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CHARACTERISATION OF EDIBLE MUSHROOM COLLECTED FROM NATURAL HABITATS IN BUNDELKHAND

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CERTIFICATE

The research work embodied in this thesis entitiled "**Characterisation of Edible Mushroom Collected from Natural Habitats in Bundelkhand**" has been carried out in the School of Life Sciences, Jawaharlal Nehru University, New Delhi. This work is original and has not been submitted so far, in part or in full, for the award of any other degree or diploma of any University.

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1. INTRODUCTION

Indian sub-continent is very rich in diversity of habitat, culture as well as ethnic communities. There are colourful tribes like Naga, Mizo, Khasi etc. in north east; Oraon, Santhal, Bhil etc. in central India; and Kadar, Irula, Chenchu in the south. They number more than 55 million, and their vocation range from hunting-gathering, nomadism to societies using most modern techniques of agricultural production. Due to expansion of means of communication and socio-political processes, isolation of tribal areas has broken down.Some of the tribes are getting absorbed into modern society as a result of cultural contact. Industrialization has opened up new avenues of earning livelyhood, thereby abandoning the customary vacations.

All the modernising forces that have made their inroads at present to pose an imminent danger of loosing this rich varied knowledge of ages of the past and it is most likely to be lost to the humanity for all times to come due to changes in paradigmic perceptions.

The habitat and environment, where the isolated simple societies lived and used their folklore about plants and animals to preserve nature, is fast disappearing on account of exegenous interventions like construction of big water reservoirs, opening of mines, unscientific and persistent exploitation of plant wealth to the extent of converting some of them as 'endangered', therefore ethnobotanical records are indespensable to determine the status of taxa, and also to bring out the extent of depletion of plant resources. This in turn can help in formulating policies and measures for the conservation of environmental resources and to develop and improve the quality of the life of the tribals. We cannot check the modernisation drive but we have to ensure their smooth transition according to the pace agreed upon by the communities themselves from one cultural pattern to another in this conflict between tradition and modernity. Only then the tribals can be saved from disaster.

In many tribal regions, tribals have changed their food and other societal habits resulting in adverse impact on health that has been glaring. This impact is also reflected in declining trend of the demographic profile. At present

these people are deprived of their food choices due to depletion of the forest resources and destruction of forest areas or, may be, due to deliberate choice on their part on account of the civilizing effect. These tribals suffer from malnutrition and are susceptical to diseases. Influences that they were not suseptible in the past; their survival till now underlines this.

Recently chemical analysis of some of these conventional wild edible foods has been done and most of them were found to be exceptionally rich in good quality protein, vitamins and minerals. Such wild edible plants known in the past as "famine food" for the urbans offer good opportunity to develop them as an alternative source of food. Moreover, their yield will have to be improved through selection programmes and these have to be popularised among the people of all types who are prone to malnutrition and cultural prejuidices.

Among the wild edible foods, edible fungi occupy an important place in food habits of various people of the world and of India. Many edible fungi are found growing wild in nature. Some of these have also brought under extensive cultivation for large scale consumption. At present many fungi are being cultivated in about 100 countries with an annual production of 3.763 million MT. Production of edible fungi is concentrated mainly in three geographical regions: about 55% in Europe, 27% in North America and 14% in East Asia. Five genera, namely *Agaricus, Lentinus, Volvariella, Pleurotus* and *Auricularia* have contributed about 88% of total world production of edible fungi during 1989-90. Of the total edible fungi cultivation, about 85% is consumed by G-6 countries. Rest of the world consumes the remaining balance of 15%. Average per capita consumption of edible fungi in major consuming countries during 1990 varied from 2.42 kg to 7.06 kg; maximum being in Germany followed by Netherlands, Canada, France, U.K., Sweden, USA, and Italy in this order. Fresh edible fungi are maximum consumed in Netherlands and preserved ones in Germany. Of the commercially cultivated fungi, average per capita consumption in India is less than 20 gms.

In India, many edible fungi are being cultivated commercially and some of the Indian edible fungi are collected from their natural habitats for consumption by local people and some are dried and exported e.g. Morels. However, many fleshy and edible fungi of India, those that dry with difficulty

and are nutritionally rich, remain to be exploited e.g. *Termitomyces* sp. and *Truffle* sp.

Edible fungi, better known as mushrooms, are good source of high quality proteins and are rich in vitamins and minerals. Mushrooms contain 20-35% protein on dry weight basis. This amount is higher than that is present in vegetables and fruits; it is also of high quality. There are twenty amino acids found in different plants, of which ten are essential for human growth to be derived from variety of food items. It may be noted that out of these ten essential amino acids nine are found in mushrooms alone! Besides this, lysine and tryptophan, the two essential amino acids that are absent in cereals are abandant in mushrooms. Such quality of mushroom is best suited for faster and better child growth. Mushrooms contain good amount of vitamin C and vitamins of B complex group (thiamine, riboflavin and niacin). They are rich in Potassium (K), Phosphorus (P) and Sodium (Na) and contain low but available form of Iron. Potassium to Sodium ratio (K:Na) is very high in mushrooms which is desirable for patients of hypertension. Mushroom is low calorie food with very little fat and has no starch; it is very low in sugars. Cholesterol is absent and ergosterol is present in mushrooms.

Like Angiosperms many edible fungi too have certain medicinal properties and have been traditionally used in Chinese and Japanese traditional medical systems for their tonic properties. In recent years there has been an upsurge and revival of this aspect of mushroom food all over the World and cosmetic products and tonic beverages have also been produced in China from *Ganoderma* mushrooms. Pharmaceuticals worth $ 700 million are produced annually in Japan alone from *Lentinus*, *Coriolus, Schizophyllum* and *Ganoderma.* Anti-tumor effects of certain mushrooms have also been reported. In this context it is the extracts of various edible fungi like *Lentinus edodes, Flammulina velutipes, Pleurotus ostreatus, Agaricus bisporus, Pholiota nameko, Tricholoma matsutake* and *Auricularia auricula* that have been tested.

Other than the medicinal properties, edible fungi as supplimentary food shall play important role in next century when total cereal production in the world would be insufficient to feed increasing population.

It is important for the future of mushroom industry to develop superior

commercial strains from wild type diversity that has arisen out of long evolution through mutation, recombination and natural selection. There is a world wide threat to mushrooms germplasm extinction due to destruction of natural habitats and material as companies want to use present but not invest in future for their profits.

Indian mushroom industry is totally dependant on exotic varities of mushrooms. Button mushroom (*Agaricus bisporus*) is native to Europe where it grows wild and is collected by the people during season. Using modern growing techniques it is being widely cultivated by the western countries. But in the era of patenting of bioresources under world trade organisation (WTO) and Trade Related Intellectual Property Rights (TRIPs) it may not be economical for other than European countries to grow this mushroom at large scale due to massive royalty imposed by the particular country to have global monopoly over the mushroom trade.

Thus, it becomes esential to find some new varities of mushroom that are found wild in India and develop growing technique so that such varities could be grown in Indian conditions without expensive infrastructure to achieve climatic conditions like of Europe (say 16-18 degree celcius fruiting temperature) costly within controlled conditions. It would be economical enough if mushrooms that grow in rainy season in India are grown inside the growing houses using simple equipments like room coolers alone maintaining room temperature 25-27 degree celcius keeping high humidity and carbon dioxide level equal to that of atmosphere. If this is achieved then expensive chiller machines and carbon dioxide metres could be done away with and cost of production could be substantially reduced. Moreover, product patented shall be of additional advantage for being compititive in the world market.

It may be noted here that all domesticated mushrooms in the world at one time or the other have been collected from the wild and were invariably selected over the years for their superior quality. In India also, a rich reserve of edible wild fungi is available.

Present work is related to such a small effort primarly based on ethno-mycological studies in the field and later in the laboratory to achive above mentioned objective i.e. domestication of ethno-mycological food.

2. LITERATURE REVIEW

I. Ethnobotanical Studies In Edible Fungi

Probably the only work on ethno-myco-medicine 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 man-plant-culture relationships, ethnic groups 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 into detail of the traditional practices of the tribals of Bundelkhand.

Ethnometereology is deeply linked with the minds of the tribals looking for edible fungi in the jungles. So far work on the Ethnometereology 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 taxanomy of *Agaricaceae* as a whole to locate the position, characterstics 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, Lactarieae* 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 photo-graphs.
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 *Octojuga*).

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 poi-sonous 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) attemted 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 indicted 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 synthatiic 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 *Agaricalaes* 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 iin 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 choosen 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 them 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 upto 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 nicrome-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 rapdily 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 Bebbington 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 speciemen. 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 speciemens 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. *Termytomyces* 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 speciemens 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.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 Goan 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* (baloon 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 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).

Himachal Pradesh and Jammu & Kashmir states are rich in fungal diversity. There are reports on different genera of edible fungi by different authers but any indepth and detailed studies on *Termitomyces* sp and related folklore is lacking. Sagar and Lakhanpal (1989, 1989a, 1990, 1991, 1993) have extensively reported on fungi of family Boletaceae from N.W. Himalayas. Lakhanpal (1994) and Lakhanpal et al. (1986) have reported on Boletes and other fleshy fungi from N.W. Himalayas, in general. Abraham and Kaul (1980,1981,1985,1988) have reported large and fleshy fungi specifically from Kashmir. Kaul and Kachroo(1974) also gave general account of common edible mushroom of Jammu and Kashmir. Fleshy fungi of Himachal Pradesh has been explored by Sharma and Jandaik (1977,1978a), Sharma and Munjal (1977)and Sharma et al (1986). Heinemann (1968) reported some of the Agaricus from coniferous Himalayas. In fact, the very first attempt to illustrate Indian fungi specially from Himalayas was made by Hennings way back in year 1901.

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 Mussourrie. 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 upto some extent. The most commonly available mushrooms in the area include *Termitomyces* sp. (Kumar, Shukla and Agrawal, 1991). These mushroom, 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.

North-Eastern Hills offer much condusive conditions for growth of numerous flora in the region including many edible fungi (Verma et al.,1987). They reported that many edible fungi are found in the region growing on the forest-floor either in mycorrhizal association with higher plants or on decaying woods and other plants debris, white-ant's nest, plant and animal residues etc. They reported differnt species of *Termitomyces* viz. *Termitomyces clypeatus* (Heim), *Termitomyces eurrhijus* (Berk.), *Termitomyces microcarpus* (Berk. & Br.) and *Termitomyces robustus* (Beeli) Heim. The information was nicely supplimented with the ethnic information.

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.

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

An edible fungi *Calocybe indica* from Rajasthan has been illustrated in detail by Doshi and her associates (Doshi, 1993; Doshi et al.,1988,1989, 1993, 1994.) They reported improved cultivation techniques with increased biological efficiency. They also simplified the technique to suit the growers. Other than *Calocybe indica* they also studied other edible fungi.

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.

Rajgopal, Rao and Varma (1981) investigated the association of several species of fungi in the worker termite gut *Odontotermes obesus* (Rambur) from nothern 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 reported by Paul, Saxena and Varma (1993).

The heterotrophic microbial activity of *Odontotermes obesus* gut and mound soil of the semiarid zone of Delhi, was examined by employing 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 *Odentotermes 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, *Odentotermes 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 deploymerizer. The cellulase activity (both C_x and C_1) 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.

VII. Termites And Termite-Related Fungi 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 on 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).

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 heimi*, *Microcerotermes championi*, *Odontotermes obesus* and *Heterotermes heimi.*

The termite *Odontotermes horni*.W. belongs to the family Termitidae and order Isoptera. It is a subter-ranean termite that 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 soldiers in 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 transvaalensispy* (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 that *Macrotermes gilvus* (Hagen), *Macrotermes malaccensis* (Haviland) and *Odontotermes 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 fulyiginosus Heim*), respectively. Key to genera and species of fungus growing termites has been made by the authers with detail of termite mound ecology. They have also studied chemical composition of the fungus and the fungi on the combs.

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 X 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.

VIII. Genus *Termitomyces* And Its Taxonomy

Termite grown fungi, *Termitomyces* sp. are of special interest in this work, which are abandant in Asia and Afica continent. Taxonomic study of fungus growing termites has been done by Nit and Yupa (1986). Three species of *Termitomyces* were collected at the early rainy season from Animal reserve at Pukhiew in North-Eastern province of Thailand. One of them was found to be a new species. All descriptions reported here were based on the observations on fresh material and anatomical descriptions were based on free hand vertical sections mounted directly in 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 had hypothesised that termite - fungus symbiosis originated with the establishment of Mycorriza. 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 fungl tissues, the arrangement and organization of which can be used to described fungl species in the same way as any other taxonomically suitable features. their charecteristics are well conserved (Agerer et al., 1990). It has been shown that there can be species-specific differences in ability to colonise 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 their fruit bodies formation.

X. 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% with the exception of that *Pleurotus* sp. that 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 in 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 part of mushroom. Trace mineral content varied from species to species and mong various 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.

3. BUNDELKHAND :

THE LAND AND IT'S TRIBAL PEOPLE

I. The Land

Bundelkhand is the area of our study of wild mushrooms. Bundelkhand is composed of six districts of southern Uttar Pradesh adjoining the border of northern Madhya Pradesh into which one district of the region falls. Districts comprising Bundelkhand are: 1. Jhansi, 2. Banda, 3. Jalaun, 4. Hamirpur and 5. Lalitpur. Recently a new district, named Shambhuji Shivaji Maharaj, has been carved out from district Banda. (see Fig.I)

The land is not fertile. Most of the land is barren and is classified as nutrient deficient and fallow apart from being rocky in nature according to standard classification of soils. Though good number of tributaries of Yamuna river viz. Ken, Chambal, Payaswani and Bagain traverse through the region there is no proper irrigation system developed so far. Thus, entire region gives a deserted look and agriculture is totally Monsoon dependent; in other words- a single Monsoon failure and the region is famished and scarsity bound inspite of all measures taken in this respect.This dependency on the one hand makes people rough and tough in their culture (Bundelkhand has always been a region of traditional fights amongst clans as immortalised by the legedary Rani of Jhansi during the battles of 1857 and yet earlier by the epic *Alha-Udal*) and on the other nature dependent in the diversifiication of their food resources.

The farthest stretch of southern most part is bordered by the 'tail' of Vindhyan range of mountains. Following is the agro-climatic data of the region:

Average Temp.(Deg.C)		**Rainfall (mm.)**		**Relative Humidity(%)**	
Min.	Max.	Min.	Max.	Min.	Max.
8	48	820	1043	26	96

Kol and *Bhil* are native to forest on the borders of U.P. and M.P. Today, inspite of the fact that they have been living in the area since time immemorial and have been refered to in the *Puranas* and have mentioned by such travellors as Al-Beruni, many of these people have migrated to other areas in search of job. Those who remain earn their livelihood by breaking the stones of mountain for road contractors and selling forest produce collected during the day in the afternoon and in the evening to the people in the open market. They are hired by local businessmen for stone breaking. They are also hired for collection of *Tendu* leaves from forest used for making *Bidis* by contractors who exploit them fiercely.

III. Historical and cultural importance of the area

Historical and cultural record of Bundelkhand is very impressive. Districts of Mahoba and Jhansi are known for producing great warriors and kings. Chandela kings were ruling in Mahoba and Tikamgarh. Chandelas built famous Khajuraho temples. Alha and Udal were two great tribal warriors under Chandelas. Tribal kings were ruling in other near by regions. In district Banda remnants of many forts in the forests and on the hills speak volumes of unrecorded history of the area. In fact, there are numerous small and big forts found in the entire Bundelkhand, indicating that there were many territories of local tribal kings who were often engaged in fighting with each other for power and wealth. The fort of Kalinger is known as an invincible fort. Jhansi is well known in the history. Lalitpur and Hamirpur are only two districts of Bundelkhand which are of little importance.

District Banda was extensively traveled and many interesting observations were made by us. River Ken of Banda Produces a stone called Sarjar. This stone when cut shows beautiful scenery like mountain, forest, river etc. These stone are exported as gems. Forty kilometer from Banda is a place called Gonda where remnants of a fort like structure and a temple are present. The temple is made of glossy and smooth red stones. There are many Khajuraho like stone idols scattered in this area and also some interesting pictures and a map in side the temple. It is said that there is huge wealth inside or under these remnants. The Department of Archeology and Ancient Indian History of BHU

at Varanasi has had sent its staff and gold detecting machines twice to the area to unearth the wealth but to avail. From a distance, the machines indicated the presence of gold in certain direction but when they reached near the remnants, the machines failed in sending the signals. The archeological staff also did some excavations on suspected area but could not make any breakthrough. Two security guards are still posted in the area of the remnants. Near Gonda there are two places called Rasin and Marfa. In Rasin there is a fort on a hill, probably of a tribal king. Here also are found old idols and caves. In Marfa too there is a huge fort on the top of a mountain. There is a huge statue of Lord Shiva and many idols carved on the stone plates in the entire fort of Marfa. It is said in folklore that this fort and the fort of Kalinger were built overnight !

Kalinger is 34 kilometers west to village Badausa (district Banda). It is a historical fort and important place of Lord Shiva, mentioned in Puranas. Out side the Kalinger fort there is found a soft stone which is said to be oozing stone. If a part of this stone is cut off, it (the rock) regains its original shape after some time ! There is another evidence of soft stone in a place called Bharatkoop 45 kilometer in the east of Kalinger. It is said that younger brother of Lord Rama, Bharat, came here to convince his brother to woo him to cancel his exile and accept the throne of Ayodhya. Both brothers met each other and the scene was so emotional and painful that even the stones were melted. The stone under the feet of Rama and Bharat was melted and their foot print were marked on it which are still Present.

Yet another evidence of soft rock is available, 20 km away from Bharatkoop, called Janaki Kund where foot prints of Sita, wife of Lord Rama, are present. A fourth evidence of soft stone is at Sphatic Shila (stones like a gem Sphatic), where two pairs of foot prints of Rama & Sita are marked on a stone.

In fact, Bharatkoop and Janaki Kund are part of famous and holy place Chitrakut where Lord Rama stayed for twelve years of his *Vanvas* (exile). Once upon a time, Chitrakut was very rich in fauna and flora. Even today, many herbal drugs are procured from the mountains of Chitrakut. Ambani group is establishing a big center for research on traditional medicines found in these area. This area has been center of penance for many Saints and Sages viz. Atri,

Sarbhang, Satikshan, Maandav, Balmiki, Sati Ansuya etc. Chitrakut is 30 km. in the east of district Banda. There is a place called Rajapur, 42 km. in the east of Chitrakut where great Hindi epic *Ramayana* was composed by Goswami Tulsidas who was native to this place. Rajapur is situated on the bank of river Yamuna. A tributary of Yamuna, called river Payaswani originates from mountains of Chitrakut near a place known as Sati Ansuya. At 10 km. south-east of Sati Ansuya is a famous place known as Gupt Godavari. There are two caves on this site. From one of the caves, originates a river (Godavari) which disappears under the Peepal tree (*Ficus religiosa*). There are beautiful and extremely smooth carvings on the side wall of the cave.These carvings are throne like on which people can sit. It is said that these were built for Lord Rama by tribals of Chitrakut. Seven kilometers in the east of Chitrakut there is a old palace as a picnic spot. The palace earlier belonged to king Peshva (19th century) who created this palace for his enjoyment. There are only remnants of the palace. On the top of the palace is a tomb on which nude idols of male and female have been carved on the stone. This is known as mini Khajuraho. Khajuraho of district Tikamgarh is very well known in history. And there is noting left of the original people of Jhansi that need to be mentioned.

4. MATERIALS AND METHODS

It was planned to study different parameters, along with diiferent folklore associated with wild edible fungi, viz. span of life, shelf life, fresh and dry biomass, germination rate, sporulation rate, fruit body characteristics, fresh weight, dry weight etc., culture the wild edible mushroom and then try to cultivate the edible fungi under different climatic conditions viz. range of temperature, both high and low; humidity requirement; nutritional requirements etc. But due to certain constrains involved in it the work was mainly confined to tissue culturing the fungi in the lab and field studies attempting to verify various folklore claims.

Field Visits: Ethnobiological Practices in Bundelkhand

The area is rich in ethnobiological folklores. There are various sayings in the area that need scientific interpretation. First hand Information from the tribals and local people was mostly collected using battery operated pocket sized microcassette recorder, Sony, Japan and on spot photographs were taken with the help of automatic Kodak Croma camera, Japan.

There is a prevailing folklore in the area that the Holy Basil (*Ocimum sanctum*) should not be raised by replanting after uprooting a younger herb; rather the herb should be raised from seeds. It is said that if the herb grows from its seeds, the herb is rich in *Gunas* (qualities) whereas the herb is poor in qualities if propagated by replantation. It seems logical if we compare three ways of plant propagation: A plant will have more vigor if grows from seed(s) due to new combinations of genes whereas plant raised by tissue culture or replantation would not be so. Also, if propagated many times through tissue culture or replantation it will loose its vigor slowly.

It has been observed that the holy basil raised by replantation possesses bigger leaves whereas those grown from seeds have smaller leaves. It is experienced by local people that smaller leaves of Holy Basil are more effective in curing common cold than the bigger leaves. A single leaf of holy

basil every day with Prasad is said to keep body healthy !

Holy Basil is worshiped by females every day. Local folklore asserts that the herb exerts its major effects on female health. It is quite likely that the aroma of the plant influences female body in a way different from its effect on males. The latest aroma therapy being practiced in USA and western countries probably supports this contention of the tribals.

Another plant widely used by local people is Henna (*Lawsonia enermis*). A paste of leaves of this plant is applied on palms and feet as motifs during the month of *Sravana* and during marriages. This is prevalent almost throughout the country. It is believed that this plant protects females' palm and feet from waterborne microbial infections as they very often bare foot. Another evidence for antiseptic property of Henna comes from use of Henna as blood purifier. Henna leaves if taken regularly along with leaves of Neem (*Azadirachta indica*) and Amla (*Embilica officinalis.*) is said to purify blood. This use of three plants in combination are known to tribals of this area. Another evidence of uses of plants in combination comes from black pepper. Black pepper (*Piper nigrum*) cures different diseases when used in combination with different plants. Black pepper is widely used with leaves of Holy basil to make hot liquor extract to cure viral infection of common cold. Black pepper along with leaves of a wikd plant Sihor, as known locally is given to a person bitten by a rabid dog. Thus black pepper along with this wild herb gives protection against rabies virus. Tribals of Bundelkhand bring leaves of an unknown plant from Marfa hill, ancient site of Saint Balmiki, and give to asthmatic patient along with black pepper. Many patients of asthma are said to have been cured with this recipe. Thus one plant cures different ailments when used in different combination. Ayurveda also illustrates this principle and it is known as *Anupaan Bhed.* Charak has written one full chapter on dosage in *Charak Samhita.* Two compounds when administered together in different quantity they give a particular response and if the same two compounds taken in equal dosages they give different response. For example, when milk and curd are consumed together, each in varying quantities, the person may suffer from abdominal disorders. But if milk and curd are mixed together in equal quantity and consumed in small dosage it cures minor ailments. This is the principle behind use of *Panchamrit* during *Puja-Katha* (rituals).

Most of the plants are used individually to cure certain diseases or wounds e.g. juice of a plant is used to turn head hairs black from brown color. They use two herbs in series to turn gray hairs black. Juice of leaf is used to cure *migraine*, type of headache in which case the juice is poured through one nostril of the side of migraine etc.

There are some treatments that do not involve uses of plant products. Tribals treat sciatica like pain of legs with simply tying a piece of hollow bone from phallenge of owl. My sister-in-law who complains sciatica like pain in her left leg, gets miraculous relief when the hollow bone piece of owl is tied to her leg near the ankle. The pain disappears with in seconds ! This bone she had purchased from a local tribal. The pain is so serious some times that she starts screaming but the moment her leg comes in contact with the bone, the pain disappears instantly.

Tribals of Bundelkhand know a termite resistant plant and the juice of the plant is used as anti-termite treatment to the soil and wood for construction of houses.Tribals and villagers conserve nature through plants and animal worship.

Folklores on Agriculture

Tribals and villagers of the area have pointed out that food grains and vegetables are losing their original taste and flavor due to shifting from green manure to chemical fertilizers. They feel that if this trend continues, a time will come when soil will lose all its strength and not even a single grain will germinate. Thus they feel that famine is eminent in India due to massive and careless uses of chemical fertilizers to increase yield to meet ever increasing demand for food grains. When yield of food grains increases by such magnitude, it is an indication of sudden fall in grain productity, claim tribals and villagers. Interestingly, it seems logical if we compare this folklore with J-shaped growth curve of a population (food grain in this case) and warnings of Food and Agricultural Organization on massive uses of chemical fertilizers by developing countries points in this direction.

Tribals and villagers of Bundelkhand supplement their diet with a wild fungus, locally known as *Garajain*, during rainy season. They collect this

edible wild fungus (mushroom) from termite mounds. They claim that this wild fungus increases virility of man and also has cancer curing property. Other medicinal uses claimed are curing various heart problems. It is felt that it's consumption causes clear motion thus may be used to cure irritable bowl syndrome (IBS). There are various myths and beliefs associated with this wild fungus, *Garajain.*

Myths Regarding Mushrooms Found In The Region

The most common Mushroom and probably the only edible fungi found in the area is the one that grows on termite mounds. Locally, it is known as *Garajain* i.e. one that grows due to thunder in the sky or *Badalon Ki Garaj*; hence the name *Garajain.* Following sayings about the *Garajain* fungi are widely current in the area :

* *Garajain* are long, creamy, fleshy structures with clubtype cap forming upper most end and are always obtained from "the house of termite" (termitarium) located under a termite mound;
* The color of the 'house of termites' is light brown with whitish impres sions;
* *Garajain* are found for 2 to 3 consecutive years during rainy season at one place
* Thundering (*Badalon Ki Garaj*) and lightning is essential for the appearence of *Garajain* above ground;
* *Garajain* germinate early in the morning and are collected the same morning. The cap of *Garajain* opens next day (after 24 to 48 hrs.);
* The cap of *Garajain* expands in day light/sun-light. The caps also open when kept under a shed. (It is likely that light and air causes swelling of cap that latter opens);
* The maximum size of cap seen are of the size of of an average *Amla.*
* The tribals also cited another example of bamboo shoots that also grow with sudden bursts induced by thundering/lightning; without lightning/thunder ing the bamboo "sprout" in the hot month of *Jyehtha* (June) also.These nodes sprout up to 3 to 4 feet in one go. It is said that one should not sit under the bamboo plants specially on the bamboo nodes during thundering/lightning in rainy season.

[If true, induction for sprouting of bamboo shoots from lightning /thundering indicates the presence of LRE or Light Responsive Elements (genes) / SRE, Sound Responsive Elements (genes) in bamboo. Sprouting of bamboo shoots in the hot months points to heat shock proteins.]

* *Garajain* "sprout" from termite mound after first few rains when heat from inside of a termite mound has been released.
* Termite comb (termatorium) may also be found at places other than the termite mounds e.g. in apparently plain agricultural fields, in the partition of crop field (*merh*), under the land giving deserted look etc.
* The main site of *Garajain* is big and old termite mounds harboring termite combs.
* After giving flushes of *Garajain* termite comb gets absorbed.
* *Garajain* grow maximum during *Magha Nakshatra* in rainy season according to traditional Indian calendar.
* If soil of termite mound is applied on head while taking bath (many local people use clay soil as soap for taking both), all hairs shall fall and the person shall become bald after a certain period.
* This edible fungus is collected from termite mounds in amounts of tens of kilogram per mound !
* *Garajain* are also found in the month of *Magh* (January) if lightning / thundering occurs but they are not long enough to reach outer surface of the mound. These *Garajain* are collected by putting one's hand deep in side the termite mound.
* In the initial stage these *Garajain* are of the size like of a mustard grains (pinheads) and white in color and are affixed on a hive like structure (termite comb or termatorium) made of soil. This hive like structure is very light in weight.
* Whenever there is lightning / thundering during rainy season these grow very fast (attain a length of more than 30 cm. within a day) and are seen over the mound in numerous numbers.
* *Garajain* start growing from *Nag Panchami* in the month of *Sravana* and are available upto the month of *Ashwin*;
* *Garajain* can not be stored for more than 24 hours; storage beyond this period spoils the stuff,

* The termite comb can be stored, it can be carried from one place to the other. As this will not get support of the termites during replacement, it would not produce *Garajain.*
* The *Garajain* found in the month of *Sravana*, show the caps of smallest size (Pea size); when these arecollected in the month of '*Bhadra*',the cap sizes are bigger and when collected in the month of *Ashwin*, the size of buttons seen are of plum size.
* Cooking time for *Garajain* is long; about an hour. It is prepared like mutton and cooked till its flavor and contents are extracted in the gravy. Only fullycooked *Garajain* should be consumed.
* These *Garajain* are also found in Madhya Pradesh e.g. in Bilaspur, Raipur, Raigarh, Katni. There these are known with different names - *Futu* in Bilaspur, *Pihri* in Katni, *Bhuifore* in Gorakhpur.

There are other kinds of wild edible mushrooms also, found in Madhya Pradesh. These are known as *Paira futu* (tiny brown color mushroom that grows naturally on rotten piles of paddy) and *Patras futu* (roughly rounded shape, brown- black in color, highly tasty, unpalatable when brown color turns yellow).

* *Bhuifore* is found in the termite mounds when there is a female termite inside. If only male termites are inhabiting the colony, the *Bhuifore* will not germinate and it will remain dormant even for 5 to 6 years.
* The *Garajain* grow only on comb like structure prepared by termites;
* The water of rain is not necessary. Had it been so, *Garajain* would have been found throughout rainy season;
* Maximum diameter of the button has been seen to grow up to the diameter of a small orange;
* As *Garajain* sprout due to thundering and lightning there is no certain time for their sprouting. They are found at any moment, e.g. morning, afternoon, evening, or night.

I. Collection of Samples

A small field laboratory was established at village Badausa, district Banda of Bundelkhand for collecting wild edible fungi, *Termitomyces* sp. and for

studying the fungus behavior of the locally abundant termite mounds *Odontotermes obesus* (Rambur). Fields surrounding this village and villages of other districts of Bundelkhand were extensively surveyed. Termite mounds were found primarily in elevated areas such as along the roadsides, elevated dividers of the agricultural lands, in vacant lots of the fields. The most striking observation was that almost all the mushroom producing termite mounds were found under or near the woody and thorny deciduous trees and shrubs viz. *Acacia tora*, *Aegle marmilose*, *Ziziphus zuzuba,Carissa carandas* and other woody and latex producing trees and shrubs. It was seen that the termite tunnels penetrated upto the roots of these plants and the termitaria were found near the root branches. Very often termitaria were found either buried just attached to the main root or adjacent to root branches. Mounds were of varying heights, the tallest was found upto three feet and were seen in clusters.

Many nests were systematically and completely excavated and their contents were measured, analyzed and sampled. The careful excavation of a termitarium required just three to five minutes. Excavations were continued until no more combs were found and the royal chamber was located. Sample of freshly collected workers, soldiers, nymphs, fungus garden and termitophiles were preserved in 70% ethanol for later study and dissection. Portions of combs, termites and surrounding soil were stored in petri dishes, for more detailed laboratory study. These were incubated in darkness at 25 ± 2°C in the laboratory.

Ethological observations of fungus growing termites were carried on in the field. Unfortunately this has not been done by earlier workers except Batra and Batra (1966), leading to considerable confusion and speculation in the literature and many misidentifications or nonidentifications of fungi or of termites.

Samples were oven dried as air drying was difficlt due to quick infections of molds. The temperatures of nest, fungus garden and field soil were recorded by simple thermometer.

II. Collection of spores (spore print)

To make a spore print, the stipe of a fresh mushroom was cut off squarely with the edge of the gills and the pileus and was placed down on a piece of glossy black paper. The pileus was then covered with a broad mouthed tumbler to prevent it from drying out too quickly. After a few hours the pileus was lifted and the mass of spores, which in the meantime had been deposited on the paper in the form of a spore print, was kept for further examination and the color determination.

In addition to variation in color, spores of many species also give an amyloid reaction with iodine and this test was also done in the laboratory for the classification of the Agaricales, described later in this chapter. Another character niticiable of these spores is the external ornamentation of the spore wall which was also studied.

III. Germplasm Conservation

Mushroom samples, after collection from field, were stored in 20% brine. The tissues were alive even after one year at 4°C. The color was near natural during this period of storage. The only change visible was slight reduction in the diameter of mushrooms without any wrinkling and the fragrance was lost.

Deposit of voucher specimens

For micromorphological investigations, the fungi and termites were fixed in formalin acetic alcohol (FAA) and also dried in oven. Fresh samples were stored in 20% brine as well as dried at room temperature. If the samples were dried without salt during period of high humidity, those were spoiled due to mold infestations. Wild mushroom sample were taken to Dr. A. K. Sarbhoy, Curator, Indian Agricultural Research Institute (IARI), Pusa, New Delhi. The sample was identified as *Termitomyces eurhizus* (Berk.) *Rajapa eurhizus* (Berk.) Singer. Voucher specimens of the mushroom are at the Mycology Museum, IARI, New Delhi. Herbarium Collection index Number (HCI No.) given to voucher specimen is **42,303**. It will be worthwhile to note that samples dried in oven had lost their natural colour and were turned brown whereas those dried in brine were light in colour maintaining almost natural colour (see Fig.A).

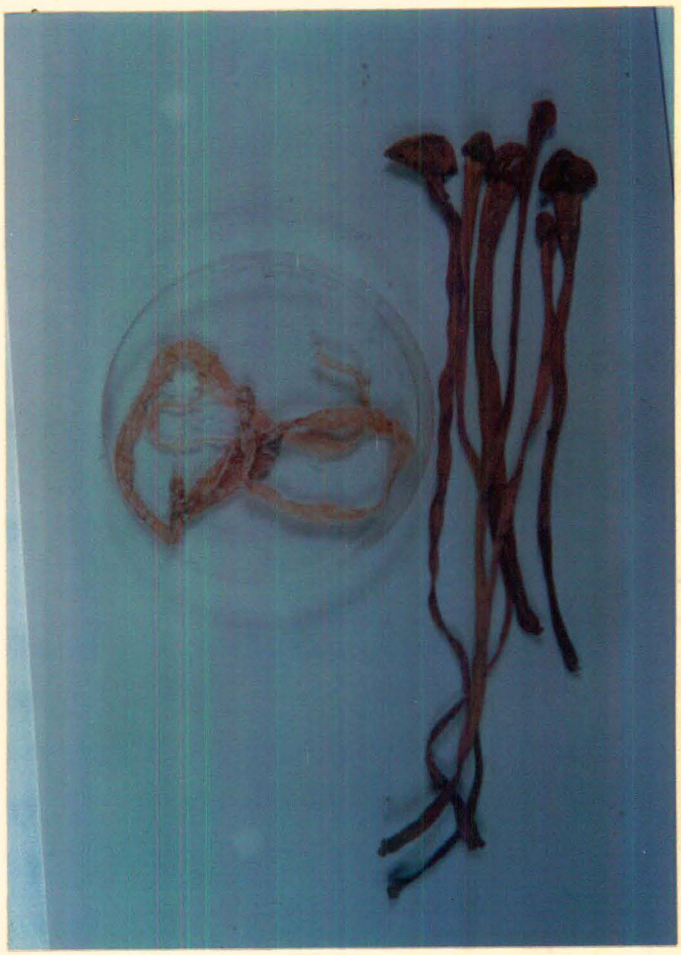


Fig. A: Petri dish containing brine preserved specimen. Next to it are oven dried specimens. Please note the difference in colour.

IV. Staining The Samples

Microchemical reactions

Melzer's reagent (Moser, 1978), the most important reagent for the study of *Agaricales* was used in following composition:

KI	1.5 gr.
Iodine	0.5 gr.
Water	20.0 gr.
Chloral hydrate	22.0 gr
	44.0 gr.

The preparation was first weted for a few seconds in ammonia (concentrated NH_4OH), then the ammonia was completely removed with filter paper, and a large excess of Melzer's reagent was added in order to compensate for any alkaline reaction still prevalent immediately around the fragment examined. The medium was homogenised after about 20 minutes and then examined.
The observed reaction is called amyloid or pseudoamyloid if positive; and inamyloid, if negative. The amyloid reaction is nearly black in some cases, in others it is a slight pallid grayish with a livid shade, with many intermediate shades between the two; pseudoamyloid is a positive reaction if the final color obtained is brown to purplish brown. Inamyloid walls are yellow to nearly hyaline.

V. Mycorrhizal Studies

Mycorrhizal studies were done to see whether any mycorrhiza-like relationship was evident in *Termitomyces* sp. Hyphae were taken from the base of the stipe and upper portions and stained in Trypton blue (0.01%) and Aceto-carmine, separately. Both these stains are widely used to study fungi exhibiting mycorhizal relationships. Tissues were fixed on the slide after pressing with the thumb and heating gently. Tissue were mounted with lactophenol, covered with cover glass and sealed to make permanent slides.

Spores of *Termitomyces eurhizus* were directly mounted with lactophenol (phenol,lactic acid and glycerol) and observed under the Orthoplan Microscope.

Photographs of the slides were taken with the help of the same Orthoplan microscope using Fuji Chrom color film (100 ASA).

The fresh tissues of round tuber like fungi collected from JNU campus, New Delhi, were also examined under the microscope after staining the fungal mycelia in the above mentioned stains and the photomicrographs were taken.

VI. Tissue Culture Of *Termitomyces eurhizus*

We used some techniques and methods of plant tissue culture to culture and grow the wild fungi *Termitomyces eurhizus*.

Laboratory for Aseptic Inoculation

Culture Room

Standard dust-free room with double doors for keeping or incubating the culture under controlled temperature, light and humidity was used for culturing the fungus in striile conditions. The temperature around 25 ±2°C and the relative humidity of above 50% was maintained in the culture room. Apart from culture room, cultures were incubated in computerised growth chamber to see the effects of minor changes in temperature and humidity. A shaker for suspension culture or single cell culture in moving liquid medium was also used in some studies.

Instruments, Chemicals and other Materials

Instruments routinely used for culture work viz. Laminar air flow; Hot air oven for drying the washed glass goods; Refrigerator for storing various thermolabile chemicals like vitamins, hormones, amino acids, casein-hydrolysate, yeast extract, coconut milk, etc. Stock solutions of salts were also kept to prevent variation; Distillation Plant; Weighing Balance: pan balance and electronic balance; pH Meter for the measurement and adjustment of pH of the nutrient medium; Vacuum Pump for filtering liquid media, sugar solution etc. through filter apparatus using air suction; Autoclave for sterilization of nutrient media, glass goods, instruments, etc.; Working Tables for preparation of medium; Heater for heating or warming the medium to dissolve agar or to melt the agarified medium; Microscope simple, compound, inverted microscope, binocular dissection microscopes were essential for various purposes. Orthoplan microscope fitted with a camera for taking photomicrograph; Microtome for

sectioning the tissue; wooden Racks for keeping the various chemicals etc.

Glass Instruments

Measuring cylinder, conical flack, pipettes, beakers are required for preparation of media. Fungal tissues were grown in wide-necked Erlenmeyer conical flask (100 ml, 150 ml, 250 ml etc.), culture tubes (25 mm in diameter and 150 mm in length), petri plates (50, 90, 140 mm in diameter), screw-capped universal bottles (20 cm^3 capacity). Pre-sterilized, disposable plastic wares were used in order to culture tissues.

Culture Medium and the Preparation of Stock Solution

From time to time, many workers (Murashige and Skoog, 1962; White, 1963; Gamborg et al., 1968; Nitsch and Nitsch, 1951; Schenk and Hildebrandt, 1972 etc.) have proposed the composition of a nutrient medium for the growth of plant tissue. But no one has so far reported to have applied the techniques and media of tissue culture used for higher plants to grow wild fungi. we attempted to culture and grow the wild fungi *Termitomyces erhizus*. As no single medium is capable of maintaining optimum growth of all plant tissues, the most suitable medium was determined by trial and error to grow this fungus. Proposed composition of MS culture medium has been modified to stimulate the growth of *Termitomyces eurhizus*. The composition of the media finally used is given as follows:

Constituents	**Murashige and Skoog (1962)**
Macro-nutrients	
$MgSO_4 \cdot 7H_2O$	370
$CaCl_2 \cdot 2H_2O$	440
KNO_3	1900
$NH_4 \cdot NO_3$	1650
KH_2PO_4	170
Micro-nutrients	
$MnSO_4 \cdot 4H_2O$	22.3
$ZnSO_4 \cdot 7H_2O$	8.6
$CuSO_4 \cdot 5H_2O$	0.025
$AlCl_3$	0.025
KI	0.83
H_3BO_3	6.2
$Na_2MoO_4 \cdot 2H_2O$	0.25

Iron source	
$FeSO_4 \cdot 7H_2O$	27.85
Na_2EDTA	37.25
Carbohydrate source	
Sucrose	30-50 g^{l-1}
Vitamins	
Myo-inositol	100.0
Nicotinic acid	0.5
Pyridoxine HCI	0.5
Thiamine HCI	0.1
Amino acid source	
Glycine	2.0

The most widely used plant tissue culture medium was formulated by Murashige and Skoog (1962), commonly called MS medium; the procedure for the preparation of stock solution of MS medium is given below:

Constituents present in original medium	**Amount (mg/L) to be taken for stock solution (X20)**	**Amount (gm) of stock (ml)**	**Final volume**
NH_4NO_3	1650	33.0	
KNO_3	1900	38.0	1000
$CaCl_2$, $2H_2O$	440	8.8	
KH_2PO_4	170	3.4	
$MgSO_4$, $7H_2O$	370	7.4	

Stock Solution of Macro-Salts (X20)

To make 1,000 ml of this stock solution, we dissolved the salts one after another in 800 ml of double distilled water (DDH_2O) and then the volume was made up. The solution was filtered and stored in refrigerator (10-16°C).

Stock Solution of KI (X1,000)

Dissolved 83 mg of KI (0.83 mg/L present in original medium) in 100 ml of DDH_2O. Stored in refrigerator (10-16°).

Stock Solution of Iron (X200)

At first we dissolved 745 mg of Na_2 EDTA (37.25 mg/L in original) in 75 ml boiling double distilled water, then added gradually 557 mg of $FeSO_4$ $7H_2O$ (27.85 mg/L in original). Kept on a magnetic stirrer for at least 1 hr. in hot condition until the color of the solution changed to golden yellow. Finally made the volume to 100 ml and stored in refrigerator (5°C). This solution specifically was kept in an amber colored bottle.

To make 100 ml of this stock, dissolved the salts sequentially, one by one in 80 ml of DDH_2O and made up the final volume. Filtered and stored the solution at approximately 5°C in a refrigerator.

Stock Solution of Meso-Inositol (X500)

Dissolved 1 gm meso-inositol (100 mg/L in original) in 20 ml DDH_2O. Stored at 0°C and used up within 15 days.

Stock Solution of Micro-Salts (X1,000)

Constituents present in original medium (Value expressed in mg)	**Amount (mg/L) to be taken for stock solution**	**Amount (X100) of stock (ml)**	**Final volume**
H_3BO_3	6.2	620	
Na_2MoO_4, $2H_2O$	0.25	25	
$CoCl_2$, $6H_2O$	0.025	2.5	100
$CuSO_4$, $5H_2O$	0.025	2.5	
ZSO_4, $7H_2O$	8.6	860	
$MnSO_4$, $4H_2O$	22.3	2230	

Stock Solution of Glycine (X1,000)

Dissolved 40 mg glycine in 20 ml of DDH_2O. Stored at O°C and used up wthin 15 days.

Stock Solution of MS for three Vitamins (X1,000)

Constituents present in original medium	Amount (mg/L) to betaken for stock solution	Amount (X50) volume (ml) (Value in mg)	Final Storage temperature (°C)	Duration of storage (in days)
Thiamine HCL	0.1	5		
Nicotinic acid	0.5	25	50	0 15
Pyridoxine HCL	0.5	25		

Stock Solution of Hormones

These stock solutions are not specific for MS medium. The stock solutions of hormones are of general nature and were used in different media at different combinations and concentrations.

Auxins and cytokinins are not directly dissolved in water. So they were at first made soluble in water miscible solvents and then water was added to get the final volume. Stock solutions of auxins and cytokinins were prepared according to need.

Preparation of Culture Medium

To make 1 liter of MS medium—

(i) Dissolved 30 gms cane sugar in 200 ml DDH_2O. Mixed 1-2 gms activated charcoal and filtered through filter paper, and set inside the Buchner funnel fitted on a special conical flask with small side arm attachment. Filtering was done by using a suction pump.

(ii) In another flask DDH_2O was taken and added in sequence to the appropriate amount of stock solution as follows—

Stock solution of macrosalts	50 ml
Stock solution of microsalts	1 ml
Stock solution of KI	1 ml
Stock solution of Fe-EDTA	5 ml
Stock solution of MS 3 vits	1 ml

Stock solution of Glycine	1 ml
Stock solution of meso-inositol	2 ml

Desired concentration of auxin and/or cytokinin are added from stock solution according to the formula—

$$\frac{\text{Desired concentration}}{\text{Stock concentration}}$$

= amount (ml) of stock solution to be taken for one liter medium.

If the quantity of the medium is less than one liter, then hormones are added using another formula—

$$\frac{\text{Required concentration X Volume of medium}}{\text{Stock concentration X1,000}}$$

= amount (ml) of stock solution to be added.

(iii) Poured filtered sucrose solution and salt, vitamins, amino acid, hormone solution mixture into a one liter measuring cylinder. Made the final volume to one liter with DDH_2O. Mixture was Shaken well to mix up uniformly.

(iv) Adjusted the pH of the liquid medium 5.6-5.8 with the aid of 0.1(N) HCI or 0.1(N) NaOH. This operation was done using the pH meter.

(v) Added 5% to 8% agar to the liquid medium to make solid medium. Heated this medium to 60°C to dissolve the agar completely. Liquid medium was also used for culture.

(vi) Dispensed the culture medium into culture tube (20 ml/tube) or wide mouth conical flask (25-40 ml/flask). Inserted non-absorbent cotton plug wrapped with gauge cloth. Covered the plug wrapped with the help of brown paper and rubber band/Aluminium foil.

(vii) Medium was finally sterilized by autoclaving.

Sterilization Procedure

(a) Sterilization of non-living Articles:

The routine sterilization procedure of non-living articles such as nutrient medium, glass goods, distilled water, instruments (wrapped with brown paper) was done by autoclaving under steam at a pressure of 15 lb/in^2 and a temperature of 120° for 15 minutes.

An alternative method of sterilizing glass goods and instruments used was by heating in an oven at 150°C for 3-4 hrs.

(b) Sterilization of Tissue:

(1) Thoroughly washed the tissue in tap water and then immersed it in 5% v/v solution of liquid detergent such as 'Teepol' for 10-15 minutes. Then washed the material thoroughly in tap water and finally in distilled water. This step was done inside a laminar air flow normally used for plant tissue culture

(2) Dipped the tissue in 70% ethyl alcohol for 60 seconds.

(3) Immediately transferred the material into an autoclaved jaw bottle and poured 0.1% mercuric chloride ($HgVl_2$) 5-10% sodium hypochlorite (v/v) solution. Kept them for 10-15 minutes. During that period, the bottle was frequently swirled for shaking so that all surfaces of material came equally in contact with sterilant.

(4) After 10-15 minutes, the sterilant was decanted and the tissue was thoroughly washed with several changes of autoclaved distilled water to remove all traces of sterilant.

(5) Now the tissue is ready for culture.

Incubation of Culture

(1) After inoculating the tissue onto the culture medium, cultures were incubated on culture rack at 25°C temperature.

(2) Culture tubes were placed at 30-45° inclined position.

(3) Illumination was provided by cool-white fluorescent light placed about 18

inches above the culture to give a light intensity of 4 - 10 x 10^3 lux, for 16 hours, at different intervals.

(4) To see the effects of absence of light i.e. darkness, the light was put off and whole rack was covered with a black cloth.

De-Differentiation and Re-Differentiation Media

To develop dedifferentiation and redifferentiation media various combinations of certain growh factors were used in different concentrations.

Finally de-differentiated tissues of wild fungi were first transferred to different De-differentiation media, then onto redifferentiation media. For raising of pure culture of Wild mushroom, the following conventional culture media were also tried:

Wheat Extract - Agar Medium : Thirty two grams wheat grains were boiled with one liter of distilled water for about 2 hours and filtered after 24 h. Twenty grams agar was then added to a liter of filtrate and boiled. The pH of the medium was adjusted at 6.5 and about 5 ml of medium was filled is each test tube. These test tubes were then sterilized at 15 lb psi for 20 to 30 minutes.

Lambert's Agar Medium : Ten grams glucose, 0.5 g magnesium sulfate ($MgSO_4$, $7H_2O$). 1.9 potassium dihydrogen phosphate (KH_2PO_4) and 20 g agar were added to one liter of distilled water. The remaining process is the same as described above

Malt Extract Agar Medium : Twenty grams malt extract and 20 g agar was added to one liter of distilled water and boiled for 5 minutes. The remaining process is the same as described above.

Compost Extract Agar Medium : Five hundred grams ready synthetic compost is boiled with 2 liters of distilled water for about 2 h and filtered after 24 h. Thirty five grams agar was then added to two liters of filtrate. The pH of medium was adjusted at 6.5-7.0 and about 5 ml medium was filled in each test tube and sterilized at 20 lb psi for 1 h.

Potato Dextrose Agar Medium : Two hundred and fifty grams peeled potatoes were boiled in one liter of distilled water till these became soft and

filtered. To the filtrate, 20 g dextrose and 20 g agar were added and volume of the liquid was raised to one liter. Remaining process is the same as mentioned in the first three media.

Termitomyces sp. collected from Bundelkhand and the tuber like fungus collected from JNU campus, New Delhi were brought to laboratory and their tissues were inoculated in the above mentioned media to prepare pure culture of these fungi.

VII. Spore culture

For raising of mono or multispore culture, spores were collected from a large sized healthy mushroom with membrane (veil) still intact under sterile conditions. The mushrooms after the surface disinfection process was mounted on a wire stand over a petri dish under glass beaker already sterilized in an oven at 160°C for about 2 h and cooled. The spores are discharged and deposited on the petri dish on the opening or rupturing of the membrane in a thick mass known as spore print. The spores were used for direct inoculation of wheat extract agar medium, Lambert's agar medium and various dedifferentiation (DD)media prepared for this purpose.

Spore suspension was prepared in sterilized distilled water. One ml of spore suspension containing more than one hundred spores was mixed in each test tube containing about 5-7 ml of already sterilized wheat extract agar, Lambert's agar liquid medium (45°C) and DD media. The inocula are then incubated at 28°C for spore germination for about 2 weeks.

The single spore (monospore) cultures are raised by the following method: One ml of spore suspension containing about 15 to 20 spores was transferred with the help of sterilized pipette in a 90 mm petri dish. Sixteen ml of liquid agar medium was also earlier poured in each petri dish containing spore suspension and stirred clock-wise slowly to distribute the spores evenly on the petri dish surface. The plated petri dishes were turned upside down after the solidification of the medium and some grain spawn were placed in the lid. Eleven days after spores sowing and continuous incubation at 28°C, the first mycelium of germinated spores became visible. The lid containing spawn was

exchanged with a sterilized lid at this stage. The monosporous culture was cut with very little medium and transferred to the fresh sterilized media filled in tubes. The screening of single spore cultures was done after 2 weeks for fluffy, appressed or strandy types. The latter was found best for preparation of master culture or stock cultures.

VIII. Range of Temperature Tolerance, Span of Life Cycle, Shelf Life, Germination Rate and Sporulation Rate

Field were extensively surveyed during rainy season to spot *Termitomyces* sp growing on termite mounds. As and when those were spotted, the atmospheric temperature and temperature inside the mounds were recorded with tthe thermometer to know the range of temperature favourable for growth of *Termitomyces* sp.

It remains to establish span of life cycle of *Termitomyces* sp in the field due to lack of possible procedure to measure it. *Termitomyces* sp collected from fields were stored at room temperature to see the changes in it. To study germination rate fresh spores of the sample were tried for raising monospore culture as explained above.

Sporulation rate was studied taking freshly harvested *Termitomyces* sp. In the field laboratory the stipe was cut at the base of the cap. Such caps were placed on a black paper and covered with glass beaker and were allowed to dischrge their spores. After every two minutes of cap opening black paper was replaced with a fresh piece of black paper. This was repeated untill the spores discharge stopped and caps were opened to their full extent. All these papers were collected for spores calculation. A micrometer was placed inside the eye piece of a binocular microscope. One such black paper with discharged spores was placed below the objective lens. Spores of one square millimeter area were taken out directly on hemocytometer and stained with KI solution. Number of spores were counted and total number of spores on each paper was claculated multiplying with the area of a circle. Sporulation rate was calculated as number of spores per minute and total number of spores per fruiting body.

Basic biological parameters viz. fresh weight, dry weight, total protein etc. were studied by Sunanta et al. (1985) hence these biochemical analysis were avoided to save time from such exercises and time was invested to study other aspects of this fungus.

IX. Cultivating The Wild Fungi To Study It's Life-cycle

These cultured tissues were transferred to various redifferentiation media and compost extract agar media,to study life cycle of the fungus. Different abiotic conditions were provided in automatically controlled computerised Growth Chamber. The parameters of diferent abiotic conditions and growth media are discussed in the following chapter of Results and Discussion.

5. RESULTS AND DISCUSSION

I. Field Study

a. Field observations

Extensive exploration of the field yielded interesting observations and many of which were found to confirm the myths on the Garajain (*Termitomyces eurhizus* as identified by A.K.Sarbhoy of Indian Agricultural Research Institute, New Delhi and described in previous chapter) :

- the fruiting bodies of the fungus in nature were found only in the rainy season i.e. July and August (months of *Sravana* and *Bhadra*, according to Hindi calender) after thundering and lightning;

- these were found in the area where strength of thundering and lightning was very powerful and were not found much away from the center of thundering/lightning where intensity of thundering and lightning was poorer;

- maximum quantity of the fungal sporophores were found in the *Magha Nakshatra* which comes under the month of Bhadra and is accompanied which generated maximum and most powerful thundering/lightning;

- the fruiting bodies were alway collected from mound of the termites (Odontotermes obesus).

Fungus grower *Odontotermes obesus* build nests or termitaria in the soil. The nests of this mound-building termite may be divided into two parts: the nest itself which is primarily underground; and the above-ground part called the mound (Fig.1 and Fig.2) The mound generally is a conical structure of hard clay, with or without buttresses. It usually contains air-filled shafts leading to the "fungus garden" (Fig.3) containig small combs in individual cavities. Normally there are no openings to the outside air, but inside the upper wall, there are thin-walled cupulate pits through which air may diffuse with minimal water loss. These shafts ordinarily are covered at the surface except during the nuptial flight of alates in the rainy season, through which winged termites

Fig. 1 and 2: Figure 1 showing the emergence of fungus fruit body from the termite mound; note that the earth contains passage through which stalk emerges. Figure 2 showing the stalks emerging out the termitorium.

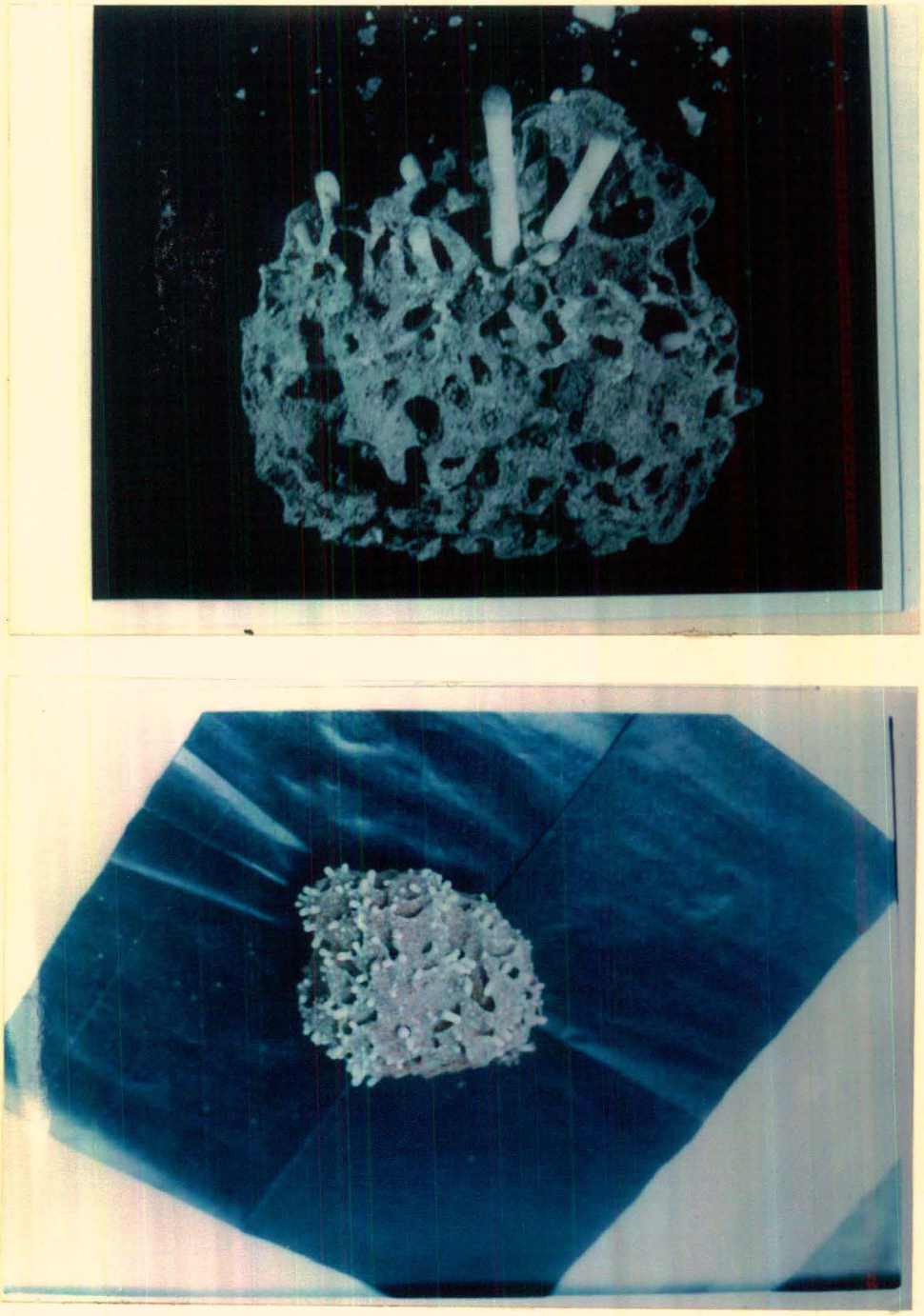


Fig. 3 A-B: Fungus garden with emerging stalks.

Fig 4: Above ground portions of the fruit body. Compare the above ground part with under ground stalk length and thickness. Note that the above ground part is thicker than the under ground part. Com pare the thickness of the above ground part with the same underground portion (fig. A)

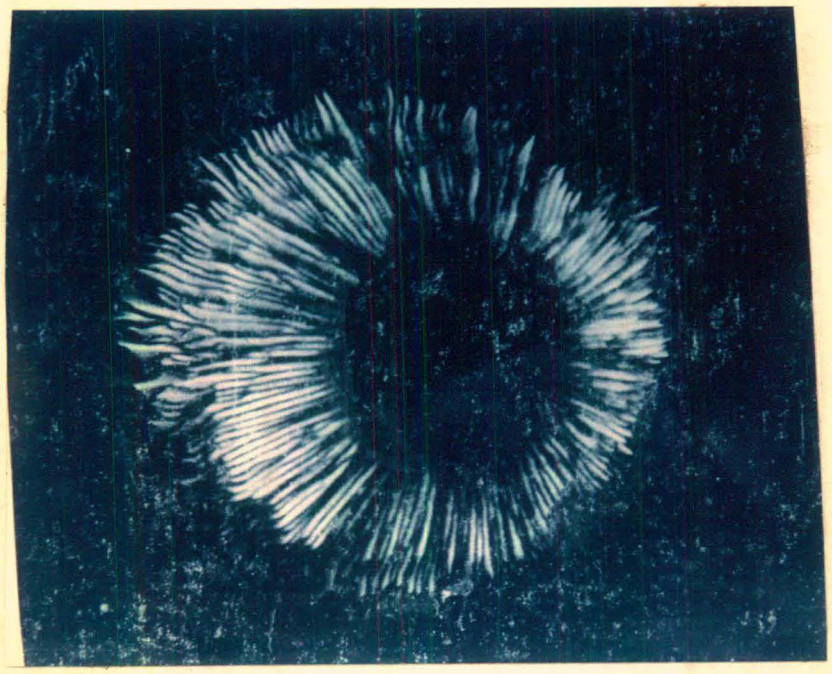


Fig 5: Spore print of normal fruit body

come out and fly before finding a new place to enter the soil or in the inactive nests where they have been damaged by human or animal activity. The most unusual finding was the occurrence of normal comb in a nest lacking the royal pair and having only about 100 termites. And this was found after at least one flush of *Termitomyces* from the termitarium of *Odontotermes* was produced. Nests of this species usually contain up to 90,900 individuals.

b. Soil moisture and minerals in termitaria

It was observed that though the soil of the termitorium and linling of the chambers surrounding the fungus garden may be alkaline, the fungus garden itself was acidic (pH 4.50 to 6.85; Table No.1.)

As airspaces in soil that can support vegetation are saturated with water vapor, it may be expected that air surrounding fungus gardens is also saturated. In Bundelkhand, it was found that during the winter rains, although the mound and adjacent soil gained free water, there was no increase in water content of the combs, indicating saturation.One reason for the high pH of mound soil in *O. obesus* may be due to mineral deposits caused by evapotranspiration, often visible as a thin white layer inside ventilation shafts. It is also known that these species as well as others bring soil and moisture from several meters' depth. As per regional horticultural department, salt concentrations in subsoil are high in the barren lands of Bundelkhand .The nature of the association of fungus-growing, mound-building termites and trees in part may be understood in terms of water and minerals brought to the surface. *Odontotermes obesus* combs freshly exacavated were drier (55% moisture) than the compost used for commercial button mushroom (66% moisture; Table No.1.)

Table No.1

pH And Moisture In Different Fungus Gardens Collected Randomly From Different Regions

Comb Nos.		# 1	# 2	# 3	# 4	# 5	# 6
pH	Comb	4.50	5.60	6.50	4.75	5.15	6.25
	Mound	7.10	7.10	8.00	7.50	8.10	8.25
Moisture	Comb	51%	52%	51.5%	51.5%	52.5%	52%
	Mound	86%	85%	84%	85%	87%	86%

c. Temperature in the nest

Movement of water under the influence of a temperature gradient is a major factor in the water regime of soils. Heat is generated by the termites and fungal metabolism in the combs, which may create a temperature gradient that assists termite activity in bringing up subsoil water and probably inducing fruiting bodies formation of the fungi. Nests with large, centralized fungus gardens may become warmer relative to their surroundings than small nests, or nests having many small combs in numerous scattered chambers. Temperatures were measured by long soil temperature measuring thermometers kept at the center of the comb and out side to see the temperature gradient inside the termitorium (Table No.2.) *Termitomyces* sp were found growing from 22 degree Celcius to 32 degree Celcius temperature. The average temperature was calculated to be 27 degree Celcius. RH was all the time above 85%.

Table No.2

Temperature (degree Celcius) in the mounds and combs

Termitoria	# 1	# 2	# 3	# 4	# 5	# 6	# 7
Mound	25.2	27.0	25.3	26.1	25.8	26.4	25.8
Comb	27.3	29.3	27.1	27.2	28.3	27.6	27.6

d. Structure of the fungus garden

Freshly exacavated fungus combs- collectively called the fungus garden, lodged in closed bowl like chambers (Fig.2)- are soft, light in weight, moist, dark brown colored sponge-shaped masses of fibers and root tissues of near by woody plants, shrub or grasses (Fig.6). Thus, they are built of finely divided vegetative especially plant root tissues and proportionately a very small amount of insect remains and sometimes also insoluble inorganic particulate matter (Batra & Batra,1967.) The entire mass is permeated with the mycelia of *Termitomyces* and *Xylaria* and at places contain large masses of ungerminated conidia of the former. *Xylaria* is a large genus and its latest taxonomic treatment is that of Martin (1970). *Xylaria* species are mostly saprobic or weekly parasite on woody

Fig. 6: Exposed fungus garden in association with exposed roots in the centre of the pit.

Fig. 7: Lump of undifferentiated cells on de-differentiation medium.

plants. In *Xylaria*, the stromata are usually epixylous i.e. growing upon wood, but some species produce them on sawdust, leaf mold, dung, or in the soil. In contrast to superficial growth of *Termitomyces*, the growth of *Xylaria* is within the comb. This conclusion is based on embeding and sectioning by Batra (1966), and thus invisible to the unaided eye. White, cottony mycelia soon turning gray to gray brown, develop rapidly on the combs removed from active nests and forms upright initials of stromata within 24 hours at 25°C.

II. Laboratory Observation

Maintaining cultures of *Odontotermes obesus* for detailed behavioral study is difficult, primarily due to rampant growth of *Xylaria* on the combs when these combs are removed from termitaria and exposed to air. Termites of all types, including the royal couple and those working in the comb, ordinarily live for only a few days when taken from large termitaria. There has been very little study of communication, division of labor and feeding habits of fungus-growing termites, probably due to the difficulty of maintaining laboratory colonies and the lack of techniques to observe their life inside undisturbed, intact nests.

a. Development and cultural characters of *Termitomyces*

Most of the growth of *T. eurhizus* is on the surface of the comb in the form of effused mycelium and raised spherules. These are the most distinctive feature of a fungus garden. The spherules originate as upright wefts of few intertwining hyphae which repeatedly branch sympodially and stain intensely towards their distal, enlarged ends. When these spore -bearing stalk-parts are cultured under controlled conditions on DD media the spherules of *Termitomyces eurhizus* begin to enlarge, remain white, produce a limited amount of aggregates loose cells (Fig.7). The *Xylaria* never appeared in any of the cultures thus obtained from fungus gardens. On DD medium mycelia of *Termitomyces eurhizus* gave numerous fruit body primordia similar to those found on the termite combs. This is the major achievement in the attempt to culture wild fungi *Termitomyces eurhizus*. (Fig. 8 and 9 and Table No.3.)

The same media was also tried for the Tuber like mushroom that was collected wild (Fig.10 and 11). The media gave pure culture and tuber like

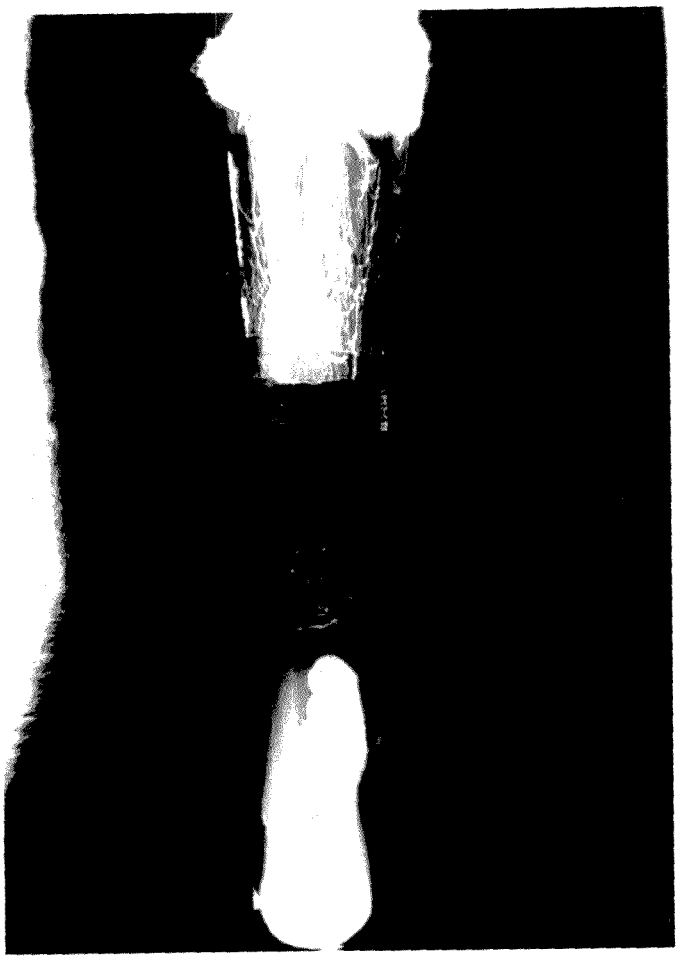


Fig. 8: De-differentiated cell mass under low magnification.

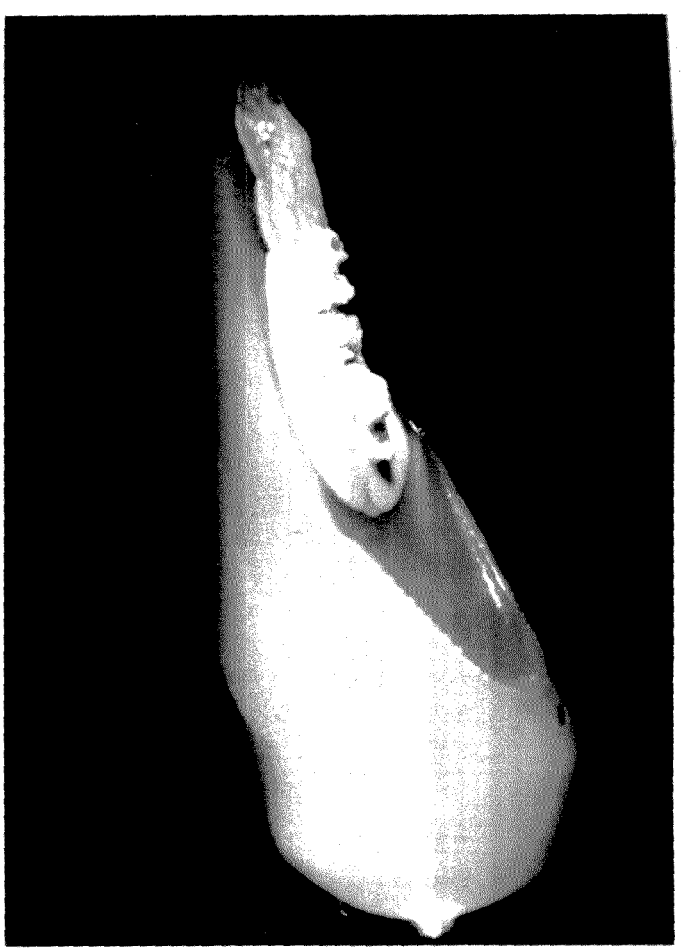


Fig. 9: The material in Fig. 8 under high magnification showing formation of bud primordia of Termitomyces.

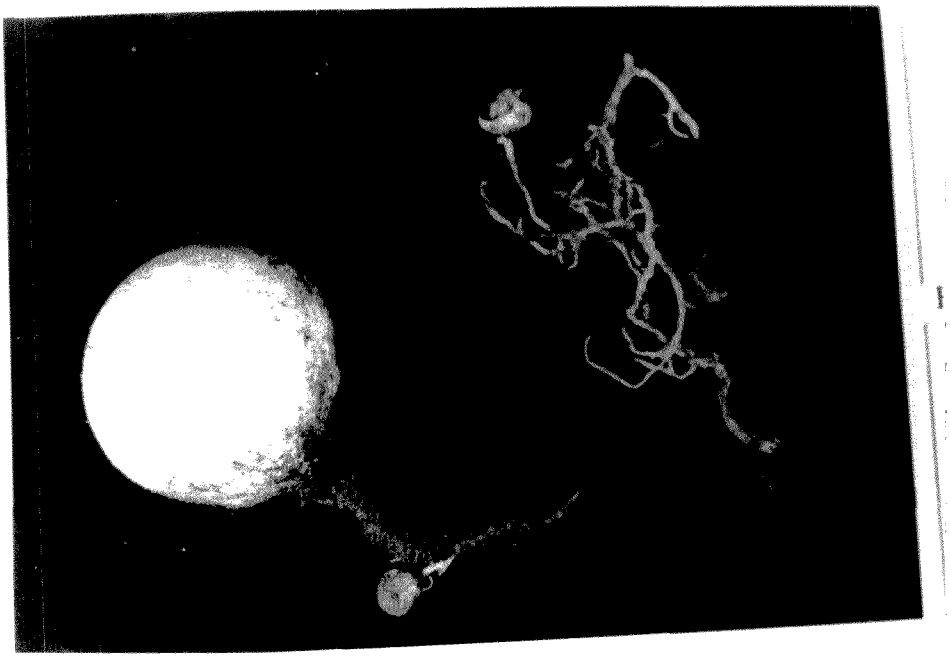


Fig 10: The tuber like mushroom full grown and attached yet growing underground fruit bodies. The upper root portion was originally attached to the underground portion of fruit body. White part of fruit body was above ground , rest underground.

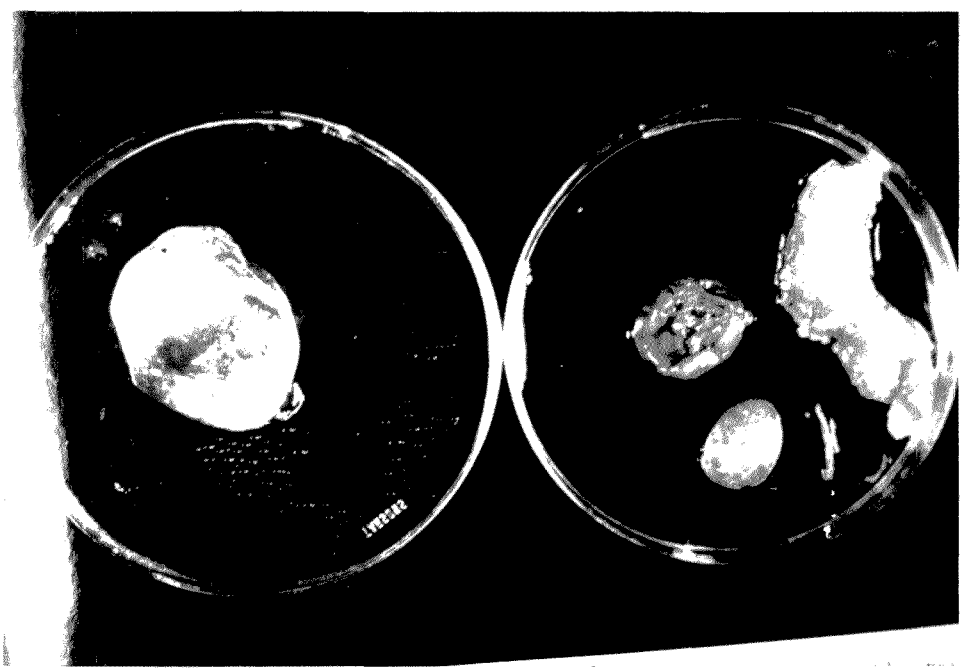


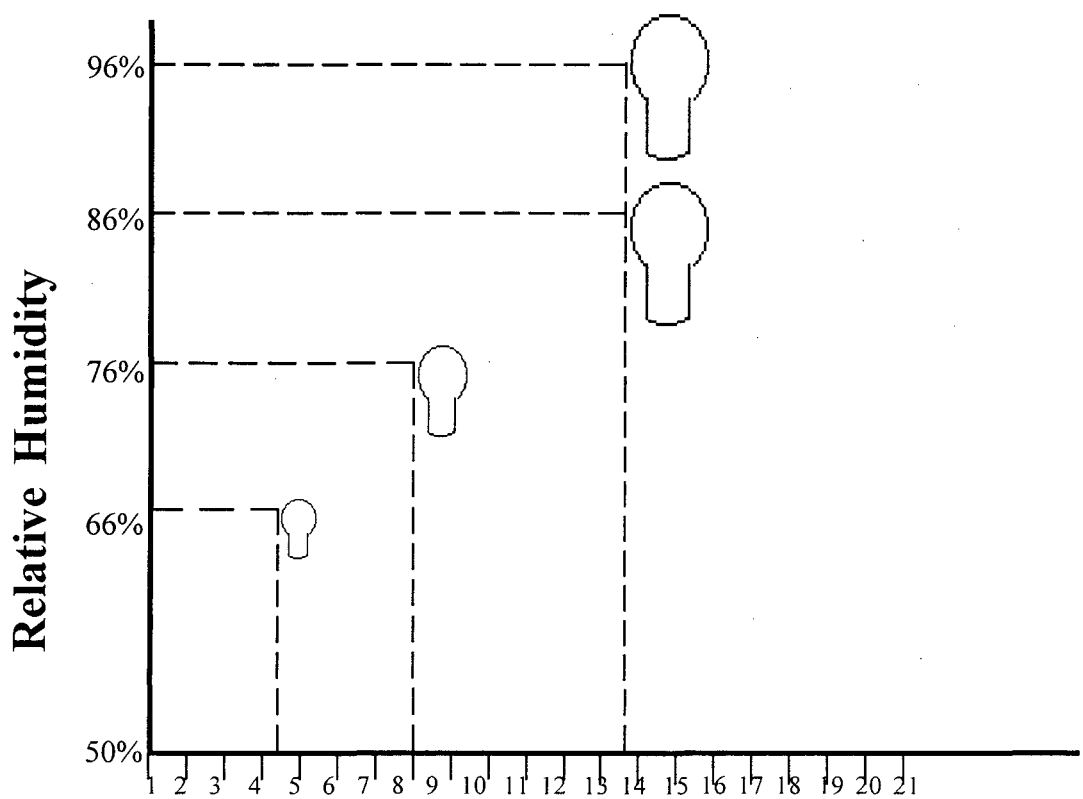
Fig. 11: Different tuber like mushroom from different sites show the morphology of the fruit.

primordium in the test tubes (Fig.12 and 13). Cap diameters were taken as Indicators of growth of the fungi.

Table No.3

Culturing Wild Fungi Under Controlled Temperature (27 Degree Celcius) And pH (5.5) With Varying Atmospheric Humidity (RH) At Delhi

Test Tube No.	# 1	# 2	# 3	# 4	# 5	# 6
Cap Diameter						
RH 66%	4mm	5mm	5mm	4mm	4mm	4mm
RH 76%	7mm	8mm	8mm	9mm	8mm	8mm
RH 86%	12mm	14mm	13mm	15mm	14mm	15mm
RH 96%	12mm	14mm	14mm	14mm	14mm	15mm



Mean Diameter in mm

(Graph Showing Effects of RH on Growth of Bud Primordia of Wild Fungi Under Controlled Conditions)

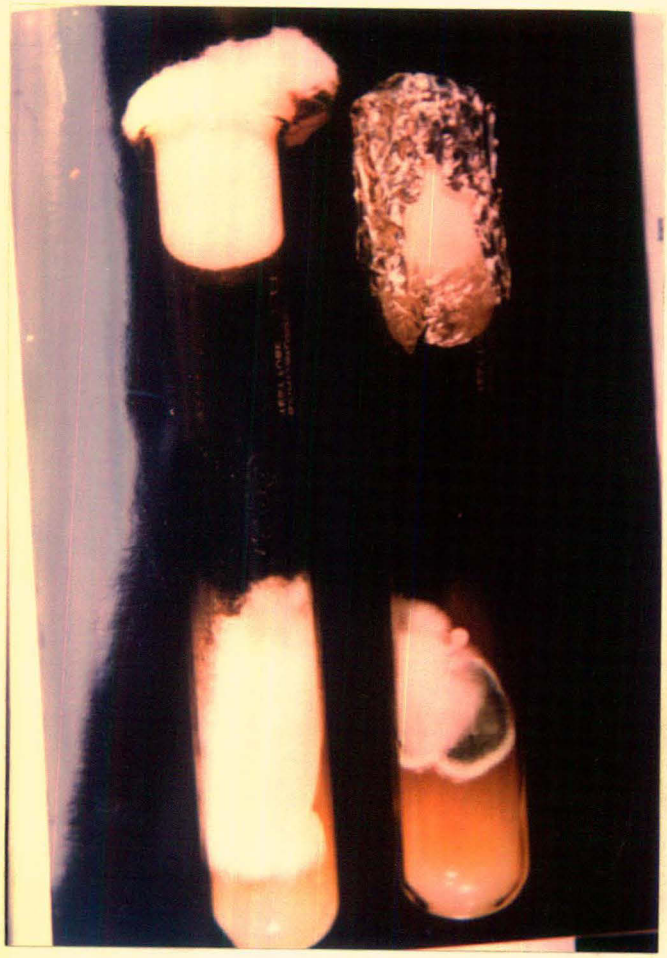


Fig. 12: Pure culture of the tuber like mushroom. Note absence of any infection in the DD medium. Nearby tube contains infection showing the contrast.

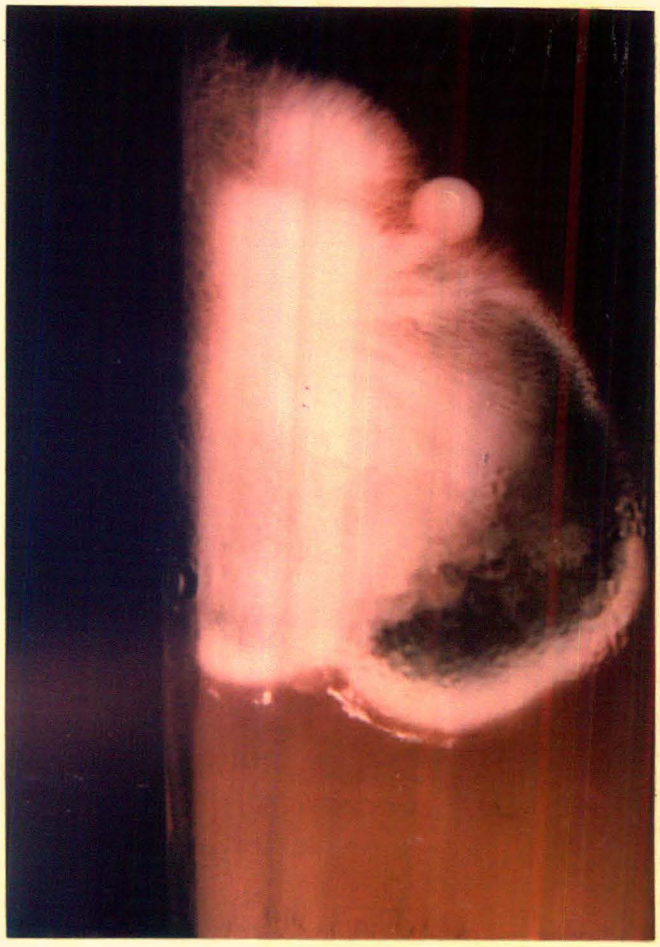


Fig. 13: Enlarged content of the infected tube in Fig. 12. Please note the formation of fruit body primordium.

It is evident from above table that after 86% RH there was no increase in the growth of the fungi with further increase in RH. Thus it is established that the suitable condition for this fungi to grow is at 27 degree Celcius temperature, pH about 5.5 and Relative Humidity about 86%.

b. Nutritional requirements of *T. eurhizus* and *X. nigripes*

Preliminary growth studies on DD media, with and without vitamins, revealed that *T. eurhizus* and *X. nigripes* required a mixture of vitamins. Similarly, it was determined that they could use NH_4NO_3 as the sole source of nitrogen even though they grow somewhat better on media with organic nitrogen (lactalbumen). Our basal medium had the following composition : NH_4NO_3 1 g (except in case of testing for other nitrogen sources) : KH_2PO_4 0.90 g; $k_2H\ PO_4$, 0.70 g; $MgSO_4\ 7\text{-}H_2O$, 0.75 g; glucose, 20 g (except in case of testing for other carbon sources); yeast extract powder, 0.3 g; distilled water, 1 liter. The pH of the medium after autoclaving was 6.4. Except for the addition of yeast extract and glucose in place of cellulose powder or cotton fibers, it is the same medium as used by Marsh (1953) in studies on fiber decomposition by microorganisms. All experiments were performed at least twice and in each case three replicates for each treatment were set up. Vitamin requirement experiments were conducted with the basal medium solidified with 2% agar in acid cleaned glassware with usual precautions. The medium used received a vitamin mixture to yield the following final concentration (micro liter/l); biotin, 5; pyridoxine, 100; riboflavin, 100; thiamine, 100. Experiments were performed at room temperature, 25 ± 2° C, since preliminary observations at 5°C to 35° C., with 5°C. interval, gave good growth at around 25° C. Plant growth regulators were used in different concentrations.

Termitomyces eurhizus is a relatively slow growing fungus on the basal medium, as compared with *Xylaria nigripes*. It grows just as well on cell free filtrate of basal medium from which 14-day-old *Xylaria* mycelium has been harvested, indicating that some nutrients, growth factors, or both are available to it. *Xylaria* on the other hand did not grow so well on similar filtrates of *Termitomyces* cultures *in vitro*. *Xylaria* is heterotrophic for biotin and thiamine; *Termitomyces* is heterotrophic for thiamine. Both fungi grew well on basal

medium with carboxylmethyl cellulose or Walseth cellulose as carbon sources, with a distinct clearing zone in the latter medium. To a limited extent *Xylaria* grew on Marsh's medium (but *Termitomyces* did not) with cotton fiber as the carbon source.

c. Span of life cycle and shelf life:

As life cycle was not completed under in vitro conditions, this could not be studied in the lab. In the field also it remains to establish span of life cycle of *Termitomyces* due to lack of possible procedure to measures it.

Termitomyces sp collected fresh from fields have no shelf life. These were getting spoiled even after 12 hours of harvesting. Their caps even started opening 4-6 hours after their harvesting from termite mounds.

d. Germination rate and Sporulation rate:

Spores failed to germinate in a suspension medium. It may be that these were exhibiting spore dormancy or they need certain factors for their germination. It is quite likely that termites provide these factors within the mounds.

Sporulation rate as per calculation was found to be very high. It was found that *Termitomyces eurhizus* produced 768 million spores in one day. There was no spore discharge after 24 hours of cap opening. The average rate of sporulation was 32 million spores per hour. Study of mechanism of spore discharge was not taken in to consideration. It is possible that termites either maintain the mycelium culture within their comb for years or collect the spores each year to preserve them as stock for mycelium culture. If this is true, as it seems to be, it is extremely challenging task for the scientists to find out the mechanism of mycelium/spore conservation and preservation by the tremites in "open" condition in the nature.

III. The Physiology Of Fruit-Body Development

Much remains to be learnt about the control of morphogenesis in Hymenomycetes fruit-bodies. *A. bisporus* which fruits well only on compost, the other species

fruit readily even on synthetic liquid media and this enables the chemical composition of the medium to be varied. By changing the level of CO_2 and humidity of the atmosphere and the light intensity it is possible to control the differentiation of the fruit-body. The effects of nutrition will not be considered here.

a. Light

Light intensity, duration and wavelength are important components of any treatment. Light may have an effect on induction of sporophore initials, a tropic effect, or an effect on fruit-body form, e.g. ratio of stipe length to pileus diameter. In *Termitomyces eurhizus* fruiting occur in darkness as observed in the field. Total darkness may be the reason for long i.e. etiolated stalks that come out of the mound through opening and open their spore bearing parts only after being in light (Fig.4; also see Fig.1). This conclusion is based on observation showing exterme miniaturisation of the whole plant growing on the mouth of a broken shaft in light in which case the spore print was small but in all details got the same pattern as the normal one. (Fig.5)

Sensitivity of fruiting of *Agaricus bisporus* to light occurs after about seven days growth and exposure to white light as brief as 1 sec. at 250 foot-candles or 5 sec. at 0.1 foot-candles was effective (K.K. Tan, 1978.) Thus the folklore that claims role of lightning/thundering in fruit body formation seems logical. But this lightning is effective only after establishment of mycorrhiza which appears to be main factor for fruit body formation. Had the lightning been the only factor for fruit body formation all the spores shed on the surface of mound would have germinated. Further increase in duration of light had no additional effect on the numbers of sporophores produced.

It has long been known that low light intensity, or absence of light, may result in sporophores of curious shape, often with elongated stipes and poor pileus development that closely match with *Termitomyces eurhizus* which has very long stipe. Termite comb on which fruit body primordia are formed is located deep in the soil, some thinglike at the base of a dark and hollow sphere. After termite establishes symbiotic relation with the fungus,lightning (and thundering) induces superfast growth of fruit bodies. The fruit body elongates towards the source of light. The elongation continues till it reaches the outer

surface of the mound on which fruit bodies of various length were found and the length of the stipe were matching the depth at which combs were found in side the mound.The fruit bodies do not attain their whole length in one go but elongate with every flush of light. When fruit body primordia emerges out, its elongation process comes to halt. Of the fruit body, twenty one cm.(Fig.14) in length, only about an inch was visible over the mound and rest was under the mound in complete darkness. A freshly emerged sporophore had no distinct pileus. Light is necessary for pileus formation. The pileus becomes distinct and enlarged after it has stayed in light for some time (Fig.15 and 16).

Light also stimulates directional growth of the stipes of *Termitomyces eurhizus*. In previous photographs it may be noted that from somewhat spherical fungal comb fruit body primordia are diverged in all directions and internally there are ingeniously designed pores in the mound in all directions. Sporophores emerged through these pores left specially for this purpose. These pores open internally are covered from out side with extremely thin layer of soil which protects the colony from attack of ants and allow the air to diffuse inside. This layer the elongated structures are able to break through.

b. The aeration complex

(1) **CO_2 concentration** : The CO_2 content of the atmosphere may have a profound influence on sporophore development. In *Agaricus bisporus* the concentration of CO_2 in the substratum of commercial compost beds rarely falls below 0.3%, about 10 times the normal atmospheric concentration and may rise to 20% or higher during the growth of the mycelium. Concentration above 1.5% stimulated stipe elongation but prevented cap expansion. Normal sporophore development occurs at about 0.2% CO_2 (Tschierpe, 1959; Tschierpe & Sinden, 1964). It has been suggested that CO_2 level within the compost would stimulate stipe elongation which would carry the cap into regions where the CO_2 concentration was sufficiently low to allow cap expansion and spore release. Expansion of the cap and release of spores within the compost would clearly be disadvantageous. These findings match well with the *Termitomyces eurhizus* as observed in nature. Inside the termite mound CO_2 concentration is supposed to be very high due to high metabolism of tiny termites as it is known that smaller the animal higher the metabolism; thus higher the consumption of oxygen (taken in through aeration shafts), higher the release of carbon dioxide

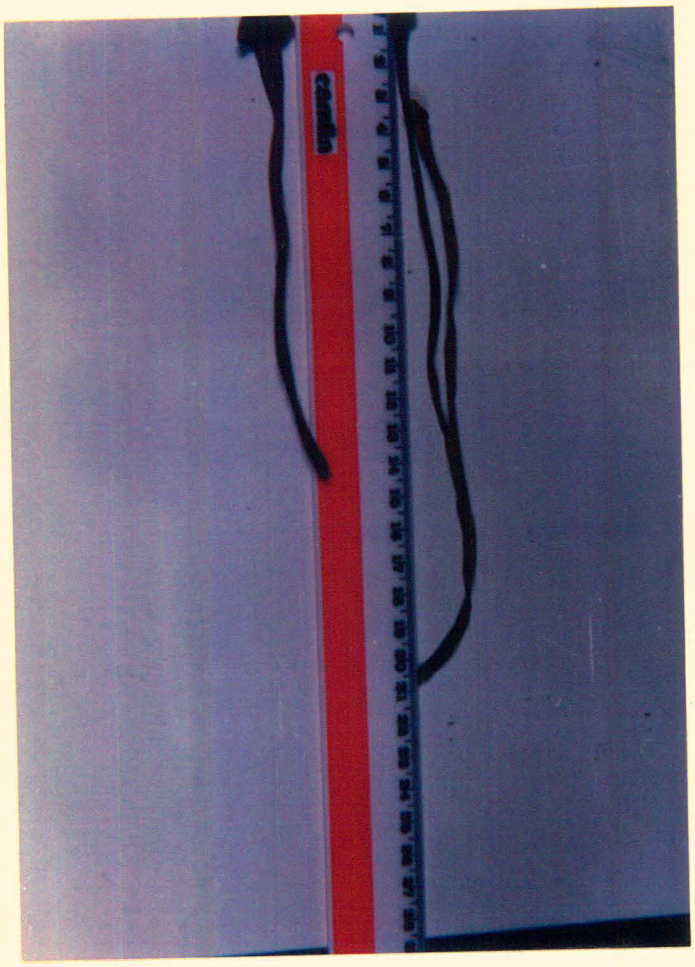


Fig.14: Showing the relationship between the fruit body and the stalk. Please note that difference in the thickness stalk above and underground.

Fig. 15: Freshly removed fruit bodies of Termitomyces from underground mound.

Fig. 16: The same as in figure 15 after two hours exposure to light. Please note the swelling of fruit body part and in some cases opening of it.

inside the mound. When size of the colony is of 90,000 tiny individuals, the input of fresh air and output of carbon dioxide can be imagined. Thus pileus which is in the form of fruit body primordia inside the mound where CO_2 concentration is supposed to be very high, starts enlarging when reaches out towards fresh air outside the mound. The presence of light and low CO_2 levels are necessary for normal sporophore development (Plunkett, 1956). Low carbon dioxide concentration for fruiting of *Termitomyces* is well in conformity with the cultivation of button mushroom where after pinning, a lot of fresh air is suddenly allowed inside the growing houses through air handling units. It is this fresh air that results in flush of buttons (mushroom.) In all probability, as CO_2 concentration is supposed to be high due to metabolism of cultured mycelia, all the previous attempts to grow this fungus in test tubes have failed. Also, ofcourse, the mysterious symbiotic (mycorrhiza) relation was not established in the test tube cultures due to our ignorance about this relation in case of various fungi as yet.

(2) **Humidity and evaporation:** In controlled conditions the rate of evaporation from developing sporophores may affect sporophore shape. In some experiments with other agarics it was found that cap diameter is reduced as transpirational water loss is decreased. Increased transpiration results in more rapid translocation rates into the fruit-body as indicated by the movement of dyes (Plunkett, 1956, 1958). Transpiration in highly humid environment (during rainy season) protects soft cells from desiccation and helps discharge spores. That is why *Termitomyces* sp. were found in nature in full growth when relative humdity in the atmosphere was reached at about 86% and temperature was optimum.

c. Temperature

There is usually a range of temperature between which sporophore development occurs. Increase of temperature within the lower part of this range hastens development. For *Coprinus cinereus* the optimum temperature for mycelial growth is about 37°C, but fruit bodies are not formed above about 30°C (Kinugawa & Furukawa, 1965). Similarly, during cultivation of *Agaricus bisporus* in controlled conditions compost bed temperature is kept at 28-29°C and to induce fruit bodies temperature is lowered suddenly to 16-18°C. The temperature inside the termite mound was 27-28°C when there were no sporophores on the

comb and the sporophore were discovered when the atmospheric temperature was 24-25°C during day time.

d. Fruit-body expansion

The rapid expansion of mushroom fruit-bodies is a well-known phenomenon. The force of expansion can be considerable. *Coprinus atramentarius* is capable of cracking asphalt paving and Buller (1931) showed that *C. sterquilinus* can lift a weight over 200 grams; many times its own weight. Studies of the growth of various agarics, e.g. *Agaricus bisporus* (Bonner et al., 1956), *C. cinereus* (Borriss, 1934) have shown that the final rapid stage of expansion is due almost entirely, if not entirely, to cell extension, i.e. of a number of cells originally laid down in the young primordium. The most rapid zone of extension is in the zone of the stipe immediately beneath the cap. Fig.1 of *Termitomyces eurhizus* shows that the same is true for this fungus too. It is clearly visible that the fungus emerges from under a depth of three feet in the soil. The soil of the mound was considerably hard and the pores built by the termites had also collapsed and mud was blocking the pores. The emerged portion of the sporophores seen are the pileus and the stipe just under pileus. There is an increase in elasticity of the stipe cells, but this seems hardly adequate to explain the 50-fold increase in growth rate measured during the final stages of stipe extension. Possibly, there is an increase in the permeability of the stipe cells to water. There is evidence that extension of the stipe is under hormonal control and that the maturing gills produce a hormone which induces cell extension. In our experiments the role of combination of various hormone like compounds in inducing the fruit bodies formation was found correct while using innovated DD media.

IV. Fungal Differentiation : Use Of DD Medium

Above mentioned findings based on studies on other Agaricales and observations in the field prompted us to to develop a suitable medium, using as test media used for cytodifferentiation of higher plants, to see the effects of different variables.

Cytodifferentiation occurs either spontaneously or under the stimulus of

specific nutritional or hormonal factors. Hence the conclusion that it is not conditioned by a single regular event. A number of chemical and physical factors have been shown to have profound effect on cytodifferentiation. All these factors affect the cytodifferentiation qualitatively and quantitatively.

The effect of chemical factors on organogenesis, especially those of phytohormones, has been studied in explant from a large number of species. The concept, as propounded by Skoog and Miller (1957), that induction of organogenesis would require, above all, the addition to culture medium of an appropriate balance of known phytohormones such as auxin and cytokinin has not proved to be so in many experimental materials. In a few cultured tissue, the endogenous regulatory complex can be adjusted to the required balance of phytohormones by an exogenous supply of auxin, cytokinin or gibberellin either separately or in combination. Generally high concentration of cytokinin brings about shoot bud initiation, whereas high levels of auxin favors rooting. Therefore, to obtain organogenesis, different permutations and combinations of hormones are supplemented in the culture medium.

Certain phenolic compounds have also been shown to induce shoot initiation on tobacco callus (Skoog and Miller, 1957.) It is mentioned that these compounds actually inactivate the auxins and consequently rise in the physiologically effective level of cytokinins which ultimately bring about the initiation of shoot buds. The use of auxin inhibitor or auxin antagonist via culture medium also cause the induction of shoot bud. Effect of phenolic compounds on fruiting body formation in wild types & mycorrhiza has been reported (Molitoris, 1979).

i. Plant Hormones

Of the many factors that influence Organogenesis *in vitro* the most important single factor seems to be the phytohormones. In their classical experiments with cultured stem pith tissue of tobacco (Skoog and Miller,1957) who demonstrated that different types of organogenesis can be achieved by varying the concentrations of auxins and cytokinin in the culture medium. When the concentrations of cytokinin are high relative to auxin, shoots are induced; when the concentrations of cytokinin are low relative to auxins, roots are reduced; and at intermediate concentrations the tissues grow as unorganized callus. This basic concept has

been used by a number of investigators to regenerate a wide variety of dicotyledonous .plants.

Auxin at low concentration stimulates xylogenesis in higher plants. There is an inverse relationship between the degree of xylem differentiation and auxin concentration. The exogenous cytokinins in combination with an auxin do markedly increase the quantity of tracheary elements. But in some cases, kinetin shows some inhibitory effect on xylogenesis e.g. *Goleus* stem callus, callus tissue of *Helianthus* and *Linum*. Therefore, the exact role of cytokinin is not clear and remains to be determined in several forms of tissue system. When this principal was applied to *Termitomyces eurhizus* it was found that interplay of both the hormones bring morphogenetical changes in finally differentiated tissues of this fungus. However, just addition of these two hormones in a particular concentration is not enough, some other factors were needed to bring morphogenetical changes in the fungus.

Gibberellin interacting with auxin is effective in cell enlargement and differentiation but not when used alone.

The sugar, particularly sucrose, in the medium is extremely essential for cytodifferentiation. Even the effect of auxin on cytodifferentiation seems to be closely dependent on the presence of sugar in the medium. Sucrose as an energy source is very important in cytodifferentiation. Concentration above 4% favored balanced cytodifferentiation.

There are few reports on effect of physical factors i.e. light, temperature etc. on differentiation of *Termitomyces* sp.

ii. Light

In general light has proved to be inhibitory in morphogenesis although, it can be replaced by cytokinin. The response to light varies depending on the nature and source of the tissue, being inhibitory in some and promotive in others. In the case of *Termitomyces* light was not required in the initial stage of fruit body primordia formation but in the later stage of maturation of sporophore light was necessary as observed in the field. This was found true in experiments with various DD media.

iii. Quality and Intensity of Light

The quality and intensity of incident light on culture may play an effective role in the promotion of organogenesis. Studies on spectral light on organogenesis reveals that the blue region of the spectrum promotes shoot formation and red light induces rooting. The treatment of blue light followed the treatment of red light also stimulates the organogenetic phenomenon. Hence the nature of organogenesis can be regulated by exposure to light of different wave length. This sort of action of light on organogenesis will help us in understanding the action of lightning on *Termitomyces* organogenesis. In some culture, artificial fluorescent light favored cytodifferentiation. Role of electromagnetic waves in the form thundering, along with lightning, reaching to termite comb remains to be established. Long back in 1932 F.H. Johnson had suspected the role of electromagnetic waves on fungi.

iv. Temperature

Most tissue cultures were grown successfully at temperatures around 25°C, but the usual environmental temperatures of the species concerned should be taken into account.

The nature of vascular differentiation is influenced by temperature conditions-whereas high temperature (35°C) proves stimulatory to xylogenesis and formation of compact wood, as in Jerusalem artichoke (*Helianthus tuberosus*), low temperature causes the development of un-differentiated new tissue. The fungus *Termitomyces* grows in tropical regions where average temperature and humidity are very high. This fact gives a valuable clue for requirement of high temperature for cytodifferentiation and when cultures were kept in different temperatures, along with many other variables in multiple numbers, results were positive.

v. Insect hormones and pheromones

During breeding season minute termites become conspicuous and enlarged due to increased activity of insect hormone(s). The pheromones of termite maintain cohesiveness among the individuals of large colony. It is very probable that

symbiosis between *Termitomyces* and *Odontotermes* could be maintained by termite pheromones and/or hormones. So far no work in this respect has been reported any where. However. Total absence of infection inside the termite mound underlines the role such elements secreted by termites play as disinfectants. They may have a yet unstudied role in differentiation & growth of fungi cultivated inside the mound by such chemical substances.

V. *Termitomyces* AND *Odontotermes* SYMBIOSIS: MYCORRHIZAL HYPOTHESIS

Mycorrhizae once thought to be exception in nature are actually the rule, with the roots of both wild and cultivated plants.

It is interesting to note that Hacskaylo (1972) has suggested that most mushrooms, including Agaricales, are ectomycorrhizal fungi and not the saprobes many consider them to be. Endomycorrhizae and ectomycorrhizae are two types of mycorrhizae and a third type that is more or less a combination of the first two.

The factors determining exactly what mycorrhizal fungus is associated with what host plant are not fully understood. Based on some previous studies on fungus growing termites, conditions under which mycorrhizae develop, the observations during extensive field visits and experiments in the laboratory it may be postulated that termites (*Odontotermes obesus*) collect spores of fungi (*Termitomyces eurhizus*) and the root tissues of a nearby deciduous woody plant and bring these together to establish a mycorrhiza like relationship between mushroom and root tissues. In this instance not the root as a whole which is typical for all mycorrhizal associations, but root cells which are some how kept alive in isolation, play a part.

Usually the insects ultimately depend upon higher plant materials for their nutrients but, lacking the ability to break these down and digest these themselves, allow fungi to do so and then feed on the fungal mycelium. When the insects move from one habitat to another the fungi are taken along by them. Obviously efficient mechanisms have been evolved by both partners in this process of symbiosis to ensure successful transmission of the fungi.

Wood, whether living, moribund, or dead, provides a favorable habitat for many insects which not only bore into it to create an abode but also utilize it as a food source. It has been pointed out that woody tissue is a particularly unpromising food source for insects in that its main components, lignin and cellulose, are resistant to degradation by insect enzymes. In addition, woody substrates are deficient in sterols and vitamins, compounds which insects cannot synthesize for themselves (Baker, 1963). The success of many wood-inhabiting insects is due to the evolution of symbioses between them and fungi inside or outside their system which enable all their nutrient requirements to be satisfied. The symbiotic fungi are sometimes housed and grow inside specialized host organs but they always remain saprotrophic and extracellular, and are never biotrophic within living host cells.

i. Deriving the facts for Mycorrhiza hypothesis fron symbiotic fungi of ambrosia beetles

Symbiotic fungi which grow on tunnel walls of ambrosia beetles or xylomycetophagous beetles (wood-borers but not wood-feeders) act as their primary food source (francke-Grosmann,1967). After pupation young adults feed for a time on the fungal layer, then cease feeding and void their digestive tracts. They pass out of the tunnels, fly to a fresh site, excavate a new tunnel and mate.

It has been categorically stated that each beetle species is associated with a particular ambrosia fungus, or particular complex of fungi, which is essential for normal development of the brood.

The beetles do not culture the fungi 'by intent', but it is likely that ambrosia fungi cannot colonize wood in the absence of their insect partners and that some will not grow in the absence of adult beetles. The simplest possible picture of nutritional behavior of the fungi in nature is one where they require lipid from insect secretions together with a suitable nitrogen source. Wood fragments impregnated with uric acid and voided by the beetles could satisfy the latter requirement in at least some fungi. Those fungi that are heterotrophic for vitamins may be supplied with these by the auxiliary fungi growing within the ambrosial mycelium.

Mycorrhizal fungi increase the solubility of minerals in the soil, improve the uptake of nutrients (nitrogen, potassium and phosphorus) for the host plant, protect the host's roots against pathogens, produce plant growth hormones and move carbohydrates from one plant to another. It appears that in return the mycorrhizal fungi obtain carbohydrates from their hosts.

Fungal symbionts also have certain nutritional characteristics that are determined by the source and derivation of their nutrients. Three modes of nutrition are possible: saprotrophic, necrotrophic and biotrophic. A single fungus may show two or even all threes kinds of nutrition during its life cycle, and changes in mode are frequently caused by changes in ecological conditions.

According to previous studies, some termites maintain fungi within their nests but it seems that these have little food value, particularly for large colonies of the insects. The fungi are certainly eaten, although this occurs relatively infrequently, and they perhaps supply vitamins or other growth factors rather than major energy-rich compounds (Grassé, 1945; Luscher, 1949, 1951; Hartzell, 1967). An additional function of these fungi might be to produce and maintain a favorable microclimate within the nest, where high humidity and high temperature are necessary. In contrast, species of a tribe of myrmicine ants, the Attini, culture saprotrophic fungi which in nature provide their sole and complete diet. The symbiosis is unique in that the ants employ a complicated procedure of husbandry to maintain and feed their fungi in specially prepared subterranean gardens within their nests (Weber, 1966, 1972a, b). The queens and their broods live in these gardens where the fungi are grown on either leaf fragments, which are cut and transported to the nests, or on particles of organic matter mixed with insect excrement showing the presence of the same factor. Those ant fungi that have so far been identified are Basidiomycotina, for example, species of *Auricularia, Agaricus* and *Lepiota,* together with, possibly the pyrenomycetous genera *Daldinia* and *Xylaria.*

Termite *Odontotermes obesus* cut the growing root hairs of near by plant, shrub, or grasses (Fig.6) and transport these to their nests along with spores of *Termitomyces* (Agaricales of Basidiomycotina) and conidia of *Xylaria.*

Ectomycorrhizal fungi when symbiotic are biotrophic but have closely related free-living saprotrophic counterparts (e.g. biotrophic *Termitomyces* with saprotrophic *Xylaria*) counterparts which inhabit the same regions of the soil, i.e. the litter or humus layers, but which never form symbioses with plant roots (Meyer, 1963).

No ant fungi can apparently exist in a free-living state outside the gardens so that their association with the ants is probably obligate (Weber, 1956a,b; 1957).

Within the gardens of any ant species, growth of only one species of fungus takes place, even though conditions in the gardens are suitable for the development of other fungi and alien spores must, in all probably be brought in continually on tissue fragments or the bodies of workers. The maintenance by the ants of a luxuriant fungal monoculture in apparantly non-sterile conditions is remarkable, but how they achieve this is not clearly understood. Foreign molds may be physically weeded out to some extent but their eradication by this method is obviously impossible. Fungistatic and bacteriostatic factors may be present in anal liquid or saliva which prevent growth of contaminants but the evidence for these is conflicting (Weber, 1957). Some species do, however, secrete phenyl acetic acid from their metapleural glands and this compound has antibiotic effects on at least some bacteria and fungi (Maschwitz, Koob and Schildkneckt, 1970). Termites were seen on the surface of the mound during dawn and dusk. It is most likely that they visit to the surface to collect spore of the fungus which had shed from the fruiting bodies of the fungus. These spores are otherwise unable to reach protected and deeply situated termite comb.

The fungi and the seeds of the shrub or the tree spread on the soil, with the means of air, water and animals. The spores germinate and give rise to mycelia. These mycelia do not grow further until they come in contact with the seed(s) of the specific plant. The seed(s) germinate and the seedling(s) grow and start developing mycorrhiza. Mycorrhiza may develop in the same year or later depending upon the plant. The termites operating under the soil find roots of these plants and cut and collect root cells in the nearby place. They start building their termitarium in the same place due to easy availability of root cells.

When termite combs were taken out from their mound and kept in open, numerous forest ants instantly appeared near the comb harboring termites. These ants were found to be in feverish search for termites. It seemed as if termites were extremely fragrant and tasty to the forest ants. One ant could captured live and running termites, alate or non-alate, and hauled them to their own nests, instantaneously. In fact, ants rob the termites and the termites were seen to be absolutely helpless. This indicated that termites *Odontotermes obesus* possess and emit pheromone like substances which are released in the environment. It seems very likely that these substances are released over the cultured spores and mycelia of *Termitomyces*. A freshly exacavated termitarium was brown-blackish in color. The color disappeared when it was kept for few hours and the termitarium was turned to copper color. It seems that some sort of volatile compound, secreted by termites, was present in the termitarium which evaporated on standing outside the mound. It is also likely that termites keep the comb moist with their salivary secretion to provide sufficient moisture to spores and mycelium for their germination and fruiting body formation.

The symbiotic fungi utilize the cellulose in the plant tissues supplied to them by the ants and provided the insects with complete carbohydrate and nitrogen-rich diet in the form of fungal cytoplasm and vacuolar sap. The ants supply nitrogen to the fungi in the from of amino acids in fecal material (Martin and Martin, 1970a). Rectal fluid, in addition to nitrogen compounds, contains proteases and these release additional nitrogen from the substrate on which the fungi are growing which the latter can then utilize.

The fungi can synthesize at least some B-group vitamins, but there is no direct evidence that they produce sterols. Not all endosymbionts are however autotrophic for vitamins and some partially fulfill their requirements for biotin or thiamine. In addition to providing vitamins and possible sterols, the endosymbionts may also play an important role in their host's nitrogen metabolism. For instance, symbiotic larvae of *S. panicea* require only arginine, leucine and threonine in their diet for normal development. Asymbiotic larvae require all essential amino acids plus glycine and tryptophan (Pant, Gupta and Nayar,1960). It is significant that many of the mycorrhizal fungi which have been isolated and grown in culture require an external supply of thiamin or of other growth-substances (Melin,1946; Melin and lindeberg, 1939; Melin and Norkrans,1942; Melin and Nyman, 1940,1941).

Possibly the most important determinant of mycorrhizal (ectomycorrhizal) structure is the species of fungus that is associated with any particular host. Since more than one fungus may be involved in mycorrhiza formation on the root system of a single tree, mycorrhizal morphology, even on an individual host, may not be constant (Meyer, 1966). Occurrence of *Termitomyces* and *Xylaria* together with root cells may be said to have mycorrhizal relationship.

Some mycorrhizal fungi produce cellulase, hemicellulase and amylase in culture (Lamb,1974). In addition some mycorrhizal fungi may provide their host with auxines, cytokinins, gibberellins and growth regulating metabolites (Slankis,1973). Gut of worker termite, *Odontotermes obesus* possesses many fungi and bacteria and many enzymes like cellulase and carboxylesterase have been isolated from mid gut of these termites (Rajgopal,Rao and Varma, 1981; Paul, Sarkar and Varma, 1986; Sreerama and Veerbhadrappa, 1991). Thus it is very likely that with help of these enzymes and their pheromones,worker termites help establishing mycorrhiza like relationship and thus mycorrhizal hypothesis gets another evidence in favor.

Roots of tree invaded by a mycorrhizal fungus may die earlier than uninfected ones (Masui, 1927). *Termitomyces* grow on dead root cells which has been digested by *Xylaria*. The disappearance of starch from the root cells has been observed in a number of examples (Burges, 1936). Similarly, in termite comb cellulose was depleted after a flush of *Termitomyces* or after formation of stromata of *Xylaria*. Carbon content was much lower than the nitrogen (low C:N) after such flushes (Batra and Batra, 1966.) Changes in the appearance and staining reactions of nuclei occur in invaded cells resembling those of the nuclei of rust-infected cells.

It is probable that root exudates play a major role through providing nutrients for fungal growth on or close to the root surface, and specific organic compounds which may enable a mycorrhizal fungus to distinguish between a potential host and a non-host species (Melin,1963). Thus this postulation that termite build their nest near those plant that give some sort of exudation (gum or resin) and cut root hair cells of these plants to prepare their nests and exudation of these plants help establishing a mycorrhiza like relationship between root tissues and *Termitomyces*, seems to be true. The cones of termite

mound were very hard and heavy. Comb particles were so tightly fixed to each other as if some strong adhesive was mixed to these particles while building the cones of the mound. It seems logical that any structure would not be so hard if water wet soil particles are kept together purely by soil adhesion. However, it may be pointed out that after heavy rain the surface of the mound changed its texture for visibly granular to smooth, indicating that the saliva of termite that was used to produce adhesive or gum like substance brought by termites from plants was water soluble to considerable extent.

The roots of both deciduous and coniferous forest trees almost invariably from mycorrhizae (Rayner,1934). Some mycorrhizae are also formed using roots of grasses. The termite mounds and deciduous trees were always found close to each other in the field. The most striking observation was that almost all mushroom producing termite mounds were found under or near the woody and thorny deciduous trees viz. Gum acacia tree, wood apple, elephant apple, Indian plum, mango and many other latex producing wild shrubs (Fig.17).

Termite tunnels were found penetrating upto the roots of these plants and the termitaria were found near the root branches. Termite combs were also found near the roots of many grasses (Fig.1 and 6).

Young lateral root are infected by way of root hairs or piliferous layer and the fungus at first actually penetrates the cortical cells to produce mycorrhizal complex (Melin, 1922; Melin, 1923). In the case of *Termitomyces*, root hairs of the plants or their very young roots are cut by termites and brought to nests where conditions for mycorrhiza are provided. Termite comb or termitaria were extremely delicate and of very light weight, like a ball made of a crumpled paper. If we prepare an artificial comb of this size using sawdust, it will have almost the same weight. Comb of the humus, of this size, will be heavier and comb of the soil very heavy.

In typical mycorrhiza, the mycelium inside the cells soon disintegrates, leaving a network of hyphae in the intercellular spaces (the Hartig net) from which hyphae grow out between the epidermal cells and form a dense mantle surrounding the rootlet. The cortical cells of the infected roots are usually enlarged as compared to those of an uninfected root of similar age. Cells belonging to the outer layers contain tannin as termites cut root tissues and store

Fig. 17: Exposed fruit bodies in a fungus garden underground. Please note the location in relation to the tree near by.

them in their nests. When we studied termite nests applying mycorrhizal techniques there were no Hartig net, as found in typical mycorrhizae.

Previous studies on mycorrhizae have revealed that infected roots may be brightly colored and are usually shorter and thicker than uninfected ones They are devoid of root hairs the function of which must necessarily be assumed by the hyphae. In Fig.18. showing an excavated termite comb attached to root of a wild shrub it can be seen that the root is bright red in color, shorter and thicker.

The fact that fruit-bodies of certain Basidiomycetes tend to be formed near trees of a particular species or group of species has long been known. The mycelium of some of these fungi is responsible for the formation of mycorrhizae. Actual organic connection between fruit-body and mycorrhiza has been demonstrated in some cases and the formation of typical association can be brought about if the tree seedlings are grown with the appropriate fungus in the soil. By such methods it has been established that many species of agarics, of *Boletus*, of subterranean Basidiomycetes such as *Rhizopogon* sp. and other truffles, form ectotrophic mycorrhizae in association with trees (Melin, 1923; Modess, 1939; Peyronel, 1921; Sappa, 1946). A particular fungus is usually associated only with a few species of trees and conversely a particular tree is associated with a limited number of species of fungus. This is what exactly has been observed for *Termitomyces eurhizus.*

ii. Conditions under which Mycorrhizae Develop

Soil conditions determine which fungus will form mycorrhizae with which particular plant and also influence the frequency of the formation of mycorrhizae. In general, Mycorrhizae are most frequent in poor, infertile soils, but where the soils are relatively fertile with adequate supplies of nitrates and ammonium salts, fewer mycorrhizae are formed (Melin, 1917).

Generally, the effects of infection are greatest when plants are grown in nutrient-poor soils; the mycorrhizal response usually being reduced or eliminated if nutrients are added to such soils. This seems to explain the observed fact that after some time when soil improves due to termite action in bringing inmeials to surface from depth with water, the termites shift their mounds to yet poorer sections.

Fig. 18: Termite comb in association with the roots of a young plant both exposed. Please note the redish colour of the plant root. R is equal to rooy, C equal to comb.

Fig. 19: Exposed fungus garden with emerging stalks. Please note the aeration shaft near by leading to termitorium.

The soil where termite mound were seen was nutrient deficient and at all the sites were found plants showing xerophytic characters such as plants with thorns, tiny leaves, dark colored leaves and glossy and single long tap root etc. Termite mounds and combs were also found in places where grasses were abundant in all cases.

Drainage also influences the development of mycorrhizae; since in poorly drained soil poor aeration prevents the growth of the fungal partner. Termite mounds were found primarily in elevated areas such as along the roadsides, elevated dividers of the agricultural lands,in vacant lots of the fields. The mountainous shape of termite mounds indicate that there are no chances of water logging on these mounds, rather it flows off from the bases of the mounds. All mounds were upto the height of two to three feet and there were drainage like tunnels surrounding the site of the termitaria (Fig.19).

VI. The Physiological Significance Of Termite-Established Mycorrhizae

The problem of the nature of the mycorrhizal relationship has attracted attention since the presence of the fungal partner with trees and shrubs etc. was first demonstrated. It is usually considered to be an example of symbiosis in which the activities of either partner benefit the other. The higher plant obviously benefits from the presence of the fungus in most of the examples which have been investigated. Seeds of many orchids either fail to germinate or produce abnormal seedlings in the absence of the mycorrhizal fungus.

It is probable that the actual benefit obtained by the higher plant is a combination of a number of factors, such as the possible ability of the fungus to fix atmospheric nitrogen, the breaking down of the organic nitrogenous compounds present in humus into a from available to the plant (Melin, 1917; Melin, 1923), the efficiency of the mycelial mantle in the absorption of mineral salts (Stahl,1900), which is correlated with the increased absorbing area of the infected, compared with uninfected, roots (Hatch,1937) and/or by the probable synthesis of growth-substances or other organic compounds by the fungus or by the inactivation of plant-retarding substances in the soil.

Further studies of the physiology of the fungal partner and of its effect on germination of seeds and subsequent seedling growth are obviously needed before a complete explanation of the relationship is possible.

It was observed by us in the field that there was an almost complete colony of termites mostly with young ones in the termitarium, before the formation of fruit body primordia of mushrooms. The number of adult termites was seen reduced in the termitarium when luxuriant growth of fruit bodies of the fungi had taken place. It appeared, as if most of the termites have migrated somewhere leaving behind the maturing sporophores on the comb.

The fruiting bodies of the fungus were collected by us after lightning and thunder during rain and the most interesting folklore in this respect was noted that after lightning these fruit bodies suddenly appear like water bubbles on the mound. The fruiting bodies were not found after about two months of rain when intensity of thundering and lightning becomes weak.

VII. Stains, Macrochemical Colour Reactions And Chemical Analysis

Absorption of specific dyes is not a direct expression of the chemical constitution of the various parts of the plant tissue. Yet in certain cases the absorption of the dye is different in different organs and different in different parts of hyphae or sporal walls, etc. This so-called metachromasy is not the same in the same organs of all Agaricales and Kuhner, Singer and Heim have used this fact as the basis of taxonomic as well as organographic differentiations, i.e. for the characterization of groups in the classification of the Agaricales and for the characterization of certain specific types of organs. These metachromatic colorations like the chemical reactions which also have been introduced into agaricology in recent years, are only characters and are not pretended to be more than that.

It is, probable, or almost certain, that the amyloidity of spore ornamentations or spore walls is not based on the presence of the same substances in all cases where a "positive" reaction with an iodine reagent is obtained.

Yet, if an argument concerning a taxonomic question based on chemical characters is only one part of a series of reasons that support, for example, the affinity of two taxonomical groups, the chemical character should not be disregarded on theoretical grounds. The overemphasis put on a chemical character alone is not justifiable even if the chemical identity of the reactions in each case could be demonstrated by analytical means. In most cases color, odor, taste and gelatinosity are characters without a fully explored chemical basis and morphology alone dose not provide complete guidance either. It is therefore necessary to use, with the utmost caution but without blind reluctance, all available characters, the more the better.

Metachromatic stains

Cresyl blue mounts of spores of some genera allow the observation of the endosporia because of a selective coloration that results from ultrafiltration of the dye solution by the apisporium in such a manner that the endosporium is dyed reddish and therefore stands out enough to be rather conspicuous even in cases where it is not very strongly developed. In other genera of the Agaricaceae, the endosporium whether strongly developed or not does not show such an effect in cresyl blue mounts. This differentiation has been shown to be of great help in the subdivision of this family, as an additional spore character to be used together with the iodine reaction and the germ pore.

In species of *Termitomyces* the hyphae of the stipe become red, thus contrasting with the normal coloration of hyphal walls with cresyl blue which is pale violet, pale blue pale violet, pale, or practically nil (Fig.20, 21 and 22). The cortical layer of the stipe should be disregarded for this purpose.

The hyphe of *Termitomyces eurhizus* taken from the basal part of the fruiting body stained blue with Trypton Blue (0.01%) and mounted with lactophenol whereas hyphae of upper regions of the fruiting body were stainless. Trypton Blue stains hyphae of mycorrhizal fungi blue thus giving another confirmation for mycorrhizal nature of *Termitomyces eurhizus*.

Similarly, when this Trypton Blue was applied to tuber like mushroom collected from near the root of *Jasminum arborescens* in JNU campus, New Delhi it also gave blue color as it gives for any other mycorrhizal fungi (Fig.23).

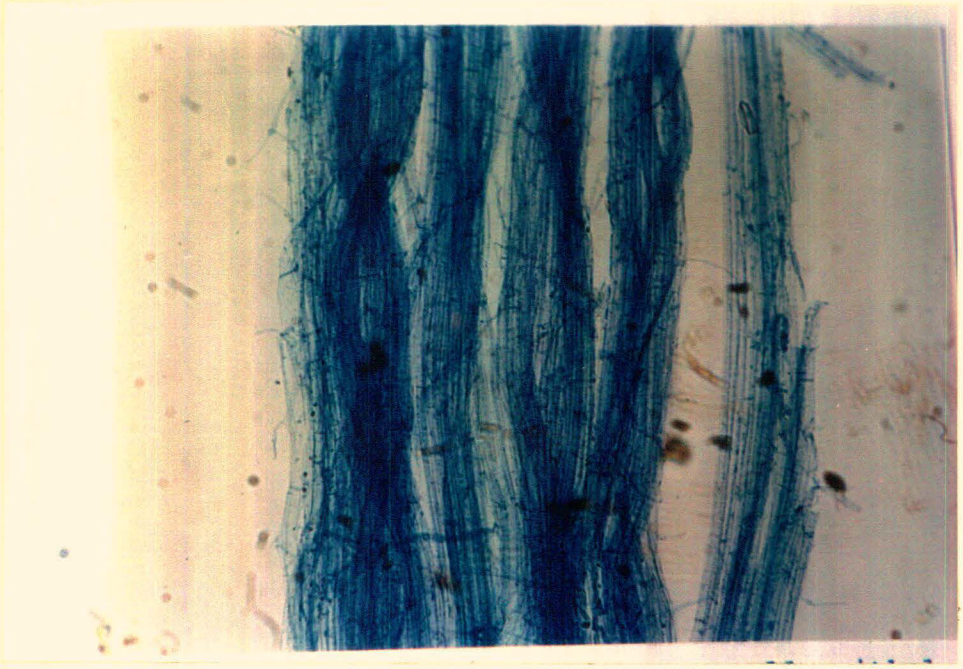


Fig. 20: Blue stained hyphae showing septate cells with large amount of weakly stained bodies (stained with cresyl blue).

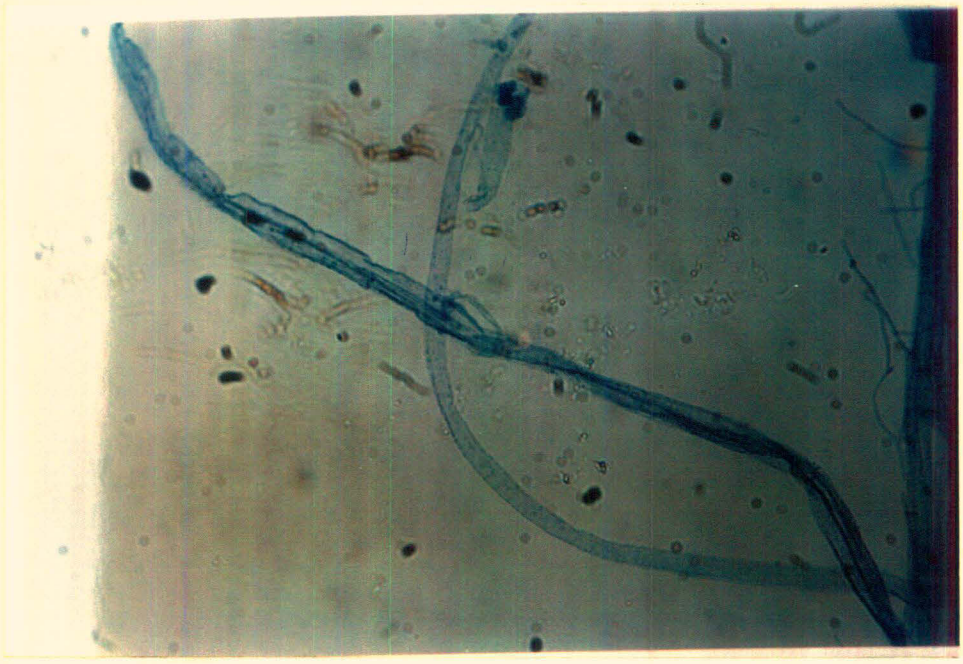


Fig. 21: The same as in Fig.20 with two hyphae separated from the mass.

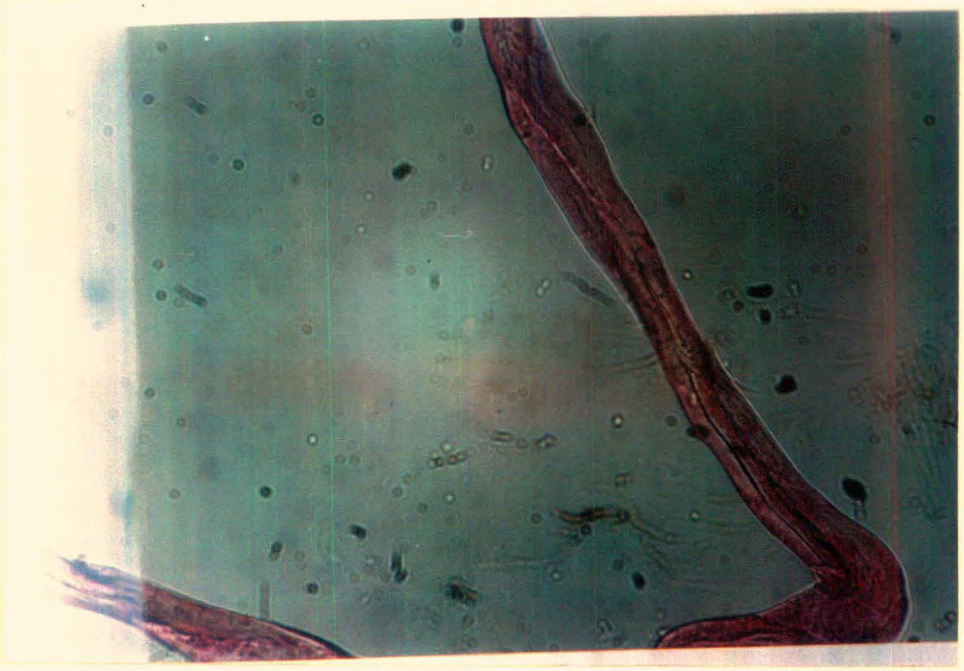


Fig. 22: The same as hyphae in cresyl blue showing change of colour in basal part, colour in the stalk strips.

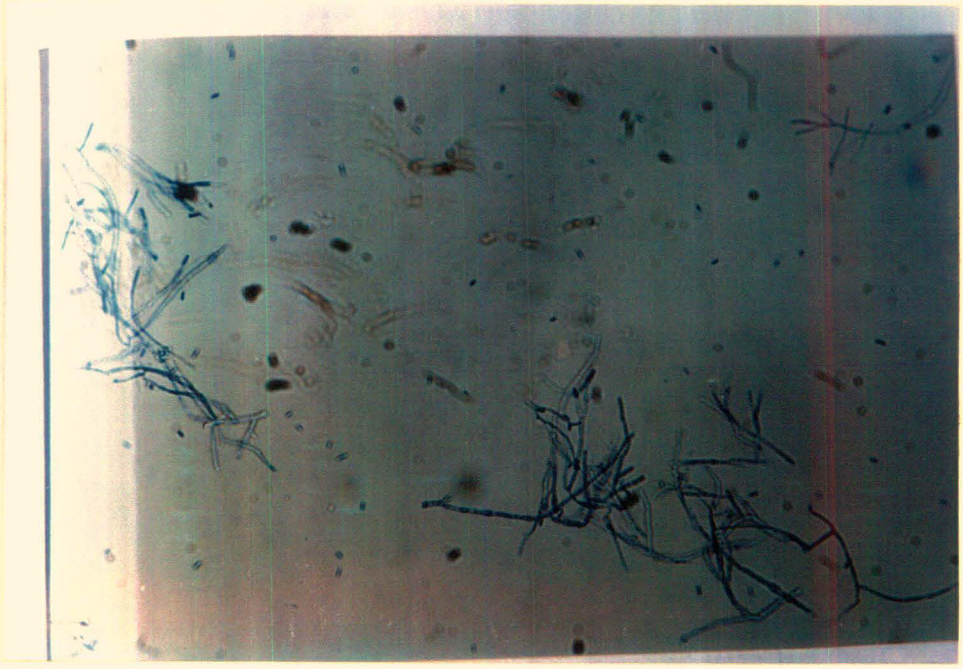


Fig.23: Tryptan blue stain in case of tube like undifferentiated mushroom showing mycorrhizal nature.

For routine preparations of an unknown agaric, it is customary to use ammonia-mounts; first without any dye. It is not wise to start the study of a species with stained material. Only as a second step, in order to get clearer pictures, the same preparation may be dyed with phloxine 2 % alcoholic solution, which is stable in ammonia or even KOH mounts. Phloxine is, however, taken up by the interior of the hyphae more than by the walls and for the walls cresyl blue is as good as any other dye for a first try. During ammonia-mounts KOH is preferred tough species like *Termitomyces* whose dense tissue are not mercerized easily by NH_4OH. However, for a study of fine structure such as diverticulation of epicuticular hyphae, pigment encrustations and spore ornamentations, KOH is inferior to NH_4OH.

VIII. Taxonomy

The Basidiomycetes differ from all other fungi in that they produce their spores, called basidiospores, on the outside of a specialized, spore producing structure, the basidium. Basidiospores are generally uninucleate and haploid, although binucleate, homokaryotic basidiospores do not appear to be uncommon. Basidiospores are the result of plasmogamy, karyogamy and meiosis, the last two of which occur in the basidium. Thus, a definite number of basidiospores (usually four) is typically produced on each basidium.

Basidiomycetes are extremely valuable in nature, forming mycorrhizal relationships with both cultivated and noncultivated plants.

Somatic Structures:

The mycelium of the Basidiomycetes consists of well-developed, septate hyphae (Fig.22) that penetrate the substratum and absorb nourishment.

The mycelium of most Basidiomycetes passes through three distinct stages of development-the primary, the secondary and the tertiary-before the fungus completes its life cycle.

Although not applicable in all groups, microscopic analysis of the hyphal types comprising basidiocarps has become an important means of identifying fungi and establishing relationships among different species. This approach is referred to as the mitic system

Both spore shape and color, as well as spore surface markings, are important taxonomic characteristics in some groups of Basidiomycetes, e.g. the Agaricales.

After applying staining techniques for the purpose of taxonomy, following keys were followed for conformation of our collectedspecies which was originaly identified by Dr. A. K. Sarbhoy, Curator, Mycology Museum, Indian Agricultural Research Institute, New Delhi.

Agaricales - a general account

The largest group of the basidiomycetes is Hymenomycetes which includes many of the well-known toadstools, bracket fungi, fairy clubs, jelly fungi and the like. The basidia in their case are often arranged in a palisade-like fashion to form a hymenium witch is fully exposed at maturity as against Gasteromycetes where it is enclosed. Three large orders have been distinguished : Agaricales, Aphyllophorales and Tulasnellales. The first two orders have holobasidia whilst the Tulasnellales have heterobasidia (the group is sometimes termed as Heterobasidiae). An important distinction between the Agaricales and Aphyllophorales is that the fruit bodies of the Agaricales are fleshy, being usually composed of thin-walled hyphae which inflate. Such construction is termed monomitic. In contrast the fruit bodies of many Aphyllophorales are often more complex, being composed of thin-walled generative hyphae, which may be accompanied by either thick-walled unbranched skeletal hyphae, or by thick-walled much-branched binding hyphae or both. Their construction may thus be monomitic, dimitic or trimitic.

In many Agaricales the developing hymenophore is surrounded by one or more veils, but these are not present in the Aphyllophorales. A further distinction is that in the Agaricales the nuclear spindles in the basidia are transverse (chiastobasidia) whilst in the Aphyllophorales from with longitudinally orientated spindles (stichobasidial) occur.

Traditionally all the gill-bearing Hymenomycetes were placed in single family, the Agaricaceae.However, modern taxonomic treatments have resulted in their subdivision into more homogeneous families. Agarics are mostly

saprophytic and many agarics form mycorrhiza with forest trees (Harly, 1969). Most of the fleshy forms are edible and some are specially cultivated for food through some are poisonous whilst other have hallucinogenic properties (Heim & Wasson, 1958; Singer, 1962; Heim, 1963).

Fruit-bodies of the agarics arise from a dikaryotic mycelium which may be short-lived or perennial. In some cases the individual hyphae may be aggregated into mycelial strands or rhizomorphs. The general from of an agaric fruit body is umbrella-shaped, with a central stalk or stipe, supporting a cap or pileus with numerous radially arranged gill or lamellae on the lower side of the cap. The hymenium covers the face of the gills or line the tubes. The young fruit-body is enclosed in a mass of tissue called the universal veil which is broken as the pileus expands leaving a basal cup-like vulva and sometimes scales on the pileus . In some agarics the hymenium may be protected by a partial veil stretching from the edge of the cap to the stem. The arranangement of the veils reflects the development of the fruit-body.

The tissue of the sporophore in the Agaricales is psedoparenchymatous, consisting of an aggregation of hyphae. The hyphae are usually thin walled and dikaryotic (generative hyphae) and may or may not bear clamp connections. The fruit-body expands due to inflation of the cells. Two main types of gill structure have been distinguished: Aequi-hymeniiferous and Inaequi-hymeniiferous.

The Agaricales have basidiocarps which are fleshy to subfleshy or rarely, almost leathery (if latter, the hymenophore is not poroid). In small basidiocarps the texture maybe membranous, pliant, or fragile. The hymenophore is smooth (rarely) to lamellate (typically) or poroid (less frequently). The basidia are two- to four- to eight-spored and one-celled at maturity. The spores are forcibly discharged from the sterigmata at maturity.

IX. Termitophilic Fungi - *Termitomyces*

Heim (1942) gave following characters of Termitophilic fungi, *Termitomyces* sp. which are found in their natural habitats of Tropics of Asia, Africa and the South Pacific.

Type species: *Termitomyces striatus* (Beeli) Heim. (Syn.: Rajapa *sing.)*.

Charecters: Habit of the carpophores collybioid to pluteoid; usually rather fleshy and large; with prominent, often very sharply differentiated umbo; stipe central; spore print pink; lamellae free to almost adnate but emarginate or with decurrent tooth; stipe with a pseudorrhiza and with a simple or double veil, or evelate. Epicutis (excepting the region on and near the umbo in certain species) consisting of repent filamentous, hyaline hyphae; hymenophoral trama bilateral in the primordium, often remaining so for a relatively long time, but in adult specimens regular and consisting of parallel, thin-filamentous hyphae; spores hyaline, inamyloid, with a hilum of the open-pore type, ellipsoid, smooth, with homogenous wall; basidia normal; cystidia present; tramal hyphae inamyloid, without clamp connections, not gelatinized. Tramal system strictly monomitic. the primordia develop in the holes of termite nests.

He described following species found in different natural habitats-

1. African species:
T. citriophyllus Heim; *T. fuliginosus* Heim (perhaps a variety of the following); *T. robustus* (Beeli) Heim; *T. striatus* (Beeli) Heim (perhaps a variety of *T. schimperi* (Pat.) Heim; *T. congolensis* (Beeli) sing. (perhaps a variety of *T. letestui* or *T. schimperi*); obviously also *T. globulus* Heim & Gooss. and *T. clypeatus* Heim.

2. Asiatic species (possibly all identical with each other):
T. eurhizus (Berk.) Heim; T. cartilagineus (Berk.) Heim; and the following species (types seen); *Flammula janseana* Henn. & Nym; *Flammula filipendula* Henn. & Nym., obviously also *Agaricus (Pluteus) rajap* Holtermann.

Singer (1945) also studied termitophilic fungi in their natural habitats - Tropical areas (Africa and Asia) and also in North America and gave the term *Podabrella* Sing.:
PODABRELLA Sing.

Type species: *Collybia microcarpa* (Berk. & Br.) Hoehnel senus Hoehnel, (Syn.: Termitomyces subgen. *Praetermitomyces* Heim.).

Characters: Habit of carpophores mycenoid-collybioid; pileus with an epicutis consisting of thin, repent, parallel, hyaline, smooth, filiform hyphae; hypodermium consisting of somewhat thicker hyphae which are contracted at the septa, likewise hyaline (the whole fungus with very little or no pigment) and elongate; lamellae subfree to adnate, thin, intermixed with lamellulae; spore print pale rose color in the type species; spores hyaline under the microscope, smooth, rather thin-walled, inamyloid, ellipsoid to ovoid; basidia rather small, normal in all regards,4-spored; cystidia usually none; edge of the lamellae homomorphous (or with spares cheilocystidia); trama of the hymenophore regular; stipe solid, rather thin, without distinct pseudorrhiza, rather soft, rising from small globulose white bodies which are ejected from termite nests by the termites [these bodies represent the primordium of the species("mycotetes", according to Heim)] context fleshy, unchanging, consisting of hyphae which are devoid of clamp connections, nonamyloid.

P. microcarpa is most probably hemi-angiocarpous, certainly not gymnocarpous according to Heim whose observations are interpreted by Reijnder as showing bivelangiocarpous development.

Species described by Singer (1945) are as follows:

P. microcarpa (Berk. & Br.) sing. (Entoloma Sacc; Collybia, Hohnel; Gymnopus, Van Overeem; Termitomyces, Heim); possibly also here: *P.alba* (Peck) Sing. (Collybia, Peck), see above.

Singer's State of knowledge on *Podabrella* : The type species is fully known. A second species has been admitted, with certain reservations, by Singer, but this, *P. alba* (Peck) Sing. is not known with regard to several characters essential for its final disposition. The spore print of this would hold true for more numerous observations and for thicker layers of the spore mass. Furthermore, it is not known whether this species is ectomycorrhizal and what the development of the carpophores is. If the spore print is never pink and the development not hemiangiocarpous, this species might either enter section *Adusta* of *Tricholoma* (if ectomycorrhizal) or else enter the genus *Gerronema* where it would be out of place because of the non-decurrent lamellae; it would also be out of place in *Pleurocollybia* since the structure of the cutis of the pileus appears to be somewhat different and the spore too large. In either case, an emendation of the diagnosis of the respective genus would necessary.

Characters: Habit of carpophores mycenoid-collybioid; pileus with an epicutis consisting of thin, repent, parallel, hyaline, smooth, filiform hyphae; hypodermium consisting of somewhat thicker hyphae which are contracted at the septa, likewise hyaline (the whole fungus with very little or no pigment) and elongate; lamellae subfree to adnate, thin, intermixed with lamellulae; spore print pale rose color in the type species; spores hyaline under the microscope, smooth, rather thin-walled, inamyloid, ellipsoid to ovoid; basidia rather small, normal in all regards,4-spored; cystidia usually none; edge of the lamellae homomorphous (or with spares cheilocystidia); trama of the hymenophore regular; stipe solid, rather thin, without distinct pseudorrhiza, rather soft, rising from small globulose white bodies which are ejected from termite nests by the termites [these bodies represent the primordium of the species("mycotetes", according to Heim)] context fleshy, unchanging, consisting of hyphae which are devoid of clamp connections, nonamyloid.

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X. Limitations To Identification Of *Termitomyces*:

Heim considers *Podabrella* a subgenus of *Termitomyces*. The two genera are undoubtedly closely related. However, in *Podabrella* (e.g. *Praetermitomyces* Heim) the primordia do not develop within the termite nests and the carpophores are devoid of pseudorrhiza and veil; the epicutis of the pileus is always and in all parts a cutis rather than a trichodermial palisade or a hymeni-from structure; the pigment is rather scanty or absent in the carpophores and the latter are much smaller than in the species of *Termitomyces* proper. Horak (1968) on the other hand, wishes to leave the possibility open that Rajapa, based on *Agaricus eurhizus* Berk. is not congeneric with *Termitomyces*. Young specimens of *T. eurhizus* seemed to me to maintain a bilateral or subbilateral structure longer than other species of the genus, e.g. *T. cartilagineus*, but *T. congolensis* showed a more distinct bilateral trend as well as *T. letestui*, whereas different material of *T. eurhizus* gave different results. There is apparently as much variability as there is in other Tricholomataceae with slightly bilateral trama (e.g. *Flammulina*) and since the character appears to be somewhat variable as soon as the primordial stage is passed, Singer (1945) considered that the genus *Termitomyces* can be divided into two according to bilaterality. On the other hand, the type of bilaterality often encountered in *Termitomyces* is of an entirely different kind as compared with that of *Amanita* and is also not comparable with that of *Limacella*; thus, there is no reason why the genus should be maintained in the Amanitaceae. However, the position of *Termitomyces* is still not finally settled. More cytological and EM data on the spores are in order inasmuch as Pegler & Young have found that the hilum is of the open-pore-type, a rather unusual feature in the Clitocybeae, inasmuch as the Rhodoteae have likewise pinkish spore print and bilateral hymenophoral trama but a spore hilum of the nodulose type. For these reasons, the *Termitomycetinae* are here maintained as an "ad int." taxon.

On the bases of following findings, the so called *Termitomyces eurhizus* seems to be a transitory species between *Termitomyces* and *Podabrella*:

1. Spore print white spores pink; (In *Podabrella* spore print is rose in color and spores are hyaline under the microscope; In *Termitomyces* spore print is white as in fig.24 and spores are pink as in fig.25). Spore hilum is of open pore type similar to *Termitomyces* (see fig.25).

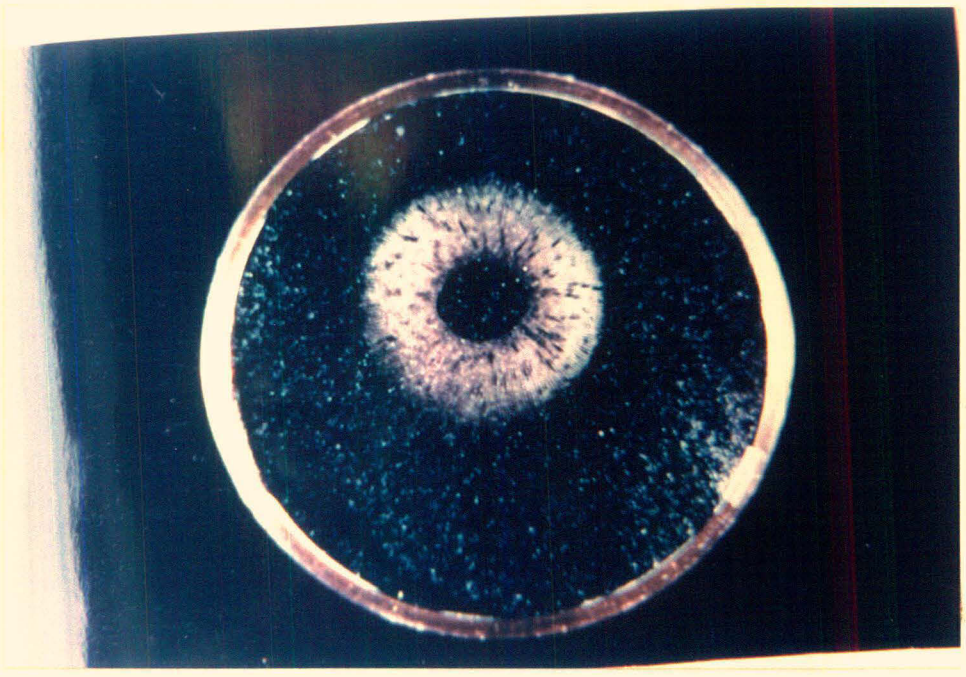

Fig. 24: White spore print of Termitomyces on black paper showing pink coloured spores in the white back ground.

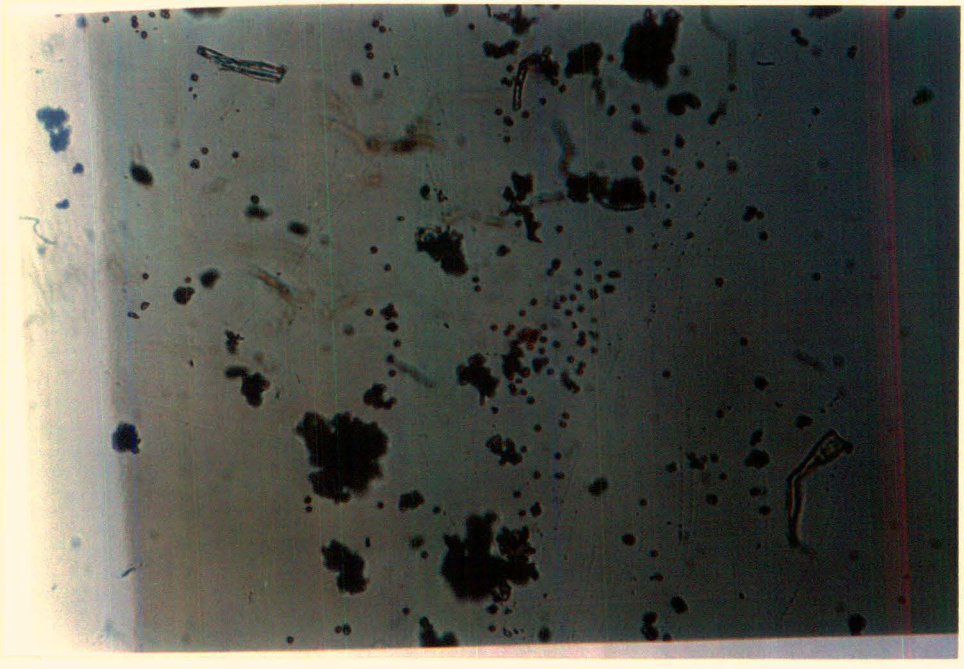

Fig. 25: Pink spore colour of the Termitomyces spores enlarged.

2. Fruit body primordia do not develop within the termite mound, carpophores are with pseudorrhiza; (In *Podabrella* the primordia do not develop in the termite nests and carpophores are devoid of pseudorrhiza). Thus here it shows similarity, in part, with *Podabrella* (Fig.1).

Moreover, affinity with *Agaricus* can also not be ruled out.Thus, it is high time to give a new nomenclature to this fungi.

3. Even Singer (1945) did not know whether his *Podabrella* (syn. *Termitomyces*) is ectomycorrhizal. However, on the bases of previous studies, our field observations and few experiments we feel that this particular fungus, transitory between *Termitomyces* and *Podabrella*, possesses mycorrhiza like features with root cells in the nests of termites.

Based upon spore print, spore color, fruit body primordia characteristics, mycorrhizal analysis we also feel that *Termitomyces* sp could be varients of *Agaricus bisporus* which when captured by termites grow as *Termitomyces* in dark termite mound with certain factors provided by the termites and some unknown mycorrhiza like complexes involved with it!

XI. Significance Of Termitophilic Fungi

When Heim (1942) restudied this relationship, he came to the conclusion that the termites are compelled to rid their nests of this fungus and use it only occasionally for food; the larvae are not fed on *Termitomyces*, at all. The practical importance of these fungi consists therefore exclusively in their value for human consumption. On the other hand, Singer (1945) who identified this fungus as *Podabrella* believed in it's role in the biology of many termite species.

In our opinion, termitophilic fungi might have following functions in their subterranean habitat:

These are either necessary for mycorrhizae formation with the seedlings of those wild plants near which termite colonies are found, to help them grow, which in turn may be used for termites' meal; OR

Termites grow these fungi to feed their young ones; OR

Termites grow these fungi to protect, in some way, the lives of all individuals of the colony during rainy seasons in Tropical areas ! Protection may be in the form of excess moisture absorbption by these fungi from termite comb which may otherwise become extra soft and broken in rainy season due to high atmospheric moisture!

CONCLUSIONS

1. With large volume of Ethnobotanical data generated over the past more than a century, it is important that folk-lore and tribal claims regarding various plants are experimentally verified and observations interpretted to seperate facts from fiction in order to facilitate rational utilisation of Ethnobotanical data for national development. In total absence of *Ethnomycological work in Bundelkhand region, ours is the first attempt to study termite related fungi of the region and its in vitro culture under controlled conditions.*

2. Large volume of prejudices regarding edibility or otherwise of fungi exists between the tribals on the one hand and the non- tribal rural and urban population on the other which can be understood only on the basis of their emperical experience and knowledge base regarding fungi as such. It is also seen that popular perception that the termites spoil the soil and should be killed if present, is not based on observed facts and literature. *It is suggested that the termites in fact. slowly improve the soil and when the soil is improved, the termites move to another spoy with relatively poor soil.*

3. *It is shown that weak brine solution with apropriate storage conditions preserves the collected speciemens and, from this tissue, new de-differentiated culture of mycelium can be obtained in laboratory conditions.*

4. It is shown that *in vitro* culture of termite-associated fungi under aseptic conditions is possible though due to problems related to constant and continuous supply of fresh air, maintanence of apropriate oxygen-carbondioxide gradient, constant temperature and light-dark balance and culturing of termites in lab conditions for periods long enough to complete the full cycle of the fungi from spore to spore it has not been possible to obtain fruiting bodies in.vitro by us as well as anywhere in the world.

5. *We are of the opinion that in nature Termite-associated fungi grow after establishing mycorrhizal complexes. In absence of this complex it is conceptually not possible to cultivate the fungus till maturity and, we think, this absence of mycorrhizal complex is an important factor in failures to domesticate termite-associated fungi.*

6. *With the help of some synthetic media it is possible to cultivate termite-associated fungi in vitro at least up to mycelial stage, opening the way for further detailed studies on the basis of readily available live material.*

7. Our observations in nature suggest that the folk-lore about the emergence of termite-related fungi is substantially correct; other claims of diverse nature yet need verification under defined conditions.

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