than in aspartic acid and glutamic acid while equal with leusine (Appendix 10c).

Termitomyces robustus

The effect of different amino acid sources on linear growth of *T. robustus* was varied significantly with change in sources at 5% level of significance. Among them, serine proved to be the maximum linear growth with somewhat thin mycelial density while aspartic acid showed the least growth with thin mycelial density ($p<0.05$). The control treatment showed intermediate growth with somewhat thick mycelial density. Glutamic acid was also observed with somewhat thin mycelial density showed the medium growth (Figure 42d).


Linear growth amino acid
Fresh weight amino acid
Dry weight amino acid
a. Amino acid T. albuminosus
Mean growth (cm)/g
6.00
4.00
2.00
0.00
Error Bar: +/- 1 SE


Linear growth amino acid
Fresh weight amino acid
Dry weight amino acid
b. Amino acid Source of T. mammiformis
5.00
4.00
3.00
2.00
1.00
0.00
Error Bar: +/- 1 SE

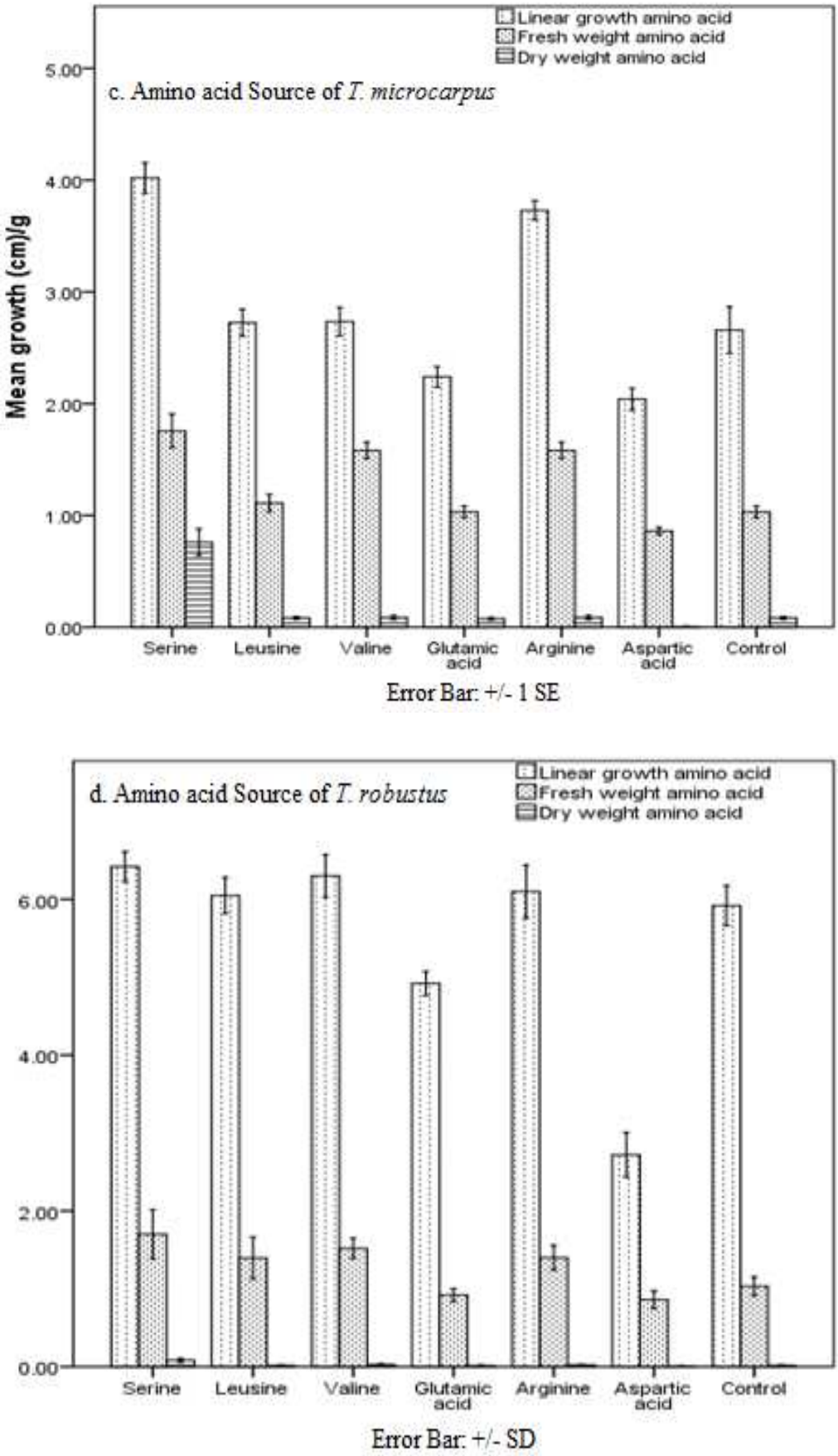


Figure 42: Myceliall growths of *Termitomyces* on different amino acid sources; One-way ANOVA, Tukey HSD Test ($p<0.05$), multiple range and mean test.

However, fresh and dry weight of mycelium in liquid medium also varied significantly with change in amino acid sources at 5% level of significance. Maximum fresh weight was observed in serine while minimum of aspartic acid. However, in control was better than in aspartic, glutamic acid. Similarly dry weight of mycelium in liquid medium was observed maximum in serine, while minimum in aspartic acid. However of control was better than in arginine, aspartic acid and glutamic acid, while equal with leusine (Appendix 10d).

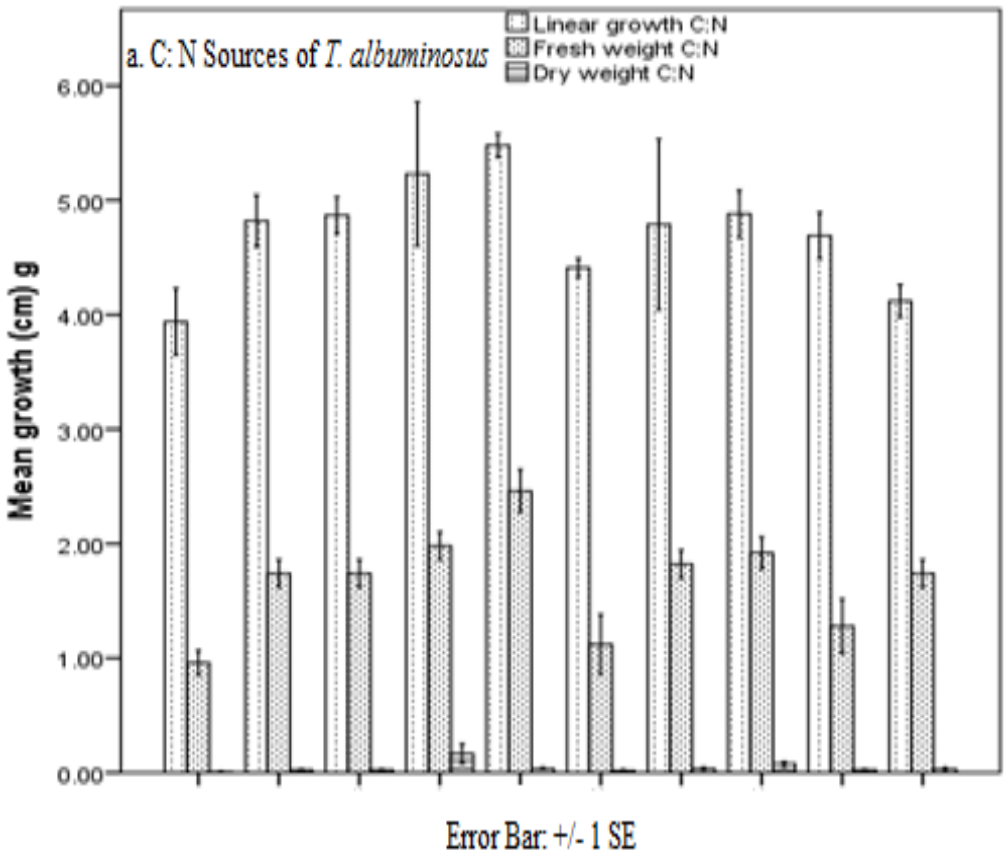
6.4.5 Effect of (C: N) ratio sources on *Termitomyces* spp.

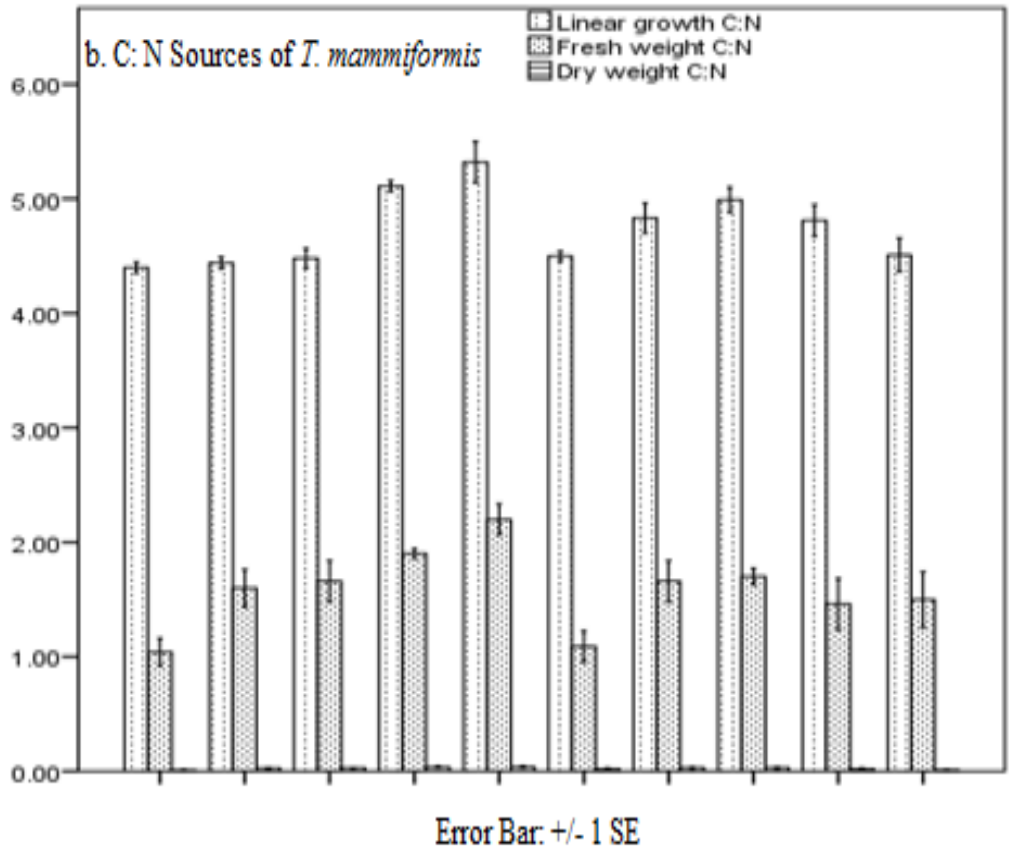
Termitomyces albuminosus

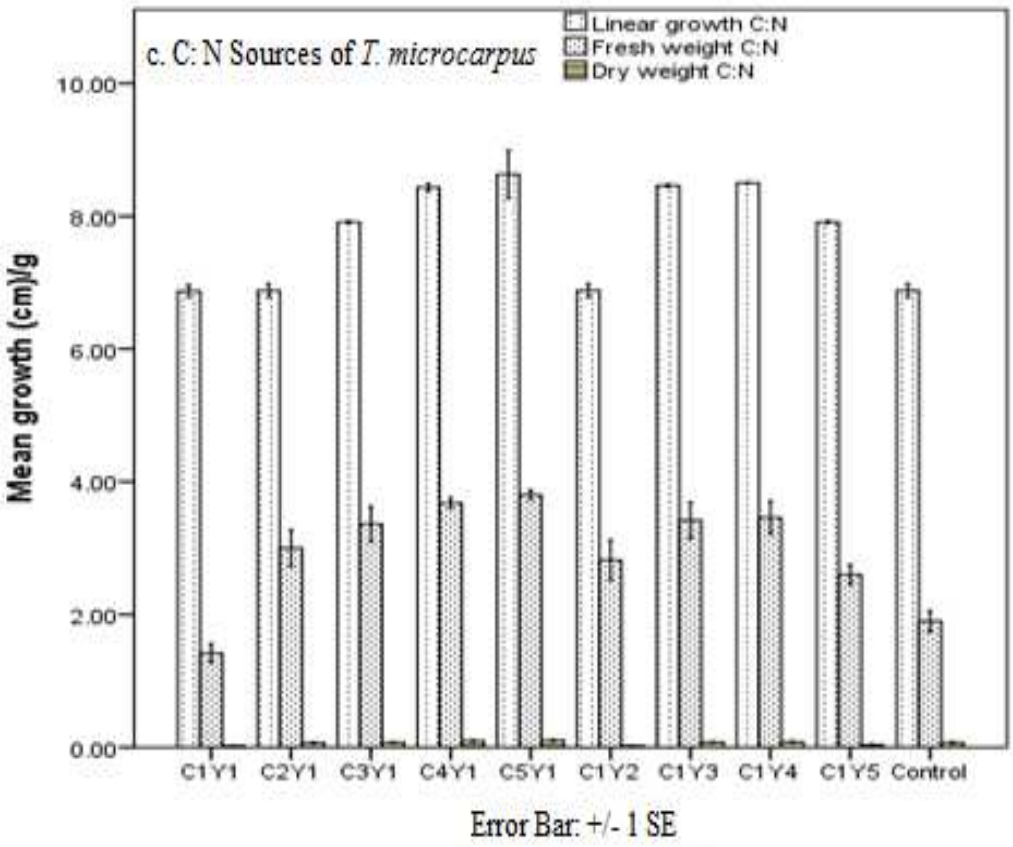
The combination of carbon and nitrogen in various proportions showed that the linear growth rate increase with the proportional increase in carbon up to C: N ratio of 5:1 ($p<0.05$). Control and C: N ratio 1:1 showed the least growth with somewhat compact and thin mycelial density respectively. In the same manner, when the ratio of nitrogen was increased, growth rate also increased gradually up to the C: N ratio of 1:4. So, the best growth observed was when the C: N was 1:4 and 1:5 respectively (Figure 43a, Photo plate 5). However, control was observed better linear growth than 1: 1. But the effect of different C: N sources on linear growth were did not vary significantly with change in sources at 5% level of significance. It might be due to the agitation, lack of orbital incubation or continuation back up.

However, both fresh and dry weights of mycelium in liquid medium were varied significantly with change in nitrogen sources at 5% level of significance. Maximum fresh weight was observed in C_5: N_1, while minimum of C_1: N_1. However of control was observed better than in 1: 1, 1: 2 and 1: 5 while equal with 2: 1, and 3: 1.

Similarly maximum dry weight was observed in C_4: N_1 and minimum of C_1: N_1. However of control was observed better than in 1: 1, 2: 1, 3: 1, 1: 2 and 1: 5 while equal with 5: 1, 1: 3, and 1: 4 (Appendix 11a).


a. C: N Sources of T. albuminosus
Linear growth C:N
Fresh weight C:N
Dry weight C:N
Mean growth (cm) g
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Error Bar: +/- 1 SE


b. C: N Sources of T. mammiformis
Linear growth C:N
Fresh weight C:N
Dry weight C:N
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Error Bar: +/- 1 SE



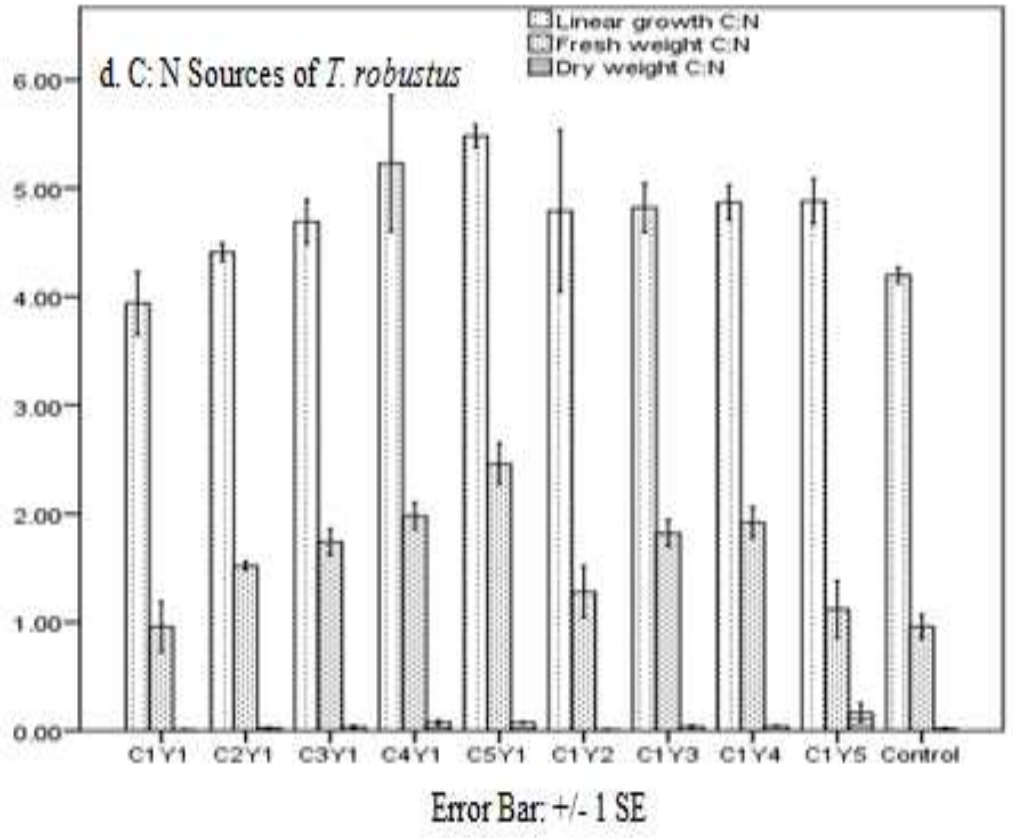


Figure 43: Myceliall growths of *Termitomyces* spp. on different C: N ratio sources; One-way ANOVA, Tukey HSD Test ($p<0.05$), multiple range and mean test.

Termitomyces mammiformis

The effect of different C: N sources on linear growth of *T. mammiformis* were varied significantly with change in sources at 5% level of significance. The combination of carbon and nitrogen in various proportions showed that the linear growth rate increase with the proportional increase in carbon up to C: N ratio of 5:1 for *T. mammiformis* ($p<0.05$). Control and C: N ratio 1:1 showed the least growth with somewhat compact and thin mycelial density respectively. In the same manner, when the ratio of nitrogen was increased, growth rate was also increased gradually up to the C: N ratio of 1:4. So, the best growth observed was when the C: N was 1:4 and 1:5 respectively (Figure 43b). However, control was observed better growth than 1: 1, 2: 1, 3:1, and 1: 2.

However, both fresh and dry weights of mycelium in liquid medium were varied significantly with change in nitrogen sources at 5% level of significance. Maximum fresh weight was observed in C_5: N_1, while minimum of C_1: N_1. However of control was observed better than in 1: 1, 1: 2 and 1: 5.

Similarly maximum dry weight was observed in C_5: N_1 and minimum C_1: N_1. However of control was observed better than in 1: 1(Appendix 11b).

Termitomyces microcarpus

The effect of different C: N sources on linear growth of *T. microcarpus* were varied significantly with change in sources at 5% level of significance. The combination of carbon and nitrogen in various proportions showed that the growth rate increase with the proportional increase in carbon up to C: N ratio of 5:1 ($p<0.05$). C: N ratio 1:1 showed the least growth with somewhat compact and somewhat thin mycelial density respectively. In the same manner, when the ratio of nitrogen was increased, growth rate was also increased gradually up to the C: N ratio of 1:4. So, the best growth observed was when the C: N was 1:4 and 1: respectively (Figure 43c). However, control was observed better growth than 1: 1 and equal was observed in 2: 1, and 1: 2.

However, both fresh and dry weights of mycelium in liquid medium were varied significantly with change in nitrogen sources at 5% level of significance. Maximum fresh weight was observed in C_5: N_1 while minimum of C_1: N_1. However of control was observed better than in 1: 1.

Similarly maximum dry weight was observed in C_5: N_1 and minimum in C_1: N_1 and C_1: N_2. However of control was observed better than in 1: 1, 1: 2, and 1: 5 while equal in 2: 1 and control (Appendix 11c).

Termitomyces robustus

The combination of carbon and nitrogen in various proportions showed that the linear growth rate increase with the proportional increase in carbon up to C: N ratio of 5:1 ($p<0.05$). C: N ratio 1:1 showed the least growth with somewhat compact and somewhat thin mycelial density respectively. In the same manner, when the ratio of nitrogen was increased, growth rate was also increased gradually up to the carbon-nitrogen ratio of 1:4. So, the best growth observed was when the C: N was 1:4 and 1:5 respectively (Figure 43d). However, control was observed better linear growth than 1: 1. But the effect of different C: N sources on linear growth were did not vary significantly with change in sources at 5% level of significance. It might be due to the agitation, lack of orbital incubation or continuation back up.

However, both fresh and dry weights of mycelium in liquid medium were varied significantly with change in nitrogen sources at 5% level of significance. Maximum fresh weight was observed in C_5: N_1 while minimum of C_1: N_1 and control. However of control was observed equal growth with 1: 1.

Similarly maximum dry weight was observed in C_1: N_5, while minimum in C_1: N_1. However of control was observed better than in 1: 1 and 1: 2 (Appendix 11d).

6.5 Discussion

The world-overview: Termitophilous mushrooms (*Termitomyces* species) on which the present investigations have been carried out, represent some of the well-known wild edible mushrooms which have been also investigated taxonomically in Europe, Africa, Asia and North America and other parts of world by number of investigators (see pp 13, Taxonomy: the world scenario). Besides taxonomy there are some passing references about their edibility and sociobiological aspects as well.

Nepalese perspectives: Among the indigenous edible species of Nepal, *Termitomyces* spp. appears to be highly suitable for commercialization purpose. In view of this it was planned to investigate domestication of pure culture of growth of these fungal

mycelium of these mushrooms with the sole purpose to realize their nutritional potential and to conserve the interesting ones for subsequent use in human welfare. So, as conserve some of these, pure-cultures of *T. albuminosus* (Berk.) R. Heim, *T. mammiformis* R. Heim, *T. microcarpus* (Berk. & Broome) R. Heim and *T. robustus* (Beeli) R. Heim have been raised on PDA medium for semisolid medium/ PD medium for submerge culture. So as to focused on optimizing the cultural media for the mycelial growth of these species, a preliminary step for domestication of these edible species by pure culture technique on lab and was the first step in context of Nepal.

Study on the mycelial growth: Study on the mycelial growth of these four species was done comparatively to assess the effect of different nutritional factors on the growth of these species. Media optimization was done using the protocols proposed by Chandra and Purkayastha (1977) and Shim, *et al.,* (2005a). For the growth and reproduction of most fungi, the culture media must contain sources for carbon, nitrogen, sulphur, phosphorus, vitamins and certain trace or metallic elements (Kaul, 1999). Hence, various nutrients were compared to identify their efficiency as the source of these elements for the mycelial growth of *T. albuminosus, T. mammiformis, T. microcarpus* and *T. robustus.*

Carbon sources: *T. albuminosus, T. mammiformis, T. microcarpus* and *T. robustus* showed that different preference for carbon sources for its metabolism. However, utilization varied from one carbon sources to another. In this study, maltose and manitol were observed to be the most effective carbon sources while lactose the least effective for mycelial growth of this mushroom (Figure 36a-d). According to Cochrane (1958) the ability of an organism to utilize carbohydrate depends on type of enzymes proposed by the organism. This result showed that four species of *Termitomyces* produces enzymes that utilize maltose and fructose better than any other carbon sources. Shim, *et al.,* (2005b) who reported this result is in line with the work of that maltose was screened suitable for mycelial growth while lactose was the least mycelium growing carbon source for *Cystoderma amianthinum* var. *ruigosoreticulatum* (F. Lorinser) Bon. According to Manjunathan and Kaviyarasan (2011), *Lentinus tuber-regium* (Fr.) Fr. Utilizes dextrose as the most effective carbon source while lactose was the least effective one for the mycelium growth. Ayodele

(2008) found that, in *Psathyrella atroumbonata* Pegler; glucose was the best carbon source which was followed by maltose, starch and mannitol in that order. The least growth was observed in lactose and control. Chandra and Purkayastha (1977) reported that most of the tropical edible macrofungi were in favor of utilizing glucose than other carbon sources. Jayasinghe, *et al.,* (2008) found that dextrose was the best carbon source for mycelial growth of *Ganoderma lucidum* (Curtis) P. Karst. This was closely followed by galactose and fructose which were not considerably different from each other. Jonathan, *et al.,* (2006) found that in *Pleurotus florida* Singer, the most supportive sugars were among the monosaccharides. Aldohexose (glucose) stimulated greater biomass yield than ketohexose (fructose) under the same conditions. Generally, complex sugar and sugar alcohols produced little biomass with the exception of dextrin and mannitol. He attributed the little amount of mycelial production with polysaccharides and sugar alcohols to their complex nature since hydrolytic enzymes would be required to convert polysaccharides and sugar alcohols to simple sugar before they will enter respiratory pathways. Mannitol (a sugar alcohol) also supported good biomass yield of *P. florida.* The best carbon source suiTable for promoting mycelial growth of *Lignosus rhinoceros* (Cooke) Ryvarden was glucose but with somewhat compact mycelial density according to Lai, *et al.,* (2011). Additionally, fructose and mannose also recorded a high radial mycelial growth rate. The combination of these three carbon sources indicated that *L. rhinoceros* preferred monosaccharaides. Shim, *et al.,* (2005b) found that for *Cystoderma amianthinum* the favorable carbon source promoting mycelial growth was fructose. Imtiaj, *et al*., (2008) found that, in *Schizophyllum commune* Fr., the suitable mycelial growth was found in dextrin and fructose; however the lowest growth of mycelium was obtained in lactose, mannose and sorbitol.

Nitrogen sources: Among the organic and inorganic nitrogen sources used during this study, these mushroom utilized organic nitrogen better than inorganic nitrogen. The best growth was found in yeast extract (Figure 37a-d). This finding was similar to that of Ayodele (2008), who reported that *Psathyrella atroumbonata* Pegler showed preference for organic nitrogen than inorganic nitrogen and the best yield was in yeast extract while the least was found in sodium nitrate and ammonium nitrate. Manjunathan and Kaviyarasan (2011) also found that *Lentinus tuber-regium* (Fr.) Fr.utilized organic nitrogen better than inorganic nitrogen, yeast extract being the best

for its mycelial growth in the same line. They suggested that the stimulatory effect of yeast extract in their study may have been due to its amino acids, protein and vitamins. According to Adebayo, *et al.,* (2011), comparatively organic nitrogen supported optium production of mycelial by *Pleurotus ostreatus* (Jacq.) P. Kumm. when inorganic nitrogen was used poor biomass growth was reported. Urea had been recorded as the best nitrogen source for biomass production by *P. ostreatus.* Jayasinghe, *et al.,* (2008) reported that *Ganoderma lucidum* (Curtis) P. Karst. showed optium mycelial growth on ammonium acetate, glycine, arginine and calcium nitrate. Jayasinghe, *et al.,* (2008) observed that inorganic nitrogen sources also enhanced the mycelial growth of *G. lucidium* Jonathan, *et al.,* (2006) found that in *Pleurotus florida* (Jacq.) Singer, inorganic compounds supported moderate biomass production. The best biomass yield was found with ammonium nitrate closely followed by potassium nitrate. Among the complex nitrogen compounds, yeast extract and casein hydrolysate supported significant biomass; Lai, *et al.,* (2011) observed that the best nitrogen source was potassium nitrate for promoting mycelial growth of *Lignosus rhinoceros* (Cooke) Ryvarden. Imtiaj, *et al.,* (2008) found that the most suitable nitrogen source were calcium nitrate, potassium nitrate, and alanine, and the most unsuitable were ammonium phosphate, histidine, urea and arginine for mycelial growth of *Schizophyllum commune* Fr., Shim, *et al.,* (2005a) found that the best nitrogen source was glycine for *Macrolepiota procera* (Scop.) Singer. However, in this study, urea, despite being an organic nitrogen source, showed the least support for the mycelial growth of the mushroom under study.

Vitamin sources: The best vitamins for the mycelial growth of were thiamine of all species of *Termitomyces*, among the different vitamin sources used. In this study, control treatment with somewhat compact, ascorbic acid with somewhat thin mycelial density and folic acid with somewhat thin mycelial density showed the least growth (Figure 38a-d). This finding was in line with the reported of Ayodele (2008), who proved that thiamine was the best vitamin, followed by nicotinic acid and riboflavin and the least mycelial growth was observed in folic acid for *Psathyrella atroumbonata* Pegler. This observation was also in line with the report of Landers (1964) who found that thiamine stimulated mycelial growth of *Cercospora arachidicola* Hori in liquid culture. Manjunathan and Kaviyarasan (2011) also proved that thiamine was the best among the vitamins followed by biotin and togoferrol for

Lentinus tuber-regium (Fr.) Fr. Adebayo, *et al.*, (2007) found that riboflavin and pyridoxine promoted the growth of mycelial for *Schizophyllum commune* Fr. Adebayo, *et al.*, (2011) observed that among the vitamins tested, ascorbic acid and folic acid had the highest stimulatory effect on mycelial of *Pleurotus ostreatus* (Jacq.) P. Kumm. Pokhrel, *et al.*, (2009a) reported that riboflavin and thiamine were the most stimulatory vitamins for mycelial growth of *Lyophyllum decastes* (Fr.) Singer.

Aminoacid sources: Out of six amino acids tested, serine proved to be the best amino acid for the mycelial growth of *Termitomyces albuminosus* (Berk.) R. Heim, *T. mammiformis* R. Heim, *T. microcarpus* (Berk. & Broome) R. Heim and *T. robustus* (Beeli) R. Heim, while aspartic acid showed the least growth. Arginine, Leucine and glutamic acid showed similar growths as the control with arginine showing thin mycelial density, and lucine and glutamic acid showing somewhat thin mycelial density (Figure 39a-d). According to Ayodele (2008), Asparagines proved to be the best amino acid followed by aspartic acid for *Psathyrella atroumbonata* Pegler. According to Chandra and Purkayastha (1977), amides such as asparagines and aspartic acid have been employed in increasing mycelial growth and fruiting body production in *Agaricus bisporus* (Lange) Imbach. Hayes (1981) observed that higher and lower concentrations of amino acids are found to be either ineffective or inhibitory for the mycelial growth of mushrooms. According to Manjunathan and Kaviyarasan (2011) glycine proved to be the best amino acid followed by L-ornithine monohydrochloride for the mycelial growth of *Lentinus tuber-regium* (Fr.) Fr. Hayes (1981) reported that higher and lower concentrations of these amino acids were found to be either ineffective or inhibitory for the mycelial growth of mushrooms. Adebayo, et al., (2011) observed that some of the amino acids used during his study had a stimulatory effect on mycelial yield of *Pleurotus ostreatus* (Jacq.) P. Kumm. Optium mycelial growth was recorded when glycine was in used. Jonathan et al. (2006) found that in *Pleurotus florida* Singer, although all the twelve amino acids used in his study enhanced biomass production, the most stimulatory amino acid was tryptophan. Pokhrel, *et al*., (2009a) found that proline and glutamic acid showed significant increase in mycelial growth than other amino acids tested in *Lyophyllum decastes* (Fr.) Singer.

C: N sources: It was observed that the mycelial growth of the fungi under study increased with the proportional increase of carbon in comparison to nitrogen up to C:

N ratio of C_5: N_1. Control treatment and C: N ratio C_1: N_1 showed the least growth with somewhat compact and somewhat thin mycelial density respectively during this study. When the ratio of nitrogen was increased, growth was also increased gradually in the same manner, up to C: N ratio C_1: N_4. So, the best growth observed was when the C: N ratio was C_5: N_1 (Figure 40a-d). Lai, *et al.,* (2011) reported that C: N ratio of C_{10}: N_1 was favorable for mycelial growth of *Lignosus rhinoceros* (Cooke) Ryvarden. Notably, no growth occurred at C_{40}: N_1 of C: N ratio. Shim, *et al.,* (2003) observed that the optimum C: N ratio suitable for favorable growth of *Paecilomyces fumosoroseus* (Wize.) Brown and Smith were on culture media which were adjusted to C: N ratio of C_{40}:N_1. Shim, *et al.,* (2005b) found the C: N ratio of C_{30}:N_1 was the best for the mycelial growth of *Cystoderma amianthinum* var. ruigosoreticulatum (F. Lorinser) Bon. Chandra and Purkayastha (1977) observed C: N ratio of C_3:Y_1, C_5:N_1 and C_1:N_3 were favourable for the growth of *Lentinus subnudus* Berk., *Volvariella volvacea* (Bull.) Singer and *Termitomyces eurrhizus* (Berk.) R. Heim respectively. According to Manjunathan and Kaviyarasan (2011), carbon to nitrogen ratio of C_1:N_3 and C_1:N_5 were the best for the mycelial growth of *Lentinus tuber-regium* (Fr.) Fr. Ayodele, (2008) found that the C: N ratio of C_4:N_1 and C_1:N_4 supported best growth of *Psathyrella atroumbonata* Pegler.

6.6 Conclusion

In view of their popularity with rural masses and capability to utilize lignocellulosic substrates there is a need to pursue this work further for cultivating these so as to understand their biological efficiency of converting agro-forest by-product and other such substrate available in plenty in different parts of the country. Through domestication and bulk production, their availability for masses will increase many times besides adding to their revenue and at the same time this will result in reduced pressure for their bulk hunting from nature. This is how the dual purpose of meeting the human demand and conservation aspects can be targeted simultaneously which is so vital for conservation of the natural ecosystem and sustainability. That's why there is need to domesticate these mushrooms. In view of this few of them including, *Termitomyces albuminosus*, *T. mammiformis*, *T. microcarpus*, and *T. robustus* have been cultured and their mycelium assayed for capability to degrade the lignocellulosic substrate.

The account presented in this thesis has shown that the mycological resources of the country are far from fully investigated. In view of their composition, mushroom needs to be used directly in diet so as to promote health, taking advantage of the additive and synergistic effects of all the bioactive compounds present in them. Therefore, wild edible mushrooms of *Termitomyces* spp. need to be domesticated for their large scale production and subsequent use as natural nutrition sources.

After the investigation under the pH 6 and the temperature 25 ± 2°C, The present study demonstrated that *Termitomyces albuminosus* (Berk.) R. Heim, *T. mammiformis* R. Heim, *T. microcarpus* (Berk. & Broome) R. Heim, and *T. robustus* (Beeli) R. Heim, showed differential preference for various nutrient sources used during the study. Among the different nutrient sources tested, the ones for the growth of the fungi optimal medium (Appendix 12) under study were:

- (Maltose) Malt extract is the best carbon source for the myceliall growth of the *T. albuminosus* and *T. robustus* in semisolid and liquid medium. Whereas Maltose is the best carbon source for growth of the *T. mammiformis* in semisolid and liquid of dry weight and peptone in fresh weight. Similarly Manitol is the best carbon source for *T.microcarpus* in semisolid and Maltose and Dextrose on liquid condition is the best growth.
- In case of *T. mammiformis* in fresh weight on submerged yield peptone were the best otherwise, all the species in semisolid and liquid condition Yeast extract of nitrogen source is the best for myceliall growth.
- In case of *T. albuminosus* and *T. mammiformis* in fresh weight on submerged yield D-Priotine were the best otherwise, all the species in semisolid and liquid conditiont Thiamine was the best vitamin sources for linear growth.
- Glutmic and Asprtic acid are the best amino acids sources for the linear growth of *T. albuminosus* in both medium (fresh and dry weight) respectively, otherwse serine was the best in all three species in both medium.
- C_4:N_1 and C_1:N_5 are the best C: N ratio source for the myceliall growth of the *T. albuminosus* and *T. robustus* respectively in dry weight of liquid condition, otherwise, C_5:N_1 was the best in all species in semisolid and liquid medium.

CHAPTER 7

7. CONCLUSION AND IMPLICATION

7.1 Conclusion

Termitophilous fungi (termite's mushroom) are a monophyletic group of tropical gilled mushrooms belonging to the single genus *Termitomyces*. They are unique, obligatory symbiont, grows in close and intimate association with termites. It contains higher dry matter, protein and fiber; but contain lower amount of fat and carbohydrates. They are also rich in important minerals, like phosphorus, potassium, calcium, copper, manganese, zinc, magnesium, sodium and iron. Besides nutritional value, the species possess high medicinal value. Evaluated phytochemicals, substantial amount of these compounds are found. It is a good source of bioactive compounds of this underutilized termite mushrooms present in wild state. The research carried out revealed nineteen taxa, (fourteen species, three forma and two newly proposed species) with food and medicinal significance, with the new addition (seventeen) of microfungal flora of Nepal and (two) proposed to the science as well. All the species has a wide distribution in throughout the Nation. Its distribution depends on the termites, constructed substrate, ecological aspects of interaction between the termite and *Termitomyces* and its mutualistic association. Soil texture, pH, OM, and chemical micro or macro nutrient composition played as the major role on termites nesting, fungus comb deposition under soil surface. So the edaphic factors have a major influence on termite distribution and abundance. Hence it needs to conserve in situ condition to previent from going to extint. In this regards its pure culture in lab maltose of carbon source, yeast extract of nitrogen, Thiamine of vitamins, serine of amino acid and C_5: N_1 of C: N sources are the optimal medium for the myceliall growth for this mushroom.

7.2 Implication

More than 50% of the species of the genus *Termitomyces* recorded worldwide occur in Nepal, Its documentation in different social, cultural and ethno medicinal practices in different parts of the nation are very important to sensitize the communities. It plays a vital role in enrichment of the socio-economic life of the rural marginal people. This knowledge provides enough background to appreciate the diversity and

their relevance in ecosystem maintenance in general and human welfare in particular. They play important roles in ecosystem replenishment and perform a wide variety of ecological roles. It creates an enthusiasm towards intensive exploration on these mycoflora.

Due to high concentration of diverse phytochemicals it is used in drug development. So that it is useful to future workers to select a group of mycoflora having similar chemical constituents to isolate biologically active principle or prepare remedies for particular case. It plays an important role for the process and product developments in environmental biotechnology.

So it provides in depth information about this genus for further studies in the field of systematics and creates attraction to the mycologist, entomologist and environmentalist to the research thus, the study sites need extensive exploration, and it is virgin place for the exploration of diversity, nutrients and growth of termite's mushrooms.

REFERENCES

Aanen, D.K., Eggleton, P., Rouland-lefevre C., Froslev, T.G., & Rosendahl, S. (2002). The evolution of fungus growing termites and their mutualistic fungal symbionts. *Procedings of the National Academy of Sciences,* **99,** 14887-14892.

Aanen, D.K., & Boomsma, J.J. (2005). Evolutionary dynamics of the mutualistic symbiosis between fungus-growing termites and *Termitomyces* fungi. In Insect-Fungal Associations: Ecology and Evolution, F. Vega and M. Blackwell, eds. Oxford University Press, Pp191-210.

Aanen, D.K., Vera, I.D.R., Henrik, H.D.F.L., Jannette, M., Wilhelm, Z.D.B., Bernard, S.C.R.L.F. & Jacobus, J.B. (2007). Patterns of Interaction specificity of fungus-growing termites and *Termitomyces* symbionts in South Africa. *BMC Evolutionary Biology,* **7,** 115-125.

Abbott, L.G., & Rude, R.K. (1993). Clinical manifestations of magnesium deficiency. *Miner Electrolyte Metab,* **19,** 314-322.

*Abe, T. (1989). *Ecology of termites; a hand book of tropical eclogy*. Tokyo University Press, Tokyo, Pp.151 (In Japanese). (Not seen in original).

Acharya, K. (2012). *A Study on Myceliall growth of Amanita chepangiana, a wild edible mushroom, on Differetnt nutrients sources*. A Master's thesis submitted to the Central Department of Botany, Tribhuvan University Nepal.

Adebayo, T.B.C., Ogunjobi, A.A. & Afolabi, O.O. (2007). Physiochemical Studies on *Schizophyllum commune* (Fries) a Nigerian Edible Fungus. *World Applied Sciences Journal,* **2**(1), 73-76.

Adebayo, T.B.C., Jonathan, S.G., Popoola, O.O. & Egbomuche, R.C. (2011). Optimization of growth conditions for myceliall yield and exopolysaccharride production by *Pleurotus ostreatus* cultivated in Nigeria. *African Journal of Microbiology Research,* **5**(15), 2130-2138.

Adejumo, T.O., & Awosanya, O.B. (2005). Proximate and mineral compositionof four edible mushrooms species from south western Nigeria. *African Journal of Biotechnology,* **4**(10), 1084-1088.

*Adhikari, M.K. (1996). *Biodiversite des Basidiomycetes au Nepal: etude systematique et Biogeographique.Specialite Ecologie-Mycologique*. These du

Doctorat de L'Universite present devant 1'Universite Paul Sabatier, Toulouse, France. no. d'ordre 2309, 205 p. (Not seen in original).

Adhikari, M.K. (2000). *Mushrooms of Nepal*. Edited by G. Durrieu, Published by Kamala S. Adhikari, Olivier Laurence, Mycosphere, Singapure and Sano, Nakoshizu, Japan & Kawai, Japan. 236 p.

Adhikari, M. K., (2009). *Researches on the Nepalese Mycoflora (Revised Account on the History of Mycological Explorations)*. Adhikari, K. S., Alka Basti Marga, Kathmandu, Nepal. 82 p.

Adhikari, M.K., Devkota, S. and Tiwari, R.D. (2005). Ethno mycological knowledge on uses of wild mushrooms in western and central Nepal. *Our nature,* **3**(1), 13-19.

Ahmad, M. (1965). Termites (Isoptera) of Thailand. *Bulletin of the American Museum of Natural History,* **131,** 1-111.

Ainsworth, G.C. (1971), *Ainsworth and Bisby's, dictionary of fungi*. CMI, Kew Surrey, England.

Akpaja, E.O., Omoanghes, S., Isikhuemhen, & Okhuoya, J.A. (2003). Ethno-mycology and uses of edible and medicinal mushrooms among the Igbo people of Nigeria. *Journal of Medicinal Mushrooms,* **5,** 313-319.

Aletor, V.A., & Aladetimi, O.O. (1995). Compositional studies on edible tropical species of Mushrooms. *Food Chemistry,* **54**(3), 265-268.

Alexopoulos, C.J., & Mims, C.W. (1993). *Intoductory Mycology*. 3rd edn, WEL, New Delhi, India. 632 p.

AOAC, (1995). *Official Methods of Analysis*. (7.007 13th Edition). Association of Official Analytical Chemist, Washington, D.C.

Arasmus, U. (1995). *Fats that heal fats that kills.* Alive Books, Burnaby, BC, Canada, 1-465 p.

Arnolds, E. (1991). Decline of ectomycorrhizal fungi in Europe. *Agriculture, Ecosystems and Environment,* **35,** 209-244.

Aryal, H.P. (2009). Role of Fungi in Soil Fertility and crop Production.2065. B.S., *Alangkar*. Nepal Student Union, Paklihawa Campus, Tribhuvan University, Nepal. 71-72 p.

Aryal, T.R. (2009). Mushroom poisoning problem in Nepal and its mitigation. *Fungi*, **2**(1), 44-46.

ASTM (American Society of Testing and Materials). (1995). *Annual Book of ASTM Standards*, Designation D4972 - 95a: Standard Test Method for pH of Soils.

Atri, N.S., Saini S.S., & Kaul, G. (1995). Taxonomic studies on North Indian Agarics – The genus *Termitomyces. Mushroom Research,* **4**(l), 7-10.

Atri, N.S., & Lakhanpal, T.N. (2002). Conservation of Mushroom Biodiversity *Indian Journal of Mushroom,* **19** (18), 1-9.

Atri, N.S., & Kaur, H. (2003). Wild mushrooms collection and identification. *Mushroom Research,* **14,** 56-59.

Atri, N.S., Kaur, A., & Kour, H. (2005). *Systematics and Sociobiology of Termitophilous mushrooms from Punjab*. In: Dargan, J.S, Atri, N.S and Dhingra, G.S, eds. The Fungi-Diversity and Conservation in India. Bishen Singh and Mahendra Pal Singh: 159-182.

Atri, N.S., Saini, S.S., Gupta, A.K., Kaur, A., Kour, H., & Saini, S.S. (2010). *Documentation of wild edible mushrooms and their seasonal availability in Punjab*. In: Mukerji, K.G. and Manoharachary, C. eds. Taxonomy and ecology of Indian fungi. I. K. International Publishing House Pvt. Ltd. New Delhi: 161-169.

Atri, N.S., Sharma, S.K., Joshi, R., Gulati, A., & Gulati, A. (2012). Amino acid composition of five wild *Pleurotus* species chosen from North West India. *European Journal of Biological Sciences,* **4**(1), 31-34.

Ayodele, S. M. (2008). Studies on the vegetative growth requirements of *Psathyrella Atroumbonata* Pegler, an edible mushroom in Nigeria. *African Journal of Plant Science,* **2**(5), 028-033.

Ayodele, S.M., Akpaja, E.O., Adamu, Y. (2009). Proceeding of the 5th International Medicinal Mushroom Conference, Nantong, China. Some edible and medicinal mushrooms found in Igala land in Nigeria and their sociocultural and ethnomycological uses, 526-531 p.

Balfour-Browne, F.L. (1968). Fungi of recent Nepal expedition. *Bulletin of the British Museum (Natural History) Botany Series,* **4,** 99-141.

Bano, Z. (1993). Food value of mushrooms. *Gram Prandyogiki*, **3,** 224-225.

Bano, Z. (1976). The nutritive value of mushrooms. In: *Proceeding of First Symposium on survey and cultivation of edible mushroom of India*. Reg. Res. lab. Srinagar, 2**,** 148-160.

Baraza, L.D., Joseph, C.C. Moshi, M.J., & Nkunya, M.H.H. (2007). Chemical Constituents and Biological Activity of three Tanzanian Wild Mushroom Species. *Tanzania Journal of Science,* **33,** 1-7.

Barros, L., Ferreira, M.J., Queiros, B., Ferreira, I., & Baptista, P. (2007). Total phenols, ascorbic acid, beta-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chemistry,* **103**(2), 413-419.

Batra, L.R., & Batra, S.W.T. (1966). Fungus-growing termites of tropical India and associated fungi. *Journal of Kansas Entomological Society*, **39,** 725-738.

Batra, L.R., & Batra. S.W.T. (1979). *Termite-fungus mutualism. In: Insect-Fungus symbiosis* (Batra L.R., Ed) 117-163, wiley, New York.

Bels, P.J. & Pataragetvit, S. (1982). *Edible mushrooms in Thailand cultivated by Termites*. Chinese University Press, Hong Kong.

Bhandary, H.R. (1980). Notes on some macrofungi of Nepal. *Journal of Natural History Museum,* **4,** 23-32.

Bhandary, H.R. (1984). *Mushrooms*. 33. In T.C. Majapuria, Nepal Nature's Paradise, Bangkok, White Lotus Co. Ltd. 235-246.

Bhandary, H.R. (1991). Some edible and medicinal fungi from Dumre to Manag, Mustang and Pokhara. *Journal of Natural History Museum,* **12**(1-4), 47-60.

Bhattarai, A. (2013). *Ethnomycological study of Macrofungi in Sukhaura Hariyali Community forest, Makrahar VDC, Rupandehi, Nepal*. A Master's thesis submitted to the Central Department of Botany, Tribhuvan University Nepal

Bhavani, D.S., Nair, M.C., & Meon. M.R. (1980). *Termitomyces robustus,* an addition to Indian edible mushroom. *Kavaka,* **8,** 53-54.

Bignell, D.E., & Eggleton, P. (2000). *Termites in ecosystems*. *In Termites: Evolution, Sociality, Symbioses, Ecology*. T. Abe, D. E. Bignell and M.Higashi, eds. Kluwer Academic Publishers, London. 363-388 p.

Bilgrami, K.S., & Verma, R.N. (1981). *Physiology of fungi*. Vikas Publishing House Pvt. Ltd., New Delhi, India.

Bill, G.F., & Cotter, H.V.T. (1989). Taxonomy and ethnomycology of *Lactarius* Sec. Dapetes (Russulaceae) in Neal. *Memoirs of the New York Botanical Garden,* **49,** 192-197.

Bose, S.R., & Bose, A.B. (1940). An account of edible mushrooms of India. *Science & Culture,* **6**,141-149.

Bother, W.J., & Eicker, A. (1991). Cultural studies on the genus *Termitomyces* in South Africa. **1**. Macro- and microscopic characters of Basidiome contexst cultures. *Mycologia Reserch,* **95,** 435-443.

Bother, W.J., & Eicker, A. (1992). Nutritional values of *Termitomyces* myceliall protein and growth of mycelium on natural substrate. *Mycologia Research,* **96**(5), 350-354.

Bougher, N.L., & Katrina, S. (1998). *Fungi of Southern Australia*. University of Western Australia Press, Nedlands.

Brunner, I., Brunner, F., & Laursen, G. (1992). Characterization and comparision of macrofungal communities in an *Alnus tenuifolia* and an *Alnus crispa* forest in Alaska. *Canadian Journal of Botany,* **70**, 1247-1258.

Buol, S.W. (2003). Formation of soils in North Carolina. Soil Science Society of North Carolina. Papers Commemorating a Century of Soil Science. 31-56.

Calonje, M., Mendoza, C.G., Galan, B. and Novaes, M.L. (1997). Enzymicactivity of the mycoparasite *Verticillium fungicola* on *Agaricus bisporus* fruit body cell walls. *Microbiology,* **143,** 2999-3006.

CBS. (2001). *Central Bureau of Statistics*. Statistical year book, National Planning Commission Secretariat, His Majesty's Government of Nepal.

Chakravarty, D.K. & Khatua, D.C. (1979). *Termitomyces microcarpus*-a new Indian edible mushroom. *Current Science,* **48,** 364-365.

Chandra, A., & Purkayastha. R.P. (1977). Physiological studies on Indian mushrooms. *Transactions of British Mycological Society,* **69,** 63-70.

Change, S.T. & Miles, P.G. (2004). *Mushrooms Cultivation, National Value, Medicinal effect and Environmental impact*. Second edition. CRC Press, Boca Raton.

Chang, S.T., & Miles, P.G. (1993). Mushrooms-trends in production and technological development. *Genenic Engineering of Biotechnology & Monitoring,* **41**/**42,** 73-84.

Cheng, D.Y., Zhou, L.W., Yang, Z.L., Wen, H.A., Bau, T., & Li, T.H. (2010). A revised checklist of edible fungi in China. *Mycosystema,* **29**(1), 1-21. jwxt@im.ac.cn; ISSN1672-6472, CN11-5180Q.

Christensen, M., Devkota, S., & Bhattarai, S. (2008a). Use of wild edible mushrooms in the Annapurna conservation area, Nepal. *Journal of Mycological & Phytopatholigical Society, Nepal*, **1,** 1-6.

Christensen, M., Bhattarai, S., Devkota, S., & Larsen, H.O. (2008b). Collection and use of wild edible fungi in Nepal. *Economic Botany*, **62**(1), 12- 23.

Christensen, M., Devkota, S., & Bhattarai, S., (2008c). Use of wild edible mushrooms in the Annapurna conservation area, Nepal. *Biodiversity Conservation,* **18,** 2205-2221.

Ciulei, I., (1982). *Practical Manuals on the Industrial Utilization of Chemical and Aromatic Plants.* Methodology for Analysis of Vegetable Drugs. 1st eds., Ministry of Chemical Industry, Bucharest.

Cochrane, V.W. (1958). *Physiology of Fungi*. John Wiley and Sons, New York.

Cowan, M.M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology* (Review), **12,** 564-582.

Darlington, J.P.E.C. (1994). *Nutrition and Evolution in Fungus-growing Termites*. In nourishment and evolution in insect Societies. Edited by Hunt. J. H. Nalepa C. A. Boulder: Westview Press. 105-130 pp.

Darlington, J.P.E.C., Zimmerman, P.R., Greenberg, J., West-berg C., & Bakwin, P. (1997). Production of metabolicgases by nests of the termite Macrotermes Jeanneli in Kenya. *Journal of Tropical Ecology,* **13,** 491-510.

De Boer, W., Folman, L.B., Summerbell, R. C., & Boddy, L. (2005). Living in a fungal world: impact of fungi on soil bacterial niche development. *FEMS Mycrobiol Review,* **29,** 795-811.

De, K.A. (2011). Provisional macroscopic key to the edible mushrooms of tropical Africa. *MycoAfrica,* **4**, 1-19.

Demain, A.L., (1999). Pharmaceutically active secondary metabolites of microorganisms. *Applied Microbiology and Biotechnology,* **52**(4), 455-63.

Denchev, C.M., Denchev, T. T., & Assyov, B. (2013). Introduction to Mushrooms. In: Identification and sustainable exploration of wild edible mushrooms in rural area (Eds.) Denchev C.M., Venturella G. and Zervakis G. MYCOTa Intereuropean cooperation networks (MYCOTION) Pp. 1-363

Deng, T.F., Chen, C.R., Cheng, M.L., Pan, C.Y., Zhou, Y., & Mo, J.C. (2008). Differences in cellulose Activity among different castes of *Odontotermes formosanus* (*Isoptera*: *Termitidae*) and the symbiotic fungus *Termitomyces albuminosus*. *Sociobiology*, **51**, 697-704.

Devkota, S. (2005a). *Mushroom Diversity in Lumle (Kaski) and Study of Clavariales from Lumle and Vicinity of Kathmandu Valley, Nepal.* A Master's thesis Submitted to the Central Department of Botany, Tribhuvan University, Nepal.

Devkota, S., Tiwari, R.D., Manandhar, V.K., & Adhikari, M.K. (2005b). Some wild mushrooms collected from Lumle, Kaski, Nepal. *Bulletin of Deptment of Plant Resource,* **26,** 10-15. Kathmandu, Nepal.

Devkota, S. (2006). Yarsagumba [*Cordyceps sinensis* (Berk)*;* Traditional utilization in Dolpa district, Western Nepal. Our Nature. *An International Biological Journal,* **4,** 48-52.

Devkota, S. (2008). Distribution and Status of Highland Mushroom: A Study from Dolpa, Nepal. *Journal of Natural History Museum*, **23**, 51-59.

Dietz, W. (2005). Physical activity recommendations: Where do we go from here? *Journal of Pediatrics*, **146,** 719-720.

Dighton, J. (2003). *Fungi in ecosystem processes*. Marcel Dekker, New york.

Dighton, J., White, J.F., & Oudemans, P. (2005). *The Fungal Community: Its Organization and Role in the Ecosystem*. CRS, Boca Ratan, FL.

Dix, N.J., & Webster, J. (1995). *Fungal Ecology*. Chapman and hall, London.

Dixon, P.A. (1983). Reproductive capacity of *Termitomyces striatus*. *Transactions of the British Mycological Society*, **80,** 131-139

*Dobermez, J.F. (1972). *Mise au Point d'une Methode Cartographique d'Etude des Montagnes Tropicales. Le Nepal, Ecologie et Phyogeographic*. Thesis, Universite Scientifique et Medicale, Grenoble. (Not seen in original).

Dobermez, J.F. (1976). Exploitation and prospects of medicinal plants in eastern Nepal. *In Mountain environment and Development*. Swiss Associations for Technical Assistance in Nepal.

Duur, K.A., Vera, I.D.R., Henrik, H.D.F.L., Jannette, M., Wilhelm, D.B.Z. Bernard, S., Corinne, R.L.F., & Jacobus, J.B. (2007). Patterns of interaction specificity of fungus-growing termites and *Termitomyces* symbionts in South Africa. BMC. *Evolutionary Biology*, **7,** 115-125.

Ebeling, W. (1975). *Urban Entomology*. University of California Press, Berkeley, California, 695 p.

Elliott, J.M. (1971). *Some methods for the statistical analysis of samples of benthic invertebrates.* Sci. Publ. 25. Freshwater Biol. Ass., Ambleside, Westmorland.

Enyiukwu, D.N., Awurum, A.N., Ononuju, C.C. & Nwaneri, J.A (2014). Significance of characterization of secondary metabolites from extracts of higher plants in plant disease management. *International Journal of Advance Agriculture Research,* **2**, 8-28.

Eswaran, A., & Ramabadran. R. (2000). Studies on some physiological, cultural and post harvest aspects of oyster mushroom, *Pleurotus eous. Tropical Agricultural Research,* **12,** 360-374.

Evans, W.C. (1997). *Trease and Evans Pharmacogonasy.* 14th Edn. W.B. Saunders Company Limited, Singapore.

Evelyn, B.R., Maxima, E.F., Delia, B. Rodriguez, A., & Jaime, A.F. (2006). Phytochemicals and functional foods. Current situation and prospect for developing countries *Segurança Alimentar e Nutricional, Campinas*, **13**(1), 1-22.

FAO/WHO. (1989). Protein quality evaluation. Report of the joint FAO/WHO expert consultation. Food and Nutrition Paper no. 51. *Food and Agriculture Organizations and the World Health Organization*, Rome.

FAO. (2012a). *World hunger and poverty facts and statistics*.

FAO. (2012b). *Food security remains an issue in Nepal*. Rome.

Fasidi, I.O., & Olorunmaiye, K.S. (1994). Studies on the requirements for vegetative growth of *Pleurotus tuberregium* (Fr.) Singer, a Nigerian mushroom. *Food Chemistry*, **50**(4), 397-401.

Ferreira, I.C.F.R., Barros, L., & Abreu, R.M.V. (2009). Antioxidants in wild mushrooms. *Current Medicinal Chemistry,* **16,** 1543-1560.

Ferreira, I.C.F.R., Vaz, J.A., Vasconcelos, M.H., & Martins, A. (2010). Compounds from wild mushrooms with antitumor potential. *Anti-Cancer Agents Medicinal Chemistry,* **10,** 424-436.

Field, J.A., & Lettinga, G. (1992). *In Plant Polyphenols: Synthesis, Properties Significance*. Hemingway RW, Laks E. (Eds). Lenum Press: New York, 673-692.

Forest, Act. (1993). Ministry of Forest and soil conservation. HMGN/USAID.

Freudenberg, K.S. (2011). A Manual for CRS Field Workers and Partner. Maryland, USA.

Frendenberger, K.S. 2011. A Manual for CRS Field Workers and Partner. Freudenberger 228, W. Lexington Street, Baltimore, Maryland 21201-3443 www.crs.org

Fries, E. (1821 & 1832). *Systema mycologicum*, sistens fungorum ordines, genera et species. Vols. I–II, Lund, Sweden: Berlingiana; Vols. III and Index, Greifswald, Germany: Ernesti Mauritiji.

Frøslev, T., Aanen, D.K., Laessoe, T., & Rosendahl, S. (2003). Hylogenetic relationships in *Termitomyces* and related genera. *Mycological Research*, **107,** 1277-1286.

Frøslev, T., (on-line): The genus *Termitomyces*. http://www.mycokey.com/ BurkinaFaso/Termitomyces.htm.

Gbolagade, J.S., Fasidi, I. O., Ajayi, E.J., & Sobowale, A.A. (2006a). Effect of physico-chemical factors and semi-synthetic media on vegetative growth of *Lentinus subnudus* (Berk.), an edible mushroom from Nigeria. *Food Chemistry,* **99,** 742-747.

Gbolagade, J., Sobowale, A., & Adejoye, D. (2006b). Optimization of submerged culture conditions for biomass production in *Pleurotus florida* (Mont.) Singer, a Nigerian edible fungus. *African Journal of Biotechnology,* **5**(16), 1464−1469.

Gbolagade, J., Kigigha, L., & Ohimain, E. (2007). Antagonistic effect of extracts of some Nigerian higher fungi against pathogenic microorganisms. *American Eurasian Journal of Agricultural & Environmental Science*, **2** (4), 364-368.

Ghorai, S., Banik, S.P., Verma, D., Chowdhury, S., Mukherjee, S., & Khowala, S. (2009). Fungal biotechnology in food and feed processing. *Food Research International*. Review.1-11 p.

*Gómez, L.D. (1995). Una nueva especie neotropical de *Termitomyces* (Agaricales: Termitomtcetaceae). *Revista de Biologia Tropical*, 42, 439-441. (Not seen in original).

Good, R. (1953). *The Geography of the Flowering Plants*. Longmans Green and Co., New York.

Groff, J.L., Gropper, S.S., & Hunt, S.M. (1995). *Advanced Nutrition and Human Metabolism*. West Publishing Company, New York. Minneapolis. 124-146.

Gurib, F.A., (2006). Medicinal plants: Traditions of yesterday and drugs of tomorrow. *molecular aspects of Medicine,* **27,** 1-93.

Gupta, U.C. (1979). Some factors affecting the determination of hot-water-soluble boron from podzol soils using azomethine-H. *Canadian Journal of Soil Science,* **59,** 241-247.

Hagen, T. (1961). *Nepal, The Kingdom in the Himalayas*. Oxford & IBH Publishing Co., New Delhi (3rd edition, 1971).

Hao, Q.P., Jin, F.Y., Yue, M.W., Tian, Y.Z., & Hong, F.W. (2008). Diversity analysis of soil dematiaceous hyphomycetes from the Yellow River source Area. *Journal of Zhejiang University Science,* **6**, 64-69.

Harkonen, M., Saarimaki, T., & Mwasumbi, L. (1995). Edible mushroom of Tanzania. *KARSTENIA*, **35,** Suppl., 92 p.

Härkönen, M. (2002). Mushroom collecting in Tanzania and human (Southern China): inherited wisdom and folklore of two different cultures. *Tropical Mycology,* **1,** 149-165.

Harkonen, M.T.N., & Mwasumbi, L. (2003). *Tanzanian Mushrooms*. Edible, harmful and other fungi. Botanical Museum Finnish Museum of Natural History.

Harmon, M.E., Franklin, J.F., & Swanson, F.J. (1986). Ecology of coarse woody debris in temperate ecosystems. *Advanced Ecological Research,* **15,** 133-320.

Hawker, E.L. (1957). *Physiology of Reproduction in Fungi*. Cambridge University Press. London.

Hawksworth, D.L. (1974). *An introduction to the principal of taxonomy and nomenclature in fungi and lichen*. CMI, England.

Hawksworth, D.L. (1991). The fungal dimention of biodiversity: magnitude, Significance and conservation. *Mycological Research,* **95,** 641-655.

Hawksworth, D.L. (2001). The magnitude of fungal diversity: the 1.5 million species revisited. *Mycological Research,* **105,** 1422-1432.

Hawksworth, D.L., Kirt, P.M., Sutton, B.C., & Pegler, D.N. (1995). *Ainsworth and Bisby's Dictionary of Fungi* 8th ed. International Mycological institute CAB International, Wallingford, Oxon.

Hayes, W.A., & Hand, P. (1981). Interrelated studies of physical, chemical and biological factors in casing soil and relationships with productivity in commercial culture of Agaricus biosporus Lange. *Mushroom Sci*., **11,** 177-181.

He, F.J., & Mac, G.G.A. (2001). Fortnightly review: Beneficial effects of potassium. *British Medical Journal,* **323**(7311), 497-501.

*Heim, R. (1941). Études descriptives et expérimentales sur les agarics termitophiles d'Afrique tropicale. *Mémoires de l'Académie des Sciences de l'Institut de France*, **64,** 1-74, pls 1-10. (Not seen in original).

*Heim, R. (1942a). Novelles etudes descriptives sur les agaris termitophiles d' Afrique tropicale. *Archives de Muse'um National d'Historie*, **6**(18), 1-60. (Not seen in original).

*Heim, R. (1942b). Les champignons des termitieres. Nouveaux aspects d'un problem de biologie et de systematique generales. *Review of Science*, **3205,** 69−86. (Not seen in original).

*Heim, R. (1942c). Nouvelles etudes descriptive sur les Agarics termitophiles d'Afrique tropicale. *Archives du Muse'um National d'Histoire Naturelle,* **6**(18**),** 107-166. (Not seen in original).

*Heim, R. (1951). Les *Termitomyces* du Congo Belge recueillis par Mme M. Goossens-Fontana. *Bulletin du Jardin Botnique de I' Etat*., **21,** 205-222. (Not seen in original).

*Heim, R. (1958). *Termitomyces* Flore Iconographique des Champignons du Conge, Brussels. Fasc. *Jardin Botanique naturelle Belgique.*, **7**, 139-151. (Not seen in original).

*Heim, R. (1977). *Termites et Champignons. Les termitophiles d'Afrique Noire at d'asie Meridionale.* Paris, France: Societe Novelle des Edition. Boubee 11, Place Saint-Michel. Paris-6. (Not seen in original).

Hellawell, J.M. (1978). *Biological Surveillance of Rivers: a biological monitoring handbook*. Water Research Centre, Stevenge Lab, Stevenage, England.

Hibbett, D.S., Binder, M., Bischoff, J.F., Blackwell, M., Cannon, P.F., Eriksson, O.E., Huhndorf, S., & James, T. (2007). A higher-level phylogenetic classification of the Fungi. *Mycological Research*, **111,** 509-547.

HMG/PCSCBS, Nepal. (1988). *Statistical year book of Nepal*. HMG Planning Commission Secretariate Central Bureau of Statistics, Ramshahpath, Thapathali, Kathmandu.

HMG/MFSC. (2002). *Nepal Biodiversity Strategy*. Ministry of Forest & Soil Conservation, Kathmandu, Nepal.

Hoff, J.A., Klopfenstein, N.B., Tonn, J.R., McDonald, G.I., Zambino, P.J., Rogers, J.D., Peever, T.L., & Carris, L.M. (2004). *Role of woody root-associated fungi in forest ecosystem processes: recent advances in fungal identification*. Forest Service, Rocky Mountain Research Station.

Hooker, J.D. (1854). *Himalayan journals* 1-2. J. Murray. London.

Imazeki, R. Otani, Y. & Hongo, T. (1988). *Illustration of fungi of Japan.* YAMA-KEI publishers Co. Ltd. Tokyo. Japan. In proceeding of National Conference on Mushrooms NCMRT Solan

Imtiaj, A.C.J., Lee, G.W., Kim, H.Y., Shim, M.J., Rho, H.S., Lee, H.S., Hur, H., Lee. M.W., Lee, U.Y., & Lee. T.S. (2008). Physiochemical requirement for the vegetative growth of *Schizophyllum commune* collected from different ecological origins. *The Korean Society of Mycology*, **36**(1), 34-39.

Inamdar, E.K., Yeole, R.D., Ghogare A. B., & Souza. N.J.D. (1996). Determination of biologically active constituents in *Centella asiatica. Journal of Chromatography A.*, **742,** 127-130.

Isiloglu, M., Merdivan, M., & Yilmaz, F. (2001). Heavy metal contents in some macrofungi collected in the North western part of Turkey. *Environmental Contamination and Toxicology*, **41,** 1-7.

Jackson, M.A. (1997). Optimizing nutritional conditions for vthevliquid culture production of effective fungal biological control agents. *Journal of Industrial Microbiology & Bictechnology,* **19,** 180-187.

Jagadish, K.L., Gunasundari, D., Hemalatha, M., Shenbhagaraman, R., & Kaviyarasan, V. (2010). Antioxidant and phytochemical potential of wild edible mushroom *Termitomyces reticulatus*: Individual cap and stipe collected from south eastern part of India. *Intrnational Journal of Pharmaceutical Sciences Resources,* **1**(7), 62-72.

Jamalludin, Goswami, M.G., & Ojha, B.M. (2001). *Fungi of India* (1989-2001). Forest Pathology Division. Tropical Forest Research Institute, Publisher Pawan Kumar, Scientific Publishers, Jabalpur.

Jayasinghe, C., Imtiaj, A., Hur, H., Lee, G.W., Lee, T.S., & Lee. U.Y. (2008). Favorable Culture Conditions for Myceliall Growth of Korean Wild Strains in *Ganoderma lucidium*. *The Korean Society of Mycology,* **36**(1), 28-33.

Jiskani, M.M. (2001). *Energy potential of mushrooms*. The DAWN Economic and Business, Review, Oct. 15-21, 2001. P. IV.

Johnson, R.A., Thomas, R.J., & Wood, T.G. (1981). The inoculation of the fungus combs in newly founded colonies of some species of *Macrotermitinae* (*Isoptera*) from Nigeria. *Journal of Natural History,* **15,** 751-756.

Jonathan, G., Ayodele, S., & Damilola. A. (2006). Optimization of sub-merged culture conditions for biomass production in *Pleurotus florida* (Mont.) Singer, a Nigerian edible fungus, *African Journal of Biotechnology,* **5**(16), 1464-1469.

Jordan, C.F. (1985). *Nutrient cycling in tropical forest ecosystems*. Wiley, Chichester, West Sussex.

Joshi, K., & Joshi, A.R. (1999). Ethnobotanical study of some wild mushrooms of the two valleys (Kathmandu and Pokhara) of Nepal. *Ethnobotany,* **11** (1&2), 47-56.

Joshi, K., & Joshi, A.R. (2008). Ethnobotanical study on some lower plants of the central development Region, Nepal. *Ethnobotanical Leaflets,* **12,** 832-40.

Joshi, A.R., & Joshi, K. (2005). *Ethnobotany and Conservation of Plant Diversity-Status, Bibliography and Agenda for Sustainable Management,* Rub Rick, Kathmandu.

Kabasa, J.D., Olila, D. Okethwangu, D., Munishi, P.K.T., & Kisovi, I., (2006). Nutritive and Nutriceutic Potential of Indigenous Bubaala Mushrooms *Termitomyces microcarpus* From the Lake Victoria Basin. *African Journal of Animal and Biomedical Science*, **1**(1), 92-96.

Kaul, T.N. (1992). *Conservation of Mushroom Resources of India*. Introduction and Action Plan, World Wide Fund for Nature-India, New Delhi.

Kaul, T. N. (1999). *Introduction to Mushroom Science (Systematic).* Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, India.

Kendrick, B. (1985). *The fifth kingdom*. Waterloo Mycologue Publication. 364 p.

Kharel, S. (1999). New record of *Lentinellus ursinus* (Fr.: Fr.) Kuhner. In abstracts of *Third National Conference of Science and Technology*. Organized by RONAST, Kathmandu, Abs: BSC 003: 64.

Kharel, S., & Rajbhandary, S. (2005). Ethnomycological knowledge of some wild edible mushrooms in Bhardeo, Lalitpur, Nepal. *Journal of Plant Science.*, **1,** 45-48.

Khowala, S., Ghosh, A.K., & Sengupta, S. (1992). Saccharification of xylan by an amyloglucosidase of *Termitomyces clypeatus* and synergism in the presence of xylanase. Applied Microbiology and Biotechnology, **37,** 287-292.

Khush, R.S., Becker, E., & Wach, M. (1992). Amplification polymorphisms of the cultivated mushroom *Agaricus bisporus*. *Applied Environmental Microbiology*, **8,** 2971-297.

Kibby, G. (1979). *Mushrooms and Toadstools.* Oxford University Press, London.

Kirk, P., Cannon, P.F., Minter, D.W., & Stalpers, J.A. (2008). Ainsworth & Bisby's *Dictionary of the fungi* 10th edn. CAB International, Wallingford, U.K.

Kjeldahl, J.Z. (1883). A new Method for the determination of nitrogen in organic bodies. *Analytical Chemistry*, **22**(1883), 366.

Klimczak, I., Malecka, M., Szlachta, M., & Gliszczy, A.S.W. (2007). Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. *Journal of Food Composition and Analysis*, **20,** 313-322.

Korb, J. (2003). Thermoregulation and ventilaton of termite mounds. *Naturwissenschaften*, **90,** 212-219.

Kosakul, T., Boasri, A., Chalermpongse, A. & Kuhiran, M. (2007). Genetic diversity of *Termitomyces* in central Thailand using isozyme markers. *The journal of Scientific Research,* Chulalongkorn University, **32**, 63-72.

Krieger, H. (1967). *The Mushroom Handbook*. Dover. New York.

Kunugawa, K., & Furukawa, H. (1965). The fruit body formation in collybia velutipes induced by lower temperature treatment of one short duration. *Botanical Magazen*, **78**, 256-273.

Kumari, D., Reddy, M.S., & Upadhyay, R.C. (2011a). Nutritional composition and Antioxidant Activities of eighteen different wild *Cantharellus* mushrooms of north-western Himalaya. *Food Science and Technology International.* **17**(6), 557-67.

Kumari, D., Reddy, M.S., & Upadhyay, R.C. (2011b). Antioxidant Activity of three Species of wild mushroom *Cantharellus* collected from north-western Himalaya, India. *International Journal of Agricultur and Biology.* **13**(3), 415-418.

Kuo, M. (2006). *Making spore prints*. Retrieved from the Mushroom Expert. Com Web site: http://www.mushroomexpert.com/spore_print.html.

Kurasawa, S.L., Sugahana, J., & Hayashi, J. (1982). Studies on dietary fibre of mushroom and edible wild plants. *Nutritional Report International*, **26,** 167-173.

Lai, W.H., Murni, M.J.S., Fauzi, D., Abas, O.M., &. Saleh, V.N.M. (2011). Optimal Culture Conditions for Myceliall Growth of *Lignosus rhinoceros*. *The Korean Society of Mycology,* **39**(2), 92-95.

Landers, K.E. (1964) Growth of *Cercospora arachidicola* in a glucose-agar-asparagine-thiamine medium. *Phytopathology*, **54**, 1236-1239.

Leelavathy, K.M., Flower, S.L. & Suja, C.P. (1983). The Genus *Termitomyces* in India. *Science and Cultivation Technology of Edible fungi*, 402-406 p.

Leelavathy, K.M., little, F., & Suja, C.P. (1985). The genus *Termitomyces* in India. *Indian Mushroom Science,* **2,** 402-407.

Lee, S.S., Chang, Y.S., & Noraswati, M.N.R. (2009). Utilization of macro fungi by some indigenous communities for food and medicine in Peninsular Malaysia. *Forest Ecology and Management,* **257,** 2062-2065.

Lepš, J., & Šmilauer, P. (2003). *Multivariate analysis of Ecological data using Canoco.* Cambridge University Press, Cambridge.

Lindequist, U., Niedermeyer, T.H.J., & Julich, W.D. (2005). The pharmacological potential of mushrooms -Review. *Evidence Based Complementary Alternative Medicine,* **2** (3), 285-99.

Lindsay, W.L., & Norvell, W.A. (1978). Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Science Society of America Journal*, **42,** 421-428.

Linnaeus, C. (1753). *Species Plantarum*. Exhibitentes Plantas Rite Cognitas ad Genera Relatas, cum Differentiis Specificis, Nominibus Trivialibus, Synonymis Selectis, et Locis Natalibus, Secundum Systema Sexuale Digestas, ed. 1. Laurentius Salvius, Stockholm, Sweden. Facsimile published 1957-1959 as Ray Soc. Publ. 140 and 142. The Ray Society, London.

Lloyd, C.G. (1808). *Mycological notes. Mycology*. Cincinnati, Ohio: Llyod Library & Museum 1898-1925.

Lodge, D.J. (1992). *Nutrient cycling by fungi in wet tropical rainforest. In: Whalley AJS (ed) Aspects of tropical mycology*. Cambridge University, University of Liverpool.

Lowy, B. (1974). *Amanita muscaria* and thunderbolt legend in Gutemala and Mexico. *Mycologia,* **66,** 186-190.

Makonde, H.M., Boga, H.I., Osiemo, Z., Mwirichia, R., Stielow, J.B., Goker, M., & Klenk, H.P. (2013). Diversity of *Termitomyces* associated with Fungus-Farming termites assessed by cultural and Culture-Independent Methods. www.plosone.org **8**(2), 8-10.

Manandhar, N.P. (2002). Ethnomedicinal plants diversity and their conservation in Nepal. In: *Recent Progress in medicinal plants.* (eds.) Singh, V.K., J.N. Govil and G. Singh. Publ. Studium Press LLC, USA. **1,** 41-46.

Manandhar, K.L. (2003). *Mushroom Cultivation Technology for women's Income.* Proceeding of International conference on women, Science & Technology for poverty Alleviation, Organized by Women in Science and Technology (WIST) Kathmandu, Nepal.

Manandhar, V.K., & Adhikari, M. K. (2009). The family Sclerodermataceae: ectomycorrhizal fungi from Nepal. *Bulletin of Deptarment of Plant Resource*, **31,** 29-34.

Manjula, B., (1983). A revised list of the Agaricoid and Boletoid basidiomycetes from India and Nepal. *Proceedings of the Indian Academy Science* (*Plant Science*), **92,** 81-213.

Manjunathan, J., & Kaviyarasan, V. (2010). Studies on the growth requirements of *Lentinus tuberregium* (Fr.). an edible mushroom of Middle-East. *Journal of Scientific Research,* **5**(**2**), 81-85.

Manjunathan, J., & Kaviyarasan, V. (2011). Optimization of mycelial growth and antimicrobial activity of new edible mushroom, *Lentinus tuberregium* (Fr.). Tamil Nadu., India. *International Journal of Pharm Tech Research,* CODEN (USA): IJPRIF ISSN: 0974-4304, **3**(1), 497-504.

Manning, K. (1995). Food value and chemical composition. *The biological and Technology of cultivated mushrooms.* John Wiley and Sons, New York.

Manzi, I., Gambelli, L., Mariconi, S., Vivanti, V., & Pizzoferrato, I. (1999). Nutrients in edible mushrooms: an interspecies comparitive study. *Food Chemistry,* **65,** 477-482.

Manzi, I., Aguzzi, A., & Pizzoferrato, I. (2001). Nutritional value of mushrooms widely consumped in Italy. *Food Chemistry*, **73**(3), 321-325.

Masamba, K.G., & Kazombo, M.R. (2010). Determination and comparison of nutrient and mineral contents between cultivated and indigenous edible mushrooms in Central Malawi. *African Journal of Food Science*, **4** (4), 176-179.

Mendal, L.B. (1898). The chemical compostion and nutritive value of some edible American fungi. *American Journal of Physiology*, **1,** 225-238.

Merwin, H.D., & Peech, M. (1950) Exchangeability of soil potassium in the sand, silt and clay fractions as influenced by the nature of the complimentary exchangeable cations. *Soil Science Society of America Proceedings,* **15,** 125-128.

Miller, O.K. (1984). *Mushroom of North America*. E. P. Duttom Publishing Company Inc. New York.

Miyamoto, T., Igarashi, T., & Takahashi, K. (2000). Liblin degrading ability of litter decomposing Basidiomycetes from Picea forest of Hokkaido. *Mycoscience,* **41,** 105-110.

Moordian, A.D., & Thurman, J.E. (1999). Drugs therapy of postprandial hyperglycemia. *Drugs,* **57,** 19-29.

Morris, B. (1986). Notes on the genus *Termitomyces* Heim in Malawi. *Society of Malawi Journal,* **39,** 40-49.

*Mossebo, D.C., Amougou, A., & Atangana, R.E. (2002). Contribution a let du genre *Termitomyces* (Basidiomycetes) au Cameroun: Ecologie et systematique. *Bulletine De la Mycologique de France*, **118** (3), 19. (Not seen in original).

Mueller, G.M., Schmitt, J.P., Leacock, P.R., Buyck, B., Cifuentes, J., Desjardin, D.E., Halling, R.E., Hijorstam, K., Iturriaga, T., Larsson, K.H., Lodge, D.J., May, T.W., Minter, D., Rajchenberg, M., Redhead, S.A., Ryvarden, L., Trappe, L.M., Walting, R., & Wu, Q.X. (2007). Global diversity and distribution of macrofungi. *Biodiversity and Conservation.* **16,** 37-48.

Mukherjee, S., & Khowala, S. (2002). Regulation of cellobiase secretion in *Termitomyces clypeatus* by Co-Aggregation with Sucrase. *Current Microbiology,* **45** (1), 70-73.

Mukherjee, S., Chowdhury, S., Ghorai, S., Pal, S., & Khowala, S. (2006). Cellobiose from *Termitomyces clypeatus*: Activity and Secretion in Presence of Glycosylation Inhibitors. *Biotechnology Letters*, **28**(21), 1773-1778.

Mukhopadhyay, A., Hazra, P.P., Sengupta, T., Ghosh, A.K., & Sengupta, S. (1997). Acetyl esterase production by *Termitomyces clypeatus*. *Biotechnology Letters*, **19**(2), 159-161.

Mukiibi, J. (1973). The nutritional value of some Uganda mushrooms. *Acta Horticulture*, **33,** 171-175.

Munyanziza, E., & Ngaga, Y.M. (2006). Survey of Edible Mushrooms of the Miombo Woodlands through Economic Incentives in two Selected Villages, Iringa Tanzania. *Discovery and Innovations Journal*, **18**(4), 304-310.

Murray, R.K., Granner, D.K., Mayes P.A., & Rodwell, V.W. (2000). *Harper's Biochemistry*. 25th Edition, McGraw-Hill, Health Profession Division, USA.

Murrin, F. (1996). *Fungi and insects*. In The *Mycota* VI, Human and animal relationships, edited by Howard, D. H. and Miller, J. D. springer-Verlag Berlin Heidelberg.

Nabubuya, A., Muyonga, J.H., & Kabasa, J.D. (2010). Nutritional and hypocholesterolemic Properties of *Termitomyces microcarpus* mushrooms. *African Journal of Food Agriculture, Nutrition and Development,* **10**(3), 2235-2257.

Nakalembe, I., Kabasa, D., & Olila, D. (2009). Indigenous knowledge and usage of wild mushrooms in Mid–Western, Uganda. *African Journal of Animal Biomedical Science,* **4**(2), 63-73.

Natarajan, K. (1975). South Indian Agaricales I. *Termitomyces. Kavaka,* **3,** 63-66.

Natarajan, K. (1977). A new species of *Termitomyces* from India. *Current Science,* **46**(19), 679-680.

Natarajan, N.K. (1979). South Indian Agaricales: *Termitomyces heimii. Mycologia,* **71**(4), 853-855. URL: http//www. jstor.org/Stable/375920/

Newbound, M. (2009). *Fungal diversity in remnant vegetation patches along an urban to rural gradients.* Ph.D. thesis. University of Melbourne, Melbourne.

Nobre, T., Eggleton, P., & Aanen, D.K. (2010). Vertical transmission as the Key to the colonization of Madagascar by fungus termites? Proceeding of the Royal Society B: *Biological Science*, **277,** 359-365.

Nobre, T., & Aanen, D.K. (2012): Fungiculture or termite husbandry? The ruminant hypothesis. *Insects*, **3**, 307-323.

Oboh, G., & Shodehinde, S.A. (2009). Distribution of nutrients, polyphenols and antioxidant activities in the pilei and stipes of some commonly consumed edible mushrooms in Nigeria. *Bulletin of Chemical Society of Ethiopia*, **23**(3), 391-398.

Ogundana, S.K., & Fagade, O. (1981). The nutritive value of some Nigerian edible mushrooms. In: *Mushroom Science XI*, Proceedings of the Eleventh International Scientific Congress on the Cultivation of Edible Fungi, Australia. 123-131p.

Ohenoja, E. (1988). Effect of forest management procedures on fungal fruit body production in Finland. *Acta Botanica Fennica*, **136**, 81-84.

Okwu, D.E. (2001). Evaluation of chemical composition of indigenous species and flavoring agents. *Global Journal of Pure and Applied Science,* **7**(3), 455-459.

Okwulehie, I.C., & Odunze, E.T. (2004). Evaluation of the Myco-chemical and mineral composition of some tropical edible mushroom. *Journal of sustainable Agriculture and environment,* **6**(1), 63-70.

Okwu, D.E. & Ohenhen, O.N. (2010). Isolation and characterization of Steroidal Glycosides from the leaves of *Stachytarpheta Jamaicensis* Linn Vahl. *Der Chemica Sinica*, **1** (2), 6-14.

Oliveira, P.D. (2009). Mushroom poisoing (Review article). *Medicina Interna,* **16**, 232-238.

Olsen, S.R.C., Watanabe, C.V.F.S., & Dean, L.A. (1954). *Estimation of available Phosphorus in soil by extraction with sodium bicarbonates*. U.S.D.A., Washington, D. C. circ. No. 939, 1-9 p.

Otieno, N.C. (1969). Further contributions to knowledge of termite fungi in East Africa: the genus *Termitomyces*. *Sydowia,* **22,** 160-165.

Pacioni, G. (1985). *The Mcdonald's encyclopedia of mushrooms and toadstools.* McDonald & Co. Ltd. London.

Packham, J.M., May, T.W., Brown, M.J., Wardlaw, T.J., & Mills, K.A. (2002). Macrofungal diversity and community ecology in mature and regrowth wet eucalpt forest in Tasmania: A multivariate study. *Australian Ecology,* **27,** 149-161.

Pandey, N. (2008). *Mushroom Diversity in central Nepal: An ethnomycological Approach.* A Doctorate thesis Submitted to the Central Department of Botany, Tribhuvan University, Nepal.

Pearson, K. (1990). On the criterion that a given system of deviations from the probable in the case of a correlated system of variables is such thatit can be reasonable supposed to have arisen from random sampling. *Philosophical magazine* series 5, **50**(302), 157-175.

Pegler, D.N., & Vanhaecke, M. (1994). Termitomyces of Southeast Asia. *Kew Bulletin*, 49**,** 717-736.

Phillips, R. (1981). *Mushrooms and other Fungi of Great Britain and Europe.* Pan Book Ltd. London.

Pichayangkul, S. (2004). *Termitomyces* sp. *Volvariella volvacea* and Fusant. *Hybrid mushrooms*. 2nd. Bangkok.

Piearce, G.D. (1987). The genus *Termitomyces* in Zambia. *The Mycologist,* **1,** 111-116.

Pokharel, C.P., Yoshimoto, H., Iida, S., & Ogha, S. (2009a). Myceliall growth and fruit body formation of *Lyophyllum decastes* in Livestock Compost. *Journal of Faculty of Agriculture, Kyushu University,* **49**(2), 273-282.

Pokharel, C.P., Yadav, R.K.P., & Obga, S. (2009b). Effect of physical factors and synthetic edia on myceliall growth of *Lyophyllum decastes*. *Journal of Ecobiotechnology,* **1**(1), 46-50.

Poudel, R. (2012). *Optimization of cultural media for myceliall growth of Volvariella taulorii (Berk. & Broome) Singer, an edibe mushroom of Nepal*. A Master's thesis Submitted to the Central Department of Botany, Tribhuvan University, Nepal.

Purkayastha, R.P., & Chandra, A. 1975. *Transactions of the British Mycological Society,* **64**(l), 168.

Pushpa, H., & Purushothama, K.B. (2010**).** Antimicrobial activity of *Lyophyllum* an edible wild mushroom. *World Journal of Agricultural Science,* **6(**5), 506-509.

Rajak, R.C., & Rai, M.K. (2005). Edible Tribal Mushroom Resources of Central India and their Ethnological Aspects. *International Journal of Medicinal Mushroom,* **7**(3), 458.

Ramesh, N.M.B.V., Saraswathy, A., Balakrishna, K.B., & Akshmanaperumalsamy, P. (2002). Antimicrobial and phytochemical studies of *Swertia corymbosa. Fitoterapia*, **73,** 160-164.

Ramsbottom, J. (1954). *Mushrooms & toadstools*. Collins 14 ST Jame's Place London.

Rana P. & Giri A. (2008). Ethnomycological uses of some wild mushrooms in Sagarmatha National Park, Nepal. In: Medicinal Plants in Nepal: An Anthology of Contemporary Research. (eds. P.K.Jha, S.B. Karmacharya, M.K.Chetri, C.B. Thapa and B.B.Shrestha). *Ecological Society* (ECOS), Kathmandu, Nepal. 140-146 p.

Rayner A.D.M., & Boddy, L. (1988). *Fungal Decomposition of Wood.* John Wiley and Sons, Chichester.

Rawla, G.S., Arya, S., & Sarwal, B.M. (1983). Species of *Termitomyces* Heim from Chandigarh. India. *Bibliography of systematic Mycology*, **91**, 13-21.

Reid, D.A. (1975). *Termitomyces umkowaani* Contr. *Bolus Herbarium*, 7: 118.

Reid, D.A. (1990). The *Leucocoprinus badhamii* complex in Europe: species which redden on bruising or become green in ammonia fumes. *Mycological Research*, **94** (5), 641-670.

Rijal, A. (2011). Surveving on Knowledge: Ethnobatany of Chepang Community from mid hill of Nepal. A Journal of Plants, People and applied research*: Ethnobotanical* R*esearch and Applications*, **9,**181-215.

Rinaldi, A., & Tyndalo, V. (1972). *The Complete Book of Mushrooms*. Cresent book, New York.

Roberts, C., Ceska, O, Kroeger, P., & Kendrick, B. (2004). Macrofungi from six habitats over five years in Clayoquot Sound, Vancouver Island. *Canadian Journal of Botany*, **82**, 1518-1538.

Rossman, A., Tulloss, R.E., O'Dell, T.E., & Thorn, R.G. (1998). *All Taxa Biodiversity Inventory of Fungi in a Costa Rican Conservation Area.* Parkway Publishers, Boone, North Carolina.

Rouland, L.C. (2002). *Symbiosis with fungi*. In Termites: Evolution, Sociality, Symbioses, Ecology. T. Abe, D.E. Bignell and M. Hihashi, eds. Kluwer Academic Publishers, Dordrecht. 289-306 p.

Rupasinghe, H.P., Jackson, C.J., Poysa, V., Berado, C.D., Bewley, J.D., & Jenkinson, J. (2003). Soyasapogenol A and B distribution in soyabean (Glycine Max L. Merr) in relation to seed physiology, genetic variability and growing location. *Journal of Agricultural and Food Chemistry*, **51**(20), 5888-5894.

Saarimäki, T., Härkönen, M., & Mwasumbi, L. (1994). Tanzanian mushrooms and their uses 3. *Termitomyces singidensis*, sp. nov. *Karstenia*, **34**, 13-20.

Saccardo, P.A. (1882 & 1931). *Sylloge Fungorum Omnium Hucusque. Cognitorum*, **1**, 26. Pavia.

Sacherer, J. (1979). The high altitude ethnoboyany of the Rolwaling Sherpas: a contribution of Nepalese studies. *Journal of Research Centre for Nepal Asia Studies,* **6,** 46-64.

Sagara, N. (2000). *Symbiotic association between animal and mushrooms*. In (A hand book of mushrooms edited kinugawa, K. and M. Ogawa). Asakurasyten, Tokyo, 284-289 p.

Saini, S.S., & Atri, N.S., (2000). Collection and Study of Agarics. An Introduction. *Indian Journal of Mushroom,* 18 (1&2), 1-5.

Sands, W.A. (1970). The association of Termites and fungi. In*: Biology of Termites I.* Krishna K. and Weesner F.M. eds. 495-524. Academic Press. New York.

Sawhasan, P., Worapong, J., & Vinijsanun. T. (2011). Morphological and Molecular Studies of selected *Termitomyces* species collected from 8 Districts of Kanchanaburi Province, Thailand. *Thai Journal of Agricultural Science,* **44**(3), 183-196.

Scalbert, A. (1991). Antimicrobial properties of tannins. *Phytochemistry,* **30,** 3875-3883.

Schmit, J.P. (2005). Species richness of tropical wood-inhabiting macrofungi provides support for species energy theory. *Mycologia,* **97,** 751-761.

Schuurman, G.W. (2005). The influence of edaphic factors and plant diversity on detritivore composition: Termites and decomposition in semi and glasslands and woodlands of the Okavango Delta, Africa. Document: Gre-3-52-55, University of Washington, Seattle, WA 98195, USA, 2 p.

Sellmeyer, D.E., Schloetter, D.E., & Schloetter, M. (2002). Potassium citrate prevents urine calcium excretion and bone resorption induced by high sodium chloride diet. *Journal of Clinical Endocrinol Metab,* **87**(5), 2008-12.

Sensarama, P. (1996). Emergency Food in Kautilya's Arthsastra. An ancient Sanskrit work. In *Ethnobiology in human welfare*. Ed. Jain S.K. Deep Publ. New Delhi. 20-21.

Sensarma, P. (1998). *Ethnobiological Information In Kautiliya Arthasastra.* Naya Prokash, Calcutta. 138 p.

Shankar, K., & Shrestha, P.P. (1999). *Climate*. Nepal's nature Paradise. Eds. By T.C. Majupuria and R. Kumar. M. Devi, Gwalior, 39-44 Pp.

Shim, S.M., Lee, K.L., Im. K.H., Lee, U.Y., Lee, M.W., & Lee. T. S. (2003). The Characteristics of cultural conditions for the myceliall growth and fruiting body formation of *Paecilomyces sinclairii. The Korean Society of Mycology,* **31**(1), 8-13.

Shim, S.M., Oh, Y.H., Lee, K.R., Kim, S.H., Im. K.H., Kim, J.W., Lee, J.U., Shim, Y.O., Shim, M.J., Lee, M.W., Ro, H.S., Lee, H.S., & Lee, T.S. (2005a). The Characteristics of cultural conditions for the myceliall growth of *Macrolepiota* procera. *The Korean Society of Mycology,* **33**(1), 15-18.

Shim, S.M., Oh, Y.H., Lee, K.R., Kim, S.H., Im, K.H., Kim, J.W., Lee, U.Y., Shim, J.O., Shim, M.J., Lee, M.W., Ro, H.S., Lee, H.S., & Lee, T.S. (2005b). The cultural conditions affecting the optical myceliall growth of *Cystoderma amianthinum*. *The Korean Society of Mycology,* **33**(1), 15-18.

Shingh, R., & Shingh, U.C. (2005). *Modern Mushroom Cultivation*. Shyam Printing Press, Jodhpur.

Sibounnavong, P., Cynthia, C.D., Kalaw, S.P., Reyes, R.G., & Soytong, K. (2008). Some species of macrofungi at Puncan, Carranglan, Nueva Ecija in the Philippines. *Journal of Agricultural Technology* **4**, 105-115.

Singer, R. (1986). *The Agaricales in modern taxonomy* (4th edition), Bishen Singh and Mahendra Pal Singh, Dehradun. 981pp.

Singh, S.C. (1966). Mushrooms. *Deorali,* **4,** 15-31.

Singh, S.C. (2007). Status of Mushroom Cultivation in Nepal with reference to wrld production and consumption trends. In: *Mycological Research and Mushroom Production in Nepal* (eds.) Singh S. C., Adhikari M.K. and Tiwari R.D. The Mycological and Phytopathological Society, Nepal (MAPSON). Pp 1-19.

Singh, S.C., & Nisha. (1973a). Notes on some Cercosporae of Nepal. *Journal of Tribhuvan University,* **8, 91**-99.

Singh, S.C., & Nisha. (1973b). Experimental cultivation of pleurotus in Nepal. *Journal of Tribhuvan University,* **8**, 81-85.

Singh, S. C. & Nisha. (1974). *Exobasidium butleri in Nepal. Indian Phytopathology,* **27,** 387-389.

*Singh, S.C., & Shrestha, R. (1986). Two New Records of Fungi for Nepal. *Himalayan Research and Development.* India, **5**, 71-72. (Not seen in original).

Singh, R., & Bhuju, D.R. (2006). Development of Science and Technology in Nepal. *Science, Technology and Society,* **6**(1), 159-178.

Siwakoti, M., & Siwakoti, S. (1998). Ethnobotanical uses of plants among the Limbu of Morang District, Nepal. *Ecoprint*. **59** (1), 79-84.

Smith, J., & Steinemann. T.L. (2000). Vitamin A deficiency and the eye. *International Ophthalmology Clinic*, **40**(4), 83-91.

S-PLUS, (2000). Professional Edition for Windows, Release 2. MathSoft, Inc.

Srivastava, B., Dwivedi, A.K., & Pandey, V.N. (2011). Ethnobotanical survey, distribution survey, distribution and utilization of *Termitomyces* in Gorakhpur Forest Division. *Plant Science Feed*, **1**(3), 28-33.

Stainton, J.D.A. (1972). *Forest of Nepal*. John Murray Ltd. London. 181 p.

Stearn, W.T. (1960). Allium and Meliola in the central and western Himalaya. *Bulletin of British Museum* (*Natural History*) *Botany*, **2**(6), 159-191.

Subba, S.K. (1975). *Effect of Carbon and Nitrogen Sources on growth of Choanephora infundibulifera (Curry) D.D. Cunn*. A Master's thesis Submitted to the Central Department of Botany, Tribhuvan University, Nepal.

Suman, B.C., & Sharma, V.P. (2007). *Mushroom Cultivation and Uses*. Bharat Printers (Press), Jodhpur.

Swan, L.W., & Leviton, A.E. (1962). *Herpetology of Nepal*. Proceeding of the California Academy of Science, **32**(60), 103142.

Swapna, S., Syed, A., & Krishnappa, M. (2008). Diversity of macrofungi in semi evergreen and moist deciduous forest of Shimoga district-Karnataka, India. *Journal of Mycology and Plant Pathology,* **38,** 2-26.

Tang, B.H., Wei, T.Z., & Yao, Y.J. (2005). Type revision of three *Termitomyces* species from India. *Mycotaxon*, **94,** 93-102.

Tang, B.H., Wei, T.L., & Yao, Y.J. (2006). Revision of *Termitomyces* species originally described from China. *Mycotaxon*, **95**, 285-293.

Taprab, Y., Johjima T., Maeda, Y., Moriya, S., Trakulnaeamsai, S., Naparatnaraporn, N., Ohkuma, M., & Kudo, T. (2005). Symbiotic fungi produce laccases

potentially involved in fungus combs of fungus growing termites in Thialand. *Applied and Environmental Microbiology*, **71**(12), 7696-7704.

ter Braak, C.J.F., & Smilauer, P. (2012). Canoco reference manual and user's guide: software for ordination, Canoco 5. Biometris, Plant Research International, The Netherlands and Czech Republic.

Thimmaiah, S.K. (1999). *Standard method of biochemical analysis*. Kalyani Publishers, New Delhi.

Thomas, R.J. (1987). Factors affecting the distribution and activity of fungi in the nests of Macrotermitinae (Isoptera). *Soil Biology and Biochemistry*, **19,** 343-349.

Tibuhwa, D.D., (2012a). Antiradical and antioxidant activities of methanolic extracts of Indigenous termitarian mushroom from Tanzania. *Food Science and Quality Management*, **7,** 13-23.

Tibuhwa, D.D., (2012b). *Termitomyces* species from Tanzania, their cultural properties and unequalled basidiospores. *Journal of Biology and Life Science.* **3**, 140-159.

Tibuhwa, D.D., Kivaisi, A.K., & Magingo, F.S.S. (2010). Utility of the Macro-Micromorphological characterictics used in classifying the species of *Termitomyces*. *Tanzanian Journal of Science*, **36,** 31-45.

Tie, Z.W., Bao, H.T., Yi, j.Y., & Pegler, D.N. (2006). *A revision of Sinotermitomyces, a synonym of Termitomyces (Agaricales)***.** *Fungal Diversity*, **21**, 225-237.

Trowell, H., Southgate, D.A., Wolever, T.M.S., Leeds, A.R., Qssull, M.A., & Jenkins. D. (1976). Dietary fibre redefined. *Lancet.*, 1, 967.

Tulloss, R. E. & H. R. Bhandary. (1992). Taxonomy and Morphology of *Amanita* and *Limacella*. http://www.amanitaceae.org/?Amanita+Chepangiana,Accessed 6th June2012.

Tulloss, R. E., T. Hongo & H. R. Bhandary. (1992). *Amanita neovoidea*-Taxonomy and Distribution. *Mycotaxon.* **95,** 235-242.

Tulloss, R. E. (1994). *Seminario sobre Amanita* (Supporting Materials for a Seminar on *Amanita*). Univ. Autón. Tlaxcala. 127 p.

Turnbull, E., & Watling, R. (1999). Some records of *Termitomyces* from old world rain forests: 731-738.

Ulziijargal, E., & Mau, J.L. (2011). Nutrient compositions of Culinary Medicinal Mushroom fruiting body and mycelial. *International Journal of Medicinal Mushroom*, **13**(4), 343-349.

Underwood, L.M. (1899). *Moulds, mildews and mushrooms: a guide to the systematic study of the Fungi and Mycetozoa and their literature*. New York, New York: Henry Holt.: 97.

Uprety, Y, Poudel, R.C., Shrestha, K.K., Rajbhandary, S., Tiwari, N.N., Shrestha, U.B., & Hugo, H.A. (2012). Diversity of use and local knowledge of wild edible plant resources in Nepal. *Journal of Ethno biology and Ethno medicine*, **8**, 16. DOI: http://www.ethnobiomed.com/content/8/1/16

Van der W., G.C.A., & Eicker, A. (1990). Species of *Termitomyces in South Africa Mycologist*, **94,** 923-937.

Vrinda, K.B., & Pradeep, C.K. (2009). *Termitomyces sagittiformis.* A lesser known edible mushroom from the Western Ghats. *Mushroom Research,* **18,** 33-36.

Vrinda, K.B., Pradeep, C.K., & Abraham, T.K. (2002). *Termitomyces umkowaani* (Cooke and Mass.) Reid- an edible mushroom from the Western Ghats. *Mushroom Research*, **11**(1), 7-8.

Walkley, A. & Black, I.A. (1934). An examination of the Degtjareff method for determining rganic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. *Soil Science*, **63,** 251-263.

Wasser, S.P., & Weis, A.L. (1999). Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: current perspectives (Review). *International Journal of Medicinal Mushrooms*, **1,** 31-62.

Wasson, G.R. (1969). *Soma-Divine mushroom of immortality XIII*, Hew Court Brace & world Inc., New York: 318.

Weber, N.A. (1966). Fungus-growing ants; a symbiotic relationship exist between an insect and a plant, involving an effevtive culturing technique. *Science*, **153,** 587-604.

Webester, J. (1970). Introduction to Fungi. (2nd Edn.) Cambridge University Press, Cambridge. New York 424 p.

*Wei, T.Z., & Yao, Y.J. (2003). Literature review of *Termitomyces* species in China. *Fungal Science*, **18**, 39-54. (in Chinese). (Not seen in original).

Wei, T.Z., Bao, H.T., Yi, j.Y., & Pegler, D.N. (2006). A revision of *Sinotermitomyces*, a synonym of *Termitomyces* (Agaricales)**.** *Fungal Diversity,* **21**, 225-237.

Wiensczyk, A., Gamiet, S., Durall, D., Jones, M., & Simard, S. (2002). Ectomycorrhizae and forestry in British Columbia: A summary of current research and conservation strategies. *British Columbia Journal of Ecosystem Management,* **2**(1), 1-19.

Winkler, D. (2004). Yartsa Gunbu-*Cordyceps sinensis*, Economy, Ecology & Ethnomycology of a fungus endemic to the Tibetan Plateau (Eds. A. Boesi and F. Cardi). *Wildlife and plants in traditional and modern Tibet: Conceptions, Exploitation and Conservation*. 34 (1/2).

Witkowska, A.M., Zujko, M.E., & Mironsuzuk-chodakowaska, I. (2011). Comparative study on wild edible mushrooms as a source of Antioxidants. *International Journal of Medicinal Mushroom*, **13**(4), 335-351.

Wood, T. (1992). *VA Mycorrhizal fungi*: *Challenges for Commercialization. In: Handbook of applied Mycology.* Fungal Biotechnology, Arora, D. K., R.P. Elander and K.G. Mukerji (Eds), Marcel Dekker, New York.

Wood T.G., & Sands, W.A. (1978). *The role of termites in ecosystem*. In: Brain MV(ed) Production Ecology of Ants and Termites, Cambridge University Press, Cambridge. 245-292 p.

Yang, T., Mo, J.C., & Cheng, J. (2004): Purification and some properties of cellulase from *Odontotermes formosanus* (*Isoptera*: *Termitidae*). *Acta Entomologica Sinica,* **11,** 1-10.

Yang, X., Skjidmore, A.K., Melick, D.R., Zhou, Z., & Xu, J. (2006). Mapping non-wood forest product (matsutake mushrooms) using logistic regression and a GIS expert system. *Ecological Modeling***, 198**, 208-218.

Zambino, P.J., & Szabo, L.J. (1993). Phylogenetic relationships of selected cereal and grass rusts based on rDNA sequence analysis. *Mycologia,* **85,** 401-441.

Zamora, C.M., & Cecilia, N.D.P.P. (1995). Natural production of wild edible mushrooms in southwestern rural territory of Mexico City, Mexico. *Forest Ecology and Management,* **72,** 13-20.

Zang, M. (1981). *Sinotermitomyces*, a new genus of *Amanitaceae* from Yunnan, China. *Mycotaxon*, **13**, 171-174.

Zang, M. (1992). Contribution to the study on the v genus Sinotermitomyces from Asia. *Mycotaxon*, **44**, 21-26.

Zhu, J.S., Halpern, G.M., & Jones, K. (1998). The Scientific Rediscovery of an Ancient Chinese Herbal Medicine: Cordyceps sinensis. Part1. *Journal of Alternative Complementary Medicine*, **4**(3), 289-303.

Zobel, D.B., Jha, P.K., Behan, M.J., & Yadav, U.K.R. (1987). *A Practical Manual for Ecology.* Ratna Pustak Distributers, Kathmandu.

Websites:

www.BiodiversityLibrary.org.com

www.IndexFungorum.com

www.Jstor.org.com

www.Mycobank.org.com

www.tropicos.org.com

APPENDICES

APPENDIX 1: Formate of Questionnaire for Ethnomycological Information.

Date: ..... Address: ..... Name: ..... Age: ..... Gender: .....
Literacy: ..... Cast: ..... Occupation: ..... Locality: .....

- Do you know about mushroom in your locality?
- How many types of mushrooms are found in your locality?
- Where do you prefer to go for collection?
- Which season is favorable for collection?
- How do you collect mushroom?
- What are the plants species and their condition on which mushroom generally grow?
- How doyou identify poisonous or edible mushroom?
- How can you minimize the poisonous nature of mushroom so that they can be suitable to eat?
- How many types are edible?
- What are the best edible mushrooms generally collected?
- Do you know any mushroom having religious/medicinal values?
- Which variety of mushroom mostly found?
- What is the local name of the particular mushroom?
- Have you ever sold mushroom?
- Which species the buyer usually prefer?
- If edible mushroom collected in large amount but nt sold completely how you do preserve them?
- Have you seen any type of mushrooms, which were existed in the past time but now are rare or extinct?
- Have you marked any type of mushrooms eaten by animals like Bear/monkeys or others?
- Have you ever eaten poisonous mushrooms unknowingly, if yes what action you undertaken then?
- Do you face the problems during mushrooms collection?
- Do you think that the mushrooms diversity should be conserved?
- What kind of vessel is used for mushroom cooking?
- How you prepare the mushroom?
- Do you have any idea of recipe of mushrooms? Only boiling/Vegetable/Pickle/Soup/ Pakauda/ Pulau?
- What is the phytogeography of observed mushroom?
    - Altitude:
    - Latitude:
    - Longitude:
    - Forest-type:
    - Humidity:
    - Soil type:

- Habitat: saprophyte/parasite/mycorrhizal
- Substrate: soil/leaves/dungs/humus/tree/stump/wood/branch
- Condition: moist/dry/open/dark/forest
- Growthpattern: alone/clusters/ring
- Fruit body: Fleshy/tough/corky/woody/leathery/membranous/cartilaginous/auricular/clavaroid /gasteroid/spathlate/round/convoluted/other
- Does the feature change? Yes/no[fruiting body]

➢ Additional information (Regarding traditional belief).

Signature

SN	Ethnic group	Local name	Uses	Remarks

APPENDIX 2: Characters coding of Nepalese *Termitomyces.*

SN	Particulars	Code No	Characters
I	Cap colour	1	White to greyish
		2	Greyish brown, paling towards margin and olive near the umbonal region
		3	Ochraceous-brown
		4	Creamy white
		5	Brown to grey towards margin, olivaceous at the apex
		6	Cream to light brown, tomentose
II	Cap surface	1	Wrinkled, smooth, glabrous,silky,viscid , rugulose, scaly
		2	Concentrically scrobiculate or radially ridged, glabrous, smooth, viscid, squamule
III	Cap margin	1	Incurved, entire, glabrous, lobed, splitting, radially straight
		2	Irregular lobed margin, Appendiculate
		3	Flat reflexed or lacerate margin
		4	Incurved when young and uplifted when mature
		5	Regular
IV	Cap Shape	1	Applanate-umbonate
		2	Plano-umonate
		3	Umbo
		4	Bullate-umbonate
		5	Gibbous
		6	Companulate-Umbo
		7	Conical to convexo-applanate
		8	Obtuse-umbo
V	Cap Size	1	Small (1-4 cm)
		2	Medium (4.1-16 cm)
		3	Large (16.1-26 cm)

VI	Perforatorium Colour	1	Olivaceous
		2	Dark grey
		3	Dark brown
VII	Perforatorium Shape	1	Umbonate
		2	Strongly spiniform
		3	Cylindrical umbonate
		4	Mmammiform and scrobiculate cone like pointed projections
		5	Small, spiniform papilla
		6	Conical, sub-mamilate continuity of cap
VIII	Gills Colour	1	White
		2	Pink
		3	Orange
IX	Gills Ornamentation	1	Free, dense, thick, adnexed
		2	Free, narrow, dense, unequal, crowded
		3	Free, entire, crowded, equal, edge, smooth margin
		4	Sinuate, crowded with lamellulae of three unequal length
		5	Free, crowded, serrulate margin
X	Stipe colour	1	White with greyish tint
		2	Buffy brown and below the pellicle grey in colour
		3	White to dirty brown
		4	Grey
		5	Concolours
		6	Silvery white
XI	Stipe surface	1	Smooth, glabrous
		2	Fibrous, smooth, glabrous, fibrillose, pellicle thick, pubesent
		3	Stuffed
XII	Stipe Shape	1	Long, cylindrical, tubular, straight, solid, fusiform solid
		2	Small, slender long, cylindrical, uniformly thick, slightly elongated below soil
		3	Conical, central, cylindrical, slightly swollen at the middle, tapering towards downward, solid
		4	Solid, Swollen at middle, bulbus base, tapering at the end
XIII	Stipe Size	1	Small (3-7.1 sq. cm)
		2	Medium (7.2-11.8 sq. cm)
		3	Large (11.9-54 sq. cm)
XIV	Annulus	1	Apical, thick, white, persistent, microscopic squamule, fugaceous, appendiculate
		2	Thin demarketion, persistent, microscopic squamule
		3	Covered by thick membranous of velar remain
		4	Absent
XV	Pseudorrhizea colour	1	Dark-brown
		2	Pale-grey
		3	×
		4	Brown

XVI	Pseudorrhizea Surface	1	Cartilaginous
		2	Smooth, glabrous, fibrous
		3	Leathery, smooth
		4	Fibrillose, stuffed
		5	×
XVII	Pseudorrhizea Shape	1	Hollow, tapering towards distal ends
		2	Solid, slender, bulbous, tapering towards distal ends
XVIII	Pseudorrhizea Size	1	Small (1.5-5 sq. cm)
		2	Medium (6-11.9 cm^2)
		3	Large (12-102 cm^2)
		4	×
XIX	Flesh Colour	1	Whitish
		2	Buffy brown
XX	Flesh Hyphae	1	Nonamyloid, thin walled, hyaline, interwoven, cylindric, easily separable from the cuticle
		2	Nonamyloid, thin walled, hyaline, septed, paralled, filamentous
XXI	Flesh Clamp Connection	1	Absent
		2	Present
XXII	Spore Print Colour	1	Pink
		2	Brown
		3	Purplish

APPENDIX 3: Comparison of macro-nutrients across Physiographic regions.

APPENDIX 3 Table 1: Comparison of macro-nutrients (g/100g) contents among the (4) species of *Termitomyces* in Tarai.

Species	**Carbohydrate**	**Protein**	**Moisture**	**Ash**	**Fiber**	**Fat**	**Dry matter**
T. albuminosus	35.00± 1.55^{b}	36.00± 0.41^{d}	10.00± 0.91^{b}	12.33± 0.53^{ab}	8.33± 0.33^{a}	2.17± 0.33^{b}	90.00± 0.91^{b}
T. mammiformis	53.00± 0.65^{d}	24.00± 0.41^{b}	7.00± 0.41^{a}	11.00± 0.41^{a}	18.00± 0.41^{b}	0.83± 0.11^{a}	93.00± 0.41^{c}
T. microcarpus	15.00± 0.41^{a}	31.00± 0.41^{c}	7.00± 0.41^{a}	49.00± 0.41^{c}	9.33± 0.33^{a}	1.33± 0.25^{ab}	93.00± 0.41^{c}
T. robustus	45.67± 1.33^{c}	22.00± 0.41^{a}	13.00± 0.41^{c}	14.00± 0.41^{b}	20.33± 0.78^{c}	1.67± 0.29^{ab}	87.00± 0.41^{a}

Means annotated by different letters are significantly different. (Tukey test after ANOVA at P = 0.05).

APPENDIX 3 Table 2: Comparison of macro-nutrient contents among the (4) species of *Termitomyces* in Siwaliks.

Species	Average weight ± SEM (g/100g)						
	Carbohydrate	**Protein**	**Moisture**	**Ash**	**Fiber**	**Fat**	**DM**
T. albuminosus	31.33± 0.73^{b}	32.67± 0.53^{d}	10.00± 0.82^{a}	10.67± 0.53^{b}	8.67± 0.53^{a}	2.17± 0.51^{b}	90.00± 0.82^{ab}
T. mammiformis	47.00± 0.41^{d}	21.00± 0.41^{b}	10.00± 0.41^{a}	8.00± 0.41^{a}	14.67± 0.53^{b}	1.25± 0.27^{ab}	90.00± 0.41^{ab}
T. microcarpus	18.00± 0.41^{a}	30.33± 0.53^{c}	12.00± 0.41^{a}	44.00± 0.82^{c}	7.33± 0.33^{a}	1.00± 0.10^{a}	88.00± 0.41^{a}
T. robustus	39.33± 0.53^{c}	19.00± 0.41^{a}	10.00± 0.41^{a}	11.00± 0.41^{b}	14.00± 0.41^{b}	0.67± 0.11^{a}	90.44± 0.38^{b}

Means annotated by different letters are significantly different. (Tukey test after ANOVA at P = 0.05).

APPENDIX 3 Table 3: Comparison of macro-nutrient contents among the (4) species of *Termitomyces* in Midhills.

Species	Average weight ±SEM (g/100g)						
	Carbohydrate	**Protein**	**Moisture**	**Ash**	**Fiber**	**Fat**	**DM**
T. albuminosus	28.00± 0.41^{b}	19.00± 0.41^{c}	12.33± 0.53^{b}	10.67± 0.53^{c}	8.33± 0.33^{b}	1.17± 0.17^{a}	87.67± 0.53^{b}
T. mammiformis	42.00± 0.41^{d}	18.00± 0.41^{c}	13.00± 0.41^{b}	5.00± 0.41^{a}	11.00± 0.41^{c}	1.00± 0.14^{a}	87.00± 0.41^{b}
T. microcarpus	21.00± 0.41^{a}	9.00± 0.41^{a}	21.00± 1.29^{c}	4.67± 0.33^{a}	5.67± 0.67^{a}	0.83± 0.13^{a}	79.00± 1.29^{a}
T. robustus	34.67± 0.78^{c}	16.00± 0.41^{b}	7.00± 0.41^{a}	8.00± 0.41^{b}	11.00± 0.41^{c}	0.67± 0.11^{a}	93.00± 0.41^{c}

Means annotated by different letters are significantly different. (Tukey test after ANOVA at P = 0.05).

APPENDIX 3 of Table 4: Comparison of macro-nutrients (g/100g) contents of *T. albuminosus* along physiographic regions.

Region	Average weight ±SEM (mg/100g)						
	Carbohydrate	**Protein**	**Moisture**	**Ash**	**Fiber**	**Fat**	**DM**
Central Mid-hill	27.00± 0.58^{a}	19.00± 0.58^{a}	14.00± 0.58^{f}	12.00± 0.58^{bc}	9.00± 0.58^{ab}	1.50± 0.29^{a}	86.00± 0.58^{a}
Central Siwalik	34.00± 0.58^{e}	34.00± 0.58^{cd}	8.00± 0.58^{ab}	11.00± 0.58^{ab}	9.00± 0.58^{ab}	4.00± 0.58^{b}	92.00± 0.58^{ef}
Central Tarai	41.00± 0.58^{f}	36.00± 0.58^{de}	10.00± 0.58^{bcd}	12.00± 0.58^{bc}	9.00± 0.58^{ab}	2.00± 0.58^{ab}	90.00± 0.58^{cde}
Eastern Mid-hill	29.00± 0.58^{abc}	20.00± 0.58^{a}	12.00± 0.58^{def}	9.00± 0.58^{a}	8.00± 0.58^{ab}	1.00± 0.29^{a}	88.00± 0.58^{abc}
Eastern Siwalik	30.00± 0.58^{bc}	31.00± 0.58^{b}	13.00± 0.58^{ef}	12.00± 0.58^{bc}	7.00± 0.58^{a}	1.50± 0.29^{a}	87.00± 0.58^{ab}
Eastern Tarai	33.00± 0.58^{de}	37.00± 0.58^{e}	13.00± 0.58^{ef}	14.00± 0.58^{c}	8.00± 0.58^{ab}	3.00± 0.58^{ab}	87.00± 0.58^{ab}
Western Mid-hill	28.00± 0.58^{ab}	18.00± 0.58^{a}	11.00± 0.58^{cde}	11.00± 0.58^{ab}	8.00± 0.58^{ab}	1.00± 0.29^{a}	89.00± 0.58^{bcd}
Western Siwalik	30.00± 0.58^{bc}	33.00± 0.58^{bc}	9.00± 0.58^{abc}	9.00± 0.58^{a}	10.00± 0.58^{b}	1.00± 0.29^{a}	91.00± 0.58^{def}
Western Tarai	31.00± 0.58^{cd}	35.00± 0.58^{cde}	7.00± 0.58^{a}	11.00± 0.58^{ab}	8.00± 0.58^{ab}	1.50± 0.29^{a}	93.00± 0.58^{f}

APPENDIX 3 Table 5: Comparison of macro-nutrient (g/100g) contents of *T. mammiformis* along physiographic regions.

Region	Carbohydrate	Protein	Moisture	Ash	Fiber	Fat	Dry Matter
Central Midhills	42.00± 0.58^{a}	18.00± 0.58^{ab}	13.00± 0.58^{fg}	5.00± 0.58^{ab}	11.00± 0.58^{ab}	1.00± 0.29^{a}	87.00± 0.58^{ab}
Central Siwaliks	47.00± 0.58^{b}	21.00± 0.58^{cde}	10.00± 0.58^{cde}	8.00± 0.58^{cde}	15.00± 0.58^{cd}	2.00± 0.58^{a}	90.00± 0.58^{cde}
Central Tarai	53.00± 0.58^{cd}	24.00± 0.58^{fg}	7.00± 0.58^{ab}	11.00± 0.58^{fg}	18.00± 0.58^{ef}	0.75± 0.14^{a}	93.00± 0.58^{fg}
Eastern Midhills	43.00± 0.58^{a}	19.00± 0.58^{abc}	12.00± 0.58^{efg}	6.00± 0.58^{abc}	12.00± 0.58^{ab}	1.00± 0.29^{a}	88.00± 0.58^{abc}
Eastern Siwaliks	48.00± 0.58^{b}	22.00± 0.58^{def}	9.00± 0.58^{bcd}	9.00± 0.58^{def}	16.00± 0.58^{de}	0.75± 0.14^{a}	91.00± 0.58^{def}
Eastern Tarai	55.00± 0.58^{d}	25.00± 0.58^{g}	6.00± 0.58^{a}	12.00± 0.58^{g}	19.00± 0.58^{f}	1.00± 0.29^{a}	94.00± 0.58^{g}
Western Midhills	41.00± 0.58^{a}	17.00± 0.58^{a}	14.00± 0.58^{g}	4.00± 0.58^{a}	10.00± 0.58^{a}	1.00± 0.29^{a}	86.00± 0.58^{a}
Western Siwaliks	46.00± 0.58^{b}	20.00± 0.58^{bcd}	11.00± 0.58^{def}	7.00± 0.58^{bcd}	13.00± 0.58^{bc}	1.00± 0.29^{a}	89.00± 0.58^{bcd}
Western Tarai	51.00± 0.58^{c}	23.00± 0.58^{efg}	8.00± 0.58^{abc}	10.00± 0.58^{efg}	17.00± 0.58^{def}	0.75± 0.14^{a}	92.00± 0.58^{efg}

Appendix 3 Table 6: Comparison of macro (g/100g) nutrients contents of *T. microcarpus* along physiographic regions.

Region	Car	Prot	Moist	Ash	Fiber	Fat	DM
C. Midhill	42.00[a]	18.00a	13.00fg	5.00ab	11.00ab	1.00a	87.00ab
C. Shiwalik	47.00[b]	21.00cde	10.00cde	8.00cde	15.00cd	2.00a	90.00cde
C. Tarai	53.00[cd]	24.00fg	7.00ab	11.00fg	18.00ef	0.75a	93.00fg
E. Midhill	43.00[a]	19.00abc	12.00efg	6.00abc	12.00ab	1.00a	88.00abc
E. Shiwalik	48.00[b]	22.00def	9.00bcd	9.00def	16.00de	0.75a	91.00def
E. Tarai	55.00[d]	25.00g	6.00a	12.00g	19.00f	1.00a	94.00g
W. Midhill	41.00[a]	17.00a	14.00g	4.00a	10.00a	1.00a	86.00a
W. Shiwalik	46.00[b]	20.00bcd	11.00def	7.00bcd	13.00bc	1.00a	89.00bcd
W. Tarai	51.00[c]	23.00efg	8.00abc	10.00efg	17.00def	0.75a	92.00efg

Appendix 3 Table 7: Comparison of macro nutrient (g/100g) contents of *T. robustus* along physiographic regions.

Regions	Car	Prot	Moist	Ash	Fiber	Fat	DM
C. Midhill	35.00b	16.00ab	7.00ab	8.00ab	11.00ab	1.00ab	93.00ef
C. Shiwalik	39.00cd	19.00cde	10.00cde	11.00cde	14.00cd	1.00ab	90.67cde
C. Tarai	50.00f	22.00fg	13.00fg	14.00fg	20.00e	2.00b	87.00ab
E. Midhill	37.00bc	17.00abc	8.00abc	9.00abc	12.00abc	0.50a	92.00def
E. Shiwalik	41.000d	20.00def	11.00def	12.00def	15.00d	0.50a	89.67bcd
E. Tarai	46.00e	23.00g	14.00g	15.00g	23.00f	2.00b	86.00a
W. Midhill	32.00a	15.00a	6.00a	7.00a	10.00a	0.50a	94.00f
W. Shiwalik	38.00c	18.00bcd	9.00bcd	10.00bcd	13.00bcd	0.50a	91.00de
W. Tarai	41.00d	21.00efg	12.00efg	13.00efg	18.00e	1.00ab	88.00abc

APPENDIX 4: Comparison of micro-nutrients across physiographic regions.

APPENDIX 4 Table 1: Comparison of micro-nutrients, (mg/100g) contents among the (4) species of *Termitomyces* in Tarai.

Species	Copper	Iron	Magnesium	Calcium	Potassium	Phosphorus	Manganese	Sodium	Zinc
T. albuminosus	4.00± 0.41[a]	24.00± 0.41[b]	209.00± 0.91[d]	48.67± 0.93[a]	2174.00± 1.08[d]	12.67± 0.53[b]	9.00± 0.41[c]	6.00± 0.41[a]	16.00± 0.41[c]
T. mammiformis	4.67± 0.53[a]	59.00± 0.41[d]	39.00± 0.41[b]	173.00± 0.41[c]	902.00± 0.41[a]	6.00± 0.41[a]	9.00± 0.41[c]	11.00± 0.41[c]	9.00± 0.41[b]
T. microcarpus	5.00± 0.41[a]	40.00± 0.41[c]	72.00± 0.41[c]	533.00± 1.29[d]	1931.33± 0.53[c]	15.33± 0.53[c]	6.00± 0.41[b]	20.00± 0.41[d]	20.00± 0.41[d]
T. robustus	4.50± 0.20[a]	13.33± 0.53[a]	32.00± 0.41[a]	102.00± 0.41[b]	908.00± 0.41[b]	11.00± 0.41[b]	4.50± 0.20[a]	8.00± 0.41[b]	7.00± 0.41[a]

Means annotated by different letters are significantly different. (Tukey test after ANOVA at P = 0.05).

APPENDIX 4 Table 2: Comparison of micro-nutrient contents, among the (4) species of *Termitomyces* in Siwaliks.

Species	Average weight ± SEM (mg/100g)								
	Copper	**Iron**	**Magnesium**	**Calcium**	**Potassium**	**Phosphorus**	**Manganese**	**Sodium**	**Zinc**
T. albuminosus	4.00± 0.41[a]	17.00± 0.41[a]	209.33± 2.11[d]	48.00± 0.41[a]	2171.33± 1.48[c]	9.33± 0.33[a]	8.00± 0.41[c]	6.67± 0.78[a]	14.00± 0.41[b]
T. mammiformis	3.67± 0.53[a]	62.00± 0.41[c]	42.00± 0.41[b]	170.00± 0.41[c]	905.00± 0.41[a]	9.00± 0.41[a]	6.00± 0.41[b]	8.00± 0.41[a]	6.00± 0.41[a]
T. microcarpus	2.67± 0.33[a]	39.00± 0.82[b]	75.33± 0.53[c]	528.33± 0.67[d]	1927.67± 0.67[b]	16.00± 0.82[b]	4.00± 0.41[a]	19.00± 0.41[b]	20.33± 0.67[c]
T. robustus	3.00± 0.20[a]	17.00± 0.41[a]	35.00± 0.41[a]	105.00± 0.41[b]	905.00± 0.41[a]	9.00± 0.41[a]	3.00± 0.20[a]	6.00± 0.41[a]	5.33± 0.53[a]

Means annotated by different letters are significantly different. (Tukey test after ANOVA at P = 0.05).

APPENDIX 4 Table 3: Comparison of micro-nutrients, contents, among the (4) species of *Termitomyces* in Midhills.

Species	Average weight ±SEM (mg/100g)								
	Copper	**Iron**	**Magnesium**	**Calcium**	**Potassium**	**Phosphorus**	**Manganese**	**Sodium**	**Zinc**
T. albuminosus	3.00± 0.41[b]	16.67± 0.53[a]	205.00± 1.55[d]	43.67± 0.53[a]	2164.00± 0.91[d]	9.00± 0.41[b]	6.00± 0.41[c]	4.33± 0.33[ab]	11.00± 0.41[b]
T. mammiformis	2.33± 0.33[d]	65.00± 0.41[d]	45.33± 0.53[b]	167.00± 0.41[c]	908.00± 0.41[b]	12.00± 0.41[c]	3.00± `0.41[b]	5.00± 0.41[b]	3.00± 0.41[a]
T. microcarpus	1.50± 0.25[a]	34.00± 0.82[c]	67.67± 0.67[c]	520.33± 1.20[d]	1919.67± 1.05[c]	12.00± 0.41[c]	2.67± 0.33a[b]	17.00± 0.41[c]	17.00± 0.41[c]
T. robustus	1.50± 0.20[c]	20.00± 0.41[b]	38.00± 0.41[a]	108.00± 0.41[b]	902.00± 0.41[a]	6.00± 0.41[a]	1.50± 0.20[a]	3.00± 0.41[a]	3.00± 0.41[a]

Means annotated by different letters are significantly different. (Tukey test after ANOVA at P = 0.05).

APPENDIX 4 Table 4: Comparison of micro-nutrient contents of *T. albuminosus* along physiographic regions.

Region	Average weight ±SEM (mg/100g)								
	Copper	**Iron**	**Magnesium**	**Calcium**	**Potassium**	**Phosphorus**	**Manganese**	**Sodium**	**Zinc**
Central Mid-hill	4.00± 0.58^{ab}	18.00± 0.58^{b}	211.00± 0.58^{cd}	45.00± 0.58^{bc}	2164.00± 0.58^{b}	9.00± 0.58^{ab}	7.00± 0.58^{abc}	5.00± 0.58^{ab}	11.00± 0.58^{ab}
Central Siwalik	5.00± 0.58^{b}	17.00± 0.58^{ab}	213.00± 0.58^{de}	48.00± 0.58^{de}	2176.00± 0.58^{e}	10.00± 0.58^{ab}	8.00± 0.58^{bcd}	9.00± 0.58^{c}	14.00± 0.58^{cde}
Central Tarai	4.00± 0.58^{ab}	24.00± 0.58^{c}	209.00± 0.58^{c}	52.00± 0.58^{f}	2178.00± 0.58^{e}	13.00± 0.58^{cd}	9.00± 0.58^{cd}	6.00± 0.58^{ab}	16.00± 0.58^{ef}
Eastern Mid-hill	2.00± 0.58^{a}	17.00± 0.58^{ab}	203.00± 0.58^{a}	42.00± 0.58^{a}	2167.00± 0.58^{c}	8.00± 0.58^{a}	5.00± 0.58^{a}	4.00± 0.58^{a}	12.00± 0.58^{abc}
Eastern Siwalik	4.00± 0.58^{ab}	18.00± 0.58^{b}	214.00± 0.58^{e}	49.00± 0.58^{e}	2172.00± 0.58^{d}	9.00± 0.58^{ab}	9.00± 0.58^{cd}	7.00± 0.58$_{bc}$	15.00± 0.58^{def}
Eastern Tarai	5.00± 0.58^{b}	25.00± 0.58^{c}	212.00± 0.58^{de}	48.00± 0.58^{de}	2173.00± 0.58^{d}	14.00± 0.58^{d}	10.00± 0.58^{d}	7.00± 0.58^{bc}	17.00± 0.58^{f}
Western Mid-hill	3.00± 0.58^{ab}	15.00± 0.58^{a}	201.00± 0.58^{a}	44.00± 0.58^{ab}	2161.00± 0.58^{a}	10.00± 0.58^{ab}	6.00± 0.58^{ab}	4.00± 0.58^{a}	10.00± 0.58^{a}
Western Siwalik	3.00± 0.58^{ab}	16.00± 0.58^{ab}	201.00± 0.58^{a}	47.00± 0.58^{cde}	2166.00± 0.58^{bc}	9.00± 0.58^{ab}	7.00± 0.58^{abc}	4.00± 0.58^{a}	13.00± 0.58^{bcd}
Western Tarai	3.00± 0.58^{ab}	23.00± 0.58^{c}	206.00± 0.58^{b}	46.00± 0.58^{bc}	2171.00± 0.58^{d}	11.00± 0.58^{bc}	8.00± 0.58b^{cd}	5.00± 0.58^{ab}	15.00± 0.58^{def}

APPENDIX 4 Table 5: Comparison of micro-nutrient (mg/100g) contents of *T. mammiformis* along physiographic regions.

Region	**Copper**	**Iron**	**Magnesium**	**Calcium**	**Potassium**	**Phosphorus**	**Manganese**	**Sodium**	**Zinc**
Central Midhills	2.00± 0.58^{a}	65.00± 0.58^{fg}	45.00± 0.58^{fg}	167.00± 0.58^{ab}	908.00± 0.58^{fg}	12.00± 0.58^{fg}	3.00± 0.58^{ab}	5.00± 0.58^{ab}	3.00± 0.58^{ab}
Central Siwaliks	4.00± 0.58^{abc}	62.00± 0.58^{cde}	42.00± 0.58^{cde}	170.00± 0.58^{cde}	905.00± 0.58^{cde}	9.00± 0.58^{cde}	6.00± 0.58^{cde}	8.00± 0.58^{cde}	6.00± 0.58^{cde}
Central Tarai	5.00± 0.58^{bc}	59.00± 0.58^{ab}	39.00± 0.58^{ab}	173.00± 0.58^{fg}	`0.58^{ab}	6.00± 0.58^{ab}	9.00± 0.58^{fg}	11.00± 0.58^{fg}	9.00± 0.58^{fg}
Eastern Midhills	3.00± 0.58^{ab}	66.00± 0.58^{g}	44.00± 0.58^{ef}	168.00± 0.58^{abc}	907.00± 0.58^{efg}	11.00± 0.58^{efg}	4.00± 0.58^{abc}	6.00± 0.58^{abc}	4.00± 0.58^{abc}
Eastern Siwaliks	5.00± 0.58^{bc}	63.00± 0.58^{def}	41.00± 0.58^{cd}	171.00± 0.58^{def}	904.00± 0.58^{bcd}	8.00± 0.58^{bcd}	7.00± 0.58^{def}	9.00± 0.58^{def}	7.00± 0.58^{def}
Eastern Tarai	3.00± 0.58^{ab}	60.00± 0.58^{abc}	38.00± 0.58^{a}	174.00± 0.58^{g}	901.00± 0.58^{a}	5.00± 0.58^{a}	10.00± 0.58^{g}	12.00± 0.58^{g}	10.00± 0.58^{g}
Western Midhills	2.00± 0.58^{a}	64.00± 0.58^{efg}	47.00± 0.58^{g}	166.00± 0.58^{a}	909.00± 0.58^{g}	13.00± 0.58^{g}	2.00± 0.58^{a}	4.00± 0.58^{a}	2.00± 0.58^{a}
Western Siwaliks	2.00± 0.58^{a}	61.00± 0.58^{bcd}	43.00± 0.58^{def}	169.00± 0.58^{bcd}	906.00± 0.58^{def}	10.00± 0.58^{def}	5.00± 0.58^{bcd}	7.00± 0.58^{b}	5.00± 0.58^{bcd}
Western Tarai	6.00± 0.58^{c}	58.00± 0.58^{a}	40.00± 0.58^{abc}	172.00± 0.58^{efg}	903.00± 0.58^{abc}	7.00± 0.58^{abc}	8.00± 0.58^{efg}	10.00± 0.58^{efg}	8.00± 0.58^{efg}

Appendix 4 Table 6: Comparison of micro nutrients (mg/100g) contents of *T. microcarpus* along physiographic regions.

Region	Cu	Fe	Mg	Ca	K	P	Mn	Na	Zn
C. Midhill	2.00a	65.00fg	45.00fg	167.00ab	908.00fg	12.00fg	3.00ab	5.00ab	3.00ab
C. Shiwalik	4.00abc	62.00cde	42.00cde	170.00cde	905.00cde	9.00cde	6.00cde	8.00cde	6.00cde
C. Tarai	5.00bc	59.00ab	39.00ab	173.00fg	902.00ab	6.00ab	9.00fg	11.00fg	9.00fg
E. Midhill	3.00ab	66.00g	44.00efg	168.00abc	907.00efg	11.00efg	4.00abc	6.00abc	4.00abc
E. Shiwalik	5.00bc	63.00def	41.00bcd	171.00def	904,00bcd	8.00bcd	7.00def	9.00def	7.00def
E. Tarai	3.00ab	60.00abc	38.00a	174.00g	901.00a	5.00a	10.00g	12.00g	10.00g
W. Midhill	2.00a	64.00efg	47.00g	166.00a	909.00g	13.00g	2.00a	4.00a	2.00a
W. Shiwalik	2.00a	61.00bcd	43.00def	169.00bcd	906.00def	10.00def	5.00bcd	7.00bcd	5.00bcd
W. Tarai	6.00c	58.00a	40.00abc	172.00efg	903,00abc	7.00abc	8.00efg	10.00efg	8.00efg

Appendix 4 Table 7: Comparison of micro-nutrient (mg/100g) contents of *T. robustus* along physiographic regions.

Regions	Cu	Fe	Mg	Ca	K	P	Mn	Na	Zn
C. Midhill	1.50ab	20.00fg	38.00fg	108.00fg	902.00ab	6.00ab	1.50ab	3.00ab	3.00ab
C. Shiwalik	3.00cde	17.00cde	35.00cde	105.00cde	905.00cde	9.00cde	3.00cde	6.00cde	5.00bcd
C. Tarai	4.50fg	13.00ab	32.00ab	102.00ab	908.00fg	11.00ef	4.50fg	8.00ef	7.00de
E. Midhill	2.00abc	19.00efg	37.00efg	107.00efg	903.00abc	7.00abc	2.00abc	4.00abc	4.00abc
E. Shiwalik	3.50def	16.00cd	34.00bcd	104.00bcd	906.00def	10.00def	3.50def	7.00def	7.00de
E. Tarai	5.00g	12.00a	31.00a	101.00a	909.00g	12.00f	5.00g	9.00f	8.00e
W. Midhill	1.00a	21.00g	39.00g	109.00g	901.00a	5.00a	1.00a	2.00a	2.00a
W. Shiwalik	2.50bcd	18.00def	36.00def	106.00def	904.00bcd	8.00bcd	2.50bcd	5.00bcd	4.00abc
W. Tarai	4.00efg	15.00bc	33.00abc	103.00abc	907.00efg	10.00def	4.00efg	7.00def	6.00cde

APPENDIX 5: Frequency of presence of phytochemicals in *Termitomyces.*

Frequency of presence of phytochemicals in four species of *Termitomyces*. Frequency based on 27 samples of each species (all together 108) collected from different regions was analyzed.

Species	Phytochemicals															
	V	A	S	T	C	F	E	Fl	Co	An	Ant	Sa	Ta	G	P	Po
T. albuminosus	0	66.67	77.78	100	88.89	88.89	100	44.44	55.56	33.33	33.33	100	88.89	100	88.89	100
T. mammiformis	3.704	88.89	70.37	100	88.89	74.07	81.48	77.78	81.48	40.74	62.96	100	100	88.89	74.07	85.19
T. microcarpus	11.11	100	77.78	100	100	66.67	55.56	77.78	11.11	33.33	22.22	77.78	88.89	88.89	88.89	77.78
T. robustus	0	100	0	100	100	66.67	66.67	66.67	66.67	66.67	66.67	100	66.67	55.56	66.67	66.67
All	3.704	88.89	56.48	100	94.44	74.07	75.93	66.67	53.7	43.52	46.3	94.44	86.11	83.33	79.63	82.41

APPENDIX 6: Soil report in different eco-zones.

SN	Place	Plot No	Region	Alt (m)	FT	Soil source	N (%)	P	K	OM (%)	C:N	pH
1	Attariya	5	WT	190	TESF	Termites nest	0.13	356.6	2208.4	2.7	20.8	6.1
						Cashing	0.08	8.9	166.9	1.7	21.25	6.33
						Visinity area	0.11	1.2	333.1	2.2	20	5
						Forest	0.1	93.1	143.2	2.6	26	5.3
2	Chiliya	33	CT	110	CMF	Termites nest	0.25	307.6	2161	5	20	6.3
						Cashing	0.04	8.7	380.6	0.8	20	5.44
						Visinity area	0.3	1.4	370	2	6.7	5.5
						Forest	0.05	22	18	2.1	42	7.6
3	KTWLRC	57	ET	215	TDsAcF	Termites nest	2.02	796.7	1656.4	40.3	20	5
						Cashing	0.07	8.5	170.9	1.5	21,5	5.72
						Visinity area	0.07	1.7	92.7	2.5	36	6.2
						Forest	0.06	8.9	19	1.5	25	7.6
4	Amaliya	85	WS	510	STDHF	Termites nest	0.22	77.8	594.2	4.4	20	6.75
						Cashing	0.05	8.3	380.6	0.9	18	5.5
						Visinity area	0.15	2.2	94.7	1.5	10	6.1
						Forest	0.07	23.2	17	1.7	24.28	7.2
5	Thada	137	CS	1261	STDHF	Termites nest	0.21	361.2	1520	4.2	20	6.4
						Cashing	0.06	8	190.7	1.5	25	5.23
						Visinity area	0.12	1.8	97.7	1.7	15	6.25
						Forest	0.08	24.2	119.5	1.8	22.5	6.46
6	Matigadha	161	ES	507	TDRF	Termites nest	0.15	300.6	902.8	2.5	17	6.34
						Cashing	0.02	1.4	807.9	1.9	95	6.8
						Visinity area	0.09	8.9	736.6	1.8	20	4.8
						Forest	0.01	54.8	28	2	200	6.48
7	Puythan-Khalanga	187	WM	1379	Q-in-Q-Lan-F	Termites nest	0.27	139.1	1662.4	4.7	17.40	4.4
						Cashing	0.09	1.2	500	1.6	18	7.14
						Visinity area	0.25	24.2	698.2	2	8	6.4
						Forest	0.75	93.1	143.2	2.4	3.2	5.3
8	Mashyam-Simaldanda	221	CM	1400	STDHF	Termites nest	1.19	116.1	2137.2	3.7	3.10	5.88
						Cashing	0.01	2.9	546.7	1.4	140	6.22
						Visinity area	0.8	4.2	300	2.4	3	5.8
						Forest	0.9	95.1	133.2	2.6	2.88	5.3
9	Peepalbot	268	EM	1290	SCF	Termites nest	1.18	705.9	1069	3.6	3.05	6.93
						Cashing	0.03	3.2	523	1.2	40	7
						Visinity area	0.95	3.7	95.7	1.9	2	6
						Forest	0.92	32.4	13.8	2.2	2.39	7.6

APPENDIX 6: Continued...

SN	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)	B (ppm)	Av. No. of fruit	Av. wt. (gm)/ Fruit	Av Biomass yield gm
1	60	8	4.73	471.16	12.85	30	14.00	415.00
	9.4	3.6	3.24	17.46	0.64			
	42.3	18.8	3.8	36.2	1.77			
	17.66	26.724	2.6	31.29	1.051			
2	58	9	5.73	491.16	13.2	28	17.33	666.66
	13.2	2.8	3.1	37.78	0.62			
	41.4	20	5	38.4	1.25			
	11.37	22.9	2.5	38.12	0.0208			
3	57	7.5	4.21	432.04	13.85	26	17.66	690.00
	11.8	2.9	4.56	6.56	0.6			
	40.9	17.1	4.9	56.2	1.4			
	11.36	25.8	2.7	39.8	0.0205			
4	62	8.5	4.83	466.16	13.7	17	12.05	316.66
	9	3.6	3	18.4	0.57			
	43.3	19.8	3.5	38.2	1.7			
	13.38	21.9	2.8	38.14	0.02			
5	64	9.5	4.66	445.16	13.4	24	13.06	320.00
	16.8	3.4	3.3	31.38	0.86			
	45.4	22	6	40.4	1.5			
	18.33	25.632	2.9	40.28	0.8793			
6	66	16	8.5	425	13.3	25	13.83	377.10
	8.3	4.5	3.4	19.2	1.26			
	43.9	19.1	5.88	34.2	1.55			
	14.7	20.908	2.4	29.14	0.1925			
7	56	6.5	4.12	421.08	11.8	15	9.38	198.60
	17.66	3	3.42	65.34	0.55			
	54.4	6.1	4.12	117.7	1.66			
	25.2	6.5	3.6	35.2	1.051			
8	81	24	9.87	460	13.5	15	10.00	265.66
	15.5	3.3	4	67.34	1.51			
	52.2	5.2	4.36	109.4	3.06			
	29	12.7	1.6	40.2	1.0501			
9	67	18	8.75	445	13.1	20	13.08	310.53
	11.37	5.1	3.5	30.3	1.38			
	55.6	6.91	4.92	121.67	1.65			
	16.8	7.9	4.5	32.8	0.0203			

APPENDIX 7: Effect of different carbon sources on growth of *Termitomyces.*

APPENDIX 7a: Effect of different carbon sources on growth *of T. albuminosus* in semi-solid and submerged conditions.

Carbon source	Linear growth in semi-solid medium ± SEM (cm)	Growth in submerged condition Weight ± SEM (gm)	
		Fresh weight	Dry weight
Dextrose	4.71 ± 0.12^{bc}	2.66 ± 0.41^{a}	0.81 ± 0.18^{a}
Fructose	5.31 ± 0.06^{cd}	2.98 ± 0.24^{a}	0.86 ± 0.08^{a}
Lactose	3.21 ± 0.36^{a}	1.96 ±0.23^{a}	0.63 ± 0.13^{a}
Maltose	5.79 ± 0.09^{d}	3.07 ± 0.21^{a}	1.00 ± 0.16^{a}
Mannitol	4.43 ± 0.04^{b}	2.56 ±0.4^{a}	0.68 ± 0.16^{a}
Sucrose	4.75 ± 0.07^{bc}	2.80 ± 0.44^{a}	0.83 ± 0.08^{a}
Control	3.59 ± 0.22^{a}	2.32 ± 0.33^{a}	0.64 ± 0.13^{a}

Means annotated by different letters are significantly different (Tukey test after ANOVA at P = 0.05).

APPENDIX 7b: Effect of different carbon sources on growth *of T. mammiformis* in semi-solid and submerged conditions.

Carbon source	Linear growth in semi-solid medium ± SEM (cm)	Growth in submerged condition Weight ± SEM (gm)	
		Fresh weight	Dry weight
Dextrose	4.76 ± 0.07^{cd}	2.232 ± 0.29^{a}	0.678 ± 0.09^{a}
Fructose	5.44 ± 0.15^{de}	3.018 ± 0.22^{a}	0.85 ± 0.07^{a}
Lactose	3.29 ± 0.40^{a}	1.96 ± 0.23^{a}	0.67 ± 0.11^{a}
Maltose	6.01 ± 0.08^{e}	2.604 ±0.035^{a}	0.92 ± 0.13^{a}
Mannitol	4.52 ± 0.12^{bc}	2.46 ± 0.32^{a}	0.764 ± 0.16^{a}
Sucrose	4.77 ± 0.16^{cd}	2.87 ± 0.21^{a}	0.836 ± 0.05^{a}
Control	3.63 ± 0.21^{ab}	2.36 ± 0.23^{a}	0.684 ± 0.16^{a}

Means annotated by different letters are significantly different (Tukey test after ANOVA at P = 0.05).

APPENDIX 7c: Effect of different carbon sources on growth *of T. microcarpus* in semi-solid and submerged conditions.

Carbon source	Linear growth in semi-solid medium ± SEM (cm)	Growth in submerged condition Weight ± SEM (gm)	
		Fresh weight	Dry weight
Dextrose	0.784 ± 0.05^{bc}	2.18 ± 0.17^{a}	0.684 ± 0.16^{a}
Fructose	1.424 ± 0.07^{d}	2.216 ± 0.28^{a}	0.702 ± 0.16^{a}
Lactose	0.386 ± 0.04^{a}	1.566 ± 0.12^{a}	0.15 ± 0.03^{a}
Maltose	1.49 ± 0.06^{d}	1.788 ± 0.18^{a}	1.312 ± 0.10b^{a}
Mannitol	0.75 ± 0.07^{bc}	4.018 ± 0.24^{b}	0.662 ± 0.14^{a}
Sucrose	0.858 ± 0.05^{c}	2.196 ± 0.28^{a}	0.684 ± 0.16^{a}
Control	0.558 ± 0.05^{ab}	1.96 ± 0.23^{a}	0.396 ± 0.12^{a}

Means annotated by different letters are significantly different (Tukey test after ANOVA at P = 0.05).

APPENDIX 7d: Effect of different carbon sources on growth *of T. robustus* in semi-solid and submerged conditions.

Carbon source	Linear growth in semi-solid medium ± SEM (cm)	Growth in submerged condition Weight ± SEM (gm)	
		Fresh weight	Dry weight
Dextrose	5.91 ± 0.27^{bc}	1.56 ± 0.10^{a}	0.01 ± 0.00^{a}
Fructose	6.39 ± 0.16^{bc}	2.14 ± 0.13^{b}	0.02 ± 0.00^{b}
Lactose	3.69 ± 0.02^{a}	1.26 ± 0.05^{a}	0.009 ± 0.00^{a}
Maltose	6.5 ± 0.07^{c}	2.16 ± 0.06^{b}	0.02 ± 0.00^{b}
Mannitol	5.73 ± 0.08^{b}	1.5 ± 0.15^{a}	0.0098 ± 0.00^{a}
Sucrose	5.96 ± 0.12^{bc}	1.6 ± 0.04^{a}	0.02 ± 0.00^{b}
Control	3.98 ± 0.23^{a}	1.28 ± 0.05^{a}	0.0096 ± 0.00^{a}

Means annotated by different letters are significantly different (Tukey test after ANOVA at P = 0.05).

APPENDIX 8: Effect of different nitrogen sources on growth of *Termitomyces.*

APPENDIX 8a: Effect of different nitrogen sources on growth *of T. albuminosus* in semi-solid and submerged conditions.

Nitrogen source	Linear growth in semi-solid medium ± SEM (cm)	Growth in submerged condition Weight ± SEM (gm)	
		Fresh weight	Dry weight
Ammonium nitrate	6.33 ± 0.23^{c}	2.7 ± 0.34^{bc}	0.174 ± 0.05^{ab}
Calcium nitrate	6.31 ± 0.12^{c}	2.614 ± 0.14^{bc}	0.15 ± 0.03^{ab}
Peptone	6.41 ± 0.22^{c}	2.98 ± 0.30^{c}	0.174 ± 0.04^{ab}
Sodium nitrate	5.81 ± 0.15^{c}	2.36 ± 0.18^{abc}	0.138 ± 0.02^{ab}
Urea	2.72 ± 0.12^{a}	1.42 ± 0.12^{a}	0.064 ± 0.01^{a}
Yeast extract	9 ± 0.00^{d}	5.14 ± 0.21^{d}	0.244 ± 0.04^{b}
Control	4.67 ± 0.12^{b}	1.82 ± 0.08^{ab}	0.076 ± 0.02^{ab}

Means annotated by different letters are significantly different (Tukey test after ANOVA at P = 0.05).

APPENDIX 8b: Effect of different nitrogen sources on growth *of T. mammiformis* in semi-solid and submerged conditions.

Nitrogen source	Linear growth in semi-solid medium ± SEM (cm	Growth in submerged condition Weight ± SEM (gm)	
		Fresh weight	Dry weight
Ammonium nitrate	6.36 ± 0.21^{c}	2.87 ± 0.21^{a}	0.836 ± 0.05^{a}
Calcium nitrate	6.09 ± 0.25^{c}	2.232 ± 0.29^{a}	0.764 ± 0.16^{a}
Peptone	6.38 ± 0.20^{c}	3.018 ± 0.22^{a}	0.85 ± 0.07^{a}
Sodium nitrate	5.65 ± 0.21^{c}	2.46 ± 0.32^{a}	0.684 ± 0.16^{a}
Urea	2.66 ± 0.12^{a}	1.96 ± 0.23^{a}	0.67 ± 0.11^{a}
Yeast extract	9 ± 0.00^{d}	2.604 ± 0.35^{a}	0.92 ± 0.13^{a}
Control	4.43 ± 0.12^{b}	2.36 ± 0.23^{a}	0.678 ± 0.09^{a}

Means annotated by different letters are significantly different (Tukey test after ANOVA at P = 0.05).

APPENDIX 8c: Effect of different nitrogen sources on growth *of T. microcarpus* in semi-solid and submerged conditions.

Nitrogen source	Linear growth in semi-solid medium ± SEM (cm)	Growth in submerged condition Weight ± SEM (gm)	
		Fresh weight	Dry weight
Ammonium nitrate	2.72 ± 0.12^{b}	3.58 ± 0.18^{b}	0.332 ± 0.12^{a}
Calcium nitrate	2.21 ± 0.10^{a}	3.104 ± 0.06^{b}	0.222 ± 0.03^{a}
Peptone	3.24 ± 0.09^{c}	3.59 ± 0.16^{b}	0.856 ± 0.04^{b}
Sodium nitrate	2.21 ± 0.08^{a}	1.42 ± 0.08^{a}	0.084 ± 0.01^{a}
Urea	2.184 ± 0.06^{a}	1.032 ± 0.05^{a}	0.082 ± 0.01^{a}
Yeast extract	3.24 ± 0.12^{c}	4.518 ± 0.10^{c}	1.352 ± 0.08^{c}
Control	2.19 ± 0.06^{a}	1.032 ± 0.05^{a}	0.082 ± 0.01^{a}

Means annotated by different letters are significantly different (Tukey test after ANOVA at P = 0.05).

APPENDIX 8d: Effect of different nitrogen sources on growth *of T. robustus* in semi-solid and submerged conditions**.**

Nitrogen source	Linear growth in semi-solid medium ± SEM (cm)	Growth in submerged condition Weight ± SEM (gm)	
		Fresh weight	Dry weight
Ammonium nitrate	5.81 ± 0.39^{ab}	1.96 ± 0.07^{cd}	0.02 ± 0.00^{a}
Calcium nitrate	5.58 ± 0.18^{ab}	1.72 ± 0.17^{bc}	0.02 ± 0.00^{a}
Peptone	5.91 ± 0.62^{ab}	1.96 ± 0.07^{cd}	0.022 ± 0.00^{ab}
Sodium nitrate	5.4 ± 0.10^{ab}	1.42 ± 0.20^{b}	0.01 ± 0.00^{a}
Urea	4.4 ± 0.11^{a}	0.88 ± 0.03^{a}	0.0076 ± 0.00^{a}
Yeast extract	6.41 ± 0.11^{b}	2.26 ± 0.05^{d}	0.036 ± 0.00^{b}
Control	5.12 ± 0.82^{ab}	1.38 ± 0.05^{ab}	0.0082 ± 0.00^{a}

Means annotated by different letters are significantly different (Tukey test after ANOVA at P = 0.05).

APPENDIX 9: Effect of different vitamin sources on growth of *Termitomyces.*

APPENDIX 9a: Effect of different vitamin sources on growth of *T. albuminosus* in semi-solid and submerged conditions.

Vitamin source	Linear growth in semi-solid medium ± SEM (cm)	Growth in submerged condition Weight ± SEM (gm)	
		Fresh weight	Dry weight
Ascorbic acid	4.43 ± 0.27^{b}	1.68 ± 0.29^{a}	0.052 ± 0.00^{a}
D-priotine	4.69 ± 0.18^{bc}	3.66 ± 0.22^{c}	0.124 ± 0.04^{a}
Folic acid	2.72 ± 0.12^{a}	1.82 ± 0.11^{ab}	0.108 ± 0.01^{a}
Nicotinic acid	5.41 ± 0.25^{cd}	2.48 ± 0.37^{ab}	0.114 ± 0.01^{a}
Thiamine	5.62 ± 0.22^{d}	2.82 ± 0.21^{bc}	0.14 ± 0.02^{a}
Control	4.27 ± 0.12^{b}	1.42 ± 0.12^{a}	0.064 ± 0.01^{a}

Means annotated by different letters are significantly different (Tukey test after ANOVA at P = 0.05).

APPENDIX 9b: Effect of different vitamin sources on growth of *T. mammiformis* in semi-solid and submerged conditions.

Vitamin source	Linear growth in semi-solid medium ± SEM (cm)	Growth in submerged condition Weight ± SEM (gm)	
		Fresh weight	Dry weight
Ascorbic acid	4.43 ± 0.27^{b}	1.412 ± 0.27^{a}	0.0408 ± 0.01^{a}
D-priotine	4.69 ± 0.18^{bc}	3.66 ± 0.22^{c}	0.124 ± 0.04^{a}
Folic acid	2.76 ± 0.10^{a}	1.82 ± 0.11^{ab}	0.108 ± 0.01^{a}
Nicotinic acid	5.42 ± 0.25^{cd}	2.48 ± 0.37^{b}	0.114 ± 0.01^{a}
Thiamine	5.7 ± 0.19^{d}	2.82 ± 0.21^{bc}	0.14 ± 0.02^{a}
Control	4.31 ± 0.09^{b}	1.312 ± 0.15^{a}	0.0468 ± 0.01^{a}

Means annotated by different letters are significantly different (Tukey test after ANOVA at P = 0.05).

APPENDIX 9c: Effect of different vitamin sources on growth of *T. microcarpus* in semi-solid and submerged conditions.

Vitamin source	Linear growth in semi-solid medium ± SEM (cm)	Growth in submerged condition Weight ± SEM (gm)	
		Fresh weight	Dry weight
Ascorbic acid	3.25 ± 0.09^{b}	1.756 ± 0.14^{b}	0.28 ± 0.08^{ab}
D-priotine	3.81 ± 0.05^{c}	1.756 ± 0.14^{b}	0.292 ± 0.13^{ab}
Folic acid	2.72 ± 0.12^{a}	1.032 ± 0.05^{a}	0.076 ± 0.01^{a}
Nicotinic acid	4.314 ± 0.08^{d}	1.972 ± 0.17^{b}	0.654 ± 0.18^{b}
Thiamine	4.47 ± 0.13^{d}	2.18 ± 0.17^{b}	0.76 ± 0.11^{b}
Control	2.928 ± 0.10^{ab}	1.582 ± 0.07^{ab}	0.086 ± 0.01^{a}

Means annotated by different letters are significantly different (Tukey test after ANOVA at P = 0.05).

APPENDIX 9d: Effect of different vitamin sources on growth of *T. robustus* in semi-solid and submerged conditions.

Vitamin source	Linear growth in semi-solid medium ± SEM (cm)	Growth in submerged condition Weight ± SEM (gm)	
		Fresh weight	Dry weight
Ascorbic acid	5.45 ± 0.06^{b}	1.34 ± 0.05^{ab}	0.0122 ± 0.00^{a}
D-priotine	5.57 ± 0.09^{b}	1.34 ± 0.20^{ab}	0.014 ± 0.00^{a}
Folic acid	4.27 ± 0.12^{a}	0.98 ± 0.06^{a}	0.0068 ± 0.00^{a}
Nicotinic acid	5.59 ± 0.06^{b}	1.36 ± 0.02^{ab}	0.0156 ± 0.00^{a}
Thiamine	5.74 ± 0.12^{b}	1.582 ± 0.07^{b}	0.086 ± 0.01^{b}
Control	5.41 ± 0.19^{b}	1.14 ± 0.12^{ab}	0.0116 ± 0.00^{a}

Means annotated by different letters are significantly different (Tukey test after ANOVA at P = 0.05).

APPENDIX 10: Effect of different amino acid sources on growth of *Termitomyces.*

APPENDIX 10a: Effect of different amino acid sources on growth of *T. albuminosus* in semi-solid and submerged conditions.

Amino acid source	Linear growth in semi-solid medium ± SEM (cm)	Growth in submerged condition Weight ± SEM (gm)	
		Fresh weight	Dry weight
Arginine	5.92 ± 0.11^{d}	1.38 ± 0.08^{a}	0.0213 ± 0.00^{a}
Aspartic acid	4.07 ± 0.11^{bc}	1.88 ± 0.14^{a}	0.094 ± 0.03^{a}
Glutamic acid	6.1 ± 0.15^{d}	1.4 ± 0.07^{a}	0.0206 ± 0.00^{a}
Leusine	4.69 ± 0.19^{c}	1.8 ± 0.17^{a}	0.094 ± 0.03^{a}
Serine	4 ± 0.00^{b}	1.74 ± 0.24^{a}	0.062 ± 0.01^{a}
Valine	$4.6 \pm 0.22b^{c}$	1.42 ± 0.08^{a}	0.084 ± 0.01^{a}
Control	2.72 ± 0.12^{a}	1.42 ± 0.12^{a}	0.064 ± 0.01^{a}

Means annotated by different letters are significantly different (Tukey test after ANOVA at P = 0.05).

APPENDIX 10b: Effect of different amino acid sources on growth of *T. mammiformis* in semi-solid and submerged conditions.

Amino acid	Linear growth in semi-solid medium ± SEM (cm)	Growth in submerged condition Weight ± SEM (gm)	
		Fresh weight	Dry weight
Arginine	4.11 ± 0.11^{b}	1.74 ± 0.24^{a}	0.094 ± 0.03^{a}
Aspartic acid	2.32 ± 0.11^{a}	1.39 ± 0.18^{a}	0.0432 ± 0.01^{a}
Glutamic acid	2.52 ± 0.11^{a}	1.42 ± 0.08^{a}	0.062 ± 0.01^{a}
Leusine	2.88 ± 0.05^{a}	1.66 ± 0.20^{a}	0.084 ± 0.01^{a}
Serine	4.69 ± 0.19^{b}	1.88 ± 0.14^{a}	0.204 ± 0.09^{a}
Valine	4.6 ± 0.22^{b}	1.8 ± 0.17^{a}	0.094 ± 0.03^{a}
Control	2.72 ± 0.12^{a}	1.42 ± 0.12^{a}	0.064 ± 0.01^{a}

Means annotated by different letters are significantly different (Tukey test after ANOVA at P = 0.05).

APPENDIX 10c: Effect of different amino acid sources on growth of *T. microcarpus* in semi-solid and submerged conditions.

Amino acid	Linear growth in semi-solid medium ± SEM (cm)	Growth in submerged condition Weight ± SEM (gm)	
		Fresh weight	Dry weight
Arginine	3.73 ± 0.08^{c}	1.582 ± 0.07^{b}	0.086 ± 0.01^{a}
Aspartic acid	2.04 ± 0.09^{a}	0.86 ± 0.03^{a}	0.0054 ± 0.00^{a}
Glutamic acid	2.24 ± 0.09^{ab}	1.032 ± 0.05^{a}	0.076 ± 0.01^{a}
Leusine	2.724 ± 0.12^{b}	1.112 ± 0.07^{a}	0.082 ± 0.01^{a}
Serine	4.02 ± 0.13^{c}	1.756 ± 0.14^{b}	0.76 ± 0.11^{b}
Valine	2.734 ± 0.12^{b}	1.582 ± 0.07^{b}	0.086 ± 0.01^{a}
Control	2.658 ± 0.20^{b}	1.032 ± 0.05^{a}	0.082 ± 0.01^{a}

Means annotated by different letters are significantly different (Tukey test after ANOVA at P = 0.05).

APPENDIX 10d: Effect of different amino acid sources on growth of *T. robustus* in semi-solid and submerged conditions.

Amino acid	Linear growth in semi-solid medium ± SEM (cm)	Growth in submerged condition Weight ± SEM (gm)	
		Fresh weight	Dry weight
Arginine	6.1 ± 0.15^{c}	1.4 ± 0.07^{bc}	0.0196 ± 0.00^{a}
Aspartic acid	2.72 ± 0.12^{a}	0.86 ± 0.05^{a}	0.0082 ± 0.00^{a}
Glutamic acid	4.92 ± 0.07^{b}	0.92 ± 0.03^{a}	0.012 ± 0.00^{a}
Leusine	6.05 ± 0.10^{c}	1.396 ± 0.12^{bc}	0.016 ± 0.00^{a}
Serine	6.42 ± 0.08^{c}	1.7 ± 0.14^{c}	0.082 ± 0.01^{b}
Valine	6.3 ± 0.12^{c}	1.52 ± 0.05^{c}	0.028 ± 0.00^{a}
Control	5.92 ± 0.11^{c}	1.032 ± 0.05^{ab}	0.016 ± 0.00^{a}

Means annotated by different letters are significantly different (Tukey test after ANOVA at P = 0.05).

APPENDIX 11: Effect of different C: N ratio sources on growth of *Termitomyces.*

APPENDIX 11a: Effect of different C: N ratio sources on growth of *T. albuminosus* in semi-solid and submerged conditions.

Carbon: Nitrogen ratio source	Linear growth in semi-solid medium ± SEM (cm)	Growth in submerged condition Weight ± SEM (gm)	
		Fresh weight	Dry weight
C_1N_1	3.94 ± 0.29^{a}	0.96 ± 0.10^{a}	0.0058 ± 0.00^{a}
C_2N_1	4.82 ± 0.22^{a}	1.74 ± 0.12^{bcd}	0.023 ± 0.00^{a}
C_3N_1	4.87 ± 0.16^{a}	1.74 ± 0.12^{bcd}	0.023 ± 0.00^{a}
C_4N_1	5.23 ± 0.63^{a}	1.98 ± 0.12^{cd}	0.166 ± 0.08^{cd}
C_5N_1	5.48 ± 0.10^{a}	2.46 ± 0.18^{d}	0.036 ± 0.00^{ab}
C_1N_2	4.41 ± 0.08^{a}	1.12 ± 0.26^{ab}	0.0172 ± 0.00^{a}
C_1N_3	4.79 ± 0.74^{a}	1.82 ± 0.12^{bcd}	0.0356 ± 0.01^{ab}
C_1N_4	4.88 ± 0.20^{a}	1.92 ± 0.13^{cd}	0.076 ± 0.02^{ab}
C_1N_5	4.69 ± 0.20^{a}	1.28 ± 0.23^{abc}	0.023 ± 0.00^{a}
Control	4.12 ± 0.14^{a}	1.74 ± 0.12^{bcd}	0.032 ± 0.01^{ab}

Means annotated by different letters are significantly different (Tukey test after ANOVA at P = 0.05).

APPENDIX 11b: Effect of different C: N ratio sources on growth of *T. mammiformis* in semi-solid and submerged conditions.

Carbon: Nitrogen ratio	Linear growth in semi-solid medium ± SEM (cm)	Growth in submerged condition Weight ± SEM (gm)	
		Fresh weight	Dry weight
C_1N_1	4.4 ± 0.04^{a}	1.04 ± 0.12^{a}	0.0076 ± 0.00^{a}
C_2N_1	4.44 ± 0.05^{a}	1.6 ± 0.16^{ab}	0.0254 ± 0.00^{ab}
C_3N_1	4.48 ± 0.08^{ab}	1.66 ± 0.18^{ab}	0.028 ± 0.00^{ab}
C_4N_1	5.11 ± 0.04^{c}	1.9 ± 0.04^{b}	0.0358 ± 0.00^{ab}
C_5N_1	5.32 ± 0.18^{c}	2.2 ± 0.13^{b}	0.038 ± 0.00^{b}
C_1N_2	4.5 ± 0.04^{ab}	1.09 ± 0.13^{a}	0.02 ± 0.00^{ab}
C_1N_3	4.83 ± 0.13^{abc}	1.66 ± 0.18^{ab}	0.03 ± 0.00^{ab}
C_1N_4	4.99 ± 0.11^{bc}	1.7 ± 0.07^{ab}	0.03 ± 0.00^{ab}
C_1N_5	4.81 ± 0.13^{abc}	1.46 ± 0.22^{ab}	0.0196 ± 0.00^{ab}
Control	4.51 ± 0.14^{ab}	1.5 ± 0.24^{ab}	0.01 ± 0.00^{ab}

Means annotated by different letters are significantly different (Tukey test after ANOVA at P = 0.05).

APPENDIX 11c: Effect of different C: N ratio sources on growth of *T. microcarpus* in semi-solid and submerged conditions.

Carbon: Nitrogen ratio	Linear growth in semi-solid medium ± SEM (cm)	Growth in submerged condition Weight ± SEM (gm)	
		Fresh weight	Dry weight
C_1N_1	6.87 ± 0.09^{a}	1.42 ± 0.13^{a}	0.02 ± 0.00^{a}
C_2N_1	6.88 ± 0.09^{a}	3 ± 0.00^{cd}	0.07 ± 0.00^{bc}
C_3N_1	7.91 ± 0.01^{b}	3.36 ± 0.26^{cd}	0.074 ± 0.00^{bc}
C_4N_1	8.43 ± 0.05^{bc}	3.68 ± 0.08^{d}	0.096 ± 0.00^{c}
C_5N_1	8.63 ± 0.36^{c}	3.8 ± 0.07^{d}	0.104 ± 0.01^{c}
C_1N_2	6.88 ± 0.09^{a}	2.82 ± 0.30^{bcd}	0.02 ± 0.00^{a}
C_1N_3	8.46 ± 0.01^{bc}	3.42 ± 0.27^{cd}	0.076 ± 0.00^{bc}
C_1N_4	8.5 ± 0.00^{bc}	3.46 ± 0.24^{cd}	0.08 ± 0.01^{c}
C_1N_5	7.91 ± 0.01^{b}	2.6 ± 0.14^{bc}	0.034 ± 0.00^{ab}
Control	6.88 ± 0.09^{a}	1.9 ± 0.15^{ab}	0.07 ± 01^{bc}

Means annotated by different letters are significantly different (Tukey test after ANOVA at P = 0.05).

APPENDIX 11d: Effect of different C: N ratio sources on growth of *T. robustus* in semi-solid and submerged conditions.

Carbon: Nitrogen ratio	Linear growth in semi-solid medium ± SEM (cm)	Growth in submerged condition Weight ± SEM (gm)	
		Fresh weight	Dry weight
C_1N_1	3.94 ± 0.29^{a}	0.96 ± 0.23^{a}	0.005 ± 0.00^{a}
C_2N_1	4.41 ± 0.08^{a}	1.52 ± 0.03^{abc}	0.023 ± 0.00^{a}
C_3N_1	4.69 ± 0.20^{a}	1.74 ± 0.12^{abcd}	0.032 ± 0.01^{ab}
C_4N_1	5.23 ± 0.63^{a}	1.98 ± 0.12^{cd}	0.076 ± 0.02^{ab}
C_5N_1	5.48 ± 0.10^{a}	2.46 ± 0.18^{d}	0.076 ± 0.00^{ab}
C_1N_2	4.79 ± 0.74^{a}	1.28 ± 0.23^{abc}	0.0058 ± 0.00^{a}
C_1N_3	4.82 ± 0.22^{a}	1.82 ± 0.12^{bcd}	0.0356 ± 0.01^{ab}
C_1N_4	4.87 ± 0.16^{a}	1.92 ± 0.13^{bcd}	0.036 ± 0.00^{ab}
C_1N_5	4.88 ± 0.20^{a}	1.12 ± 0.26^{ab}	0.166 ± 0.08^{b}
Control	4.2 ± 07^{a}	0.96 ± 0.10^{a}	0.0172 ± 0.00^{a}

Means annotated by different letters are significantly different (Tukey test after ANOVA at P = 0.05).

APPENDIX 12: Growth of *Termitomyces* mycelium in different nutritive sources

1	**Highest and Lowest Mycelial growth of Termitomyces spp. on Carbon Source**					
Species	Highest growth			lowest growth		
	Linear growth in semisolid medium	Mass growth in submerged condition		Linear growth in semisolid medium	mass growth in submerged condition	
		fresh weight	dry weight		fresh weight	dry weight
T. albuminosus	Malt extract	Malt extract	Malt extract	Lactose	Lactose	Lactose
T. mammiform	Malt extract	Fructode	Malt extract	Lactose	Lactose	Lactose
T. microcarpus	Manitol	Malt extract	Dextrose	Lactose	Lactose	Lactose
T. robustus	Malt extract	Malt extract	Malt extract	Lactose	Lactose	Manitol
2	**Highest and Lowest Mycelial growth of Termitomyces spp. on Nitrogen Source**					
Species	Highest growth			lowest growth		
	Linear growth in semisolid medium	Mass growth in submerged condition		Linear growth in semisolid medium	mass growth in submerged condition	
		fresh weight	dry weight		fresh weight	dry weight
T. albuminosus	Yeast extract	Yeast extract	Yeast extract	Urea	Urea	Urea
T. mammiform	Yeast extract	Peptone	Yeast extract	Urea	Urea	Urea
T. microcarpus	Yeast extract	yeast extract	Yeast extract	Urea	Urea	Urea
T. robustus	Yeast extract	yeast extract	Yeast extract	Urea	Urea	Urea
3	**Highest and Lowest Mycelial growth of Termitomyces spp. on Vitamins Source**					
Species	Highest growth			lowest growth		
	Linear growth in semisolid medium	Mass growth in submerged condition		Linear growth in semisolid medium	mass growth in submerged condition	
		fresh weight	dry weight		fresh weight	dry weight
T. albuminosus	Thiamine	D-priotine	Thiamine	Folic acid	Control	Ascorbic acid
T. mammiform	Thiamine	D-priotine	Thiamine	Folic acid	Control	Control
T. microcarpus	Thiamine	Thiamine	Thiamine	Folic acid	Folic acid	Folic acid
T. robustus	Thiamine	Thiamine	Thiamine	Folic acid	Folic acid	Folic acid
4	**Highest and Lowest Mycelial growth of Termitomyces spp. on Amino acid Source**					
Species	Highest growth			lowest growth		
	Linear growth in semisolid medium	Mass growth in submerged condition		Linear growth in semisolid medium	mass growth in submerged condition	
		fresh weight	dry weight		fresh weight	dry weight
T. albuminosus	Glutamic acid	Aspartic acid	Aspartic acid	Control	Arginine	Arginine
T. mammiform	Serine	Serine	Serine	Aspartic acid	Glutamic	Aspartic acid
T. microcarpus	Serine	Serine	Serine	Aspartic acid	Aspartic acid	Aspartic acid
T. robustus	Serine	Serine	Serine	Aspartic acid	Aspartic acid	Aspartic acid
5	**Highest and Lowest Mycelial growth of Termitomyces spp. on Carbon to Nitrogen ratio (C:N) Source**					
Species	Highest growth			lowest growth		
	Linear growth in semisolid medium	Mass growth in submerged condition		Linear growth in semisolid medium	mass growth in submerged condition	
		fresh weight	dry weight		fresh weight	dry weight
T. albuminosus	C5Y1	C5Y1	C4Y1	C1Y1	C1Y1	C1Y1
T. mammiform	C5Y1	C5Y1	C5Y1	C1Y1	C1Y1	C1Y1
T. microcarpus	C5Y1	C5Y1	C5Y1	C1Y1 & C1Y2	C1Y1	C1Y1
T. robustus	C5Y1	C5Y1	C1Y5	C1Y1	C1Y1	C1Y1 & C1Y2

APPENDIX 13: List of participation on Conference/Seminar/Symposium

National

1. Participation and Paper Presentation in: Sixth National Conference on Science and Technology, 25-27 Sept.2012, Khumaltar, Lalitpur, Nepal. Entitle: "Higher-Mycodiversity in Sankarnagar Community Forest, Western Tarai region of Nepal. 2012. Abstracts, NAST, 39 p.
2. Participation and Paper Presentation in Seminar on *Modern Trends in Science and Technology*, 28-29 December, 2012, Biratnagar, Nepal. Entitle: "A survey on Mycodiversity in Namuna Community Forest, Rupandehi district, Nepal". 2012. Abstracts, 28-29 December, 2012. Biratnagar, Nepal. BOT-10, 7 p.
3. Participation in *Orientation Programme of First year of Four year B.Sc. course* in Central Department of Botany, Organized by Science and Technology (IOST), T.U., Kathmandu, Nepal, held on 17-10, January, 2013. (2069-10-4 to 6).
4. Participation and Paper Presentation in: National Conference on Biotechnology, 22-23 Nov. 2014, Khumaltar, Lalitpur, Nepal. Entitle: "Phytochemical study of Termite's Mushroom in Nepal: An Ethno mycological Approach. 2014. Abstracts, NBA, Ph-05-Or, 39 p

International

1. Participation and Poster Presentation in *International Conference, Biodiversity, Livelihood & climate Change in Himalayas*, 12-14 December 2010; Kathmandu, Nepal. Entitle: "Some Wild Mushrooms of Rupandehi District of Nepal". Abstracts, Central Department Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal. POS-7, 127 p.
2. Participation and Paper Presentation in *National Conference, Biodiversity: Changing Patterns and Conservation Strategies*, 26-28 November 2011; Gorakhpur, India. Entitle: "A Preliminary Study on Higher- Mycodiversity Prevailing in Peepaldanda Community Forest, West, Nepal". Abstracts, Department Botany, DDU Gorakhpur University, Gorakhpur-273009. Session II-70, 63 p.
3. Participation and Poster Presentation in 3rd Global conference '' Plant Pathology & Food Security'', January 10-13, 2012, Rajasthan, Udaipur-313 001, India. Entitle: "A Preliminary study on Wild Mushroom Diversity of Karhiya Community Forest, West Nepal". Abstracts, ISMPP & MPUAT, Udaipur- 313001, Rajasthan, India. Technical Session 5. PS-5.81, 120 p.
4. Participation and Paper Presentation in *XXXVI Annual Conference of the Indian Botanical Society* ''Plant Wealth and Human Welfare'', October 18-20, 2013, Gorakhpur, India. Entitle: "Phytochemical Screening of Termite's Mushroom *Termitomyces albuminosus* in Nepal". Abstracts, Department Botany, DDU Gorakhpur University, Gorakhpur-273009, U.P., India. Session I.
5. Participation and Paper Presentation in: *International Conference, on Emerging Trends in Science and Technology*" March 22-23, 2014, Biratnagar, Nepal. Entitle: "Ethnomycology of *Termitomyces* R. Heim in Nepal". Abstracts, 28-29 p.

6. Participation and Paper Presentation in: Int. Seminar on Environmental Degradation, Sustainable Development and Entrenchment of Poverty: Issues and Policy Implications. 21st-23rd August, 2014. Entitle: Termitophilous fungi in relation to different Ethnic Groups in Nepal. 1 p. Abstract Volume cum Souvenir. Dept. of History & Economics, C.K.B. College, Teok, Jorhat,Assam. 1p.

7. Participation and Paper Presentation in: Asian Plant Science Conference. 1st-3rd November, 2014, Bhairahawa, Nepal. Entitle: Termitomyces R. Heim in Nepal: An ethno mycological approach. Abstracts, Session II 81p. Organized By: Society for Applied Biotechnology, Biotechnology Society of Nepal, Association for the Advancement of Biodiversity Science and Eurasian Academy of Environmental Sciences.

8. Participation and Paper Presentation in: National Symposium on Mycological Research-Emerging Trends, Applications and Prospects and 41st Annual Meeting of Mycological Society of India, February 23-24, 2015. Entitle: Systematics of Nepalese Termitomyces. Abstracts, Section-A. AO-14, pp 61. Organized by: Deparment of Botany Punjabi University, Patiala-147 002 in collaboration with Mycological Society of India on February 23-24, 2015.

APPENDIX 14: Poster presentation on International Conference

1. Participation and Poster Presentation in *Sixth National Conference on Science and Technology*, 25-27 Sept.2012, Khumaltar, Lalitpur, Nepal. Entitle: "Higher–Mycodiversity in Sankarnagar Community Forest, Western Tarai region of Nepal. 2012. Abstracts, NAST, 39 p.

2. Participation and Poster Presentation in *3rd Global conference* '' Plant Pathology & Food Security'', January 10-13, 2012, Rajasthan, Udaipur–313 001, India. Entitle: "A Preliminary study on Wild Mushroom Diversity of Karhiya Community Forest, West Nepal". Abstracts, ISMPP & MPUAT, Udaipur- 313001, Rajasthan, India. Technical Session 5. PS-5.81, 120 p.

APPENDIX 15: List of Abstract Published

National

1. Higher-Mycodiversity in Sankarnagar Community Forest, Western Tarai region of Nepal. 2012. Abstracts, NAST, sixth national conference on Science and Technology. 25-27 Sept. 2012 Khumaltar, Lalitpur. Nepal. 39 p.

2. A survey on Mycodiversity in Namuna Community Forest, Rupandehi district, Nepal". 2012. Abstracts, Seminar on Modern Trends in Science and Technology, 28-29 Dec. 2012, Biratnagar, Nepal. BOT-10, 7 p.

3. Phytochemical study of Termite's Mushroom in Nepal: An Ethnomycological Approach. 2014. Abstract, NBA, National Conference on Biotechnology, 22- 23 Nov. 2014 Khumaltar, Lalitpur, Nepal. Ph-05-Or, 43 p.

International

1. Some Wild Mushrooms of Rupandehi District of Nepal 2010. Abstracts, Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu. Nepal, *Int. Conf. Biodiversity, Livelihood* and *Climate Change in the Himalayas*, 12−14, December, 2010. Kathmandu, Nepal. POS-7, 127 p.
2. A Preliminary Study on Higher-Mycodiversity Prevailing in Peepaldanda Community Forest, West, Nepal.2011.Abstracts, Department of Botany, DDU Gorakhpur University, Gorakhpur 273009. *National Conference Biodiversity: Changing Patterns and* Conservation *strategies*, 26-28, November. 2011. Gorakhpur, India. Sesson II-70, 63 p.
3. A Preliminary study on Wild Mushroom Diversity of Karhiya Community Forest, West Nepal 2012. *Journal of Mycology and Phytopathology*. **42:** 70. India.
4. Phytochemical Screening of Termite's Mushroom *Termitomyces albuminosus* in Nepal. Abstracts, Dept. Bot., DDU Gorakhpur University, Gorakhpur-273009, U.P., India. Session I.
5. Ethnomycology of *Termitomyces* R. Heim in Nepal. Abstracts, *Int. Conf. on Emerging Trends in Sc. and Tech*. March 22-23, 2014, Biratnagar, Nepal, 28-29 p.
6. Termitophilous fungi in relation to different Ethnic Groups in Nepal. Abstract Volume Cum Souvenir, Int. Seminar on Environmental Degradation, Sustainable Development and Entrenchment of Poverty: Issues and Policy Implications. 21st - 23 rd August, 2014. Dept. of History & Economics, C.K.B. College, Teok, Jorhat, Assam. 1p.
7. Termitomyces R. Heim in Nepal: An ethnomycological approaches. Abstracts, Asian Plant Science Conference, 1-3 November, 2014, Bhairahawa. Organized By: Society for Applied Biotechnology, Biotechnology Society of Nepal, Association for the Advancement of Biodiversity Science and Eurasian Academy of Environmental Sciences. Session II 81p.
8. Phytochemical study of Termite's Mushroom in Nepal: An Ethno mycological Approach. Abstracts, National Conference on Biotechnology, 22-23 Nov. 2014, Khumaltar, Lalitpur, Nepal. Organized by: Nepal Biotechnological Association. Ph-05-Or, 43 p.
9. Systematics of Nepalese Termitomyces. Abstracts, National Symposium on Mycological Research-Emerging Trends, Applications and Prospects and 41st Annual Meeting of Mycological Society of India, February 23-24, 2015. Organized by: Deparment of Botany Punjabi University, Patiala-147 002 in collaboration with Mycological Society of India on February 23-24, 2015.

APPENDIX 16: List of Publication

1. Aryal, H.P., Budhathoki, U. & Adhikari, M.K. (2012a). Mycodiversity in Peepaldanda Community Forest, Western Tarai Region of Nepal. ISSN 1995-8579. *Bull. Dept. Pl. Res.*, **34**, 13-17.
2. Aryal, H.P., & Budhathoki, U. (2012b). Macro-fungi of Karhiya Community Forest, western Tarai, Nepal. *Nepal Journal of Bioscience*, **2,** 93-97.

3. Aryal, H.P., & Budhathoki, U. (2012c). Survey on Mycodiversity in Namuna Community Forest, Rupandehi District, Nepal: An Ethnomycological approach. In: *Proceedings of Seminar on Modern Trends in Science & Technology*. (Eds.) Adhikari, D., Rai, S.K. and Limbu, K.P. Publ. Nepal Biological Society, Nepal Physical Society (Eastern Chapter) and Research Council of Science and Technology. Dec. 28-29, 2012. Biratnagar, Nepal. 51- 61 p.
4. Aryal, H.P., & Budhathoki, U. (2013a). Ethnomycological studies on some macro-fungi in Rupandehi District, Nepal. *Ban KO Jankari,* **23**(1), 51-56.
5. Aryal, H.P., & Budhathoki, U. (2013b). *Buchwaldoboletus lignicola* (Basidiomycetes), an Inedible Wild Mushroom New to Nepal. *Our Nature,* **11**(1), 31-35.
6. Aryal, H.P., & Budhathoki, U. (2013c). Mycodiversity at Sankarnagar Community Forest, Rupandehi District. *Nep. Jour. Sc. Tech.,* **14** (1), 75-80.
7. Aryal, H.P., & Budhathoki, U. (2013d). The Genus Amanita (Pers.) in Lumbini zone, Nepal. *Scientific World,* 11(11), 113-120.
8. Aryal, H.P., & Budhathoki, U. (2013e). Ethnomycological Study on Wild Mushrooms of Vulkepani Community Forest, Rupandehi District, Nepal. *Nepal Journal of Bioscience,* **3,** 49-53.
9. Aryal, H.P., & Budhathoki, U. (2013f). Phytochemical Screening of Termite's mushroom in Nepal. *Jour. Nat. Hist. Mus.,* **27,** 107-119.
10. Aryal, H.P., & Budhathoki, U. (2013g). *Termitomyces albuminosus* (Berk.) Heim a New Fungal Record from Arghakhnchi, Nepal. *OAKS,* **9,** 44-47.
11. Aryal, H.P., & Budhathoki, U. (2013h). Some Wild Mushrooms of Rupandehi District, West Nepal. ISSN 2091-0762. *Bibechana,* 10**,** 34-43.
12. Aryal, H.P., Budhathoki, U., & Tiwari, R.D. (2014a). *Termitomyces microcarpus* (Berk. & broome) R. Heim: A New records from Nepal. *Jour. Mycol. Pl. Pathol,* **44**(1), 13-18.
13. Aryal, H.P., Budhathoki, U., & Lakhey, P.B. (2014b). Phytochemical study of *Termitomyces robustus* (Beeli) R. Heim in Nepal. ISSN 1995-8579. *Bull. Dept. Pl. Res.,* **36,** 92−97.
14. Aryal, H.P., & Budhathoki, U. (2014c). Ethnomycology of *Termitomyces* spp. R. Heim for its medicinal importance in Nepal. *An International Journal of Medicinal Plants*. New Delhi, India. June, 2014c. 6(2): 128-137. Published by: Society for Conservation and Resource Development of Medicinal Plants, India. Print ISSN: 0975-4261. Online ISSN: 0975-6892. Article DOI: 10.5958/0975-6892.2014.00481.X
15. Aryal, H.P., Poudel, R., & Budhathoki, U. (2014d). Macro fungi from Siddababa Sacred Grove: West, Nepal. *Journal of Agriculture and Environment.* 15, 107-116.
16. Aryal, H.P., Budhathoki, U., & Tiwari, R.D. (2014e). Macrofungi in Community Managed Forest, Rupandehi District, Nepal: An Ethnomycological study. *Nepal Journal of Bioscience.* August, 2014f. **4:** 47-52. Biratnagar, Nepal.
17. Aryal, H.P., & Budhathoki, U. Phyto-Chemical Study of Termite's Mushroom in Nepal: An Ethnomycological Approach. *Journal of Plant Science & Research*. **2**(2): 121. Open Science Publications, April, 2015a. ISSN: 2349-2805. www.opensciencepublications.com
18. New Records of Termite's mushroom from Nepal. *Journal of Mycology and Plant Pathology*. Rajasthan, India. June, 2015b. **45**(2): 00-00 www.ismpp.org.in
19. Aryal, H.P., & Budhathoki, U. (2015c). Phytochemial screening of Termite's mushroom *Termitomyces albuminosus* in Nepal. Annual newsletter OAKS. Publisher: Department of Botany, DSB Campus, Kumaun University, Nainital, India. www.kunanital.ac.in

APPENDIX 17: List of Species collected from the study area**. ***denotes new taxa

PHOTO PLATE 1: *T. albuminosus* in different Carbon source

Dextrose (F) Dextrose (B) Lactose (F) Lactose (B)

Sucrose (F) Sucrose (B) Fructose (F) Fructose (B)

Mannitol (F) Mannitol (B) Maltose (F) Maltose (B)

Control (F) Control (B)

Note: F=Front side and B= Back side

PHOTO PLATE 2: *T. albuminosus* in different Nitrogen source

NH_4NO_3 (F) NH_4NO_3 (B) Peptone (F) Peptone (B)

Urea (F) Urea (B) $NaNO_3$ (F) $NaNO_3$ (B)

$Ca(NO_3)_2$ (F) $Ca(NO_3)_2$ (B) Yeast extract (F) Yeast extract (B)

Control (F) Control (B)

PHOTO PLATE 3: *T. albuminosus* in different Vitamin source

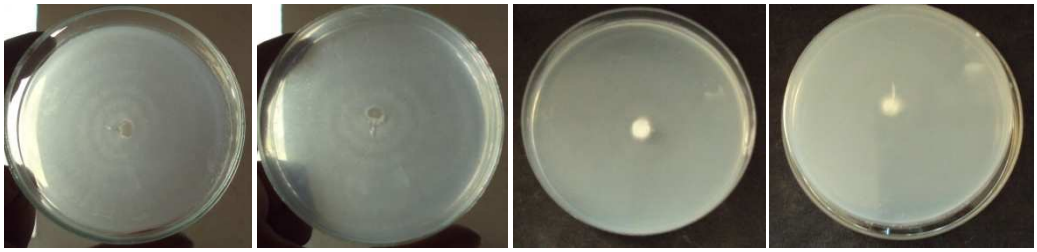
Ascorbic acid (F) Ascorbic acid (F) Nicotinic acid (F) Nicotinic acid (F)

Folic acid (F) Folic acid (F) Thiamine (F) Thiamine (B)

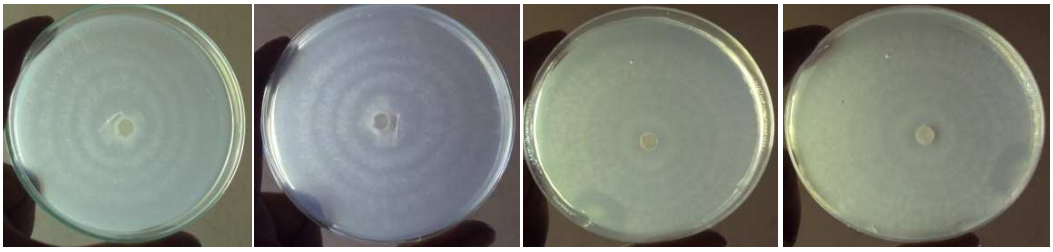
D-pirovic acid (F) D-pirovic acid (B) Control (F) Control (B)

PHOTO PLATE 4: *T. albuminosus* in different Amino acid source

Serine (F) Serine (B) Leusine (F) Leusine (B)



Valine (F) Valine (B) Glutamic acid (F) Glutamic acid (B)

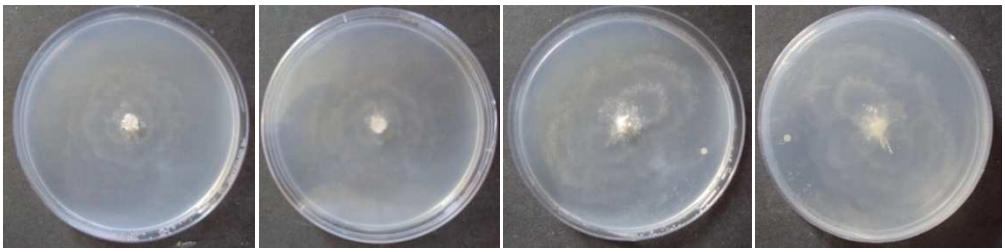


Argenine (F) Argenine (B) Aspertic acid (F) Aspertic acid (B)

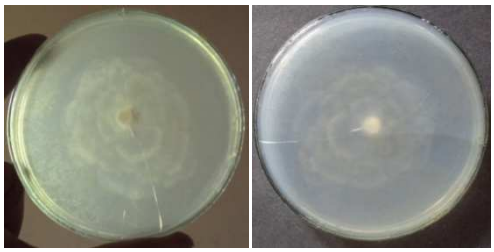
Control (F) Control (B)

PHOTO PLATE 5: *T. albuminosus* in different C: N source

C1:N1 (F) C1:N1 (B) C2: Y1 (F) C2: Y1 (F)

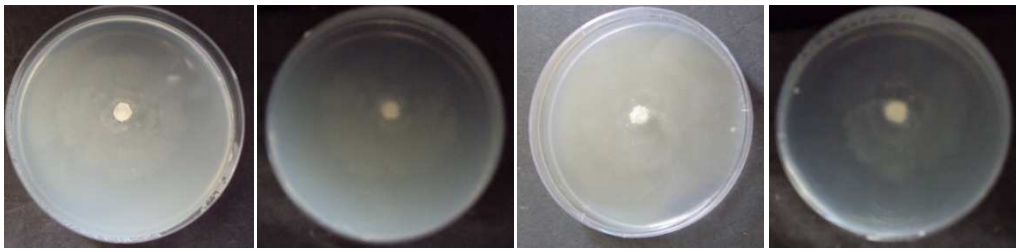


C3:Y1 (F) C3:Y1 (B) C4:Y1 (F) C4:Y1 (B)



C5:Y1 (F) C5:Y1 (B)

C1:N2 (F) C1:N2 (B) C1:Y3 (F) C1:Y3 (B)



C1:Y4 (F) C1:Y4 (B) C1:Y5 (F) C1:Y5 (B)

Control (F) Control (B)