2. LITERATURE REVIEW

I. Ethnobotanical Studies In Edible Fungi

Probably the only work on ethno-mycology from central India has been done by Rai et al. (1993). A detailed work on documentation of ethnobotanical research on the regions of Bundelkhand and other parts of the country has been compiled by Maheshwari (1987). No work is more comprehensive than the distinguished ethnobotanist S.K. Jain’s Dictionary of Indian folk medicine (1991) and his reference manual (1987) for understanding of man-plant-culture relationships, ethnic groups & ethnobotanists in India has been brought by Jain and Sudhanshu (1991). Dixit and Pandey (1984) studied folk medicines in Bundelkhand. However, so far none has gone deep in the traditional practices of the tribals of Bundelkhand.

Ethnometeorology is deeply linked in the minds of the tribals for looking for edible fungi in the jungles. So far work on the Ethnometeorology is at the very preliminary stage. No one has seriously studied the possibilities of weather forecasting, using the behavioral activities of the animals including social insects prior to arrival of a particular season. For the study of this aspect as a backgrounder to understand the termite-related fungus under study, following literatures have been referred: Early detection of swarming sites of subterranean termite Odontotermes distans Holmgren and Holmgren (Kumar, 1992); From colony foundation to dispersal flight in a higher fungus-growing termite, Macrotermes subhyalinus (Han and Bordereau, 1992); Population estimation and seasonal fluctuations of the mound building termite Odontotermes wallonensis in South India (Rajagopal, 1985). As Termitomyces belongs to Agaricaceae it would be prudent to have a look at the taxonomy of Agaricaceae as a whole to locate the position, characteristics and broad features of the mushroom as such.

II. Taxonomy Of The Agaricaceae

Singer (1936, 1939 and 1951) gave full details and keys to the genera of Boletaceae and Agaricaceae seperately, based upon the new anatomical and chemical studies.
Murrill (1910-1916) illustrated and described genera and species of tribes *Chanterellae, Lactariec*ae and part of the white-spored, rose-colored spored, ochre-spored, purple-brown to black spored genera of *Agariceae*. Remainder of white-spored forms and the pink-and brown-spored forms, including *Inocybe* and *Cortinarius* were described by C.H. Kauffman, and *Pholiota* and *Hypodendron* by L.O. Overholts.

Kauffman (1918) gave a full descriptions of all species of *Agaricaceae* known to occur in Michigan, and many genera of all species recognized in Northeastern United States. Descriptions are illustrated by excellent photographs. Following are works on white-spored genera: Harper (1921); Morgan (1906) and Murrill (1913). Harper (1913) reported ochre-or rust-spored genera of the species of *Pholiota* in the region of the Great Lakes.

Singer’s notes (1936) contains keys to the more or less green species of *Russula* in France and to the species of *Russula* associated with the birch in France and bordering countries, also key to distinguish the genera *Phyllotopsis, Dochmiopsis, Rhodotus*, and *Octofuga*).

Red-or pink-spored genera have been described by Murrill (1915) and Bohus (1945).

Key and descriptions of 16 species of Purple-spored genera of the genus *Psaliota* (*Agaricus*) in the Philippines and Hungary are given by Mendosa (1940) and Kalmar (1946), respectively.

Species of the genus *Coprinus* are balck-spored genera (Massee, 1896).

### III. Edible And Poisonous Mushrooms Belonging To *Agaricaceae*

Methods of distinguishing edible mushrooms and toadstool are described by Gibson (1895).

Atkinson (1900) studied American fungi including mushrooms and poisonous fungi. Other works on edible and poisonous fungi are: Smith (1938); Anonymous (1945); Gussow and Odell (1927).
IV. In Vitro Cultivation Of Edible Fungi

Isolation and laboratory culture of *Termitomyces cartilagineus* edible fungi at UPLB, Philippines was done by Quimio (1978). Sarot et al. (1983) attempted to grow and develop *Termitomyces* sp. from spores. Sathe and Dighe (1987) developed a method for long-term preservation of Oyster mushroom (*Pleurotus* spp.); the culture could be kept viable by this method at least for 8 years by repeated use of the same cultural practices. This method is simple, economic, requiring minimum space and needs no cryostatic or low temperature arrangements and therefore most suitable in tropical developing countries. A technique for isolating the fungus in pure culture from field collections has been developed by Trappe (1969).

Experiments on *Agaricus bisporus* have indicated that some of the growth regulator compounds viz. flurprimidol, cycocel, succinic acid, dimethylhydrazideancymedol, gibberellic acid, 6-benzyladenine, alfa-napthalene acitic acid, caffein and thiophylline, at some concentrations effected yield and size of the *Agaricus bisporus* (Halberi and Schisler, 1986).

Balazs (1988) studied changes in the microelement content of some cultivated and wild mushrooms under *in vitro* cultivation. Trials and measurements included 26 elements in different mushroom species covering both cultivated and wild. It was observed that mushroom species differed amongst themselves regarding amounts of those elements which are generally abundant in green plants; these were found to be much lower in the mushrooms. The observation is affirmed by literature available and could also be explained by the special position of mushrooms in taxonomy. Cultivated mushrooms are much poorer in almost all the elements studied than the wild mushrooms. From a nutritional point of view this phenomenon gives wild mushrooms an edge over cultivated ones and explains the popularity of wild mushrooms collected from nature. However, it gives an advantage to cultivated mushrooms in case of dieting and regulated intake of various elements. The higher microelement content of wild species deserves attention from the point of view of the daily microelement introduction by nutrients and from the point of view of genetic engineering to increase microelement content of cultivated mushrooms where such increase is considered desirable.
Chemical composition of mycelia of *Termitomyces albuminosus* (Berk) Heim, collected from nature, have been analysed by Zhao et al. (1988) using submerged cultural technique.

Botha and Eicker (1992) tried to cultivate *Termitomyces* mycelial on a number of natural substrates. However, they could not cultivate *Termitomyces* for any significant period on any of the synthetic culture media or on natural substrates. Chen et al. (1987) also tried to grow *Termitomyces albuminosus* in nursery beds with usual compost-humus composition that are used in button mushroom cultivation but failed.

It may be noted that so far all attempts at cultivating or culturing any species of *Termitomyces* either on defined medium or on natural medium used for cultivating various edible fungi for large scale harvesting have failed to give any results. Various studies on *Termitomyces* from all over the world yet remain confined to freshly collected or dried material while various other *Agaricales* not associated with termites continue to progress.

Fritsche and Sonnengberg (1988) have used wild types for breeding of *Agaricus bisporus*. On the other hand Anderson (1993) has pointed to the threat emerging for the genetic diversity in *A. bisporus*, in nature and has emphasized the need for collection and preservation of wild types for use in breeding all over the world.

V. Conservation Of Mushroom Germplasm

A number of studies have come out on the conservation of germplasm of various fungi. The most widely referred techniques used for this purpose are freeze drying (lyophilisation) and storage of fungus tissue and spores in liquid nitrogen.

Paper & Alexander, (1945), for the first time used lyophilization technique for preserving fungal cultures stored at National Research Laboratory, Peoria, U.S.A. In this process fungus tissue is preserved under vacuum in a frozen state. The suspending medium is chosen to give protection during the process and also for convenience, enabling easy filling of the ampules and vials. Such media, normally used, are skimmed milk, serum, peptone, various sugars or
mixtures of them. Some sugars have associated problems due to their behavior during freeze drying. Bubbling can also occur prior to freezing or later in the process if solutes are allowed to thaw. Overdrying will kill or in other cases cause mutation by damaging DNA. Storing the ampules at low temperature is thought to give greater longevities and minus 40 seems to be most favoured temperature permitting preservation for more than 15 years.

Atkinson and Bakerspigel (1954) developed soil storage method for germplasm conservation. In this method spore suspension is poured into sterile soil and allowed to grow for about 10 days. The cultures can then be stored, as for agar slants, at plus 4°C. Cultures treated in this way remain viable and typical for long periods.

Silica Gel method was reported by Roberts (1980). A cooled suspension in 5% skimmed milk is poured on to precooled silica gel (purified without indicator, 6-22 mesh or medium grade) in screw caped bottles and allowed to dry at room temperature until the crystals separate in about 14 days. The caps are then screwed down, and the bottles stored in a refrigerator over indicator silica gel at 4-6°C. When required a few crystals of culture on gel are scattered on agar plate to get colonies. Survival is up to about 10 to 11 years according to species is reported.

Bagg (1967) used aluminium foil and silica gel for semi freeze drying. McCartney screw cap vials are half filled with dry self indicating silica gel. A small piece of aluminium foil is then placed in each bottle so that it formed a diagonal slope at the width of the bottle. The bottles are then autoclaved for one hour. Inoculum is placed on the foil in the form of 5 mm diameter discs, six or eight to a bottle. The discs are conveniently cut using a cork borer, the end of which had been bent over 90°. The discs are lifted with a microme-wire flattened at the tip and inserted into the foil right side up. The bottles are placed in a refrigerator immediately after inoculation. The discs dried out in about two hours. In order to subculture from the foil a piece bearing an agar disc was torn away using sterile forceps. This was placed (disc slide down) on a fresh agar plate. Cultures grew rapidly from this type of Inoculum.

Slide collection system is used for preservation of dry spore masses of Agarics and Boletes. The slides of rare species of Agarics showing structural
features of various species are conserved according to this method by the authors. The slides are then numbered and accessioned in the Herbaria. A list of these are prepared which can be used for comparing the original slides.

A new technique for the preservation of fragile or rapidly decomposing diseased plant material has been developed by Bebington and Burrell (1968). According to this method the specimens are dried, either in electric oven or in a freeze drier, and then coated with undiluted clear polyurethane by dipping. The specimen are then hung up in an oven at 50-60°C. This treatment forms a hard durable and transparent coating around the specimen. However, this treatment does not permit observation of fine details and introduces unnatural lusture to the specimen. However, the thus coated material can be handled freely without fear of damage. Care is taken to ensure that the polyurethane completely covers the surface. A large range of diseased specimens can be preserved by this method with specially good results, as reported for specimens from grasses and apple leaves. For the preservation of fungus infected potatoe and pea leaves, the polyurethane is diluted (2:1) with rectified spirit, thereby resulting in much thinner coating.

VI. *Termitomyces* Reported From Various Parts Of India

*Termitomyces* as an edible termite-associated fungi is reported to be common in various parts of India. It may be noted that, due to prevailing lack of interest, the regions of India from where so far there are no reports, it should not be taken as if there is no *Termitomyces* observable in those areas, viz. Delhi, as we have observed and collected material of termite-related fungus from J.N.U. Campus, Delhi which was identified as that of *Termitomyces* by specialists in Mycology Division of IARI by Professor Dr. A.K.Sarbhoy on the basis of comparison with museum specimens in the excellent collection of Mycology Museum of IARI supervised and looked after by Dr. Sarbhoy.

With this caution in mind that negative, or absence, of results not being taken as final, let us have a look at the position of *Termitomyces* in various parts of India.
Western Ghats in the Goa sector are blessed with abundant wild mushroom flora. This rich mushroom flora of Western Ghats as represented in Goa, and the cultural habits of Goan people who have been influenced by European taste through Portuguese cultural influence is of great interest in itself and is reflected in the consumption of wild mushrooms by the people of Goa occurring in nature. The most popular edible wild mushrooms of the region belong to the species of *Termitomyces* (Termitophillic) which inhabit the termite mounds or ant hills in forest areas of Goa and Sawantwadi and grow abundantly in the monsoon season (Rao and Dhandar, 1995). The growth of *Termitomyces* can also be seen all along the Konkan region starting from Maharashtra to the Malabar region of Kerala. Their edibility must have been discovered long back in the hoary past when Kols, Mundaris and Asura tribals inhabited the Western Ghats. In Goa, these wild mushrooms collected by forest dwellers are sold in the markets of Panjim, Margao, Ponda, Mapusa, etc., during mid July-September with August as the peak period of their sale. It is estimated that about 75 to 100 tonnes of wild mushrooms are consumed by Goans every year. These mushrooms are locally called as ‘Olmi’in Goanese language. However, there are many local names given to these mushrooms on the basis of their habitat and morphology etc. Some of these are: Sringar Olmi (decorative mushroom), Fugo Olmi (balloon mushroom), Tel Olmi (oily mushroom), Ponos Olmi (jack fruit tree mushroom), Sorop Olmi (snake mushroom), Shital Olmi (rice grain mushrooms), etc. Goans are known for preparation of delicious variety of mushroom dishes like salads, pickles, pizzas, chutney, pakoras and popular pungent dish ‘olmya bhaji’ prepared in wet grounded masala using *Termitomyces* species.

This preliminary survey not only reveals the presence of abundant *Termitomyces* flora in the region but also indicates the possibility that some of them could be exploited for use in breeding/improvement programs, artificial cultivation and even for commercialisation provided we are in a position to *in vitro* cultivate the species as we yet do not know if it is the same species or there are more than one species involved in Goa.

No reports are available regarding the natural mushroom flora of Gujarat. Although mushroom species belonging to the following genera are found at various places in Gujarat during monsoons (Patel and Rafique, 1991):

i) *Pleurotus* spp.
ii) *Termitomyces* spp.

iii) *Agaricus* spp.

iv) *Lepiota* spp.

Total of 14 genera of edible fungi have been reported from Kerala. *Termitomyces* are the most commonly consumed mushroom in the state used by the tribal people and villagers of Kerala (Natarajan and Raman, 1983).

The people of Tamil Nadu collect and consume some of the edible mushrooms during the monsoon season. Different species of *Termitomyces* abundantly occur in the plains of the state during monsoon season (Natarajan, 1975). It is the most common edible species collected and consumed by the public. The tribal people residing in hilly regions collect the naturally occurring mushrooms and consume.

From Punjab three species of *Termitomyces* have been reported (Rawla et al., 1983).

Although mushroom, at large, have been reported from different parts of the states, relatively few edible species have been recorded so far. The number of exotic and indigenous edible species, their nature, nutritive values and cultivation techniques are not yet fully known except for a few selected species. Butler and Bisby (1960) have reported a large number of edible fungi in Uttar Pradesh; their list includes *Termitomyces strietus* (Pseli) Heim. from Dehradun and Massuri. We may underline that we in detail would be dealing with the same found naturally growing in another region of Uttar Pradesh viz. Bundelkhand.

Only after the establishment of the All India Co-ordinated Mushroom Improvement Project at Indira Gandhi Krishi Vishva Vidyalaya (IGKVV), Raipur in 1988, the work on survey of mushroom flora of the state was undertaken in Madhya Pradesh. There are many types of edible mushrooms naturally growing in eastern Madhya Pradesh forests which are collected, consumed and marketed up to some extent. The most commonly available mushrooms in the area include *Termitomyces* sp. (Kumar, Shukla and Agrawal, 1991). These mushrooms, as and when brought to the market, are sold as hot cakes which indicates liking of people to such naturally growing mushrooms.
However, these mushrooms are available only in rainy season for about a month or so. It is presumed that in forests of other parts of Madhya Pradesh, appreciable quantities of edible mushrooms are available, although these parts have not been surveyed.

**VII. Termites And Termite-Related Fungi In India And In The World**

Termite related fungi have been reported from various part of the world. Fungi associated with the subterranean termite *Reticulitermes flavipes* in Ontario were reported by Zoberi and Grace (1990). Fungi associated with the sand termite *Psammotermes hypostoma* have been reported from Egypt (Moharram, Bagy and Abdel 1992). West Indian drywood termite, *Cryptotermes brevis* feed and survive due to wood degradation by fungi (Moein and Rust, 1992). Some fungi like *Metarhizium anisopliae*, are entomogenous and used for biological control of termites (Haenel, 1984; Ahmad et al, 1990) but many termites and fungi also live symbiotically.

The ‘Omajowa’ or ‘Termitenpilz’ which grows in groups around the bases of tall termite mounds in Namibia, have been illustrated and described (Westhuizen and Eicker, 1991). It is identified as *Termitomyces schimperi* (Pat.) Heim. Available evidence indicates that *Macrotermes michaelseni* (SjÖstedt) is the associated termite, an association previously unrecorded. The biology and distribution of *T. schimperi* in Namibia are discussed.

Termite *Macrotermes natalensis* have been found to harbor some fungi in the hills of Nigeria (Zoberi, 1979). The morphological, physical and chemical properties of two mounds of *Macrotermes bellicosus* (Smeathman) were compared with surrounding soils in Sierra Leone (Miedema and Vuure, 1977). Bagine (1989) studied nest structure, population structure and genetic differentiation of some morphologically similar species of *Macrotermes* in Kenya (Bagine, 1989).

The pedological role of fungus-growing termites (Termitidae: Macrotermitinae) in tropical environments, with special reference to *Macrotermes muelleri* were studied by Garnier (1989).
DISTRIBUTION OF TERMITOMYCES IN THE WORLD

Termitomyces Habitat

EQUATORIAL SCALE
0 2000 4000 6000 KILOMETRES

180° 120° 60° W. 0° E. 60° 120° 180°
Mound dimensions, internal structure and potential colony size in the fungus growing termite *Macrotermes michaelseni* (Isoptera: Macrotermitinae) have been described by Schuurman and Dangerfield (1996).

Malik and Sheikh (1990) studied the effect of different relative humidities on survival and moisture loss of termite workers and soldiers *Coptotermes laciniatus, Microcerotermes championi, Odontotermes obesus* and *Heterotermes heimi*.

The termite *Odontotermes horni* W. belongs to the family Termitidae and order Isoptera. It is a subterranean termite feeds actively on humus cellulosic materials on the upper strata of the earth. Another report on association of *Odontotermes obesus* Rambur with fungi was published by Farhat (1982). Later, habit and habitat of Termite *Odontotermes obesus* Ramb. was illustrated by Pluak (1984).

Species of the genus *Odontotermes* (Isoptera: termitidae) have been reported from China (Gao, 1987).

There is variation in the size of the soldier caste of the Termite *Odontotermes Obesus* (Rambur) (Akhtar and Anwer, 1991).

Ventilation and thermal constancy play a major role in the colony of a southern African termite *Odontotermes transvaalensis* (Turner, 1994).

Fungus growing termites in Thailand were studied by Yupa (1986). Four genera; fourteenth species of the family Termitidae, subfamily Macrotermitinae were studied as follows: *Macrotermes gilvus* (Hagen), *Macrotermes carbonarius* (Hagen), *Macrotermes malaccensis* (Haviland), *Macrotermes annandalei* (Silvestri). *Macrotermes chaiglomi* Ahmad, *Microtermes pakistanicus* Ahmad, *Microtermes obesi* Holmgren, *Odontatermes longignathus* Holmgren, *Odontstermes javanicus* Holmgren, *Odontstermes feae* (Wasmann), *Odontstermes formosanus* (Shiraki), *Odontstermes proformosanus* Ahmad, *Hypotermes xenotermitis* (Wasmann) and *Hypotermes makhamensis* Ahmad. The result was found that *Macrotermes gilvus* (Hagen), *Macrotermes malaccensis* (Haviland) and *Odontostermes formosanus* (Shiraki) could grow together with fired termite mushroom (*Termitomyces* sp. No.I), flooded termite mushroom (*Termitomyces*
sp. No. II, and pale greyish brown termite mushroom (*Termitomyces fulviginosus Heim*), respectively. Key to genera and species of fungus growing termites had been made with detail of termite mound ecology. Chemical composition of the fungus combs had been analyzed and the fungi on the combs had been identified.

Though the fungi of 'termitarium' have been studied in detail the information on the fungal flora of termite gut is very limited. Role of oxygen and the intestinal microflora in the metabolism of lignin-derived phenylpropanoids and other monoaromatic compounds by termites were studied (Brune et al., 1995). They studied the metabolism of monoaromatic model compounds by termites and their gut microflora. Feeding trials performed with [ring-U-14C] benzoic acid and [ring-U-14C] cinnamic acid revealed the general ability of termites of the major feeding guilds (wood and soil feeders and fungus cultivators) to mineralize the aromatic nucleus. Up to 70% of the radioactive label was released as $^{14}$CO$_2$; the remainder was more or less equally distributed among termite bodies, gut contents, and feces. Gut homogenates of the wood-feeding termites *Nasutitermes lujae* (Wasmann) and *Reticulitermes flavipes* (Kollar) mineralized ring-labeled benzoic or cinnamic acid only if oxygen was present. In the absence of oxygen, benzoate was not attacked, and cinnamate was only reduced to phenylpropionate. Similar results were obtained with other, nonlabeled lignin-related phenylpropanoids (ferulic, 3,4-dihydroxycinnamic, and 4-hydroxycinnamic acids), whose ring moieties underwent degradation only if oxygen was present. Under anoxic conditions, the substrates were merely modified (by side chain reduction and demethylation), and this modification occurred at the same time as a net accumulation of phenylpropanoids formed endogenously in the gut homogenate, a phenomenon not observed under oxic conditions. Enumeration by the most-probable-number technique revealed that each *N. lujae* gut contained about 105 bacteria that were capable of completely mineralizing aromatic substrates in the presence of oxygen (about $10^{-8}$) bacteria per ml). In the absence of oxygen, small numbers of ring-modifying microorganisms were found (less than 50 bacteria per gut), but none of these microorganisms were capable of ring cleavage. Similar results were obtained with gut homogenates of *R. flavipes*, except that a larger number of anaerobic ring-modifying microorganisms was present ($>5 \times 10^{-3}$ bacteria per gut).
The comparative study of digestive oxidases in five fungus-growing species and its symbiotic fungus (*Termitomyces* sp.) brought new insight into the nutritive mode of several species of fungus growing termites known to have a great impact in most African ecosystems (Rouland et al., 1989, 1991). While this work stressed the importance and the variety of enzymatic activities detected in the termite workers digestive tract, their results clearly distinguished two main symbiotic mechanisms into termite nutrition, according to the ability for the symbiotic fungus to produce active enzymes. In the case of *Macrotermes bellicosus*, *Odontotermes near pauperans* and *Pseudacanthotermes militaris*, the metabolism of the fungi is characterized by a relatively higher enzymatic production (variable according to the substrates tested). These enzymes are ingested by the termite and the digestion is due to the combined action of the enzymes from the termite gut and from the fungus. In the case of *Ancistrotermes cavithorax* and *Microtermes toumodiensis*, one can question the role of the fungi as they exhibited very low enzymatic activities. The fungus protoplasm could then be a nutrition source for the termite. Possibly also, these fungi could degrade other substrates (chitin, lignin) not tested in their experiments. Their results also showed a very high oligosaccharidasic activity of *Pseudacanthotermes militaris* symbiotic fungus (*Termitomyces striatus*) which appear to coincide with a different behaviour of the termite towards its fungus comb.

Rajgopal, Rao and Varma (1981) investigated the association of several species of fungi in the worker termite gut *Odontotermes obesus* (Rambur) from northern India. They incubated supernatant extracts from the homogenized gut of worker termite, *Odontotermes obesus* (Rambur) on Rose Bengal agar medium and Czapek-Dox agar medium has shown the presence of the following species of fungi: *Cunninghamella echinulata*, *Penicillium* spp., *Fusarium moniliforme*, *Aspergillus awamori*, *A. flavus*, *A. nidulans*, *A. clavatus* and *Rhizopus stolonifer*. These fungi were not reported earlier from the termite-gut. Six mesophilic aerobic bacteria, degrading cellulose were screened from live mound soils (*Odontotermes obesus*) located in semi-arid areas. The cultural and physiological characteristics of two purified forms (*Cellulomonas* sp.) were studied by Paul, Sarkar and Varma (1985). Ultrastructural studies of the termite (*Odontotermes obesus*) gut microflora and its cellulolytic properties were worked out by Paul, Saxena and Varma (1993).
The heterotrophic microbial activity of *Odontotermes obesus* gut and mound soil of the semiarid zone of Delhi, India was examined by implying enrichments technique. The cellulose degraders along with the total bacterial population of the mounds were lowest in summer months, but no relative decline of cellulose degraders was observed in comparison to the total population. The feeding habit of *Odontotermes* was associated with the gut inhabitants *Staphylococcus*, *Micrococcus luteus*, *M. roscus* and with soil inhabitants *Bacillus* "thermoalcaliphilus" and *Cellulomonas* sp. *M. luteus* and *M. roscus* degrade various types of cellulose by producing endogenous and exogenous cellulase *in vitro*. As cellulosic detritus to bacterial biomass can be expected to constitute a significant flow of carbon and energy from plant to bacteria and therefore to animals in these ecosystems (Sarkar, Varma and Sarkar, 1988).

Two carboxylesterases (TE-I and TE-II) from the mid-gut of the termite *Odontotermes horni*. W., were purified by apparent homogeneity by means of ammonium sulfate fractionation (Sreerma and Veerabhadrappa, 1991). Later in 1993, they isolated, identified studied properties of carboxylesterases of the termite gut-associated fungus, *Xylaria nigripes*. K., from the host termite, *Odontotermes horni*. W., mid-gut carboxylesterases (Sreerama and Veerabhadrappa, 1993).

*In vitro* studies of cellulose digesting properties of *Staphylococcus saprophyticus* isolated from termite gut were established by Paul, Sarker and Varma (1986). *Staphylococcus saprophyticus* inhabiting the gut of *Odontotermes obesus* is a potential cellulose depolymerizer. The cellulase activity (both C\textsubscript{x} and C\textsubscript{r}) was extracellular and was mainly located in the culture supernatant. As the culture ages, the cellulose is concentrations of yeast extract and the CMC in the incubation medium were 0.6% and 1.5%, respectively. The pH and temperature optima for depolymerization of cellulose were 6.6 and 45°C respectively.

**VIII. Genus Termitomyces And Its Taxonomy**

Termite grown fungi, *Termitomyces* sp. are of special interest in this work, which are abundant in Asia and Afica continent. Taxonomic study of termites
on growing fungi has been done by Nit and Yupa (1986). Three species of *Termitomyces* were collected at the early of the rainy season from Animal reserve area at Pukhiew in North-Eastern province of Thailand. One of them was found to be new species. All description reported here were based on the observations on fresh material and anatomical descriptions based on free hand vertical sections mounted directly on Melzer’s Iodine. Colour terminology used was according to the Federal Standard Colors (NO 595a) U.S.A. Description of the species Pileus: 3-7 cm. broad, surface dry, waxy, orange (32169, P11); Stipe: 8-11 cm. length, 1-1.5 cm. width, tapered from apex to base, no ring and valva, surface with scale (17855, P30) solid; Gills: white (27778, P30), crowded, free, margin smooth, central; Spore: white (27886, P30), elliptical 7.5x13 micro, non-amyloid. Habit, Habitat and Distribution: Scattered around termine hill at Dry dipterocarp forest in August. Growth at temperature 28 deg. celsius and humidity 85 %; Tissue culture: Grown in Potato Dextrose Agar, Semisynthetic Agar, Malt yeast Agar and Malt Agar. The mycelia grow in dark better than under light. Common name: Walking Deer Termite mushroom.

Life cycle, morphological, physiological and cytological studies of *Termitomyces* sp. in Thailand was done by Yongyuth et al.(1978).

Wild edible mushrooms belonging to species of genus *Termitomyces* (Heim.) have been reported from many different habitats of the world: Malaysia (Vanhaeche and Abdul,1990); Japan (Otani,1979); China (Yang et al.); South Africa (Westhuizen and Eicker,1990).

**IX. Mycorrhiza And Termite**

In 1979, L. Garling (in Biotropica journal of USA) has had hypothesised that termite - fungus symbiosis originated with the establishment of Mycorrhiza. But no evidence in this regard has been produced by any one so far. Harinikumar and Bagyaraj (1994) have reported that earthworms, ants, millipeds, and termites disseminate vesicular-arbuscular mycorrhizal fungi in soil.

Studies of ectomycorrhiza (and endomycorryza) can contribute to elucidation of fungal relationships (Agerer et al., 1990; Agerer, 1990a). Ectomycorrhiza stuctures consist of fungal tissues, the arrangement and organization of which
can be used to describe fungal species in the same way as any other taxonomically suitable features. Their characteristics are well conserved (Agerer et al., 1990). It has been shown that there can be species-specific differences in ability to colonize roots depending upon age of individual trees or stands (e.g. Fleming, 1983). Furthermore, it has been shown that some ectomycorrhizal fungi can grow their hyphae within the rhizomorphs and ectomycorrhiza of other fungi (Agerer, 1990a, 1991a), suggesting that these ectomycorrhizal fungi can influence each other with respect to plant nutrition and with respect to their fruit bodies formation.

Following methods were described by Agerer et al. (1990) for preparation of ectomycorrhiza:

**Fresh ectomycorrhiza**
* Very fresh material is suitable for cryotome sections in demineralized water. Such sections are free of cytoplasmic contents and give brilliant picture of mantle structures.
* Cut preferably straight or only slightly curved ectomycorrhiza under a dissecting microscope into pieces c.3-4 mm long, include tips.
* These are placed in small flasks which contained demineralized water.

**Ectomycorrhiza fixed in FEA**
* Fix ectomycorrhiza in FEA at least for 12h, using only fresh and turgescent material.
* Cut preferably straight or only slightly curved ectomycorrhiza under a dissecting microscope into pieces c.3-4 mm long, include tips.
* Place these pieces into small flasks which contained 2% glycerin water solution.
* Leave for 2-3 hrs., shaking gently several times.

**Sections of ectomycorrhiza:**
**Preparation of the cryotome**
* Use "type C" knives.
* Choose the suitable temperature (-25°C for 2% glycerin water solution and -30°C for fresh ectomycorrhiza water). A test section should be made because ectomycorrhiza behave differently.
* Pour 2-3 drops of glycerin and water solution (demineralized water for
fresh ectomycorrhiza) on the section table to the cryotome; the amount is dependent on the dimensions of the ectomycorrhiza.
* Orientate the ectomycorrhiza in the freezing drop (horizontally for longitudinal sections, vertically for cross-sections).
* Allow the drop to freeze completely.
* Choose the thickness of the sections.
* Elevate the table until the knife touched the frozen drop.
* Pour 1-2 drops of mounting medium (lactic acid or cotton blue in lactic acid) on a microscope slide.
* Make the sections and observe the procedure through a dissecting microscope.
* Remove the section with a fine paint brush (which has been previously dipped in 2% glycerin water solution) from the knife and put them immediately in the drop of mounting medium (five sections in one drop).
* Orientate the sections with a needle under a dissecting microscope.
* Cover with a cover slip and soak away the surplus medium with a piece of filter paper.
* Seal the preparation with Entellan (use a fume hood for health reasons).
* Allow the preparation to dry overnight.

**Microtomy of embedded ectomycorrhiza:**

**Fixation**
* The ectomycorrhiza should be fixed for at least 12h in FEA; only fresh and turgescent material to be was used.
* Cut straight or only slightly bent ectomycorrhiza under a dissecting microscope into pieces 3-4 mm long. Include the tips of the ectomycorrhiza.

**Dehydration and infiltration**
* Four steps of ethanol dehydration. 20%, 40%, 70% and 80%, each of 30 min. are required.
* The infiltration solution to be prepared by mixing 50 ml basic resin with 1 bag of activator.
* Infiltration should be performed in two steps; (a) ethanol 96%: infiltration solution = 1:1 (at least 6h); and (b) pure infiltration solution (at least 24h).

**Embedding and polymerization**
* Mix the embedding medium (15 ml infiltration solution) and add 1 ml (=20 drops) of the hardener; mix thoroughly.
* Fill 0.06 ml embedding medium in the lower part of the histo-moulds with an automatic pipette.
* Transfer the ectomycorrhiza into small dishes which contain some infiltration solution.
* Take the ectomycorrhiza with fine forceps, soak away the surplus infiltration solution with a piece of filter paper and keep the ectomycorrhiza singly into the holes of the histo-moulds.
* Enough time is now available (20-30 min) to orientate the ectomycorrhiza under a dissecting microscope, horizontally for longitudinal sections, or with the tip downwards for cross-sections: as the viscosity of the embedding medium became greater with time; after 20-30 min the ectomycorrhiza does not moved any more.
* Allow the medium to polymerize at room temperature (overnight).
* Make new embedding solution.
* Lay the holders in the holes of the histo-moulds.
* Fill 1 ml of the newly mixed embedding medium with an automatic pipette through the holes of the holders.
* Allow the medium to polymerize at room temperature (at least 12h).
* Take out the holders with the embedded ectomycorrhiza.

**Sectioning**
* Give cut to the historesin block into a pyramid shape.
* Take a hard metal knife (d-grinding) and fix the block in the microtome holder.
* Add a large volume of demineralized water to pre-cleaned microscope slides.
* Removed the sections with a fine needle from the knife and keep them serially arranged into the water.
* Allow the water to dry and the sections adhered onto the microscope slides in a warm place (60°C, overnight).
* Add some drops of Entellan (used a fume hood for health reasons) to the microscope slide and lay a cover slip carefully, slowly and at first obliquely on the drops.
* Allow the preparation to dry at least overnight;
Recipes for some of the more important reagents used in mycorrhizal studies

* Cotton blue in lactic acid (Erb and Matheis, 1983); solution of 0.05 g cotton blue in 30 ml lactic acid 85-90%.
* Ethanol: 70%.
* FEA (=FAA): formaldehyde: ethanol 70% : acetic acid= 5:90:5.
* FeSO₄ (Meixner, 1975): solution of 1 g Iron(II)-sulfate in 10 ml distilled water, finally some drops of conc. H₂SO₄ should be added.
* Guaiacol (Erb and Matheis, 1983): solution of 1 g Guaiac resin in 6 ml ethanol 70%, solution is stable only for c. 1 year.
* KOH: 15% aqueous solution (w/v).
* Lactic acid: 85%.
* Melzer's reagent (Moser, 1978): solution of 0.5 g iodine, 1.5 g KI, 20 ml distilled water and 20 ml chloral hydrate.
* NH₄OH (Singer, 1986): concentrated solution.
* Pyrogallol (Singer, 1986): concentrated solution.
* Sudan III: dissolved in a water bath 1 g Sudan III in 500 ml ethanol 96% and added finally 500 ml glycerin.
* Sulfovanillin: for this reagent a few crystals of vanillin are placed close to the cover slip on one side of the preparation. After addition of one drop of conc. H₂SO₄ a piece of filter paper is laid on the other side of the cover slip. Vanillin is dissolved in the acid and the fresh reagent is drawn through the preparation.
* Toluidine blue in water (Ingleby et al., 1990): (w/v) aqueous solution.

Staining of nuclei and siderophilous granules as described by Agerer (1990):

Preparation of the ectomycorrhiza
Both fresh ectomycorrhiza and specimens fixed in FEA can be used.

Mordanting procedure
For the staining procedure c. 1/3 ml of KE is placed in small pyrex tubes of 1-3 ml capacity (Eppendorf tubes).
* Retrieve the ectomycorrhiza from the mordanting solution, remove the surplus FBV with a piece of filter paper and keep them into the KE in the Eppendorf tubes; these are kept closed, their caps should have a small
hole to prevent bursting.

* They should then be boiled for c. 5 min.

**Preparation of microscope slides**

* Pour the contents of the Eppendorf tubes onto a filter paper; remove the ectomycorrhiza with a fine needle from the filter paper or from the wall of the Eppendorf tube.

* Transfer the ectomycorrhiza directly into a drop of Hoyer's Mounting Medium (HMM) lying on a microscope slide. Peel off mantle fragments and rhizomorphs under a dissecting microscope and cover with a cover slip.

* If the mantle fragments are stained too intensely, the ectomycorrhiza should be first transferred for partial discoloration into acetic acid 50% or chloral hydrate for a short period.

* The HMM dried and the slides become permanent.

**Composition Of Various Solutions**

**Mordanting solution (FBV):**

* The following chemicals are mixed: 5 ml ferric chloride (a 10% solution in acetic acid 50%). 5 ml copper acetate (a 10% solution in acetic acid 50%). 5 ml picric acid (saturated in distilled water). 5 ml formaldehyde (saturated in distilled water).

* 1 ml lead acetate (1% solution in acetic acid 50%) are added drop by drop while stirring constantly.

* The final solution is stable for years.

**Aceto-carmine(KE):**

* Boil a few grams of carmine under reflux with 200-300 ml of acetic acid 50% for 2-3 h.

* Filter next day and the solution is ready for use.

* The solution is stable for several years.

**Hoyer's Mounting Medium (HMM):**

* Chemicals: 15g gum Arabic or gum guaiacol, 100g chloral hydrate, 10g glycerin, 25 ml water.

* Add to the water a crystal of chloral hydrate the size of a small pea to prevent bacterial growth upon the gum.

* Soak the gum in the water for about 24 h.
* Add the rest of the 100 g chloral hydrate and allow the solution stand until all material is dissolved; this takes several days.
* Add the glycerin.
* The solution is now ready for use and is stable for several years.

**X. Genus *Termitomyces* In India**

In the Indian-subcontinent Purkayastha and Chandra (1975) reported termite grown fungi *Termitomyces eurhizus* (Heim.).

In the same year Batra published his work on eating habit and manipulation of *Termitomyces* sp. by the termite *Odontotermes* sp. Later in the year 1977 he hypothesised mutualistic relationship between *Termitomyces* sp. and *Odontotermes* sp.

Chakravarty and Khatua (1979) reported *Termitomyces microcarpus* as new edible mushroom of India.

Edible mushrooms of West Bengal *Termitomyces* Heim was reported by Aich et al. (1977).

*Termitomyces* sp. from various part of India have been reported by different authors as mentioned in previous pages but none have been reported from the region of Bundelkhand.

**XI. Nutritive Values Of Commercial Mushroom And *Termitomyces* Species**

Edible mushrooms have low carbohydrate and fat content. The protein content is on average, higher than that of other vegetables. The digestibility and quality of the receptacles depend greatly on the type of mushroom and the cultivation technology. The major value of edible mushrooms in developing countries is that they can be produced, without requiring additional land, on plant remains which are indigestible for animals (Zadrazil, 1988).

Protein nutritional quality of different species of wild edible mushrooms in Thailand was investigated. The test mushrooms were *Boletus* sp., *Geaster*
sp., Pleurotus sp., Russula delica Fr., Termitomyces sp., and Tricholoma crassum. Almost all of fresh mushrooms contained 2-3% protein, but one species of Termitomyces contained rather high protein content of 6.27% determined by Kjeldahl Method. The moisture content of fresh mushrooms was about 80-90%, except Pleurotus sp. contained only 62.9% moisture. The amino acid compositions of mushroom proteins were monitored by using amino acids in various concentrations. The calculated amino acid scores showed that almost all of mushrooms tested were phenylalanine and tyrosine rich. Some mushrooms contained protein having amino acid scores of threonine and tryptophan over 100. Amino acid scores of lysine, methionine and cysteine indicated that proteins of some mushrooms were limited in those amino acids. However, these wild edible mushrooms seemed to be good sources of amino acid phenylalanine, tyrosine, tryptophan and threonine. Amino acids such as isoleucine, leucine and valine were also found rather high content in most mushrooms. The results suggested that eating various kinds of mushrooms in each meal might help receiving more nutritional quality of mushroom proteins (Sunanta et al., 1985).

Biochemical analysis, specific activities and quantitative assay revealed presence of various enzymes in the edible mushroom Termitomyces microcarpus (Berkley et Broom) Heim. (Skelton and Matanganyidze, 1981).

Edible mushrooms [Agaricus, Coprinus, Laccaria, Craterellus, Clitocybe, Boletus, Marasmius, Tricholoma, Psatyrella, Cantharellus, Hydnum, Pleurotus, Lycoperdon, Lepiota], possess certain trace elements and heavy metals like mercury, cadmium, lead, copper, zinc, nickel, selenium, arsenic (Andersen et al. 1982).

Amino acids and trace minerals of three edible wild mushrooms from Nigeria have also been studied (Alofe, 1991; Adewusi et al., 1993). Six samples of three wild edible mushrooms collected during the rainy season and identified as Termitomyces robustus, Tricholoma lobayensis, and Volvariella esculenta were studied. The pileus (cap) and stipe (stalk) of the button stage and incomplete open cap of each were separately assayed for amino acid and trace mineral (chromium, cobalt, nickel, and zinc) content. The mushroom species had similar distribution of amino acids. They all contained all the essential amino acids (tryptophan was not determined) in varying amounts. Variations in amino acid content were found to be related to the stage of development, type,
and part of mushroom. Trace mineral content varied by species and parts rather than by the stage of development. Glycine, glutamate, alanine, and aspartate were the most abundant amino acids in all the mushrooms. Cystine-cystein and methionine were the most limiting of the sulfur-containing amino acids. Zinc was found to be more than five times more abundant than any of the three other minerals. The results were presented for fresh weight only.