Review of Literature
1. Natural history of Termitomyces symbiosis

Fungi have been observed in association with various insects (Kendrick, 1991) including scale insects (Hemiptera), gall midges (Diptera), beetles (Coleoptera), aphids (Homoptera), ants (Hymenoptera) and termites (Isoptera). Among these insects, ants (fungus-growing ants, sub-family Myrmicinae) and termites (fungus-growing termites, subfamily Macrotermiteinae) are well known to have a major impact on tropical ecosystems (Cherrett et al., 1989; Wood and Thomas, 1989). About 40–60 million years before the advent of human agriculture, three insect lineages, termites, ants and beetles independently evolved the ability to grow fungi for food. Like humans, the insect farmers became dependent on cultivated crops for food and developed task-partitioned societies cooperating in gigantic agricultural enterprises. Both human farming and fungus growing termites share defining features of modern agriculture with reference to habitual planting, cultivation and harvesting. Agricultural life ultimately enabled all of these insect farmers to rise to major ecological importance.

Termitomyces was the name given by Roger Heim in 1941 to a group of agarics associated with termite nests in Central Africa and East Asia. The genus Termitomyces (Kingdom: Fungi, Division: Basidiomycota, Class: Basidiomycetes, Subclass: Agaricomycetidae, Order: Agaricales, Family: Lyophyllaceae Julich) survives as an obligate mutualistic symbiont with termites of the subfamily Macrotermiteinae. This ecosymbiosis between Macrotermiteinae and Termitomyces seems to be the result of a co-evolutionary process since the early tertiary with a single origin in the African rainforest (Aanen et al., 2002; Aanen and Eggleton, 2005).

Termitomyces are completely dependent on the termites since they have never been found free-living and they are rapidly overgrown by other fungi, when removed from the termite nest (Darlington, 1994). In turn, the termites are apparently completely dependent on Termitomyces fungi as they have never been found without Termitomyces and experiments have shown that they are unable to survive without the fungus (Grasse, 1959; Sands, 1960; Lefevre, 2000). Termite fungi cultures are distributed (Figure-1) throughout tropical and subtropical areas in Africa and Asia (Table-1). They have evolved into approximately 330 extant species, belonging to
approximately 12 genera (Kambhampati and Eggleton, 2000). They play a significant role in the decomposition of dead plant materials and have a great impact on carbon cycling in tropical ecosystems (Abe and Matsumoto, 1978; Wood and Sands, 1978).

The fungus-growing termites maintain their symbionts (*Termitomyces*) on well-protected fungus gardens or fungus combs which are built from undigested dead plant material that has quickly passed through the gut (Figure- 2, a). The fungus combs are housed in single or multiple chambers inside a mound or dispersed in the soil (Darlington, 1994; Aanen and Boomsma, 2005). A few weeks after the inoculation, innumerable conidia rich in nitrogen form many white nodules on the fungus comb (Figure- 2, b and c). The host termites feed on the mature parts of the fungus comb; the mycelium then develops on the surface of the comb and digesting the raw forage (Lefevre and Bignell, 2004). Simultaneously, termites use both the fungal tissues (mycelium and asexual fruiting bodies called nodules also called mycotetes (Figure- 2, b and c) and the degraded plant materials from the bottom of the comb, and pass it again through the intestine, where it is digested by the termites’ normal intestinal symbionts and by fungal enzymes which remain active in the intestine (Leuthold *et al*., 1989; Traniello and Leuthold, 2000). Thus, the fungus comb is a sort of flow-through composter, with new material added continually to the top and digested material continually being consumed from the bottom. The fungus combs are sites for conversion of low-quality lignified cellulose to a high quality food of simpler sugars (Rouland *et al*., 1988, 1991; Veivers *et al*., 1991).
Fig- 2: Development of *Termitomyces* on termite comb a) Termite comb along with fungus that grows on it, b and c) Fungal nodules and also referred to as "mycotetes", spherules, synnemata, conidial spheres by various other authors, d) Development of fruiting bodies from nodules (a-c Aanen, 2002; d- Pahlevanlo and Janardhana, 2012).

The exact role of *Termitomyces* in the symbiosis with the Macrotermiteinae is still being debated (Bignell, 2000; Ohkuma, 2002). Several hypotheses concerning the main role of *Termitomyces* fungi have been proposed; first, the fungi are presumed to possess the ability to degrade lignin to make cellulose more susceptible to attack by the termites’ own cellulose (Veivers *et al.*, 1991; Hyodo *et al.*, 2000; Johjima *et al.*, 2003; Taprab *et al.*, 2005). Martin and Martin (1978) and Matoub and Rouland, (1995) proposed that symbiotic fungi provide termites with cellulase and xylanases. Third, fungi serve as nitrogen-rich food, which is advantageous because the dead plant material consumed by termites contains very little nitrogen (The nitrogen contents of the mycotetes and the fungus comb range from 5.7 to 7.9% and 0.81 to 2.1%, respectively, whereas the normal range for wood is 0.02 to 0.3% (Matsumoto, 1976; Collins, 1983). Fourth, the production of heat and metabolic water (Luscher, 1949) in other hand the fungi gain advantages in:

1- Access to plant material that can easily be penetrated and has an increased surface area.
2- The provision of an optimal microclimate.
3- The selective inhibition of other fungi that are competitively superior and the prevention of microbial infections by termite secretions.
Some studies suggest that the role of the fungus may differ in different termites (Lefevre, 2000; Hyodo et al., 2003). For example, Hyodo et al. (2003) reported that the role of the fungus in species of the genus Macrotermes was mainly to degrade lignin, whereas the fungus in three other genera served primarily as food (Figure- 3).

![Scheme of the digestive symbiosis between Macrotermes and Termitomyces](image)

Fig- 3: Scheme of the digestive symbiosis between Macrotermes and *Termitomyces*, raw forage rapidly pass through the gut, which contains the usual culture of bacterial symbionts, but which is also replete with fungal spores (Turner, 2004).

There is a continual breaking down and renewal of fungus-combs in nests (Grasse and Noirot, 1957) and this communal deposition of fecal material with its consumption after a period of fungus action apparently have replaced the direct anal interchange of food commonly practiced by other termites (Harris, 1965). Occasionally, during the rainy season sexual fruiting bodies (basidiocarps with sexual spores) arise from nodules, and grow out of the top of termite colonies (Figure- 2d) (Heim, 1977). These spores are carried into newly founded nests by the first workers of the new colony on their first foraging trips (Johnson, 1981; Johnson et al., 1981; Darlington, 1994).

According to the Index Fungorum database, there are 55 names published in *Termitomyces*, distributed throughout equatorial and southern Africa and widely in Southeast Asia (Pegler and Vanhaecke, 1994), but recent studies have been described only 30 species of the *Termitomyces* (Kirk et al., 2008). Together these cover a wide
macromorphological range, including the small *Termitymes microcarpus*, which grows on ejected comb material and rarely exceeds 2 cm in cap diameter and the gigantic *T. titanicus*, with a cap diameter of up to 1 meter making it the largest gilled mushroom fruit body in the world (Piearce, 1987). All species of the genus are nutritious and have commercial value in some regions (Oso, 1975; Parent and Thoen, 1977; Batra and Batra, 1979; Ogundana and Fagade, 1982, Purkayastha, 1985), and have been used for human consumption for many years in many countries (Sangvichien and Hawksworth, 2001).

2. Role of Termite and *Termitymes* in ecosystem

Termites have been divided into 7 families, 281 genera, and about 2600 species (Kambhampati and Eggleton, 2000). Traditionally, termites also have been separated as "higher" and "lower" termites based on their symbionts. All species of the paraphyletic grouping of lower termites harbor a dense and diverse population of prokaryotes and flagellate protists (singlecelled eukaryotes) in their guts. Higher termites comprise only a single family (Termitidae with four subfamilies), but they represent more than 80% of all described species (Kambhampati and Eggleton, 2000).

**Fig- 4:** Phylogenetic relationships among the seven termite families (left) and four subfamilies of the Termitidae (Donovan *et al.*, 2000).
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The Macrotermitinae have been subdivided into 11 taxonomically well-supported genera and about 330 species (Kambhampati and Eggleton, 2000). The Macrotermitinae often dominate the termite fauna in the tropical savannas and forests of the Old World (Lefevre, 2000). Most of the diversity occurs in Africa, where 10 of the 11 genera are found (Figure-4). Fungus-growing termites have significant role in tropical and subtropical ecosystems. They play an important role in carbon mineralization, recycling process, nitrogen fixation, soil fertility and have a great impact on plant litter decomposition (Abbadie and Lepage, 1989; Jones, 1990). Fungus-growing termites consume major part of annual above ground litter fall up to 30% in savannas (Wood and Sands, 1978). Almost half the above-ground plant litter in the Western Ghats forest and bamboo groves are too recycled by termites and Termitomyces.

Fungus-growing termites play a major role in nutrient cycling as they affect the decomposition processes in most tropical ecosystems, particularly in savannas. Amongst the soil organisms, fungus-growing termites apparently play an important role in soil fertility in tropical ecosystems because of their strong impact on soil physical and chemical properties. In particular, the contribution of Termitomyces fungi on carbon mineralization is very large (Yamada et al., 2005). Several roles of Termitomyces in the decomposition of plant materials were proposed, including the provision of glycosyl hydrolases for the termite hosts (Martin and Martin, 1978; Matoub and Lefevre, 1995) and lignin (phenol) degradation during fungus comb maturation (Grasse and Noirot, 1958; Hyodo et al., 2000; Johjima et al., 2003; Taprab et al., 2005).

For example in Kenya 90% of the dead wood decomposition is mediated by termites (Buxton 1981), in Nigeria they are responsible for removing of 60% of the annual wood fall, 24% of the tree leaf annual production (Collins, 1983) and 60% of the grass litter production (Ohiaagu and Wood, 1979). Termitomyces degrades the mylospheres removing carbon in the form of carbon dioxide during the course of respiration and therefore concentrating the nitrogen. Grasse and Noirot (1958) reported that Termitomyces breaks down lignin, based on staining with safranin, and that the utility of this is to release cellulose for digestion. This lignin degradation is thought to be the major function of the fungus (Sands, 1969). Ligninases are reported
to be produced by *Termitomyces* (Rohrmann and Rossman, 1980). Laccase production by *T. albuminosus* has been detected by using syringaldazine (Batra and Batra, 1979). Several enzymes have been purified from the *Termitomyces* fungus, such as laccase (Taprab *et al.*, 2005; Bose *et al.*, 2007), phenol oxidase (Johjima *et al.*, 2003), cellulobisae (Mukherjee and Khowala, 2002), amyloglucosidase (Ghosh *et al.*, 1997) and xylanase (Matoub and Lefevre, 1995).

3. Coexistence of Macrotermiteinae-*Termitomyces*

New colonies of fungus-growing termites are usually initiated by a pair of alates that become king and queen of the society. Some termite species show vertical symbionts transmission, meaning that Macrotermiteinae workers consume the nitrogen-rich fungal nodules that grow on the combs. These nodules are unripe mushrooms that are harvested long before they reach sexual maturity (Bathelier, 1927; Grasse and Noirot, 1958; Heim, 1977; Johnson *et al.*, 1981; Leuthold *et al.*, 1989). Possibly as an adaptation to this early harvesting by termites, nodules contain asexual spores that survive gut passage and serve as inoculum for newly constructed comb substrate (Leuthold *et al.*, 1989). The continuous seeding with asexual spores allows rapid growth of a new mycelium and of new nodules, which are then consumed again (Figure- 5 and 6).

However, in most fungus-growing termite species, new colonies acquire their symbiotic fungal partners via horizontal transmission. Occasionally, sexual fruiting bodies arise from nodules and grow out of the top of termite colonies (Heim, 1977). Mushrooms growing out of the termite colonies produce large amounts of wind-dispersed basidiospores which form the inoculum of incipient colonies of fungus-growing termites (Aanen, 2006). Most likely, foraging workers of incipient colonies collect *Termitomyces* spores (actively or passively) from their direct nest environment (Sands, 1960; Johnson *et al.*, 1981; Sieber, 1983; Korb and Aanen, 2003). This seems to be the ancestral form of transmission (Korb and Aanen, 2003; Aanen and Boomsma, 2006). Asexual spores are rarely found on unripe mushrooms of other basidiomycete fungi so it is likely that the production of those spores in *Termitomyces* is an adaptation to the symbiosis with termites (Figure- 6).
**Fig- 5:** Schematic representation of the propagation of *Termitomyces* fungus within a Macrotermes termite colony. (a), consume nodules (b), unripe mushrooms, (c), nodules contain asexual, gut-resistant spores (d), new substrate (e), nodule selection by workers (f), oldest fragments (g), fungus garden are consumed by older workers (Leuthold *et al.*, 1989).

**Fig- 6:** Transmission of fungus in Macrotermitinae during colony foundation (Aanen, 2003).
Little is known on the fungal diversity and also on the biological and environmental determinants of the fructification of the different *Termitomyces* species. Although several authors (Grasse, 1958) underlined the relative importance of both internal and external factors affecting the termite society and determining its control on the fungus.

Carbon dioxide mole fractions commonly range from 0.5% to 1%, with concomitant reductions in oxygen concentrations (Darlington *et al.*, 1997; Korb and Linsenmair, 2000; Turner, 2001). The nest atmosphere is also rich in the gaseous products of the anaerobic and methanogenic bacteria that reside in termites’ hindguts, and the volatile acids, alcohols and other hydrocarbons produced by the fungi (Darlington *et al.*, 1997). The nest atmosphere is also very humid, the water vapor supplied by a high production rate of metabolic water supplemented by soil water (Weir, 1973; Darlington *et al.*, 1997; Turner, 2001).

Termites in the nest monitor local concentrations of CO$_2$, oxygen and water vapor, and any disturbance of these properties indicates a mismatch between respiration and ventilation. When a disturbance is sensed, worker termites are recruited from the nest, where they normally reside, to the mound surface, where they build new surface, excavate vent holes, or seal porous layers of soil (Turner, 2000). Consequently, the termites’ building activity alters the mound’s capture of wind energy, which feeds back onto the stimulus (the perturbation in nest atmosphere) that initiates the building in the first place. The fungal partner in the symbiosis seems to benefit the most from nest homeostasis and nest atmosphere is crucial for the success of the symbiosis.

Also in nests of fungus-growing termites, many organisms other than termites and *Termitomyces* have been found: inquiline flies (Gumming, 1996), a range of arthropods (Batra and Batra, 1979), bacteria (Hongoh *et al.*, 2006), and many fungi (Sands, 1969; Thomas, 1987). Especially members of the ascomycete genus *Xylaria* have been frequently reported from fungus growing termite nests (Rogers *et al.*, 2005; Ju and Hsieh, 2007; Okane and Nakagiri, 2007). Visible structures of *Xylaria* typically occur when termite nests are dead or decaying (Rogers *et al.*, 2005). When *Xylaria* species emerge, they cover fungus combs throughout the fungus garden with mycelium, stromata and synnemata, some with ascomal initials (Rogers *et al.*, 2005). When fungus comb from a healthy nest is incubated in the absence of termites, it is...
often covered by a vigorous mycelium of *Xylaria* within a few days (Batra and Batra, 1979; Thomas, 1987; Okane and Nakagiri, 2007).

High concentrations of CO$_2$ in the nests may provide a competitive edge for *Termitomyces* relative to non-*Termitomyces* species, as *Termitomyces* may be more tolerant of high CO$_2$ concentrations compared to other fungi (Batra and Batra, 1966). The lack of filamentous fungal contamination may also be related to why only the genus *Termitomyces* thrives on the fungus comb with termites. In other hand Macrotermiteinae excrete several fungicides (Lamberty et al., 2001; Bulmer and Crozier, 2004), such fungicides may have prevented contamination in the cultivation of fungus gardens and helped to maintain the *Termitomyces* monocultures (Moriya et al., 2005).

4. *Termitomyces* taxonomy and diversity

The first description of *Termitomyces* was given by Heim (1941) for a group of termitophilous agarics found in Central Africa he placed the genus in the Amanitaceae. Singer (1945) separated *Termitomyces microcarpus* (Berk and Broome) Heim in order to propose a new genus, Podabrella, on the basis of the epigeous habit and lack of pseudorhizal development, and to which he added some non-tropical, non-termitophilous species. Singer (1951) although later transferred it to the Tricholomataceae. Pegler (1986) accommodated the genus *Termitomyces* Heim within Pluteaceae Kotl. and Pouzar, on the basis of the *pluteoid* habit, densely crowded, free lamellae which rapidly decay, the pink spore deposit, the type of hymenial cystidia and the basidiospores which often develop a wall-structure with two teguments and the presence of an open-pore hilum (Pegler and Young, 1971). Based on the presence of siderophilous granulation detected in the basidia, cyanophilous spores, the bilateral lamellar trama of young carpophores, Singer (1986) placed them within Tricholomataceae next to Lyophyllae in its own tribus, Termitomycetaceae, due to complete absence of clamp connection. Molecular work however supports the placement of *Termitomyces* in the Tricholomataceae within or near Lyophyllae (Moncalvo et al., 2000; Kirk et al., 2001; Lefevre et al., 2002). Recent molecular studies by Matheny et al., (2007) proposed to place *Termitomyces* in Lyophyllaceae Jülich (Figure-7), and the proposal was accepted in Dictionary of Fungi 10th edition (Kirk et al., 2008). Zang (1981) examined some collections from
Yunnan Province, China, and proposed a new genus, *Sinotermitomyces*, which may be separated from *Termitomyces*.

![Phylogenetic analysis of *Termitomyces* species (Matheny et al., 2007).](image)

**Fig- 7:** Phylogenetic analysis of *Termitomyces* species (Matheny et al., 2007).

The genus *Termitomyces* is distributed throughout equatorial and southern Africa and widely in south-east Asia (Pegler, 1994). Approximately 30 species of the *Termitomyces* symbionts have been described (Kirk et al. 2008). The genus, *Sinotermitomyces* Zang, was introduced in 1981 from Yunnan Province China (Zang, 1981) and placed in family Tricholomataceae, order Agaricales (Kirk et al., 2001). As in *Termitomyces*, the genus *Sinotermitomyces* develops with an obligate symbiotic relationship with the termites belonging to the subfamily of Macrotermitinae (Aanen et al., 2005). The *Sinotermitomyces* consist of five species namely *S. carnosus* M. Zang, *S. cavus* M. Zang, *S. griseus* M. Zang, *S. rugosiceps* M. Zang and *S. taiwanensis* M. Zang and C.M. Chen (Zang, 1981, 1992; Zang and Chen, 1998).

Based on the description given by Zang (1981), the species of *Sinotermitomyces* are very close to the genus *Termitomyces* in having symbiotic association with the termites and also in their morphological characteristics, but differ in having small basidiome, mamiform perforatorium, hollow stipe, velar remains on the stipe, pileus and cystidia with protuberances. But all of these characteristics are also present in *Termitomyces* species. The recent molecular phylogenetic study also confirmed that, the genus *Sinotermitomyces* and *Termitomyces* are congeneric (Froslev et al., 2003).
The examination of type material of all the five *Sinotermitomyces* species and other additional collections shows that they are representatives of three known *Termitomyces* species. Diversity of *Termitomyces* the African species have been fairly well documented (Buyck, 1994) but this was not the case for those found in Southeast Asia. It was not until over fifty years later that Pegler and Vanhaecke (1994) reviewed numerous collections throughout the region and published a key to fourteen species, including two *Sinotermitomyces* (Figure- 9).

In China, they are found mainly to the south of the Yangtze River (Zang, 1981). With a varied geographical and climatic environment, China provides favorable conditions for *Termitomyces*. So far, 25 taxa of *Termitomyces*, including three new species, have been reported from 17 provinces within China (Wei *et al.*, 2003). In Thailand the common name for *Termitomyces* is Hed Khone. Several *Termitomyces* species are widely available; *T. eurrhizus, T. globulus, T. microcarpus* *T. albuminosus* and *T. aurantiacus* which is the most familiar. The best time for collecting *Termitomyces* is at the end of the rainy season that is during October and November (Sangvichien and Hawksworth, 2001). Several species of *Termitomyces*, especially *T. heimii* and *T. clypeatus* have been reported from Nepal based on observation of Morten Christensen (2007). Extensive works have been done in Malaysia and different species of *Termitomyces* have been recorded (Lee *et al.*, 2009). *Termitomyces heimii* and *Termitomyces indicus*, for the first time described from Madras by Natarajan (1979). Different species of *Termitomyces* were recorded from Tamil Nadu, *Himachal Pradesh*, Goa, Kerala, Jammu and Kashmir and Western Ghats of India, this much of reports show the large diversity and density of *Termitomyces* species in India. Perhaps India is the largest gene pool of *Termitomyces* in Asia (Mani and Kumaresan, 2009).

5. Biology of *Termitomyces*

Most of the higher basidiomycetes, *Termitomyces* species do not form clamp connections between adjacent cells (Heim, 1977), which makes a heterokaryon not easily distinguishable from a homokaryon. Most basidiomycetes have a heterothallic mating system (Burnett, 2003). In heterothallic mating system, a single haploid basidiospore germinates to form a haploid mycelium, this mycelium usually has a single nucleus per cell (*i.e.* a monokaryon), but sometimes multiple identical nuclei
are present within a single cell (Kues and Liu, 2000). Contact between two compatible homokaryons is followed by cell fusions and reciprocal nuclear exchange and migration through both mycelia, so that all cells in this heterokaryon have two nuclei (i.e. a dikaryon) one from each component monokaryon.

The heterokaryon can give rise to sexual fruit bodies where the basidia develop in which the nuclei fuse and enter a short diploid stage before they are under meiosis (Carlile et al., 2001; Burnett, 2003). It is usually assumed that Termitomyces fungi have a heterothallic (i.e. out crossing) life cycle (Heim, 1977), like most basidiomycetes studied so far (Raper, 1966). In this life cycle (Figure-7) spores germinate and form a monokaryon, all cells of which have a single nucleus. Two monokaryons of the same species with different mating types can fuse and form stable dikaryon, all cells of which have two nuclei, one of each monokaryon. A dikaryon can form fruiting bodies, where meiosis and spore formation takes place.

The horizontal transmission is likely to fit into this general scheme and also the four spore basidia of fruiting bodies from Termitomyces support this (Heim, 1977). Alternatively, however, some of the fruiting Termitomyces fungi might have a (secondarily) homothallic (i.e. non-out crossing) mating system. In that case, a single fungal spore is sufficient to complete a fungal life cycle. Recent study demonstrated that Termitomyces strains have a heterothallic mating system with a homokaryotic and a heterokaryotic phase (Licht et al., 2006).

Here a question arises; what type of life cycle do the non-fruiting Termitomyces species have? One possibility is that the mycelium of these species is monokaryotic and that this monokaryotic mycelium is clonally propagated by vertical transmission (Figure-8). Very few monokaryotic basidiomycetes that can produce fruiting bodies are known (Ullrich and Raper, 1975). Therefore, keeping mycelia monokaryotic could be a proximate explanation for the lack of fruiting of some fungal symbionts. Another possibility is that the non-fruiting fungi have a dikaryotic mycelium that is clonally transmitted by vertical transmission. In this case, the termites might actively prevent their fungal symbionts from fruiting, for example by eating the primordial (Figure-8). Obviously, life cycles of Termitomyces need to be studied to find out the exact possible strategies.
6. Termitomyces identification

Good scientific mushroom taxonomy studies are very important in mushroom classification since poorly done taxonomy can be fatal. Like any other living organisms, mushrooms are identified through observing their identity of their characters with naked eye, using hand lens, microscopes and more recently by the use of molecular techniques. Through these methods, protocols for describing and illustrating each species are made to guide correct identification of a particular mushroom (Bougher and Katrina, 1998).

**Phenotype characteristics:** In conventional mushroom taxonomy, general procedures are followed by examining and recording the characters of the basidiomes starting from the field. Macro-morphological characters easily observed in the field are recorded first, followed by further examination including microscopic characters observed on fresh or dried mushrooms. The taxonomy of *Termitomyces* has been mainly based on the basidiocarps and what we know is that a significant number of the strains do not fruit, or at least not regularly (Moncalvo et al., 2002). *Termitomyces* species are universally characterized based on the dome-looking shape of their perforatorium on the pileus, which is well developed, and the subterranean pseudorrhiza, which are attached to the fungal comb of the termite’s nest (Tang et al.,...
The main distinguishing features for the species are: basidiome size, pileus colour and Spore colour, presence or absence and types of Cystidia, texture, the form of the central umbo or perforatorium, and the presence or absence of velar remnants. These features are variable and depend upon the architecture, depth, size and nutritional status of the parent comb, and the resistance of soil. Great care is required to trace a delicate pseudorrhiza to its origin (Figure - 9).

The study by Pegler and Vanhaecke (1994) in Southeast Asia extensively characterized different important phenotypes of Termitomyces species that grow in this part of world (Pegler and Vanhaecke, 1994). T. microcarpus and T. indicus, which shows epigeal development, lacking a pseudorrhiza, and have aregular hymenophoral trama. Typical T. microcarpus and T. indicus are recognised by its small size and appearing in spectacular swarms on large termite mounds, or occasionally from recent termite workings extruded over the ground.

Fig- 9: Universal characters of Termitomyces species a) Termitomyces species (macro-phonotype structure) b) Termitomyces associated with termite comb in nature (Anon., 2003).
Cultural characteristics: Cultural studies of the genus *Termitomyces* Heim have been studied exclusively on mycelium obtained from comb sporodochia and the basidiome context of fruit bodies (Petch, 1913; Heim, 1977; Batra and Batra, 1979; Piearce, 1987). Heim (1977) supplied a condensed description and an illustration of the hyphal characters of *T. mammiformis* Heim cultures. Cultural similarity of *Termitomyces* species studied in Asia and Africa have been confirmed clearly by Heim (1977) and Piearce (1987), as well as Batra and Batra (1979). *Termitomyces* is very slow-growing in agar culture and may be rapidly overgrown by other fungi. This makes it very difficult to isolate from the natural habitat where many other fungus propagules are present. Many authors have found it difficult or impossible to isolate *Termitomyces* (Perch, 1906; Abo-Khatwa, 1977; Zoberi, 1979).

The study conducted by Botha and Eicker (1991) demonstrated that the mode of conidiogenesis, germination and the basic morphology of the conidiophores and conidia, as well as the branching pattern of the conidiogenous hyphae and conidial chains, are identical for all the species studied in this investigation. *Termitomyces* species do not form clamp connections between adjacent cells (Heim, 1977). Microscopic cultural similarity of *Termitomyces* species, growth characters of basidiome context cultures differ markedly, and it is possible to distinguish between cultures of different species. This may be accomplished by comparing growth characters such as mat texture, colour and odor (Botha and Eicker, 1991).

Molecular characteristics: In some cases identification to distinguish between genetically related species based on phenotypic and cultural characters had been reported to be difficult or impossible (Lee *et al.*, 2006). On the other hand this procedure is convenient, rapid accurate and requires only a small amount of sample (Lee *et al.*, 2006). Molecular approach for identification of several Basidiomycetes genera has been attempted during the recent years. In terms of molecular characterization of fungi most used genomic region is the ITS region (Gardes and Bruns, 1991, 1993; Anderson and Stasovski, 1992). The molecular characterization of genus *Termitomyces* based on nuclear ribosomal DNA sequences consisting of internal transcribed spacers (ITS1 and ITS4), 5.8S rDNA, and partial large subunit (LSU) rDNA extensively studied by Yaovapa (2002). Several works have been done
in South Africa and Asia but a lack of molecular data on Indian species is very obvious.

7. *Termitomyces* in local communities

The Yoruba people of southwestern Nigeria are rich in culture and have a lot of myths, which explain the origin and behavior of many things in their environment and daily life. *Termitomyces microcarpus* is used medicinally by Yoruba native doctors as an ingredient in the preparation of a charm supposed to bring good luck; particularly to traders they strongly believed that this has the power of promoting the sale of articles by drawing the buyers into the shop. Curatively, the Yoruba traditional doctors employ *T. microcarpus* in a medicinal preparation for the treatment of gonorrhea. The medicine, which is administered orally, is prepared by pounding a large quantity of the fruit bodies of the fungus with the pulp of the fruit of *Cucurbita pepo* Linn. Apart from this, *T. robustus* is used medicinally as a remedy for Maagun (Oso, 1975).

The Nigerian tribes use the *T. robustus* and *T. striatus* are used as pot herbs. Among some tribes in Uganda *T. microcarpus* is used for medicinal purposes, infertility enhancement and inducing appetite in certain disease conditions. *Termitomyces* species are a highly prized delicacy collected both for home consumption and for sale in local markets or along roadsides (Pegler and Vanaecke, 1994) where they command a high price. In Thailand the common name for *Termitomyces* is Hed Khone and as a much prized delicacy. Because of its unique taste and aromatic the local people of Thailand call *T. robustus* as a “king of Mushroom” flavors.

Species of the termite-fungus (genus *Termitomyces*) are a local delicacy (Faridah et al., 2002) and a favourite among all of the indigenous populations in Peninsular as well as East Malaysia. *T. heimii*, is the species that most available and commonly called ‘cendawan busut’, translated to mean mound fungus. A mycological survey of the North Luangwa National Park in Zambia revealed three species of *Termitomyces; T. titanicus, T. clypeatus* and *T. eurrhizus* (Smith, 1995). These mushrooms were sold along the roadside or used to make.
In China, *T. albuminosus* is believed to possess medicinal properties, such as strengthening the stomach, improving thought processes, preventing intestinal carcinoma and curing hemorrhoids. Phytochemical assays revealed the presence of polysaccharides, saponins, cerebrosides, coumarin and ergosterol in this fungus (Qi *et al.*, 2001). In traditional Chinese medicine, *T. albuminosus* is used to treat hemorrhoids.

8. *Termitomyces* as super food

*Termitomyces* species are well known as a food source and are considered delicacies, with many species on sale in markets and at roadsides in the old world tropics. Numerous studies on nutritional value of *Termitomyces* species has been investigated in different countries by Mukii bi (1973) in Uganda, Ogundana and Fagade (1982) in Nigeria and Parent and Thoen (1977) in Zaire. Bano, Ahmed and Shrivastava (1964) and Purkayastha and Chandra (1975, 1976) have ascertained the nutritional value of several *Termitomyces* species in India. A superfood should have the following characteristics. They are, minimally processed without nutrient enriching, have nutritional benefits not seen in other foods commonly eaten and have a high nutrient density compared to its kilojoule content. provide other bioactive compounds such as antioxidants, phenol, flavonoid, β carotene, have research linking the food to a potential reduced risk of long-term disease, low in kilojoules, with a high nutrient density, provides good nutrition without adding fat, cholesterol or sodium to the meal, as an antioxidant capacity similar to or better than, common vegetables and provides bio-active compounds that appear to improve immune function and potentially lower the risk of breast and prostate cancer.

A comparison of the edible mushrooms of the Upper-Shaba showed that *Termitomyces* had the highest protein content with values ranging from 33-45 g per 100 g dry weight (Parent and Thoen, 1977). Another study by Ogundana (1982) shows *T. robustus* and *T. clypeatus* contained 31% protein, 32% carbohydrate and 10 –14% ascorbic acid (Ogundana and Fagade, 1982). In addition to being highly nutritious, the *Termitomyces* species also have a distinctive flavour and a texture which is quite firm, not unlike chicken or meat. It is not surprising, therefore, that they are a welcome addition to the diet in many countries and can be a valuable food
supplement both in terms of variety and nutrition. It would appear that some of the *Termitomyces* species fall between most legumes and meat. Indeed, earlier studies (Suzuki and Oshima, 1976; Gruen and Wong, 1982; Zakhary *et al*., 1983) indicate that edible mushrooms are highly nutritious and compare favorably with meat egg and milk (Aletor, 1995). Tryptophan is one of the essential amino acids often limiting in the diets of developing countries, the Tryptophan content of *T. robustus* are high and this amino acid becomes more important when its sparing role for niacin is taken into consideration (Olumuyiwa *et al*., 2006). Glycine, glutamate and alanine are the most plentiful amino acids in all the *Termitomyces* species (Alofe, 1991).

Some species, such as *T. umkowaani* and *T. sagittaeformis*, contain all the essential amino acids (Botha and Eicker, 1992). Other species, lack one or more of the essential amino acids, for example in *T. reticulatus* four essential amino acids (isoleucine, leucine, methionine and cytosine) are severely limiting (Botha and Eicker, 1992); Research has linked vitamin D to a rapidly increasing number of benefits beyond healthy bones and the prevention of rickets and osteoporosis. These benefits include a decreased risk of heart disease, type II diabetes, hypertension and colorectal cancer (Dobnig *et al*., 2008; Lee *et al*., 2009). The vitamin D in mushrooms is easy to absorb (Outila *et al*., 1999; Jasinghe *et al*., 2005; Koyyalamudi *et al*., 2009). There is at least 85% retention of vitamin D in wild mushrooms after frying for five minutes (Mattila *et al*., 1999). Furthermore, there is very little loss of vitamin D2 when mushrooms are refrigerated for eight days 12 or even three months (Mattila *et al*., 1999).

Despite their high water content, fruiting bodies of the genus *Termitomyces* appear to be a great source of fibers and minerals. The high content in crude fibers, low energy value and low lipids content with high amounts of poly unsaturated fatty acids lead to the conclusion that these mushrooms would be a valuable contribution to the menus of persons under hypocaloric diet. There seems to be general agreement among consumers and scientists alike about the superior nutritive value of *Termitomyces* species in general when compared with other edible mushrooms.

Oxidation is essential to many organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen-derived free
radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, and atherosclerosis, as well as in the degenerative processes associated with aging (Wang et al., 2006). In order to reduce free radical damage to the human body, synthetic antioxidants are used for industrial processing at the present time. However, the most commonly used synthetic antioxidants have been suspected of being responsible for liver damage and carcinogenesis (Qi, 2005). Thus, it is essential to develop and utilize effective and natural antioxidants that can protect the human body from free radicals and retard the progress of many chronic diseases.

Puttaraju et al. (2006) noted the existence of varietal differences in antioxidant activities among Termitomyces species. In which T. heimii and T. mammiformis showed high antioxidant activity among 23 tested mushroom from India. Total phenol content of Termitomyces species was high and interestingly hot water extraction shows high antioxidant activity compare to solvent extraction (Puttaraju et al., 2006). In a recent study, Oyetayo (2008) using a multi mechanistic antioxidant assay which include DPPH radical scavenging, superoxide radical scavenging, hydroxyl radical scavenging and reducing power assay found that ethanol, hot water and petroleum ether extracts of Termitomyces clypeatus (Heim) had antioxidant effect comparable to synthetic antioxidant, BHT. The implication of this result is that, this edible mushroom, T. clypeatus, obtained from Nigeria may be a good source of alternative antioxidant to the synthetic antioxidants which are known to have side effect.

The study carried out by Mau (2003) on antioxidant activity of T. albuminosus and two other macrofungi that grown in Thailand showed highest activity or equal to normal synthetic antioxidant drugs. Appreciable levels of phenolic compounds have been reported in Termitomyces species that are indigenous in Nigeria (Oyetayo and Akindahunsi, 2004). Several studies on antioxidant activities of Termitomyces species clarified the capability of these Macrofungi to become a valuable source of natural antioxidant in human diets.

Termitomyces mushrooms produce a host of interesting bioactive compounds comprising lectins (Guillot et al., 1997; Wang et al., 1998), antifungal proteins (Kobayashi et al., 1992), ribosome inactivating proteins (Wang et al., 2000), ribonucleases (Ye and Ng, 2002; Wang et al., 2003), laccases (Garzillo et al., 2001),
proteases (Kim and Kim, 2001), polysaccharides (Zhang et al., 1994) and polysaccharopeptides (Wang et al., 1996). Oyetayo (2008) also found that *T. clypeatus* has appreciable level of polysaccharides. All species were characterized by high concentrations of unsaturated fatty acids amounting to 52–87% of the total fatty acid content.

The potential immunoceutical property of two common edible mushrooms, *T. robustus* and *T. striatus*, indigenous of Nigeria was demonstrated by Adewusi et al. (1993). In a recent study, Mondal et al. (2006) reported plenocytes activation of rats by polysaccharides isolated from aqueous extract of *T. striatus*. The study by Oyetayo (2008) shows *Termitomyces* species indigenous to Nigeria had been reported to possess hypolipidemic, antioxidant, immunostimulatory and cholesterol lowering properties.

In recent years mushroom polysaccharides have drawn the attention of chemists and immunobiologists on account of their immunomodulatory and antitumor properties. Polysaccharides isolated from fruiting bodies of, *T. eurrhizus*, (Mondal et al., 2006) *T. striatus*, (Mondal et al., 2006) and *T. microcarpus* (Chandra et al., 2007), have been studied extensively. An interesting endo-β (l→4) xylanase, constitutively produced by mushroom *T. clypeatus* in dextrin medium, was purified to homogeneity (Ghosh et al., 1980). Protease with a molecular mass of 30kDa was isolated from fresh fruiting bodies of the mushroom *T. albuminosus* (Zheng et al., 2011).

*T. clypeatus*, which simultaneously produces α-arabinofuranosidase with xylanase (Sinha and Sengupta, 1995), also produces acetyl esterase with these enzymes and the enzyme was equally active on pnitrophenylacetate and acetyl xylan. *Termitomyces clypeatus* has been shown to be able to produce a broad spectrum of extracellular glucosidases (cellulase, sucrase, cellobiase etc.) but also of xylanases and laccases (Ghorai et al., 2009).

The fungus *T. clypeatus* has been found to be a potential producer of a broad spectrum of extracellular glucosidases (cellulase, sucrase, cellobiase etc.), capable of hydrolysing the polysaccharides, e.g., hemicellulose, cellulose, and starch (Khowala and Sengupta, 1992; Khowala et al., 1992). Different enzymes such as endo1, 4-b-D-
Xylanase, 1, 4-b-D-Xylosidase, a-L-arabinofuranosidase, acetyl esterase, a-amylase, and amyloglucosidase were also purified from this species.

Zheng et al. (2011) had discovered a novel alkaline protease from *T. albuminosus* grown in Yunnan province (China). Alkaline proteases are degradative enzymes which catalyze the hydrolysis of proteins. Proteases not only play an important role in the cellular metabolic processes (Tremacoldi et al., 2007), but also have many applications in detergents, leather processing, silver recovery, medical purposes, food processing, feeds, the chemical industry, as well as waste treatment (Ma et al., 2007). Several polysaccharide active molecules have been isolated and reported from *Termitomyces* species, Krishnendu Chandra (2007) have reported α-(1→4) β-(1→3) glucan from *T. microcarpus* for the first time.

The chemical evaluation of *Termitomyces* species revealed the presence of polysaccharides, saponins, cerebrosides, coumarin and ergosterol in this fungus (Qi et al., 2001; Yao et al., 1998). The research work by Lu (2008) showed that *T. albuminosus* in submerged culture had potent analgesic and anti-inflammatory effects. The results obtained from this experiment supported the traditional use of this fungus in the treatment of hemorrhoids. The protein fraction (Cibacron blue affinity eluted protein (CBAEP) fraction) isolated from *Termitomyces clypeatus* have shown Antiproliferative and immunostimulatory activities in cell line as well as vivo conditions (Maiti et al., 2008). The β-glucan extracted from *T. eurrhizus* by oral administration to rats demonstrated decreased the cholesterol level in rats after treated with this extract with certain concentration (Muristu, 2011).

9. **Importance of current research**

Not much work has been done to explore the mushroom diversity in regions such as Kodagu, Karnataka, especially in *Termitomyces* species. Because of their importance to mankind has been realized for their exploration and documentation for economic benefits. This study is of great significance because multiple varieties of *Termitomyces* exist in different parts of Karnataka specifically Kodagu. Thus selection of *Termitomyces* variety becomes an important aspect to gauge the health benefits. Therefore, in this study, an attempt has been made to investigate different varieties of edible *Termitomyces*, occurring in the hilly and tribal regions of Kodagu,
Karnataka. Hence the thesis entitled “Diversity, Nutritional Value and Bioactive Principles from Termitomyces Species of Kodagu Region in Karnataka” was proposed with the following objectives as under:

- To explore and document edible Termitomyces species found naturally in Kodagu region of Karnataka.
- To evaluate Termitomyces species for their suitability for human consumption in terms of nutritional value and also to screen for valuable bioactive principles.
- To develop low cost and high yielding production method for mass cultivation of Termitomyces species.

The above three objectives have been carefully investigated by employing standard methods of scientific investigations. The results obtained were subjected to statistical analyses and compiled and presented in three different chapters. Finally, at the end of the thesis, summary and important references are provided under References section.