BIOLOGY OF TERMITOMYCES SPECIES AND STANDARDISATION OF ITS CULTIVATION TECHNIQUES

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "Biology of *Termitomyces* species and standardisation of its cultivation techniques" is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Vellayani,

20 March 1989.

CERTIFICATE

Certified that this thesis entitled "Biology of *Termitomyces* species and standardisation of its cultivation techniques" is a record of research work done independently by Smt. SREELATHA NAIR. G.S. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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INTRODUCTION

INTRODUCTION

The interaction and interdependence between termite fungus mutualism and the fungus gardens have fascinated biologists through out the world. But due to the difficulty of working with these termite growing fungus information regarding their biology remain largely anecdotal and fragmentry till today though konig (1779) made the first attempt to study the details of this mycosymbionts.

The warm humid tropical climatic conditions and diversity in soils of Kerala supports the growth and occurrence of wide variety of mushroom flora, of which many are edible. It is interesting to note that the genus Termitomyces is among the most highly prized and sought after mushroom that occur luxuriantly during the monsoon periods through out the State. But knowledge of these mutalistic macrofungi are surprisingly meagre and inadequate to facilitate their largescale production by artificial methods. No basic systematic study has been carried out in Kerala to record their basidiome macromorphology, ecology, symbiosis and proper utilization of these

esculent species. Considering these points, the present study was undertaken on the following aspects.

1. Collection and identification of different species of Termitomyces from different parts of Kerala and study on their natural distribution.
2. Detailed study on the morphology and developmental morphology of different species
3. Studies on the ecology of the locally available species and the symbiotic relationship with termites.
4. Cultural and physiological studies of different species.
5. Comparative study on the nutritive value of different edible species.
6. Studies on post harvest processing for preservation of different species.
7. Trials on artificial cultivation of promising species utilizing various organic substrates.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The occurrence of fungi in termite nests was first recorded by the German naturalist König (1779) at Tanjore in South India. He observed inside termite nests, brain shaped formations of a few centimeters diameter, which he called 'Mushroom garden Comb' or Nursery and believed the fungus as the food of young termites. Smeathman (1791) published an account of his investigations of termite nests in West Africa, where he referred the combs as Nurseries. He found that the combs were sprinkled with small white globules, about the size of Pin head which he later identified as the young stage of a species of mushroom.

More than half a century later Berkeley (1846) reported about a gill agaric, found among specimens received by him from Ceylon which was said to grow from about four feet below the surface of earth, from the termite combs. This was the common termite agaric which was eaten by natives in all the countries where it occurs which he named as Lentinus cartilagenous Berk. Berkeley (1862) again reported in the "Transactions of the Linnean society" a sclerotium from India under the name of Sclerotum stipitatum Berk and Curr., formerly named by him as Agaricus termitegena Berk, which was later identified

as Lentinus cartilagensus similar to the gill agaric obtained from Ceylon. Gibbon (1874) collected few mushrooms from Gorakhpur, India about fourteen inches in length, from the centre of the white ant hillock. He studied in detail, its habitat as well as development and observed that they appeared to emerge from a peculiar substance always found in ant-hills and which generally was taken for the ant's food. He believed that it is this substance under the combined influence of heat, dampness and darkness which make the mushrooms grow. Later those mushrooms were identified as Lentinus cartilaginaus. Möller (1893) worked on fungal gardens of leaf cutting ants which paved the way for understanding the real nature of mushroom culture in termite gardens. Holtermann (1898) reported an agaric growing upon termite comb in Ceylon, Java, Singapore and Borneo which resembled Lentinus cartilaginaus. However, he called the new species as Pluteus raiapa Holterman which was later found identical with the species Raiapa curhizus (Berk.) Sing reported from West Bengal, India.

Patouillard (1898) reported the same fungi as a termite agaric under the name Collybia radicate Pan non Rehl. He pointed out that a sponge like mass was attached

to the base of his specimen, confirming it to be a termite agaric. Hennings and Nymann (1899) redescribed Holtermann's agaric as Pholiota janseana Henn and Nymann and subsequently as Flammula janseana knowing that it was the same species. He also described the species differently as Flammula filipendula Henn and Nym., Pluteus treubianus and Pluteus bogoriensis Henn and Nym believing that the three specimens examined belonged to different species.

Karawaiew (1901) gave an account of the white spheres formed on a termite comb at Buitenzorg. Subsequent descriptions of similar fungi by several authors created a number of synonym. Hennigs (1904) while describing the fungus flora of South America, described an agaric Pluteus terrítus Henn. from termite nests in Brazil; which resembled the termite agaric Lentinus cartilagineus Berk. Tragärdh (1904) published an account of fungus growing termites in Sudan., where he gave a precise description of the "white spheres". Petch (1906) described Collybia albuminosa (Berk.) growing from the nests of various Odontotermes species in Ceylon. He also noted the association between the small mushroom Entoloma microcarpum Berk. growing from the nests of various Odontotermes species in Ceylon and also noted the

association between the small mushroom Entoloma microcarpum Berk. and Broome and the termites. Weiss (1906) from East Africa observed that the agarics on termite nests are cultivated by termites for their food. Jumelle and Perrier de la Bathie (1907) studied in detail the fungi in termite nests in Madagascar. They reported that termite combs bear white conidial spheres, upto one mm diameter and that these spheres arise from a mycelium which permeates the substance of the comb and runs over the entire surface of it.

Bose (1923) isolated Xylaria nigripes, Coprinus species and a species of Termitomyces from Indian Termitaria. Bottomley and Fuller (1923) observed the fructification of Entoloma microcarpum Berk. Broome, developing on fragments of combs which was brought up out of the nests by Odontotermes species during the rainy season in South Africa. Bethellier (1927) showed that the white nodules, which are normally made up of conidiophores and conidia on the fungus comb, are also the primordia of the agaric phase. Butler and Bisby (1931) studied Termitomyces striatus (Beeli) Heim. from West Bengal. Grasse (1937) found that the mycelium which permeates the entire comb, is mixed with finer hyphae of Xylaria species, generally considered to be saprophytic rather than symbiotic.

Bose and Bose (1940) gave an account of 20 edible mushroom species including Agaricus campestris, L. ex Fr., Coprinus comatus (Mull. ex Fr.) S.F. Gray, Termitomyces microcarpus Berk. Br. and Termitomyces albuminosus Berk. and Heim. The final break through in the study of termite fungi came with the work of Heim (1942) who created the genus Termitomyces for all the basidiomycetes symbiotic fungi on the combs of Macrotermitinae and described a number of species in the group associating them with their termite hosts, wherever possible. He also divided Termitomyces into two subgenera: Praetermitomyce for the single species Entoloma microcarpum (Berk. Br.) Heim and Eutermitomyces for the remaining species which fructify at the tips of long stipes or "Pseudorhiza" that grow out from the fungus combs, through the mounds or soil above the nest. The central part of the cap of some of these larger species is hardened, thickened and to some extent pointed as an adaptation to penetrate the soil. This structure termed as "perforatorium" by Heim is taxonomically important in the group.

Cheo (1942) established that the conidial stage described as Agarita duthei Berk. was infact very similar to the character of Termitomyces albuminosus (Berk.) Heim.

Grasse, and Heim (1950) described Termitomyces medius as a species somewhat intermediate to the two subgenera, although it is classed as Eutermitomyces. Heim (1952) considered that the size and form of the fungi are to some extent influenced by the size and position of the combs and the variations between species arise partly from differing stages of adaptation to the 'Cavernulous' condition. He observed that Odontotermes species of termites appeared to have adopted to some change in Termitomyces microcarpus. In this case, during rainy season when the fungus is about to fructify, the termites habitually shave away the outer layer of its fungus combs and spread them on the surface of the soil above the nest where the fructification occurs. He also described a new species in the group, viz., Termitomyces microcarpus f. sp. santalensis from Santal in Bihar.

Chopra and Chopra (1955) in their review of work on Indian medicinal plants mentioned the edible species of Mushrooms like Termitomyces albuminosus (Berk.) Heim. Harris (1961) reported that intensified foraging in the area where basidiospores had recently been discharged would result in reinoculation of new combs. Singer (1961) observed the development of Termitomyces to be Hemiangiocarpic. Pegler (1962) while describing the agaric

flora of East Africa had prepared an exhaustive key to the East African species of Termitomyces following the classification adopted by Heim (1967) described in detail the morphological characters of the species identified by him as associated with termites. He described in detail the morphology of eleven important species of Termitomyces. Purkayastha and Chandra (1975) collected Termitomyces eurhizus (Berk.) Heim for the first time in India from West Bengal. Natarajan (1975) described four species of Termitomyces ie., Termitomyces badius Otino, Termitomyces clypeatus Heim, Termitomyces microcarpus (Berk. and Br.) Heim and a new species of Termitomyces indicus Natarajan sp. nov. Natarajan (1977) also recorded a new species of Termitomyces from Jammu viz., Termitomyces radicatus Natarajan sp. nov. Batra and Batra (1979) while reviewing past taxonomic classification of termite fungi listed out thirty two species of Termitomyces associating them with their respective symbionts. Zoberi (1972) described in detail eleven species of Termitomyces found in Africa and Asia Natarajan (1979) again described another new species of Termitomyces, viz., Termitomyces heimii sp. nov. from Chepauk, Madras Pegler and Piearce (1981) gave an account of six popular edible

mushrooms of Zambia including a new species, Termitomyces titanicus Pegler and Piarco sp. nov. This species is reported to be the one producing the largest known basidiocarp, with pileus measuring sixty three cm to one metre in diameter. Chakkaravarthy [illegible] and Khatva (1979) described Termitomyces microcarpus as new Indian edible mushroom.

Bhavani Devi et al., (1980) reported Termitomyces robustus (Beeli) Heim as a new edible fungus for the country. Bhavani Dev (1982) also reported two species of Termitomyces viz. T. mammiformis and T. microcarpus commonly occuring in Kerala. Sathe and Daud (1980) reported T. poonensis sp. Nov. from Maharashtra; Podobrella microcarpa (Berk and Br.) Singer and T. heimii Natarajan from Karnataka; Podobrella microcarpa (Berk and Br.) singer var. major var. Nov., T. longiradicatus sp. Nov. and T. quilonsii sp. nov. from Kerala. Rawla et al., (1983) describing the Termitomyces flora of chandigarh, India recorded two new species for the country viz. T. striatus (Beeli) Heim and T. microcarpus (Berk and Br.) Heim var. Santalensis. Pegler (1986) recorded two species of Termitomyces from Sri Lanka. Leelavathi et al (1987) reported eleven species of Termitomyces from Malabar, Kerala. Sharma et al. (1977) reported Termitomyces microcorpus from Himachal Pradesh.

Ecology, Symbiosis and Isolation

Escherich (1911) suggested that fungi might maintain the high humidity required by termites and their metabolic heat creates air currents that ventilate nests. Many workers have conducted extensive studies on the ecology and symbiosis of the fungus and associated termites. Randall and Dooly (1934) noted an odour of acetic or related acid, when gut contents of termites were acidified. The secretion of soldiers of Odontotermes also had this smell and they concluded that a byproduct of cellulose digestion has come to be involved in both defence and the adaptation of Termitomyces to a habitat otherwise inimical to fungi. Kalshoven (1936) believed that fungi in nests of Macrotermes, Odontotermes and Microtermes were eaten away by termites from below. According to Grasse (1937) the fungus provided nutritional adjuvants or vitamins to the termites. Ghidni (1939) was of opinion that the comb served as the apparatus for humidity control essential for the growth of the fungus and termites. According to Mukerji and Mitra (1949) there was no significant difference in pH between termitarium of Odontotermes redimanii and surrounding soil and reported that the pH of comb generally ranges between 3.9 and 4.3. Studies conducted by Cheo (1942) also confirmed the above

observations. He noticed that the growth of Xylaria in active combs was suppressed while the growth of spherules of Termitomyces was promoted.

Lüscher (1951) suggested that the function of comb was combined with heat production maintaining a constant high temperature in the nest. Luscher (1951) also studied the carbondioxide content in the termite nest, which was found to go upto 2.7 per cent as against 0.3 per cent in the atmosphere outside. He also pointed out that the high carbondioxide inhibited spore germination and growth of some other fungi in the nest. Lilly and Barnet (1951) and Batra and Batra (1966) also corroborated the view. Heim (1952) considered Termitomyces to be a termitophilous commensal. He observed that fungi like Xylaria nigripes, Peziza, Mucor, Thamnidium, Cephalosporium, Aspergillus species etc. reported to be associated with termite hills are found only in abandoned combs. Podaxon, Gynophragmium Marasmius Omphalia, Leucocoprinus, Lepiota, Psalliota and Xerocomus which could also be isolated from termite hills are considered to be saprophytes. Sands (1956) and Ausat et al. (1960) reported that termites of all castes, ordinarily live for only a few hours when taken from large termitaries. They further showed experimentally that

fungus comb with mycelium and nodules is an important part of the diet of Odontotermes and could also prove that the termites even supplied with alternative food could survive no longer when starved. He found that combs were made of aggregations of faeces of termites that they were periodically regenerated. Heim (1962) reported that termites carry with them on their legs mushroom germs which could be found in their food and body wastes. He observed another ascomycetes fungus which he identified as Xylaria also makes it appearance on termite nests. When the nests are taken out of its natural environment, the Xylaria grows actively and eventually the explosive appearance of Xylaria takes over, chokes the basidiomycetes and destroys it. Batra and Batra (1966) noticed that the spherules and matrix of fungus comb formed the food of large nymphs, workers and alates in the termite colony. Batra and Batra (1966) studied in detail the symbiotic relationship of the fungi and termites and concluded that the defensive oral secretion of the soldiers with the addition of saliva exerts a fungistatic effect in general on fungi other than Termitomyces. They further reported that all combs excavated were acidic in pH. They further found that the temperature inside the comb was warmer in winter months and

cooler in summer months. They also observed that, the heat generated by the metabolism of both termites and fungi create a temperature gradient in the combs. Their studies also brought to light the fact that the nitrogen content of the fungus comb was higher than that of plant material compounds for its construction. They assumed that fungus combs might be a means of conserving nitrogen which in the nest is cycled repeatedly between termites and the fungus.

Zoberi (1979) conducted experiments to study the distribution of fungi in a termite hill and isolated twenty seven species representing seventeen genera. He observed that combs insulated from outer environment was found to differ in its species composition and that seasonal change exerts its influence only upon the population of fungus within the upper layers of the hill. He also speculated that the strands of Xylaria species are masticated by the termites and utilised for building new combs where as the spherules of Termitomyces are used as additional supply of food and sources of vitamins for the termites. He is also of opinion that the mycelium permeating the Pour des required microclimate within the chamber for the survival of termites. Zoberi (1979) realised the importance of cellulose

decomposition, the life process of the termites, as they derive their main source of metabolic substrate and energy from cellulose. But the Macrotermitanes species of termites do not secrete specific enzyme (cellulose) for cellulose decomposition.

Purkayastha and [illegible] Chandra (1975) succeeded in preparing the culture of Termitomyces eurhizus (Bark) Heim in a synthetic medium and identified ten amino acids from the mycelia. Ghosh and Sengupta (1978) isolated Termitomyces in a complex solid agar medium utilising dextrin soluble starch at a temperature of 28 to 32°C. They observed filament elongation upto 7-8 days. However, Zoberi (1979) recorded that white sporules did not grow artificially on any of the media tried at different temperatures. Rohrmann and Rossmann (1980) reported that Termitomyces species produces lignin degrading enzymes unlike Xylaria. He also found that Synnamata of Termitomyces were composed of 38 per cent protein which contained all the amino acids termites required. Ghosh and Sengupta (1979) studied the effect of vitamins, hormones and fatty acids on the submerged mycelial yields of some mushrooms grown in synthetic media and concluded that among plant growth harmones listed, kinetin was growth stimulatory

for Termitomyces clypeatus. Rebecca Thomas (1985) developed a selective medium to facilitate isolation of Termitomyces. The maximum colony diameter was recorded to 2.2cm. Optimum conditions of temperature and pH for Termitomyces culture associated with different termite species were also standardised. She further studied the microbial ecology of macrotermitinae nests and reported Termitomyces as the major fungus present in the comb while other fungi were present only as spores, which grew rapidly when combs were removed from nests. She also noted a substance in the extracts of food store of termites which prevents germination of other contaminant fungi.

De (1985) also reported success in getting culture of Termitomyces microcarpus from the spore deposit, using malt agar medium by exposing the slants to 0, 6, 12, 18, 24 hour light (1000 Lux) every day and then incubating them in complete darkness at a temperature of 28 ± 2°C.

Dixon (1983) conducted detailed studies on the response of Termitomyces species to rainfall and sporophore production. He noticed it could be divided into two phases, a pre-rain period of primordial initiation and a post-rain period of sporophore maturation which form eight to ten days. He also observed that a flush could be

induced during mid dry seasons by irrigating the soil surface with sufficient water and flush was seen to appear eight to nine days after irrigation.

Edibility

Edibility of Termitomyces species has been known from time immemorial. Bakshi (1951) stated that all recorded species of Termitomyces are edible and very delicious. According to Singer (1961) most of mushrooms eaten in Africa belonged to the genus Termitomyces, which were considered as superior to all other mushrooms. Purkayastha and Chandra (1975) conducted the edibility trials on mice using Termitomyces species and observed a significant increase in body weight of four week old male mice.

Nutritive value

Bano et al. (1964) estimated the protein content in Termitomyces species and reported them as a good source of leucine and isoleucine. Protein from Termitomyces contained high percentage of histidine and arginine. Mukibi (1975) studied the nutritive value of some Ugandan Mushrooms. Termitomyces was comparable with or in some cases was greater than that of many grain legumes. Ten essential amino acids were also recorded in appreciable quantities. Purkayastha and Chandra (1975) separated ten amino acids

from Termitomyces eurhizus (Berk.) and recorded it to be a better source of alanine. Guy and Thoen (1977) also estimated the proteins, fat, carbohydrates and crude fibre content of five species of Termitomyces. Rawls et al. (1983) conducted chemotaxonomic studies on three taxa of Termitomyces species by analysing the free and bound amino acids. Three of them were found to have L-arginine in common with one another in the free state and had L-leucine and L-methionine in bound state.

Preservation and Marketing

Generally Termitomyces species were preserved by smoking. OSO (1975) gave an account of the method of collection, preservation and marketing of Termitomyces species commonly occurring in several parts of Nigeria. He observed that the yoruba women after collecting mushrooms in the forest bring them in large basket to the main roads where they are displayed for purchase by passers by. They also carry the mushrooms into their village or town for sale. Mushroom collected in a day are usually cooked the same day. However, they maintain a supply over a long period of time by smoking and storing large quantities.

MATERIALS AND METHODS

MATERIALS AND METHODS

In order to study the natural distribution of different species of Termitomyces of Kerala a preliminary survey was carried out in the following localities in the fourteen districts of the State.

(1)	Vellayani	(Trivandrum)
(2)	Peyad	(Trivandrum)
(3)	Kallar and Manithooki	(Trivandrum)
(4)	Puthenthope	(Trivandrum)
(5)	Vellanad	(Trivandrum)
(6)	Varkala	(Trivandrum)
(7)	Anchal	(Quilon)
(8)	Kottarakkara	(Quilon)
(9)	Pathanamthitta	(Pathanamthitta)
(10)	Ranni	(Pathanamthitta)
(11)	Kayamkulam	(Alleppay)
(12)	Mavelikkara	(Alleppey)
(13)	Kumarakom	(Kottayam)
(14)	Kottayam	(Kottayam)
(15)	Odakkali	(Ernakulam)
(16)	Kothamangalam	(Ernakulam)
(17)	Pampadumpara	(Idukki)
(18)	Kumali	(Idukki)

(19)	Vellanikkara	(Trichur)
(20)	Mukundapuram	(Trichur)
(21)	Pattambi	(Palghat)
(22)	Chittor	(Palghat)
(23)	Kottakkal	(Malappuram)
(24)	Mancheri	(Malappuram)
(25)	Kozhikode	(Calicut)
(26)	Ferooke	(Calicut)
(27)	Panniyoor	(Cannanore)
(28)	Payyannoor	(Cannanore)
(29)	Ambalavayal	(Wynad)
(30)	Kalpatta	(Wynad)
(31)	Pilicode	(Kasargod)
(32)	Nileswaram	(Kasargod)

The periodicity of occurrence, soil type, distribution etc. were recorded and are presented in Table 1.

The species collected were identified by comparing the characters already reported in literature. Descriptions and terms followed are the same used by Heim (1962), Singer (1975), Pegler (1986) and Natarajan (1978). The glossary of terms used are also appended Appendix-I. Morphological and microscopical characters of all the specimens collected were studied and recorded in a data sheet

prepared by Nair and Devi (1984). Data sheet is presented in Appendix-II . Different measurements of pileus, lamellae, stipe, pseudorhiza etc. were taken from an average of 20 specimens. All the colour descriptions used in the present study were according to the Dictionary of Colours by Maerz and Paul (1950) and cited under results by appropriate plate number (PL).

Spore prints were taken on a white sheet of paper from freshly collected fruit bodies. The stipe was cut off, just beneath the pileus and was placed on a white paper, facing the gills downwards. A piece of moist cotton was placed inside the bell jar to maintain moisture. The whole assembly was kept undisturbed for 8-10 h to get a clear spore print. Permanent spore prints were similarly made on a sheet of white paper coated with gum arabic and kept for future reference.

The specimens were dried in a Sigg Dorrex dehydrator and there after labelled and preserved in sealed polythene bags. Specimens were also preserved by wet method using formalin and acetic acid solution. The collections were deposited in the herbarium unit of the Department of Plant Pathology, College of Agriculture, Vellayani, Trivandrum.

Agaricological tests like macrochemical and metachromatin reactions of various parts of basidiocarps were observed and recorded following the methods of Watling (1971) and Singer (1975). The tests were carried out on the surface and context of the pileus, stipe, stipe apex and base. Fresh tissues from the pileus measuring approximately one square centimetre were dissected from different parts of the sporophore with clean single edge razor blade and placed in a porcelain spot plate. Two drops of Melzer's reagent was applied and [illegible] allowed to stand for a minimum of 15 min before recording observations. The colour change was recorded and was graded as inamyloid or pseudoamyloid if negative (final colour being brown to purple brown) and amyloid if positive (final colour yellow to black). Melzer's reaction for spore mass was also detected by the same method. Reaction tests were also carried out using ferrous sulphate (3%), aqueous potassium hydroxide (3%), hydrochloric acid (1 ml) or Conc.Sulphuric acid, Phenol (2%) and Sulphovanillin. Composition of all the reagents, media and chemicals used are given in Appendix-III . Microscopic measurements of all the structures were made from mounts in low Potassium hydroxide solution at a magnification of x2000.

The identity of the specimens was confirmed by the Royal Botanic Garden, England and Centre for Advanced Studies in Botany, Madras.

Identification of the associated termites, collected from respective termite nests in all localities was done by the Commonwealth Institute of Entomology, London and Forest Research Institute, Dehradun.

Developmental Morphology:

Most common species which appeared regularly in Vellayani and Peyad in Trivandrum district was chosen for this study. Six termite nests each located in these places were carefully traced out, using handfork and trowel in order to keep the combs properly exposed. Different stages of the sporocarp present in each of the combs *in situ* were recorded. The nest cavities were then carefully covered with a clean glass sheet and the escavated soil was replaced over it so as to maintain the natural conditions to the extent possible. Regular observations at tuenty four hour intervals in all the six nests were continued for eight days and different stages of growth were recorded and described following previous descriptions adopted in Agaricus Chang, 1978 . Photographs and drawings of these different stages were prepared. Growth and

development of the sporocarps after their emergence above the soil to full maturity were also recorded.

Combs of different stages were carefully removed along with a portion of surrounding soils and transferred into sterilized glass troughs and covered with glass plates. The troughs were immediately transferred to the laboratory and kept in a B.O.D. incubator at 28°C. Sterile water was sprinkled at intervals to maintain the soil moisture. Regular observations on the further development of the sporocarps in the combs were made at 24 h intervals. The trial was continued for one week.

Ecology and Symbiosis

Six nests were systematically excavated at Puthenthope and their contents were measured and analysed. One metre deep trench was taken about 0.6 m away from edge of each mound. The trench was gradually widened, and excavation was done slowly to remove the combs. The portions of combs, termites and surrounding soil were immediately placed in sterile glass jars and sterile petridishes for more detailed laboratory studies.

Temperature

Temperature of the comb inside the termite nest and soil surroundings was determined using soil thermometer.

The thermometer was carefully inserted into the comb located in deeper layer of soil and was allowed to remain until the mercury level became steady. Temperature of surrounding soil was also measured by the same method.

Humidity

Humidity of the comb was determined using Barigo Dial type hygrometer. This instrument was placed carefully into the comb and readings were taken, after 10 min, directly from the instrument. This was repeated outside the comb also.

Moisture

Twenty g of comb material were taken in a petridish and was kept in an oven at 105°C. The weight of the comb material was recorded at different intervals until a constant weight was obtained. For determining the fungal population in the termite nests, the samples were crushed and sieved. Serial dilutions were prepared in sterile water and from 10^{-4} dilution, 1 ml was plated on Martin's rose bengal agar. Colony counts were made after incubation for three days. Three replications were maintained in each case. To determine bacterial population, 10^{-6} dilution was prepared and one ml of the solution was plated on Nutrient agar media and incubated at room temperature for three days. Three replications were maintained. To determine actinomycete

population 10^{-6} dilution was also prepared and plated on Kuster's agar. Three replications were maintained and colony counts were taken after four days of incubation.

pH

Ten g of sieved comb material were taken in a 50 ml beaker and distilled water was added while stirring. Stirring was continued for 30 min and contents were allowed to settle for about 30 min. Readings were taken directly from digital pH meter (ELICO Pvt. Ltd.) standardised against buffer solution of known pH.

Total Nitrogen content

The total nitrogen content of comb material was determined by Kjedahl method as outlined by Jackson (1968). Five g of sieved comb material were taken and digested with 12 ml of digestion mixture and 30 ml concentrated H_2SO_4 for about ½ to 1 hour till the solution became clear. A 100 ml conical flask with 5 ml of 4 per cent Boric acid solution and 2-4 drops of methyl red indicator were placed at the condenser tip. 8-10 ml of 40 per cent NaOH - $Na_2S_2O_3$ solution were then added to the distillation flask. About 15 ml of distillate were collected and titrated against 0.00904 N. HCl. The end point was indicated by appearance of a violet colour. A blank experiment with all the ingredients except the sample was also done.

Cellulose content

The cellulose content was estimated by the method of Updegraff (1963) with slight modification. Two g sample was digested with 35 ml acetic/nitric reagent in a boiling tube and mixed well. The tube was placed on a water bath at 100°C for 30 min. It was cooled to room temperature and centrifuged at 3000 r.p.m. for 30 min. The supernatant was discarded and the residue was washed with distilled water, centrifuged again, the supernatant discarded. Twenty ml of 67 per cent H_2SO_4 were then added to the residue allowed to stand at room temperature for 1 hr for complete digetion. One ml from the stock was made up to 100 ml and 0.5 ml aliquots of the diluted solution were taken in test tubes and 45 ml distilled water were added to this and mixed well and the tubes were kept in a boiling water-bath for 15 min cooled to room temperature and the O.D. was measured at 620 mm against a blank prepared identically with 0.5 ml distilled water instead of the sample. A standard was also run simultaneously using carboxy methyl cellulose.

Ash

Two g of comb material were taken in a previously weighed dish. The dish was inserted into an electric

muffle furnace and the temperature was increased to 105°C. When ashing was completed after 3 h the dish was removed and cooled in a desiccator. The dish with its contents was weighed and the differences in weight was recorded and the weight of ash was calculated in percentage.

Effect of soil moisture in the production of sporophores

A field trial was laid out during August 1985 in the coconut garden at Puthenthope where species of Termitomyces was found to occur in several solitary nests, scattered in a few sites in the garden (Plate I). Four similar sites with good concentration of termite nests were selected. Productive nests in each of the sites (plots) were identified based on emergence of mushrooms and were serially marked with field labels. Based on the continuity, these marked nests in each of the four sites were clustered in two equal groups. One group was kept as control and the other received treatment (irrigation). All the treated groups received regular irrigation with 50 lit water daily during dry periods in order to keep subsoil, sufficiently moist up to a depth of 50 cm. The control group of nests was left to natural conditions, with no artificial irrigation. Daily observation on the emergence of mushrooms in both the treated and control plots in all four sites

were recorded. The trial was continued up to the end of December, 1985. Termites collected from all locations were preserved in 95 per cent Ethyl alcohol for identification.

Growth of Termitomyces in different solid media

Growth of the fungus was studied in the following solid media incubated at room temperature.

1. Potato dextrose agar
2. Oat meal agar
3. Malt extract agar
4. Czapek's agar
5. Saboraud media
6. Nutrient agar
7. Purkayasthe's synthetic medium
8. Rebecca's selective medium

The composition of various media is given in Appendix III . Since the fungus had very little growth in stock culture, both tissues and spherules were tried in all the media.

Isolation from tissues

The different media were transferred at the rate of 100 ml in 250 ml Erlenmeyer flasks and autoclaved at 1.02 Kg/cm^2 for 15 to 20 min. After sterilization and

before solidification 2 ml of 1 per cent Ambistrin - S (Streptomycin sulphate) and one per cent Penicillin - G salt were added to the medium in each flask to inhibit bacterial growth. The stipe and pileus tissues from fresh young mushrooms were separated, surface sterilized with 95 per cent alcohol for one minute and cut into bits of 2-3 cm size. These bits were transferred aseptically into the flasks and were incubated at room temperature. Three replications were maintained in each case.

Isolation from spherules

Combs were removed carefully and small spherules were selected. The spherules were surface sterilized with 95 per cent alcohol for one minute and were aseptically inoculated in 15 ml of each media plated on sterilized petridishes. The dishes were incubated at room temperature. Three replications were maintained in each case.

Growth in Liquid media

Liquid media were prepared and 50 ml of each medium were transferred to 250 ml Erlenmeyer flasks and autoclaved at 1.02 Kg/cm^2. After sterilization the media were inoculated with spherules. Four replications were maintained in each case. The flasks were incubated at room temperature. Observations were taken after 48 h.

Effect of temperature on the mycelial growth of T.robustus

Out of the different media mentioned above, only the Rebecca's selective medium was used for this study. The medium was prepared and autoclaved at 1.02 kg/cm^2 for 15 min and poured into sterilized petridishes of 9 cm diam. at the rate of 15 ml in each dish and allowed to solidify. The spherules were taken, surface sterilized in 95 per cent alcohol, and transferred aseptically into the dishes. The dishes were incubated at four different temperatures, viz., 20°C, 25°C, 30°C and 35°C. Radial measurements of the fungal growth were recorded after 2, 4, 6, 8, 10 and 12 days respectively. Three replications were maintained for each treatment.

Effect of light and darkness on the mycelial growth of T.robustus

Fifteen ml of selective medium were plated in sterilised petridishes. Fresh spherules were selected, surface sterilized with 95 per cent alcohol, and transferred aseptically to the centre of the dishes. Six dishes were placed under ordinary light conditions and the other set of equal number wrapped with black paper and incubated in complete darkness. Both the sets were incubated at room temperature. Observations were recorded after 48 h.

Comparative efficacy of different spawn substrates in supporting the mycelial growth of Termitomyces

The following six substrates were tried for spawn production. 1. Paddy straw 2. Rice bran 3. Spent tea waste 4. Banana pseudostem 5. Saw dust 6. Wheat grain

Two hundred g of each substrate, except spent tea waste were taken and boiled in water for 10 min. After draining the excess water from the materials, clean Erlenmeyer flasks of 250 ml capacity were half filled with the substrate. Five g of calcium carbonate were mixed throughly with the grains before filling. Paddy straw and spent tea waste were filled as described earlier. Red gram powder at the rate of 5 g was added to each flask. The bottles were plugged and sterilised at 1.05 kg/cm^2 for 2 h/day for 2 days and allowed to cool down. Mycelial bits from the pure culture T.robustus were inoculated aseptically and incubated at room temperature. Visual observations of the mycelial growth of the fungus were recorded and arbiterly graded as follows on the 20th day of incubation.

Visual observations	Grade
Very good growth	xxxx
Good growth	xxx
Moderate growth	xx
Very poor growth	x
No growth	-

Effect of temperature on the mycelial growth of Termitomyces on different substrates

Erlenmeyer flasks filled with the six substrates as in the previous experiments were inoculated with the mycelial bits of Termitomyces and maintained at different temperatures, viz., 20°C, 25°C, 30°C and 35°C for 20 days. At the end of the incubation period, the mycelial growth was visually graded as in the previous experiments and recorded.

Effect of different sources of carbon on the mycelial growth of T.robustus

Rebeeca's selective medium was used as the basal medium and various mono and disaccharides in the form of dextrose, lactose and maltose were substituted for cellulose, in the selective media as to give the same percentage of carbon in each treatment. Samples without addition of any sugar were taken as control.

The medium prepared as above was autoclaved at 1.02 kg/cm^2 for 15 min, cooled and poured into sterilised petridishes, at the rate of 15 ml and allowed to solidify. Spherules were taken, surface sterilised and transferred aseptically into the centre of each dish. The dishes were incubated at room temperature and radial growth was measured at intervals of 5, 10 and 12 days.

Nutritive value

Analysis of moisture

Fifty grams of fresh mushrooms were taken in a petri-dish and dried in a Sigg Dorrex dehydrator at 70°C., till a constant weight was obtained. The loss in weight was determined and the percentage of moisture calculated. It was repeated with six samples.

Analysis of Total Free Amino acids (Moore and Stein 1948)

One hundred mg dry mushroom samples were mixed with 10 ml and 5 per cent T.C.A. (Tricholoro acetic acid) in cold. The extract was centrifuged and the supernatant made upto 10 ml. From this 0.5 ml was taken and neutralised with 0.5 N NaOH. To this 1 ml ninhydrin reagent was added and placed in a boiling water bath for 20 min. Cooled the tubes and diluted to 5 ml using diluent solvent (Equal volume of reagent grade Propanol and H_2O). The optical density of the coloured product was read at 570nm in a spectophotometer (AIMIL) against a blank of diluent solvent. A standard was run using Leucin (SIGMA).

Analysis of Crude Fibre

To 5 g powdered dried mushroom sample, in a beaker 200 ml boiling 1.25 per cent H_2SO_4 were added. The beaker was then placed in a hot plate and boiled exactly for 30min

Filtered through a clean filter pad, rinsed the beaker with 50 ml boiling H_2O washed through same filter pad, removed the pad and transferred the contents carefully into a beaker. Two hundred ml boiling 1.25 per cent NaOH were added and again boiled exactly for 30 min and filtered as above washed with 50 ml portions of water. After complete draining the residue was dried for 2 h at 130°C. Cooled in a desiccator and weighed. Ignited for 30 min at 600°C in a muffle furnace. Cooled in a desiccator and reweighed. The loss in weight on ignition was taken and the Crude fibre in the sample was calculated in percentage.

Analysis of fat (Brand 1963)

Five hundred mg of powdered dry mushroom sample were transferred to centrifuge tubes, with 20 ml alcohol: ether (3:1). The tube was placed in a constant temperature water bath at 60°C for 2 h. Centrifuged and decanted the supernatant into a graduated measuring cylinder. Refluxed the residue with 20 ml chloroform: methanol (1:1) for 1 h at 55°C centrifuged again and the combined supernatants were placed in a weighed evaporating basin and [illegible] noted the weight. The difference in weight was recorded and the fat content expressed as g/100 g D.M.

Analysis of true protein

A homogenate (1:20 w/v) of the dried mushroom sample was prepared with cold 0.1 N NaOH. The extract was digested for 10 min in a constant temperature water bath at 70°C for complete dissolution of proteins centrifuged at 3000 r.p.m. for 30 min and the supernatant made upto 10 ml. An aliquot of the homogenate was diluted 5 times, 1 ml from this was used for the estimation of proteins by the method of Lowry et al. (1951). To 1 ml sample, 4 ml reagent C was added, mixed and kept for 10 min. To this 0.5 ml diluted Folinopheral reagent was added. Volume was made up to 10 ml and allowed to remain at room temperature for 30 min for colour development. The optical density of the coloured product was measured at 660 nm in a spectro photometer (AIMIL), against a blank prepared identically using 1 ml distilled water. The protein content was calculated using standard prepared with Bovine alubmine (Sigma).

Analysis of total carbohydrates (Smith 1956)

One hundred mg of dried mushroom sample were extracted with 25 ml aqueous ethanol (80% v/v) at 100°C for 5 min. The extract was centrifuged at 300 r.p.m. for 30 min. Supernatant was collected and made up to 100 ml. From this 1 ml

aliquots were taken and the carbohydrate content was estimated by Phenol sulphuric acid method. One ml aliquot and one ml of five per cent Phenol were mixed and added 10 ml conc.H_2SO_4. The residue from alcoholic extraction was suspended in 20 ml 72 per cent H_2SO_4 extracted overnight at room temperature. After adding 80 ml water mixing and allowing to stand for 30 min the suspension was centrifuged and the supernatant was made up to 100 ml. One ml aliquot was taken from this and carbohydrate content was estimated as described previously.

Finally the residue from the above extraction was suspended in 2 ml water, mixed well and used for the determination of carbohydrate as described earlier.

A standard was run using glucose (BDH). The Carbohydrate values for the first supernatant, second supernatant and the residue were pooled to get total carbohydrate.

Amino acids

The amino acids were extracted from the tissues and determined using the method of Sinha and Cossions (1964). Five g of fresh sample of mushroom were homogenised with 50 ml ethanol (95%) and kept overnight. The extract was then filtered and the residue was washed with 50 ml of 50 per cent ethanol. The ethanol was removed from filtrate

by keeping it at 60°C which was then run through a Dowex 50 (200 mesh) column of 10 cm length and 1 cm diam. The column was washed with 75 ml of 25 per cent ammonia solution and washings evaporated to 3 ml at 60°C. The amino acids present were determined qualitatively by Thin Layer Chromatography. The solvent system used was butanol: acetic acid: water (4:1:5). The spots were developed by spraying ninhydrin solution (0.3 per cent in ethanol). Standard amino acids were spotted simultaneously for comparison of Rf values.

Studies on post harvest processing

Dehydration

One hundred g of fresh sporocarp of Termitomyces robustus were taken, pseudorhiza and bulbous portion of stipes were removed and dried under sun for 3 days to reduce the moisture content to 5-6 per cent. Simultaneously, other samples were also dehydrated keeping it in Sigg Dorrex dehydrator continuously for 24 h at a temperature of 55-70°C. The dehydrated samples were transferred to polythene bags of 100 gauge thickness and sealed. Another set of samples was kept in air tight containers. Dried ones kept opened served as control, visual observations and organoleptic tests were conducted at different periods after 3, 6, 9 and 12 months respectively.

Preservation by powdering

Samples of mushroom were dehydrated using a Sigg Dorrex dehydrator and were powdered and stored either in polythene bags of 100 gauge thickness or air tight containers. Samples kept open, served as control. Visual observations and organoleptic tests were conducted, at different periods after 2, 6, 9 and 12 months respectively. In order to allow for safe storage of fresh mushrooms, the following methods of preservation and processing were tried.

Refrigeration

One hundred g samples of freshly collected mushrooms were stored in refrigerator at 10-15°C. Samples were kept in both open and closed polythene bags. Five replicates maintained in each case. Visual observations were taken after 24, 48, 72 and 96 h, respectively.

Preservation in brine

Young mushrooms before full expansion of pileus were collected, washed in tap water and steeped in boiling water for 1-2 min. Brine of 1 to 7 per cent concentration was prepared by dissolving sodium chloride in sterile water. An aliquot of 150 ml each of the solution was transferred to clean conical flasks of 250 ml capacity

and autoclaved at 1.02 kg/cm^2. Equal quantity of mushrooms was transferred aseptically to each flask, containing the brine and were incubated at room temperature. Three replications were maintained for each treatment.

The microbial flora of the above preserved mushrooms were estimated following the serial dilution plate technique at weekly intervals for a period of 6 weeks during storage periods. Visual observations of the preserved mushrooms at different concentrations were made. A 10^{-7} dilution of brine was prepared and the bacterial, actinomycetes and fungal population from the preserved samples were estimated employing Nutrient agar, Kuster's agar and Martin's rose bengal agar respectively.

Blanching

Two hundred and fifty grams of fresh mushrooms were taken, washed well, and dipped in boiling water for five min. It was then taken out and sun dried for 3 days. The dried material was then packed in polythene bags of 100 gauge thickness and stored at room temperature and by refrigeration and observations were taken at three days interval for three months.

Hundred grams of young mushrooms were washed, cut into small pieces of about 5-10 mm thickness, boiled in

50 per cent vinegar solution for 10 min allowed to drain for 24 h and dried in sun. They were then placed in glass jars containing gingelly oil. The pieces were pressed down to eliminate air bubbles and were completely covered with oil. The jars were tightly closed and sterilized in water bath for 45 min before storing. Periodical observations on the preserved material was made as in the above cases.

Pickling

Four fifty grams of young mushrooms were washed, cut into several pieces and placed in a pan, covered with vinegar. The following ingredients were also added. Chilli powder (one tea spoon), ground ginger (one tea spoon), chopped onion (225 g) and 4 pieces of garlic. The ingredients were cooked gently until tender and simmered in hot gingelly oil. Cooled and filled in clean, sterilized glass bottles, sealed air tightly and stored.

Ketchup

Four fifty grams of fresh mushrooms were cut into small pieces, spread them out in a bowl and sprinkled six table spoons of salt covered and left for two days. At intervals, they were stirred and squashed. Then they were transferred to larger pan, adding one cup vinegar, two

table spoon chopped onion, ½ teaspoon pepper and four teaspoon oil and were gently simmered for about 1 h until it became a strong concentrate. It was then strained through a clean cheese cloth into sterilised bottle and sealed air tight. The bottle was further sterilised in water bath before being stored.

RESULTS

RESULTS

A state wide survey was conducted during the South West and North East monsoon periods in 1984-85 for the collection of Termitomyces species. The following nine species were collected from thirty two localities of the State (Fig. 1)

1. Termitomyces robustus (Beeli) Heim
2. T. heimii Natarajan sp. Nov.
3. T. clypeatus Heim
4. T. radicatus Natarajan sp. Nov.
5. T. striatus (Beeli) Heim
6. T. perforans Heim
7. T. globulus Heim Goossens
8. T. microcarpus (Bark and Br.) Heim
9. T. microcarpus var. santalensis Heim

Detailed studies on morphological and microscopic characters were carried out for identifying the specimens and the details are given separately. Their periodicity, frequency and intensity of occurrence, distribution and soil types were also observed and enumerated in the table 1 .

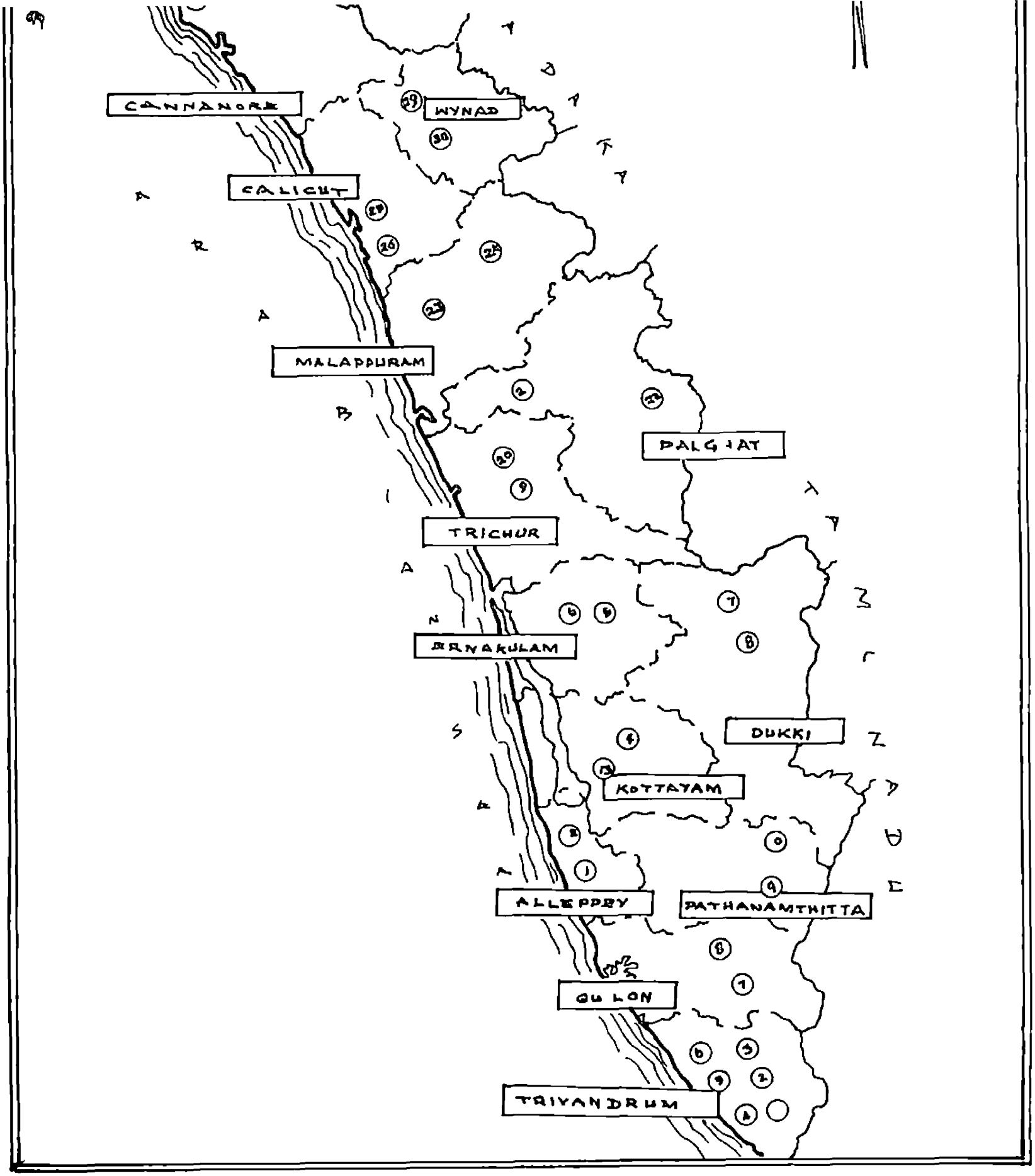
CANNANORE
WYNAD
CALICUT
MALAPPURAM
PALGHAT
TRICHUR
ERNAKULAM
IDUKKI
KOTTAYAM
ALLEPPEY
PATHANAMTHITTA
QUILON
TRIVANDRUM

Table-1

Periodicity of occurrence and distribution of *Termitomyces* spp.

Name of the species	Place of collection	District	Soil type	*Intensity and habit of occurrence	Month of occurrence
1. *Termitomyces robustus*	Throughout the State	All districts	All soil type	+	June-July September-October
2. *T. heimii*	Kallar	Trivandrum	Forest loam	+ + + +	July
	Manithooki	Trivandrum	Forest loam	+ + + +	July-October
	Ambalavayal	Wynad	Forest loam	+ + + +	July
	Odakkali	Ernakulam	Laterite	+ + + +	July-October
3. *T. clypeatus*	Vellanad	Trivandrum	Laterite	+ + +	July
4. *T. radicatus*	Peyad	Trivandum	Laterite	+ +	July
5. *T. striatus*	Pachalloor	Trivandrum	Red loam	+ +	October
6. *T. globulus*	Puthenthope	Trivandrum	Coastal sandy loam	+ + +	July
7. *T. perforans*	Mavelikara	Alleppy	Laterite	+ +	June-July
8. *T. microcarpus*	Throughout the State	All districts	All soil type	+ + + +	September-October June-July September-October
9. *T. microcarpus*	Odakkali	Ernakulam	Laterite	+ + + +	June-July
var. *santalensis*	Ponmudi	Trivandrum	Forest loam	+ + + +	September-October

* Intensity and habit of occurrence

+ + + + 50-100 and more sporocarps - Large groups
+ + + 25-50 Sporocarps - Small groups
+ + 10-25 Sporocarps - Scattered
+ 1-10 Sporocarps - Solitary

Plate - I Experimental plot

Observations showed that among the nine species collected T. microcarpus, T. microcarpus var santalensis and T. robustus were the most commonly occurring and widely distributed species through out the State. Collections revealed that they have a wide range of distribution irrespective of soil type. T. heimii showed less common distribution and their occurrence were mainly confined to the undisturbed forest soil under thick vegetation. T. globulus, T. glyreatus, T. perforans, T. striatus and T. radicatus were observed infrequent in their distribution during the season.

The data presented in the table 1 revealed that based on the intensity and habit of occurrence T. robustus and T. striatus were always found growing solitary above the hypogeal termite combs. T. microcarpus and T. microcarpus var santalensis occurred in widely scattered groups consisting of more than hundred sporocarps above the scattered termite combs. T. heimii also occurred in groups of more than hundred sporocarps growing around or on above the partly epigeal termitaria. T. glyreatus and T. globulus were noticed growing in small scattered groups of 25-50 sporocarps above the hypogeal termite combs while T. radicatus and T. perforans appeared in wall scattered groups of 10-25 sporocarps.

Plate - IIa. *Termitomyces robustus* in natural habitat

Plate - II.b

Sporocarp of *T. robustus* showing

46

The results relating to the periodicity of occurrence of different species of Termitomyces showed that among the nine species six of them abundantly occurred during the post monsoon periods viz., July and October (South West and North East) while the two species T. microcarpus and T. microcarpus form santalensis occur during both monsoon periods.

Termitomyces robustus (Beeli) Heim. in Bull. Jard. Bot; Brux 21:2110 44-46 (1951)

Schulzeria. robusta Beeli in Bull. Soc. Belg. 69: Belg. 60:75

Termitomyces fuliginosus Heim in Arch. Mus. Nat. Hist. Nat. Paris VI. 18; 118 (1942)

Sporophores solitary or scattered, found growing in the termite mounds.(Plate XIa) Pileus 6-20 cm diam., convex, planoconvex at maturity with obtusely pointed dark brownish black perforatorium (PL-14-6-CED). Surface uniformly tawny brown (PL-12-5 DCED); irregularly ridged, often glabrous; viscid when moist. Margin incurred, often lacerate and reflexed at maturity. Flesh, white and soft 5 mm thick with hyaline, interwoven hyphe of 5-10 mm. Lamellae white to pale ochraceous with a pinkish tint; free to subadnate with decurrent tooth; 6 mm broad,

Plate - IIIa

Termitomyces heimii Sporocarps in natural habitat

Plate - IIIb

T. heimii Sporocarp showing

crowded, with lamellulae; margin crenulate. Stipe 10-20 x 2-3 cm in epigeal region, smooth, white, solid, cylindric but thickening into a bulbous base below the soil and then tapering into a long subterranean pseudorhiza (16.5 x 1.8cm) with sclerotised disc, (PL-8-12-4) ending in the termitarium. (Plate IIb) Spore print pinkish cream. Basidia 25-35 x 7-8 µm, clavate bearing 4 sterigmata. Spores 8.7 - 8.2 x 4.5-5 µm, obovoid to ellipsoid, smooth. Cystidia numerous. Pleuro cystidia 26.5-26.2 x 10-14.2 µm (Fig. 2) Cheilocystidia 14.1 - 16 x 7-11.1 um variable in shape from clavate, cylindric to napi form; thin walled. Hymenophoral trama indistinctly bilateral. All hyphae lacking clamp connection.

Edibility - Excellent

Season - North-East and South-West monsoon periods 1984-'85

Distribution - Collected from different places throughout the state.

Locally known as Uppukoon, Masathandan, Milampulappan. Local people use to collect and consume during the season.

Termitomyces heimii Natarajan sp. nov.

Sporophore gregarious growing in large numbers in the forest soil. (Plate IIIa) Pileus 4.7 cm in diam., convex

crowded, with lamellulae/ margin crenulate. Stipe 10-20 x 2-3 cm in epigeal region, smooth, white, solid, cylindric but thickening into a bulbous base below the soil and then tapering into a long subterranean pseudorhiza (16.5 x 1.8cm) with sclerotized disc, (PL-8-12-4) ending in the termitarium. (Plate IIb) Spore print pinkish cream. Basidia 25-35 x 7-8 mm, clavate bearing 4 sterigmata. Spores 5.7 - 8.2 x 6.5-8 mm, obovoid to ellipsoid, smooth. Cystidia numerous. Pleuro cystidia 24.5-36.2 x 10-14.2 µm (Fig. 2) Cheilocystidia 14.1 - 16 x 7-11.1 um variable in shape from clavate, cylindric to napi form/ thin walled. Hymenopheral trama indistinctly bilateral. All hyphae lacking clamp connection.

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Termitomyces heimii Natarajan sp. nov.

Sporophore gregarious growing in large numbers in the forest soil. (Plate IIIa) Pileus 6.7 cm in diam., convex

Fig 2 Termitomyces robustus

a Habit x 3/4

b Basidiospores x 2000

c Basidia x 2000

d Cheilocystidia x 2000

e Pleuro cystidia x 2000

a
b
c
d
e
10 µm

becoming planoconvex at maturity sub umbonate with obtuse tip. Surface white to light brown (P-10-1-A) greyish brown in centre, with prominent umbo (PL-13-1-A), smooth to squamulose; viscid when moist; margin entire to lobed. Context white, 8 mm thick; hyphae flat. Lamellae free, becoming pink 6 mm broad, crowded with lamellulae; margin serrate. Reaction with Melzer's reagent inamyloid. Stipe 10-19 x 1-1.5 cm, white, cylindrical attenuating towards base; solid above ground level; white, glabrous to squamulose, hollow below the soil with long pseudorhiza (PL-82-1-A), 16-0.7 cm which ends in the termite nest. (Plate IIIb) Annulus hanging. (Plate IIIc) persistent, spore print pink. Basidiospores ellipsoid, 7-9 x 4.5 µm, hyaline inamyloid, thick walled with few refractive guttules. Basidia clavate 22.25 µm x 6-8 µm, 2-4 spored (Fig.3) Cheilocystidia and pleurocystidia absent. Gill trama regular, hyphae 2-8 x 15 µm diam. All hyphae lack clamp connections.

Edibility - Excellent

Season - South-West monsoon 1984-85

Distribution - Collected from Manithookki and Kallar forest areas. They are seen growing in large numbers in forest area in the

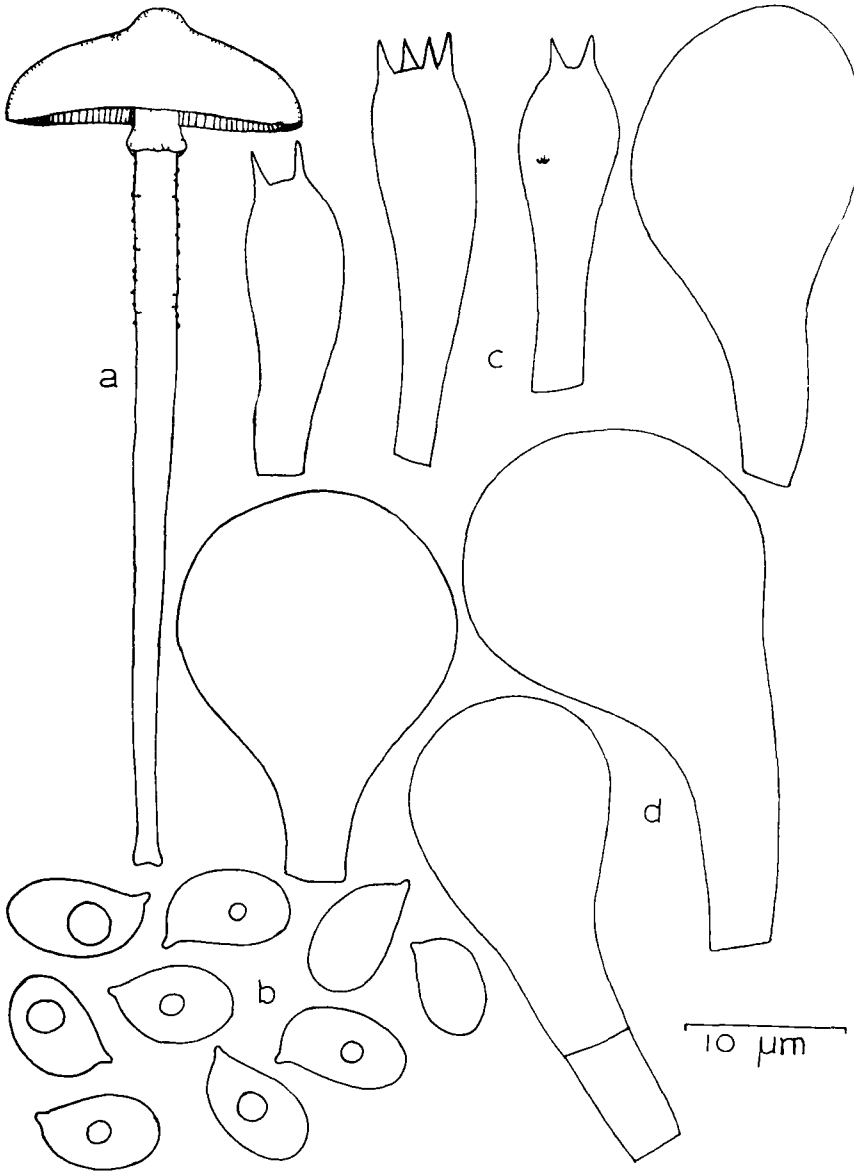

a
b
c
d
10 µm

Fig 3. Termitomyces heimi

a. Habit x 1

b Basidiospores x 2000

c. Bosidia x 2000

d. Cheilocystidia x 2000

Plate - III c *T. Reimii* stipe showing Superior, prominent annulus.

undisturbed ground under the trees. Tribal people collect them for consumption and marketing. Locally known as Perumkala.

Termitomyces clypeatus Heim, Bull. Jard. Bot. Bruxelles 21:207

Sporophore solitary or scattered on the ground. Pileus 3-6 cm diam., with very pointed perforatorium (Plate IV). Surface glabrous, light brown, with dark brown centre (PL-15-8-A), minutely striate; viscid when moist; margin lobed. Pileus surface a repent epicutis of hyphae 2-6 mm in diam., Context white 5 mm thick, hyphae 3-17 um in diam., reaction with Melzer's reagent inamyloid. Lamellae white to pale ochraceous; free, 0.5 mm broad, crowded with lamellulae. Stipe 4.8 x 0.3-06 cm, cylindrical, solid, white, becoming dark brown near the base. Base slightly swollen with 3-6 long, brownish black (3-6 x 0.8 cm) Pseudorhiza spore print pink. Basidiospores broadly ellipsoid 6-7 x 4.5 mm, hyaline, inamyloid, thick walled with prominent refractive guttules (Fig. 4). Basidia clavate, 18-21 x 6.8 µm, tetrasporic, sterigmata exceptionally long even upto 9 mm. Cheilocystidia pyriform 25-30 x 15-20 µm. Pleurocystidia rare 25-32 x 7-18 µm,

Fig 4. Termitomyces clypeatus

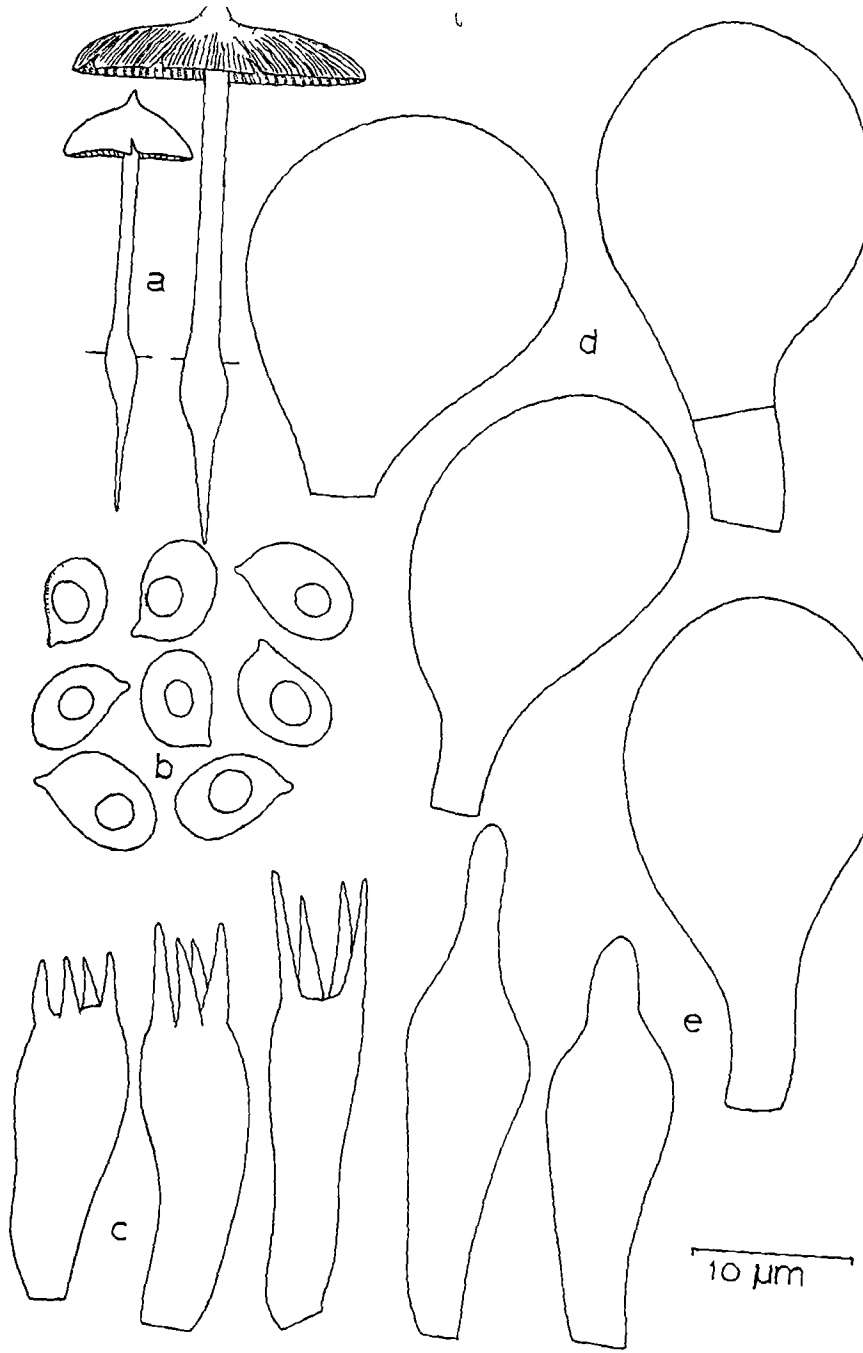
a. Habit x 1

b. Basidiospores x 2000

c. Basidia x 2000

d. Cheilocystidia x 2000

e. Pleurocystidia x 2000


a
b
c
d
e
10 µm

pyriform to ventricose, rostrate, thin walled. Gill trama regular, hyphae 8-14 mm. All hyphae lack clamp connections.

Edibility - Excellent

Season - North East and South West monsoon 1984-85

Distribution - Collected from Vellanad, Trivandrum district during May-June, 1984 and 1985. Locally known as Mullukoonu.

Termitomyces radicatus Natarajan sp. nov.

Sporophores solitary or scattered. Pileus 1.5-2.5cm in diam., convex to plano convex at maturity, sometimes uplifted with central pointed perforatorium; surface whitish grey (PL-7C-10) smooth. (Plate V) Pileal surface an epicutis of hyphae 2.5 mm broad; margin splitting and striate. Lamellulae free, white, 4 mm broad, crowded with lamellulae. Context 4 mm thick white; hyphae 2-6 um diam. Reaction with Melzer's reagent inamyloid stipe 2.5 x 0.2-0.3 cm. Cylindrical yellowish white, solid, glabrous, without annulus. Pseudorhiza 2.5 x 0.2 cm. Spore print pink. Basidiospores broadly ellipsoid, 7-8 x 4.5 µm, hyaline, inamyloid, thin walled prominent refractive guttule. (Fig. 5b) Basidia clavate, 27-30 x 6.7 µm, tetrasporic, sterigmata upto 3 µm long. Cystidia absent.

Fig 5a. Termitomyces microcarpus

a. Habit x 1

b. Basidiospores x 2000

c. Basidia x 2000

d. Cheilocystidia x 2000

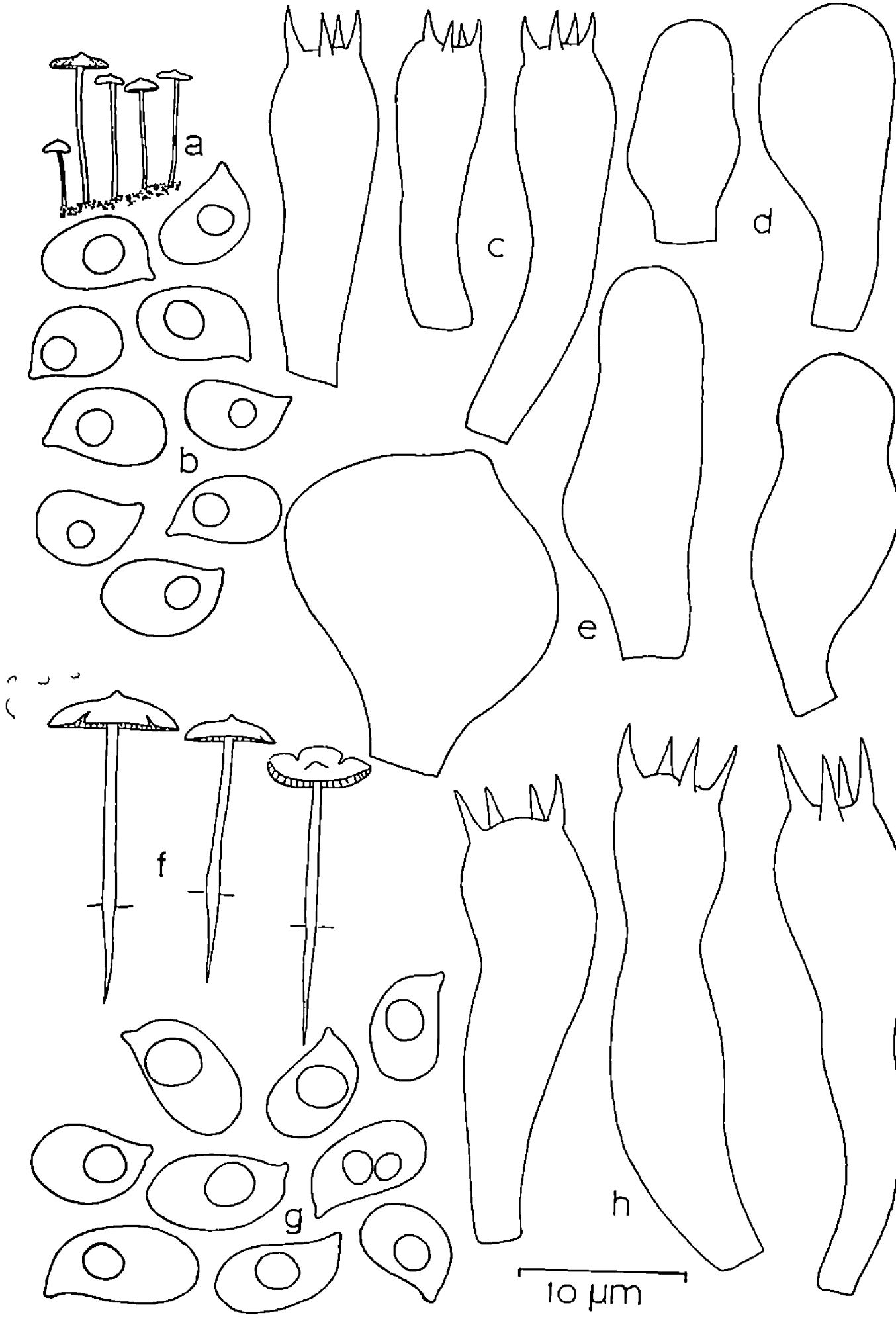
e. Pleurocystidia x 2000

Fig 5b. Termitomyces radicatus

f. Habit x 1

g. Basidispores x 2000

h. Basidia x 2000


a
b
c
d
e
f
g
h
10 µm

IV

Plate-IV Sporocarp of T. clypeatus showing spiny perforatorium

V

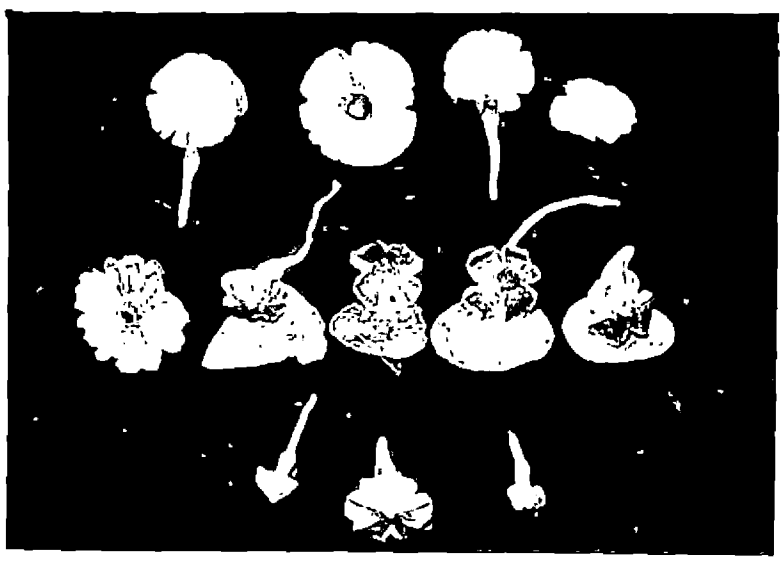


Plate - V T. radicatus

Plate-IV Sporocarp of *T. clypeatus* showing spiny perforatorium

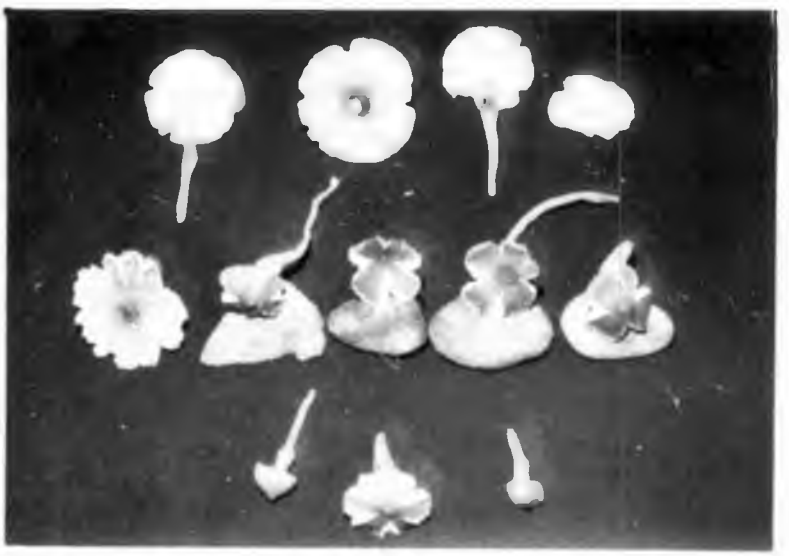


Plate - V *T. radicatus*

Gill trama regular consisting of thin walled parallel hyphae. All hyphae lack clamp connections.

Edibility - Excellent

Season - South-West monsoon period 1984

Distribution - Collected from Peyad, in Trivandrum district

Termitomyces striatus (Beeli) Heim in Mem. Acad. Sci. Institt France 64:47, tt 1-10 (1941)

Schulzeria striata Beeli in Bull. Jard. Bot. Brux 15:29,t.

Sporophore solitary or scattered in the soil under shade of coconut plantation. Pileus 8-10 cm diam., convex to flat surface smooth ivory white (PL-9-C-D) with broadly conical perforatorium; margin thick, incurved when young splitting on expansion (Plate VI) Lamellulae free, creamy white turning pale pink 3-4 mm broad, crowded with lamellulae. Stipe 18-20 x 2-2.2 cm, cylindric, white, slightly swollen in the middle and attenuated above and below, with pseudorhiza 9-10 cm long stuffed above slightly hollow below. Context 0.7 cm thick at the centre, spongy and whitish, consisting of interwoven hyphae which are inamyloid, thin walled 3-11 mm diam. Spore print pink. Spores 5.5-9 x 3-5 um, obovid to ellipsoid, hyaline, inamyloid, thin walled with few guttules. Basidia

Fig 6 Termitomyces striatus

A Habit (x½)

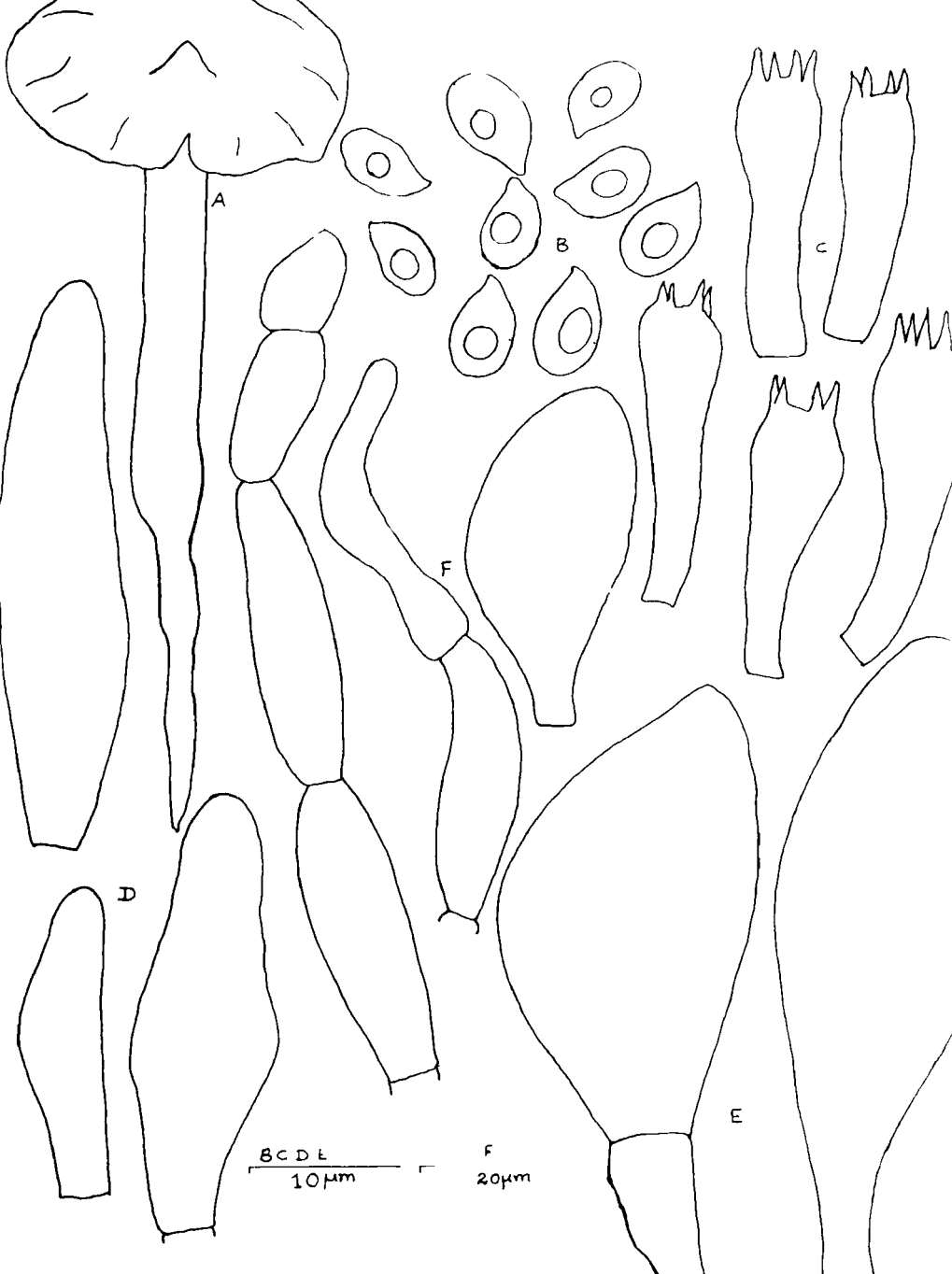
B Basidiospores (x2000)

C Basidia (x 2000)

D Cheilocystidia (x 2000)

E Pleurocystidia (x 2000)

F Pileus surface structures (x 1000)


A
B
C
D
E
F
BCDE
10µm
F
20µm

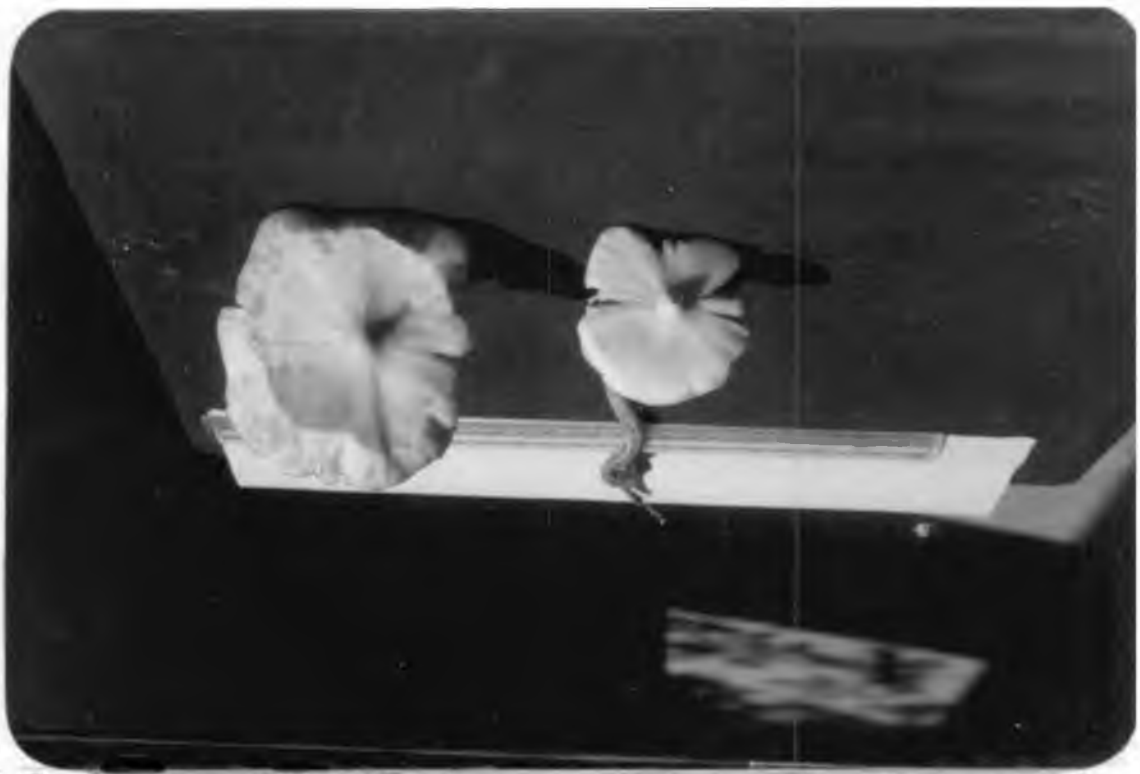


Plate - VI *T. striatus* sporocarp showing sturdy solid stipa and hollow fibrous pseudorhiza

14-25 x 5-6.5 um, clavate bearing four sterigmata. Cheilocystidia rare 25-27.5 x 10.15 µm clavate, pyriform, thin walled and hyaline. (Fig. 6) Pleurocystida not observed. Hymenophoral trama sub regular to regular, hyaline, inamyloid consisting of thin walled hyphae 2-5 mm diam. Pileal surface an epicutis of repent hyphae which are inamyloid thin walled 1.5 - 2.5 µm diam. Clamp connections absent.

Edibility - Excellent

Season - North East monsoon

Distribution - Collected from Pachalloor, Trivandrum district growing on the sides of the red soil mud fence under the coconut plantation.

Termitomyces perforans Heim in Termites et Champignons (1977)

Sporophore gregarious or scattered in small groups growing in the plain ground or in open fields. Pileus 2-2.5 cm diam., campanulate to convex, then expanding to plano convex with sharp, pointed perforatorium. Surface greyish white (PL-12-ABC) darker in the centre; dry, glabrous; margin straight, becoming deeply incised and lobed at maturity. Lamellulae free to adnexed, thin, white to pale pinkish; 2 mm broad, moderately crowded

with few lamellulae. Stipe 4-4.7 x 0.2 - 3mm., white, solid, smooth cylindric above the soil and with a base continuing as 3-4 cm long and white pseudorhiza. Context thin, white, consisting of loosely inter woven hyphae with inflated cells of 35 mm diam. Spore print pink. Spores 6.5 - 7.2 x 3.5-4.7 µm, ovoid to ellipsoid, hyaline, inamyloid, thin walled with one or more refractive guttules. Basidia 24-33.4 x 4.66-7.4 µm, clavate with 4 sterigmata. Cheilocystidia and pleurocystidia absent. Hymenophoral trama regular, hyaline; hyphae 4.4-7.5 to 16.1-19 mm diam. Pileal surface an epicutis of thin walled, hyaline parallel hyphe of 5-75 mm diam. Clamp connections absent.

Edibility - Excellent

Season - South West monsoon

Distribution - Collected from Mavelikkara and Kottarakkara during June 1984. Commonly collected and consumed by local people and is known by the local name 'Arikoomu'.

Termitomyces globulus Heim and Goossens in Bull. Jar. Bot. Brux. 21: 216, 1951

Sporophores solitary or scattered growing on plain fields. (Plate VIIa) Pileus 8-12.5 cm diam., at

Plate - VIIa *T. globulus* sporocarps with excavated termitaria in the sandy soil.

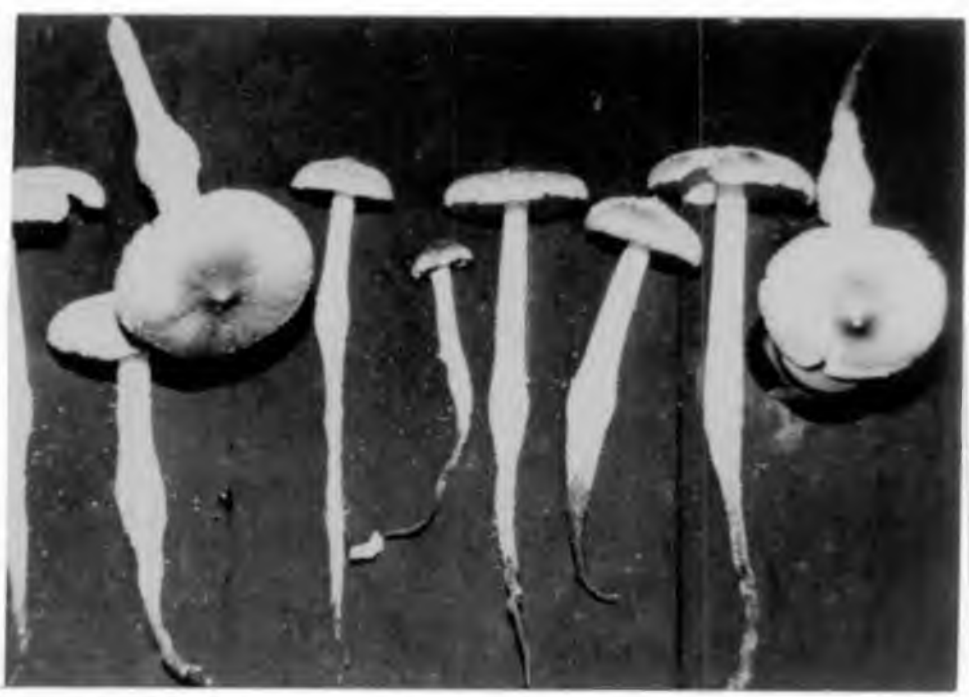


Plate - VII b *T. globulus* sporocarps showing the black long pseudorhiza, globulus pileus with perforatorium.

first globose and sometimes staying so or expanding to convex - campanulate or applanate with a poorly defined umbonate perforatarium. Surface pale dull sepia (PL-e-A-10), shining, smooth to slightly fibrillose, often cracking radially almost to the centre. Margin, thin, at first involute and remaining inflexed even after expansion. Lamellulae free, white to pink; 8 mm broad, crowded with lamellulae. Context white, 10.5-15 x 1.5-2.2 cm above ground; cylindric, solid, surface whitish, fibrous devoid of any veler structures, expanding slightly below ground level (1.7-2 cm) and then forming a long slender tapering brownish black pseudorhiza (10-13 x 0.7 cm) (Plate VIIb) Spores 6-8 x 3-4 um, obovoid, ellipsoid. Spore print pink. Basidia 26-30 x 7-8.5 µm, clavate, bearing four sterigmata. Cheilocystidia polymorphic, hyaline. Pleurocystia rare 25.35x 13.20 µm. Hymenophoral trama at first bilateral becoming subregular. All hyphae lack clamp connections.

Edibility - Excellent

Season - South West and North East monsoon

Distribution - Collected from coastal sandy loam under coconut plantation mixed with cashew trees. Puthenthope, Trivandrum district. Commonly known as "Parambinkoon".

Termitomyces microcarpus (Berk. and Br.) Heim in Mem. Acad. Sci. Inst. Fr. 64:72 (1941)

Hygrophorus obrussaeus Berk. in Hooker, Lond. Journ. Bot. 6.489 bis (1847) proparte, non H. obrussaeus (Fr.) Fr., Epicrisis, 331 (1838)

Agaricus microcarpus Berk. and Br. in journ. Linn. Soc., Bot. 11:537 (1871).

A. intermixtus Berk. and Br., loc, Cit,: 537

A. Sciolus kalchbr. in Grevillea 9:111 (1881)

Hygena, sciola (Kalchbur.) Sacc., syll. Fung. 5:297(1887)

Entoloma microcarpum (Berk. and Br.) Sacc. loc. Cit.:687

E. intermixtum (Berk. and Br.) Sacc. loc. Cit,: 692

Collybia microcarpa (Berk. and Br.) V. Hohn. in Akad. Wiss. Wien Math: natur KI. 117:993 (1908)

M. microcarpa (Berk. and Br.) Pat. in Bull SOC. Mycet. Fr.29: 213 (1913).

Gymnopus microcarpus (Berk. and Br.) Van overeem apud Heyne Natt. Pl. Nederl. Ind. ser. 2, 1:76 (1927).

M. termitum Beeli in Rev. Zool, Bot. Afric. 21:327 (1932).

Podabrella microcarpa (Berk. and Br.) Singer in Lloydia 8:144 (1945)

Termitomyces narohiensis Otiero in proc. E.Afr.Acad.2:110 (1966).

Plate - VIII

T. microcarpus sporocarps natural habitat

Plate - IX

Sporocarps of T. microcarpus var. *santalensis*

Sporophore growing in large numbers on the ground and in lawns. (Plate VIII) Pileus 0.5-3 cm diam., campanulate to convex, expanding, umbonate to papillate. Surface whitish to cream sometime pale brownish darkening ochraceous at the centre (PL-12-BCD) dry, glabrous. Margin entire, and incised. Lamellulae subfree to adnexed, thin, white to pale cream, crowded 1-2 mm broad, with lamellulae. Context thin, white consisting of interwoven hyphae 5-10 mm diam. Reaction with Melzer's reagent inamyloid. Stipe 2-4 x 0.5 slender, whitish, straight, cylindric attenuated to form a pseudorhiza which is not distinct. Spore print pink to flesh coloured. Spores 6-8.5 x 3.7-4.8 um ovoid to ellipsoid hyaline to pale straminecus, inamyloid, thin walled with one or more refractive guttules. (Fig. 5a) Basidia 20-30 x 6-8 µm, clavate. Cheilocystidia and pleurocystidia similar inconstant and often rare 16-40 x 9-16µm pyriform to cylindric. Hymenophoral trama regular. Pileal surface an epicutis of thin walled radially parallel hyphae 3-4 mm diam. All hyphae lack clamp connections.

Edibility - Excellent

Season - South West and North East monsoon

Distribution - Collected from all the localities through out the State. A common mushroom growing in large numbers during the monsoon season giving the appearance of spreading rice grain on the soil hence it is locally known as "Arikoon"; collected and consumed by the people of the State.

Termitomyces microcarpus (Berk. and Br.) Heim forma gentalensis Heim, Mem. Acad. Sci. Inst. France 64:73 (1941).

Sporophore growing in groups or scattered on the plain ground. Pileus 0.3-0.8 cm Pileus 0.3 - 0.8 cm in diam. (Plate IX) convex to plane convex at maturity with central small umbo; surface striate, glabrous, white (PL-91-A); margin entire, rarely, lobed. Lamellulae free, less crowded, whitish, thin, with few lamellulae. Pileus surface a repent epicutis; hyphae made up of cylindrical units, 25-15 x 7-13 µm. Context very thin 1 mm thick; hyphae 4-10 µm, thin walled, branched; reaction with Melzer's reagent thin inamyloid. Stipe cylindrical 1-2.5x 0.1-0.2 cm, solid glabrous without pseudorhiza. Spore print pink. (Plate X). Basidiospore short, ellipsoid, 6.7 - 4.5 µm, hyaline, inamyloid, thin walled. Basidia clavate 17.24 x 6.7 µm, tetrasporic with sterigmata up

Table-2

Stages of developmental morphology of T. robustus

Stages of development	Time in h	Morphological characters	Measurement of different structures in mm					
			Complete sporocarp	Pileus	Stipe		Pseudorhiza	
					Length	Diam.	Length	Diam.
1. Spherule	24	Globose to subglobose white, solid	1	-	-	-	-	-
2. Clove bud	48	Resembles clove bud in shape with round head and short cylindrical stalk. Pileus region dark brown in colour.	18	8	3	5	3	5
3. Primordial elongation	72	Pileus and stipe differentiated. Pileus globose brown and viscid, stalk thick, brown	28	8	15	7	15	7
					undifferentiated			
4. Pseudorhizal stage	96	Pileus distinctly campanulate cremish white with prominent brownish umbo. Stipe rudimentary. Prominent black pseudorhiza which pushes the organs upwards.	-	45	-	-	15	11

(Contd.....2)

Stages of development	Time in h	Morphological characters	Measurement of different structures in mm					
			Complete sporocarp	Pileus	Stipe Length	Stipe Diam.	Pseudorhiza Length	Pseudorhiza Diam.
5. Epigeal button stage	120	The stipe and the pseudorhiza elongates and thickens and slowly pushes the pileus region above the surface of the soil and emerges as small convex to globose structure.	-	72	18	12	98	12
6. Epigeal egg stage	144	Pileus convex, campanulate; brown, smooth and viscid with central dark raised umbo. Stipe short white, thick and solid.	-	81	28	19	125	18
7. Epigeal elongation	168	Pileus increases in diam., stipe becomes prominent elongates; white cylindrical smooth. Pseudorhiza long and tapering.	-	92	38	19	125	18
8. Mature stage	192	Sporocarps attains full size stipe elongates fully and the pileus increases in dia; convex to planoconvex, creamish brown smooth and viscid margin entire, surface with pointed dark brown perforatarium in the centre. Pseudorhiza long, tapering and sclerotised.	-	112	98	21	135	18

to 4 µm long. Gill edge heteromorphous. Cheilocystedia clavate 15.23 x 6-9 µm with large vacules. Pleurocystidia clavate to pyriform 19.25 x 2.15 µm with large vacuoles; thin walled. Gill trama regular with hyphae 3-10 µm broad.

Edibility - Excellent

Season - North East monsoon

Distribution - Collected from debris of termite nurseries seen in groups. Collected during September October 1985 from Panniyoor, Cannanore and Trivandrum districts. Commonly known as 'Arikoom'.

Developmental morphology of Termitomyces robustus

Developmental morphology of T. robustus which appeared commonly in two localities, viz., Vellayani and Peyad (Trivandrum district) were studied in detail during the South West and North West post monsoon periods. Based on the morphological differentiation, eight stages, viz., Spherules, Clove bud, primordial elongation, pseudorhizal stage, Epigeal button, Epigeal egg, Epigeal elongation and Mature stage were observed and described (Table 2). The first four stages of growth and development were hypogeal while remaining four stages were epigeal. (Fig. 7).

Fig 7 Different stages of development of Termitomyces robustus

I Spherules

II Clove bud stage

III Primordial elongation

IV Pseudorhizal stage

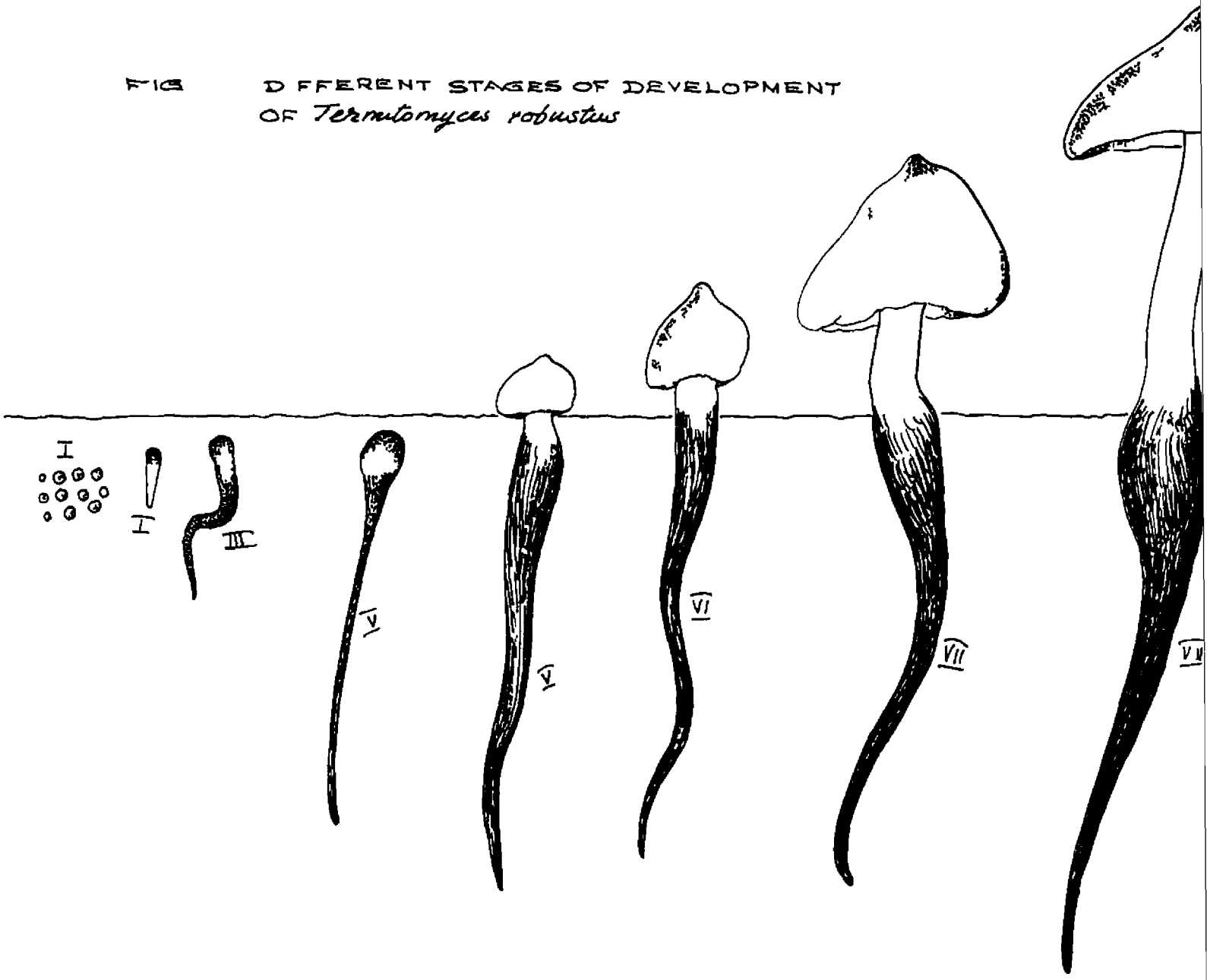
V Epigial button

VI Epigial egg

VII Epigial elongation

VIII Mature stage

FIG DIFFERENT STAGES OF DEVELOPMENT OF *Termitomyces robustus*



Excavations of termitaria from different places revealed the presence of large number of spherules all over the termitaria. This tiny spherules twenty four hours after its first appearance developed into globose, white, solid, structures of 1 mm diam. Critical observations of spherules in vertical sections revealed an undifferentiated pileus and stipe and the whole spherules appeared as a tiny knot of hyphae.

After 48 h of development the globular spherules were transformed into a shape of clove buds with round head and short cylindrical stalk. The apical globose thick region slowly developed into deep brown, smooth, solid pileus (8 mm dia.) and cylindrical undifferentiated region (stalk) consisting of stipe and pseudorhiza about 8 mm in length.

The third stage of development, viz., primordial elongation (72 h) revealed distinct thick globose dark brown, solid pileus of 9 mm diam., and a stipe with the size of 15x7 mm. During this stage the rate of growth of stipe region is more than that of pileus region.

In the pseudorhizal stage, pseudorhiza forms the predominent structure measuring 15 x 11 mm. The pseudorhiza is dark brown and thick and tapers towards base.

The pileus is comparatively small and convex with a brownish umbo.

In the epigeal button stage (120 h) the pileus emerges just above the soil as small globose to convex structure with a short rudimentary stipe. Pileus surface creamish brown, smooth, viscid when wet. The pseudorhiza dark brown, fibrous, 98 x 12 mm long below the soil.

The fifth epigeal egg stage of development (144 h) revealed the following structures. Pileus 81 mm diam., campanulate brownish, smooth, viscid when wet central dark raised umbo. Stipe short, thick, white, cylindrical, smooth and solid (28x10 mm); pseudorhiza 125x18 mm diam., near the soil level tapering towards the tip (5-8 mm), root like fibrous brownish black and sclerotised.

In the epigeal elongation stage of 168 h from the spherule stage the pileus expanded and increased to a diam., of 92 mm. Pileus dark brown in the centre with broadly spiniform perforatarium and pale brown towards the margin; margin entire. The stipe considerably elongates to a size of 38x10 mm which enlarges into bulbous base (10 mm) just below the soil level and again abruptly narrows down into a long brownish black sclerotised pseudorhiza of 125x18 mm.

The final mature stage after 192 h of development depicts the last stage of development of the sporocarp and is observed growing solitary above the termitearia. Pileus 122 mm diam., convex to plane convex, surface dark brown in centre, pale greenish brown towards margin, smooth viscid when wet. Perforatorium broadly spiniform. Margin straight, reflexed and incised in the old specimen. Stipe 98x21 mm cylindrical smooth white and solid. Pseudorhiza long, deep brown to black, fibrous, sclerotised 135x18 mm in size tapering towards the basal region which ends in termite comb.

In vitro studies on the developmental morphology of T. robustus

To study the growth of fungi on the combs, three productive termite combs were excavated, transferred in sterile glass troughs, brought to the laboratory and incubated. After three days incubation, strands of mycelia developed from the comb and grew upwards. Outward growth of mycelia from the base of the comb was first initiated by a number of robust leading hyphae, which branched at fairly wide intervals to form progressively thinner branches. Some of the branches reached to the lid to which they became appressed and grew towards all

Table-3

Temperature and humidity inside and outside the combs of T. robustus

	Inside the comb	Outside the comb
Temperature ^{o}C	29.06	28.20
Humidity (Percentage)	100	90.00

directions, in such a manner that the entire surface of the trough became covered by these creeping hyphae which later anastomosed. These hyphae were later identified as species of Xylaria. Within a period of three weeks these hyphae became tough and dark and developed into long black stalks. It was also observed that sphe rules did not establish any further growth in these combs. These results were repeatedly observed in all the combs under study.

Ecology and Symbiosis

Comparative study of the internal and external temperatures and humidity of the comb of T. eubustus

The data relating to the temperature and humidity given in the table 3 revealed that there was not much variation in the maximum and minimum temperatures inside the termitarium and in the surrounding soils. Maximum and minimum temperature recorded inside the comb and surrounding soils were 31.2, 28.1, 29.5 and 27.0°C respectively. Results showed that the combs were slightly warmer (31.0°C) relative to the surroundings (29.5°C). Maximum internal humidity of 100 per cent was recorded inside the comb while it was 88-98 per cent in the surrounding soils were 99.6 per cent and 80 per cent

Table-4

Chemical composition of termite combs in g/100 GDM

Name	Moisture	Cellulose	Carbon	Nitrogen	pH	Ash
T. heimi	8.4	17.01	36.88	0.022	4.8	5.0
	8.6	17.01	38.00	0.025	6.5	10.5
	8.8	18.03	37.92	0.026	4.3	8.5
	8.8	18.1	36.99	0.024	4.8	7.5
Mean	8.7	17.5	37.5	0.024	4.5	7.0
T. rubustus	8.9	17.02	38.50	0.026	4.5	10.5
	8.7	17.03	41.04	0.025	4.8	7.0
	8.6	17.03	40.12	0.021	4.1	8.5
	8.8	17.01	39.20	0.027	4.3	9.5
Mean	8.8	17.02	39.7	0.025	4.4	8.15

respectively. Observations showed that the combs were fully saturated with water vapour.

Chemical composition of the combs of T. robustus and T. heimii

The chemical composition of the combs of T.robustus and T.heimii given in the table 4 showed that there was no significant difference in the total moisture, cellulose, carbon, nitrogen content and pH. The moisture percentage of the combs of T.robustus and T.heimii were 8.8 and 8.7 respectively. The cellulose, carbon and nitrogen content of the combs of T.robustus were 17.02, 39.7 and 0.025g/100g DM respectively where as in the case of T.heimii it was 17.5, 37.5 and 0.024g/100g DM respectively. The pH values of the combs of T.robustus and T.heimii were 4.4 and 4.5.

Isolation of other fungi from the termitaria

Isoletions of other fungi from six samples of termitaria collected from different localities revealed the occurrence of 19 species of fungi belonging to 12 genera. The results obtained are given in the Table 5 . Among the 19 species of fungi Aspergillus and Xylaria were found to be the predominent fungi in the combs.

Table-5

Fungi isolated from the combs of T.ruhnatus

	Name of fungi
1.	Aspergillus niger
2.	A. flavus Link
3.	A. oryzae (Ahlburs) Cohn.
4.	Aspergillus sp.
5.	Penicillium sp.
6.	Stemphylium lanuginosum Hars.
7.	Fusarium semitectum (Corda) Sacc.
8.	Fusarium sp.
9.	Trichoderma sp.
10.	Xylosphera furcata
11.	X. nigripes (Klotzsch) Dennis.
12.	X. multiplex
13.	Xylosphera sp.
14.	Gliocladium roseum (Link) Bainier
15.	Mucor sp.
16.	Neurospora sp.
17.	Botryodiplodia sp.
18.	Alternaria sp.
19.	Torula sp.

Table-6

Species of termites associated with Termitomyces spp.

No.	Name of Termitomyces spp.	Name of termite spp.
1.	T. robustus	Odontotermes brunneus (Hagen)
2.	T. heimii	Odontotermes malabaricus (Holmg. and Holmg.)
3.	T. clypeatus	Odontotermes redemanni (Wasmann)
4.	T. radicatus	Odontotermes obesus (Rambur)
5.	T. microcarpus	Odontotermes obesus (Rambur)
6.	T. microcarpus var. santalensis	Odontotermes obesus (Rambur)
7.	T. striatus	Odontotermes species

Termites associated with the termitaria

Different termite species associated with different species of termitomyces were identified and listed Table 6 It was found that Odontotermes was the most common termite genera found throughout Kerala associated with these mushrooms.

Odontotermes obesus was found to be associated with the termitomyces species belonging to pratermitomyces. The rest of the termitomyces species belonging to the subgenus eutermitomyces showed speficity in association with different species of termites. O. brunneus, O. malabaricus, O. redemanni and O. species showed symbiotic association with T. robustus, T. heimi, T. clypeatus and T. striatus respectively.

Pests of Termitomyces sp.

The Amblyrrus cinctipinnis was found to be the common pest of different termitomyces sp. The beetle was found to infest and feed the emerging as well as mature sporocarps which made the sporocarps unfit for consumption.

Effect of soil moisture on the production of sporopheres of T. globulus

Studies on the effect of soil moisture for the production of sporopheres given in the table 7 revealed that

Table-7

Incidence of sporocarps in T. robustus in irrigated and non irrigated (Control) termite nests

Plot No.	No. of termite nests	No. of sporocarp emerged	
		Irrigated	Non irrigated
1	6	27	27
2	6	19	11
3	6	20	14
4	6	15	10
Total	24	81	53
Mean		3.38	2.22

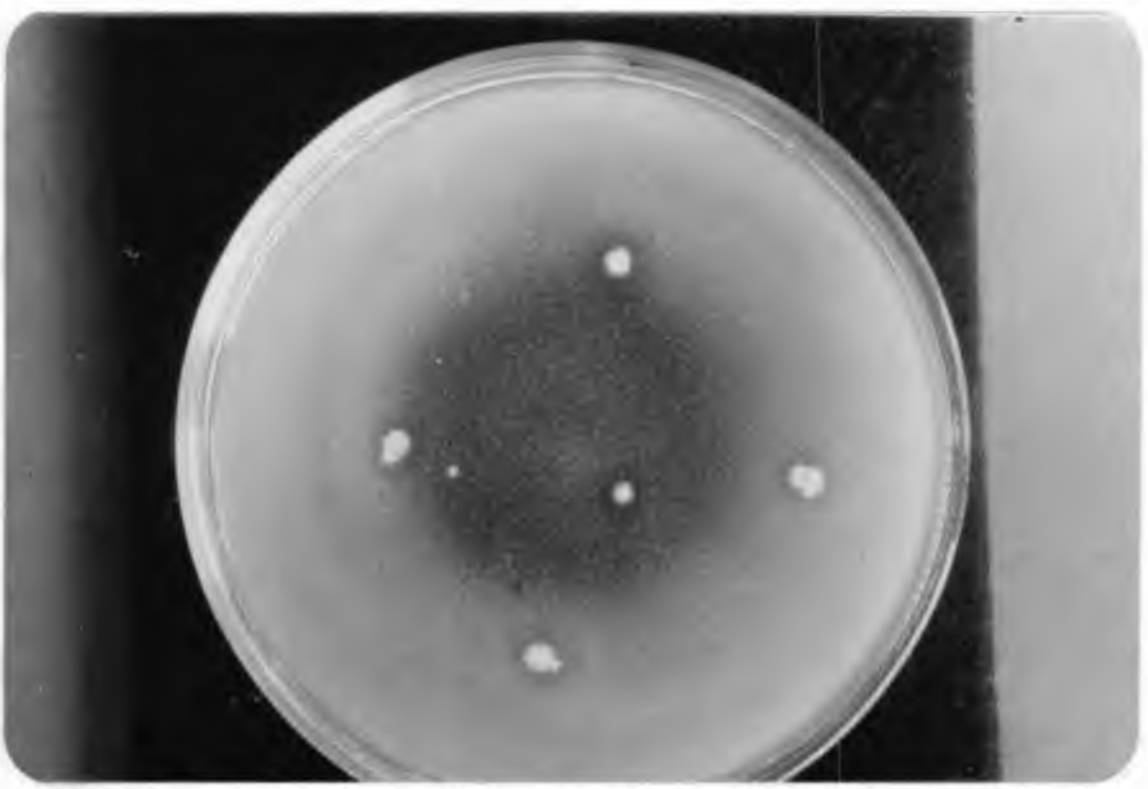


Plate-x Scanty mycelial growth of *T. robustus* on Rebecca's medium

the number of sporocarps emerged from daily irrigated plots was maximum (81) when compared to the non-irrigated plots (53).

Cultural studies

Growth of Termitomyces in different solid and liquid media

In order to study the growth character of T.robustus on different media, tissues as well as spherules were inoculated aseptically in different solid and liquid media. Mycelial growth was not observed in all the media inoculated with tissues. But scanty growth was observed in Rebecca's medium (Selective medium) at 30°C inoculated with spherules Table 8 . Maximum radial growth obtained was 16 mm (Plate X).

Effect of different temperatures on the growth

Growth of Termitomyces spherules at different temperatures was studied using the Rebecca's solid medium. The dishes were incubated at 4 different temperatures, viz., 20°C, 25°C, 30°C and 35°C. (Fig. 8) Observations showed that maximum mycelial growth of the fungus was observed at 30°C (16 mm) followed by 25°C (13 mm). No growth was observed at temperatures above 30°C and below 25°C.

Effect of different sources of carbon

In order to find out the best source of carbon for the mycelial growth, dextrose maltose and lactose were incorporated in the basal solid medium. Observations revealed that cellulose was the best source of carbon showing maximum radial growth (16 mm) followed by maltose (14 mm) and lactose (10 mm) respectively. Growth was poor when glucose was used as carbon source.

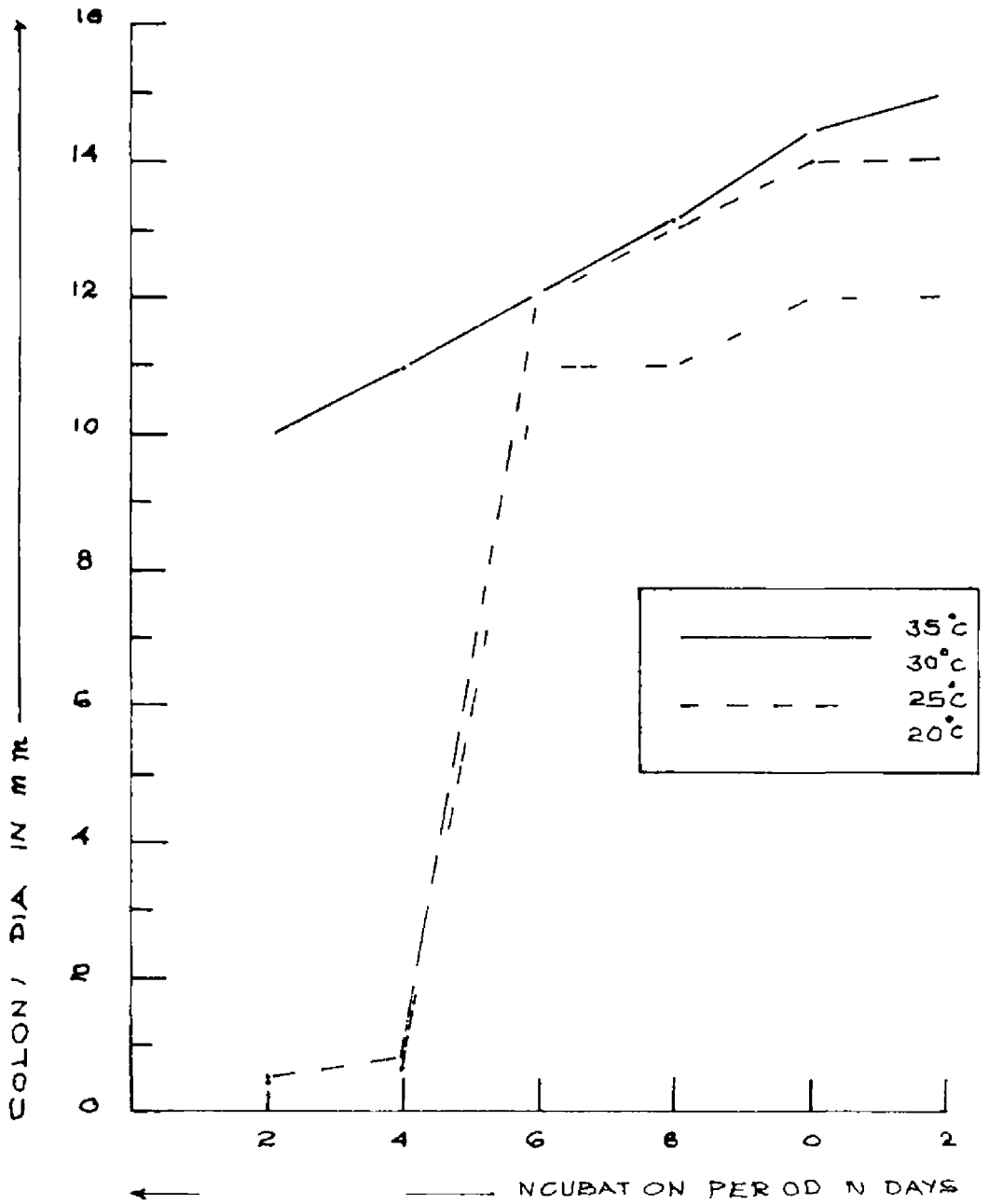
Table-8

Radial growth of T. robustus on different solid media incubated at different temperatures (in mm)

Medium	Temperature 20°C incubation in days						Temperature 25°C incubation in days						Temperature 30°C incubation in days						Temperature 35°C incubation in days					
	2	4	6	8	10	12	2	4	6	8	10	12	2	4	6	8	10	12	2	4	6	8	10	12
Potato dextrose agar	*-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oat meal agar	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nutrient agar	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Saboraud media	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Purkayastha's synthetic media	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Czapek's agar	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Richard's medium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rebecca's media	0.5	0.6	11	11	12	12	0.5	0.8	12	13	14	14	11	12	13	14	15	16	10	11	12	13	14	15

*- Nil

FIG RADIAL GROWTH OF *Termitomyces robustus* N REBECCAS MEDIUM



Effect of light and darkness on the mycelial growth

The results indicated that the rate of growth of the fungus was more or less same in the case of cultures incubated in the light and darkness. Maximum growth (15mm) was attained on the 12th day of incubation in darkness while the corresponding maximum growth observed in the case of cultures incubated in light was 14mm.

Comparative efficacy of different spawn substrates for supporting mycelial growth of fungus.

Studies conducted with six substrates for supporting the mycelial growth revealed that the substrates were covered with non-basidiomycete mycelium after 48 h of incubation.

Nutritive value of sporophore

The nutritive value of the sporophores of six species of Termitomyces was assessed and the results obtained are given in Table 9 . The true protein content was between 28.99 g/100 g and 20.49 g/100 g dry matter. Termitomyces heimii was found to have maximum protein content (28.99 g/100 g dry matter). Minimum protein content was observed in the case of T.robustus (19.84g/100g dry matter). The carbohydrate content was between 53.92g/100g and 46.1g/100g dry matter. Maximum

Table-2

Nutritive value of different Termitomyces spp.

	True protein (g/100g DM)	Carbohydrate (g/100g DM)	Fat (g/100g DM)	Crude fibre (g/100g DM)	Total free aminoacids (g/100gDM)	Ash	Dry wt.%
1. *T. robustus*	19.84	52.23	6.4	5.300	0.825	7.0	8.9
2. *T. heimii*	28.99	33.10	2.5	8.8	8.062	10.5	11.8
3. *T. clypeatus*	23.84	53.92	3.5	8.8	0.625	8.5	10.5
4. *T. radicatus*	22.30	49.1	4.2	3.1	0.838	12.0	8.9
5. *T. microcarpus*	20.4	46.1	3.8	3.2	0.82	10.81	8.1
6. *T. microcarpus* var *santaleensis*	21.4	50.01	4.8	3.2	0.741	9.27	7.9
7. *T. globulus*	22.10	49.2	3.9	3.1	9.82	11.9	8.1

Table-10

Qualitative estimation of ten essential amino acids recorded from Termitogyrus rotundus

1.	Lysine	+
2.	Histidine	+
3.	Arginine	+
4.	Threonine	+
5.	Valine	+
6.	Methionine	+
7.	Isoleucine	+
8.	Leucine	+
9.	Tyrosine	+
10.	Phenyl alanine	+

+ indicate the presence.

carbohydrate content (53.92g/100g dry matter) was observed in the case of *T. clypeatus*. The fat content ranged between 6 and 2.5g/100g dry matter and the maximum was recorded in the case of *T. robustus* (6g/100g dry matter). The crude fibre value ranged between 8.8 and 3.2g/100g dry matter. Ten essential amino acids were detected in the sporocarps of *Termitomyces robustus* and are listed in Table 10 .

Preservation

Dehydration

Visual observations of six samples of dehydrated mushrooms kept in closed polythene bags and in air tight containers preserved for a period of 3 to 12 months revealed that samples were free from microbial spoilage. The samples kept open were deteriorated within one week and damaged by maggots and moulds like *Rhizopus* and *Aspergillus*.

Powdering

Powdered samples kept in air tight containers and polythene bags remained free from any microbial spoilage for a period of ten months. The samples kept open remained only for a period of two weeks which subsequently deteriorated.

Refrigeration

Storage of fresh sporocarps of Termitomyces robustus under refrigeration revealed that the samples which were kept in open polythene bags remained fresh up to 48 h of storage. The samples started shrinking and showed brown discolouration after 72 h of storage. Organoleptic tests also showed no taste difference after cooking The sample kept for 48 h developed a bad flavour and was found to be unpalatable after cooking. Samples kept in closed polythene bags showed that after 24 h of preservation accumulation of moisture in polythene bags and oozing of dark brown liquids from the mushrooms. Such sporocarps were unfit for consumption and decayed emiting a foul smell.

Preservation in brine

Fresh mushrooms in epigeal egg stage were harvested cleaned and preserved in different concentrations of brine (1 to 7%) for six weeks. Visual observations of the preserved mushrooms at different concentrations of brine revealed that the mushrooms retained more or less original colour. Organoleptic tests of the above samples gave moderate acceptability. The data (Table 11) showing the microbial assay of preserved mushrooms conducted at

erial growth when preserved in 5, 6 and 7 per cent brine up to four weeks. The results indicated gradual reduction in bacterial population as the concentration of brine increased. Actinomycetes was absent in all the treatments throughout the experimental period.

Blanching

Visual observations on the blanched specimens packed in polythene bags kept at room temperature showed that the mushrooms remained fresh for only four days. The same sample kept under refrigeration remained fresh up to one week. The blanched specimen kept in sterilised jars remained fresh up to three months.

Pickling and ketchup

The samples which were preserved by these methods remained free of microbial attack for a period of six months. Both the preparations were palatable and had a very good taste.

DISCUSSION

DISCUSSION

The warm humid climatic conditions and diversity in soils of the state favour the luxuriant growth of a wide variety of fungal flora. No sustained effort has been made so far for a systematic study of this highly prized esculent native macrofungi. Results of the present preliminary state wide survey conducted on the occurrence of Termitomyces flora of Kerala revealed the immense potentialities of this State to support the growth of this excellent species of termite agaric. First step to exploit this protein source is the systematic collection, identification, detailed description and documentation of each species along with the place of occurrence and the seasons of its appearance. The most detailed accounts of this intriguing, paleo tropical mushroom genus Termitomyces Heim and its relationship with termites were those of Heim (1977) mainly from Central Africa and Batra and Batra (1979) in India.

For the present study of Termitomyces species of Kerala, intensive and extensive collections were made during the South West and North East monsoon periods during 1984-85 in 32 localities. Identification of the collections revealed the occurrence of nine species of

Termitomyces of which seven belong to the subgenera Eutermitomyces and the rest two pretermitomyces. Based on the salmon pink colour of the spore print it is grouped under rhodosporae (Singer 1961). The results of macrochemical tests performed on fresh materials in accordance with Hetling (1971) show no marked or consistent difference between species and was recorded as inamyloid.

The ethnomycological data of the nine species of Termitomyces were also collected as far as possible from the natives of the State.

In order to document the most common species of the State, their frequency of occurrence and abundance were recorded by collecting same species repeatedly from thirty two localities of the State with special reference to several places in Trivandrum district. Observations showed that T. microcarpus, T. microcarpus var. santalensis and T. robustus were the most common and widely distributed species throughout the State irrespective of soil type. Bhavani Devi (1982) reported the occurrence of T. microcarpus and T. robustus for the first time from Kerala. Bose and Bose (1949), Heim (1952), Natarajan (1975), Sethe et al. (1980) and

Leelavathy et a (1984) also reported the occurrence of T. microcarpus and T. microcarpus var santalensis from different parts of the country.

Though the people of Kerala used to collect and consume T. microcarpus spp. from time immemorial no systematic study has been made in the ecology and taxonomic character of this species till Bhavani Devi (1982) gave a detailed account of the mushroom flora of the State. This small fungus with a maximum basidiome size of 2.5 cm occurs in swarms over large area. Pseudorhiza and a prominent perforatorium are lacking in T. microcarpus and T. microcarpus var santalensis. But they differ mainly due to the colour variation of the pileal surface. In T. microcarpus the colour of the pileal surface ranges from greyish white to creamish white while in T. microcarpus var santalensis it is always ivory white.

Heim (1952 and 1977) has thoroughly discussed T. microcarpus (subgenus pratermitomyces) and its variants, which showed epigeal development, lacking pseudorhiza and have a regular hymenophoral trama. Typical T. microcarpus is recognised by its small size and its appearance in spectacular swarms on the ground. He observed that species of termites appeared to have adapted to some

Plate - X1a *T. heimii* growing on hard ground

Plate - X1b *T. heimii* on the termite mounds

change in T. microcarpus. In this case during rainy season when the fungus is about to fructify the termites habitually shave away the outer layer of its fungus combs and spread them on the surface of the soil above the nest where the fructification occurs. He described T. microcarpus var. santalensis from santal in Bihar.

T. microcarpus is one of the most common species collected and consumed by the natives and is locally known by the name 'Arikoon' because its appearance resembled white rice grains spread on the ground.

T. heimi. The medium sized species of Termitomyces was found to grow gregariously consisting of hundreds of fruiting bodies in a group in the forest soils of the state. Critical observations of the sporophores collected from the different localities in the forest area revealed that the termites in the undisturbed soil below the thick vegetation used to build epigeal mounds and the basidiocarps developed in and around the mounds have short pseudorhiza, (8-10 cm) compared to the basidiocarps with long pseudorhiza (25-30 cm) collected from the hard ground sites (Plate XI a and b) In the first case the termitarium was located in few centimeters below soil above the termite mounds where as in the second case it was located

at a depth of 25-30 cm. Plate XIc, Observations showed that the length of the pseudorhiza depended upon the depth of the subteranian termitaria. The above observation corroborates with that of Heim (1952) who considered that the size and form of the termite fungi were to some extent influenced by the position of the combs. Pileus of this species was fleshy with broad umbo. Stipe strong and solid with prominent annulus. Natarajan (1979) described T. heimii as a new species by substantiating the contrasting characters of T. eurhizus (Berk) Heim which closely resembled T. heimii. Present collection also resembled species of Lepiota by possessing white pileus, thick annulus and peculiar odour which are the distinguishing morphological features of Lepiota. This could be the reason why Heim (1941) and Pegler (1942) reported it as Lepiota albuminosa (Berk) Heim and Macrolepiota albuminosa (Berk) Pegler.

Though T. heimii is less common in their distribution than T. robustus and T. microcarpus and mainly confined to the forest areas, it appears to be one of the major food items during the season for the tribes and people who live in the near suburbs. 'Perumkada' is the name given to this fleshy Termitomyces species because

of its long pseudorhiza looking like a white stick. Since they occur in large numbers during the season, it forms a popular commodity of the local market.

Termitomyces robustus belonging to the subgenera Eutermitomyces is the second common and popular Termitomyces species distributed throughout the state irrespective of soil type and physiography. This large robust species is always found to occur above the hypogeal termitarium of Odontotermis hrunneus, located 20-30 cm below the soil level. Hence it is characterised and distinguished by dark spiniform perforatorium and long black, fibrous pseudorhiza. These two prominent and striking characters make the species unique. Here again observations in the present study corraborates with the observation of Heim (1952), Heim (1977), Zoberi (1979) and Bhavani Devi (1982) reported this species from Congo, Zambia and India respectively. Since this species is commonly collected and consumed by the people, it is known by several vernacular names viz., Uppukoon, Mazathandan, Nilampulappan, Perumalan etc. each revealing the distinguishing characters of the sporophore. It is interesting to note that collections of Termitomyces globulus were made from the coastal sandy loam soil in Trivandrum district.

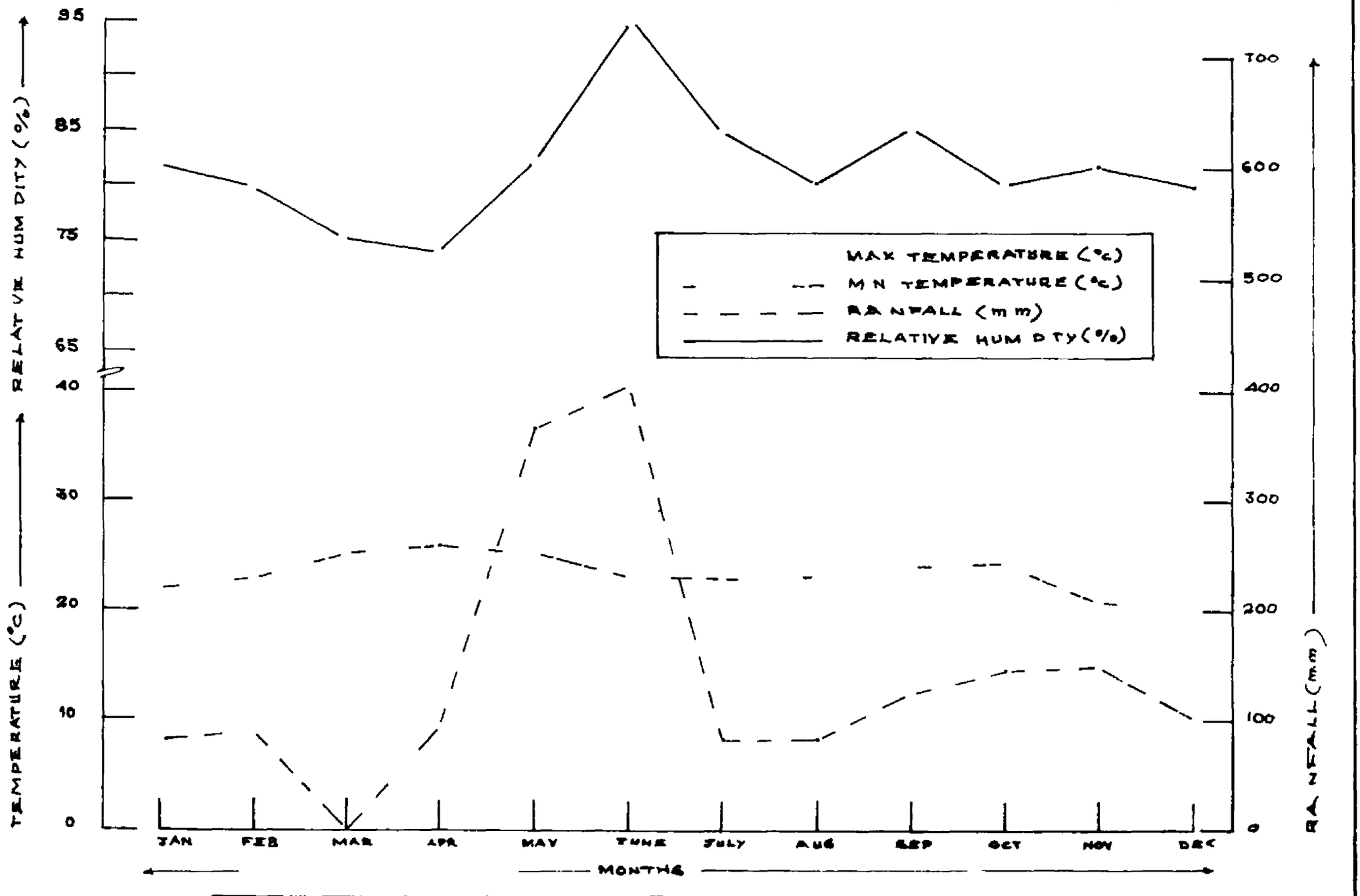
Occurrence of this fungus during the monsoon period in the sandy soil appear to have some close relationship with the vegetation of the area viz. cashew trees and coconut palms. It is observed that the termitaria Odontotermes obesus is made up of masticated cashew leaves and root bits of coconut. T. globulus has a medium sized globular pileus with a perforatorium and 10-15 cm long black, fibrous and abruptly narrowing pseudorhiza. 'Parambin koon' is the local name given to this much sought after mushroom providing delicious daily dish for the local people.

T. striatus is found to occur in red loam soil above the hypogeal termitaria. The pseudorhiza though long, is not fibrous and brownish black like T. robustus but semi hollow, creamish white and slightly tapering.

Termitomyces clypeatus, T. perforans and T. radicatus have larger pileus and moderately developed pseudorhiza than T. microcarpus. Perforatorium is very sharp and spiny in T. clypeatus broadly spiny form in T. perforans. They are locally known as Mullukoon and 'Arikoon'.

Growth, occurrence and distribution of mushroom flora in a state generally depend on rainfall and availability of suitable substrates. The results relating

FIG SEASONAL VARIATION ON THE OCCU RENCE OF *Termitomyces robustus*



to the periodicity of occurrence of all the eight species of Termitomyces showed a post monsoon maxima while T. microcarpus exhibited a monsoon maxima. Fig. 9.

Dixon (1983) who studied the influence of rainfall on the seasonal production of T. striatus observed that fruiting of tropical Termitomyces spp. occurs during the rainy season. The sporophore production of T. striatus is confined to September-November rains. He also recorded that 2-3 cm rain per day initiated a flush of sporophore production. He observed a two phase period a pre-rain period of primordial induction and a post-rain period of sporophore maturation.

Studies were conducted to observe the different stages of development of T. robustus. This species has been selected for the present study, because of its common occurrence during the season. Very little study has been conducted to know the developmental morphology of the termite growing fungus, probably due to the difficulty of maintaining the termite combs in the laboratory. The present study is the fore most attempt in the country and also elsewhere in the world.

In an attempt to study the different stages of development of T. robustus close observationswere carried out

Plate-XIC *T. heimii* showing long pseudorhiza

Plate-XII *T. robustus* Termitarium Showing White Spherules

from the mycelial ramification of the fungus in the termarteria to its epigial maturity stage. Eight stages viz., spherules, clove bud, primordial elongation, Pseudorhizal stage, epigeal button stage, epigeal egg, epigeal elongation and mature stage were observed, identified and named accordingly. Among these stages the first four stages of growth and development were hypogeal while the remaining four stages were epigeal. Termitaria excavated from different places revealed the presence of large number of pearl white, globose, solid structures called spherules or 'mycotete' (Heim 1977) all (Plate XII) over it. The spherules in vertical section showed a tiny knot of hyphae without any differenciation of pileus and stipe. (Buller 1958)

The spherules gradually developed into a clove bud stage showing slight differenciation of stipe and pileus. In this stage the fruiting body resembles the shape of clove bud without showing any differenciation of stipe and pseudorhiza. In the next stages viz. primordial elongation the anterior end of the fruiting body was stout and thick consisting the primordia of pileus and stipe while the posterior portions narrowed down to a distinct dark brown pseudorhiza, a characteristic of the genus.

Pseudorhizal stage represents the 4th stage of development of the fruiting body. In this stage the rate of elongation of pseudorhiza is more than the rate of development of pileus and stipe. As the growth of the fruiting body is negetively geotropic, it elongates upward pushing the pileus and stipe region towards the soil after 4 days of development. On the fifth day the globose pileus emerges just above the soil level, with a short solid stipe region still below the ground. This epigeal button stage is followed by the epigeal egg stage where the pileus and stipe increases in size. Epigeal egg stage is the ideal time for the harvest of the biasidiocarp, because observations showed that harvested fleshy bodies soon get spoiled due to the infestation of insects and maggots and made them unsuitable for consumption.

On 8th day the sporocarp reaches its maturity stage. The pileus expanded to a diam. of 15 cm, with pale creamish brown surface and a broad central perforatorium. Fruiting body is usually harvested by breaking the stipe at the soil level leaving the long subterranian pseudorhiza. The results of the present study revealed that the period of maturation of the

sporopheres of T. robustus may range from 7-8 days. Dixon (1982) recorded a similar observation and reported that T. stratus required a period of 8-10 days for the sporophore maturation.

In the present study an attempt was also made to study the ecology and symbiosis of Termitomyces occurring in the State. Many workers have studied in detail the ecology and symbiosis of Termitomyces sp. (Hendee 1934) 34 Batra and Batra, 1962, Sands, 1969, Ackeri, 1979).

The data relating to the temperature and humidity showed that there was no significant variation in both temperature and humidity inside the comb and surrounding soils. This findings agree well with the views of previous workers (Mukerji and Mitra, 1949, Krishna and Batra 1969, Batra and Batra (1979).

The chemical composition of the combs of T. robustus and T. heimii showed that there was no significant difference in the total moisture, cellulose, carbon, nitrogen content and pH. The combs are made by termites using almost similar plant materials. This could be the reason for the insignificant variations in the chemical composition of the combs of two species of mushrooms. The

moisture percentage of the combs of Termitomyces were 8.8 and 8.7. Airspaces in soils that can support vegetation are saturated with water vapour (Buckman and Brady, 1969), therefore it may be expected that air surrounding fungus gardens is also saturated. Few critical data are available for soil moisture movement within termite nests and the effect of temperature gradients that exist with in their nests as compared with the surrounding soils (Weir, 1975).

The cellulose and carbon content of the combs of Termitomyces sp. were much higher than the nitrogen content. This is contrary to the observations made by Batra and Batra, (1979) who found that the nitrogen content of O. obesus comb was consistently higher and the cellulose correspondingly lower than that of persumed raw materials. The higher cellulose and carbon content observed in the present study could be attributed due to the higher carbon and cellulose content of plant debrises utilised for the preparation of the combs by termites.

The pH values of the combs were in the acidic range of 4.4 and 4.5. This is in agreement with the findings of Batra and Batra (1979).

Nineteen species of fungi could be isolated from the termite combs. Among the fungi isolated, Aspergillus and Xylaria were the predominent ones. Similar observations were made by previous workers who studied this tropical mushroom. Petch (1907) isolated number of species of Xylaria from the termite combs. In the absence of termites the saprophytic fungi multiplied in the combs. Haim (1952) observed fungi like Xylaria nigripes, Penisa nigor, Thamidium, Cephalosporium, Aspergillus sp. etc. in abandoned combs. Zoberi (1979) isolated twenty seven species representing seventeen genera. He also speculated that the strands of Xylaria species are masticated by the termites and utilised for building new combs where as the spherules of Termitomyces are used as additional supply of food and sources of vitamins for the termites.

Odontotermes sp. was the most common termite genera found throughout Kerala associated with Termitomyces sp. Batra and Batra (1977) have studied and established the relationship of Odontotermes sp with Termitomyces sp in India. Batra and Batra (1966) have also studied the commensalic role of Odontotermes sp with Termitomyces sp. The association of Odontotermes sp with different species of

Termitomyces sp recorded in the present study appears to have an obligate association with termites as reported by Pisarec (1980). He has also identified the association of different species of Odontotermes and other genera with seven species of Termitomyces in Zambia. In the present study Odontotermes was the only genera associated with Termitomyces species.

The beetle Amblyrogus cinctipinnis was found to be the common pest of Termitomyces sp. Many insects of the wood eating groups belonging to Coleoptra have become nutritional specialists and posses intra-cellular symbiotic fungi. The Coleoptra beetle reported in the present study was found to infest and feed the emerging as well as mature sporocarps which might have also been resulted in symbiotic association.

The number of sporocarps emerged from daily irrigated plots were maximum when compared to the non-irrigated plots. The present results confirm and extend the general observations that fruiting of tropical Termitomyces spp. occurs during the rainy seasons. T. striatus flushes during both rainy seasons (Alasoadura, 1965 and Dixon, 1983).

Dixon (1983) observed that the production of fruit bodies of T. striatus appears to be trigerred in response to a large amount of rain. He also suggested that a flush could be induced during mid-dry seasons by irrigating the soil surface with sufficient water.

Scanty growth was observed on the solid Rebecca's medium at 30°C when Termitomyces robustus was inoculated. slow growth of Termitomyces have been reported by Heim (1977) and newly isolated cultures are generally slower than their sub isolates. Maximum growth of the fungus was observed at 30°C. Growth was less below 25°C and above 30°C. Batra and Batra (1979) made similar observations. This confirms that the optimum temperature for the growth of the fungus ranges from 25°C to 30°C on solid media supported with various nutrients which gives indigation of the importance of symbiotic relationship of the fungus with termites for proper growth and sporophore production. Ghosh and Sengupta (1978) isolated Termitomyces in a complex solid medium utilizing dextrin soluble starch at a temperature of 28 to 32°C. They observed filament elongation up to 7-8 days. However, Zoberi(1979) recorded that white spherules did not grow artificially on any of the media tried at different temperature.

Poor growth of the fungus on solid media observed in the present study emphasises the importance of the symbiotic relationships of the fungus with termites for proper growth and sporophore production.

The rate of growth of the fungus incubated in light and darkness was more or less same indicating that light and darkness had no influence on the growth of the fungus.

In the present study maximum growth of Termitomyces robustus was observed when cellulose was given as the carbon source. Termitomyces sp whose natural habitats being termit combs are capable elaborating the enzyme cellulases for the enzymatic break down of cellulose are well adapted to ligno-cellulolitic substrates. Batra and Batra (1974) observed good growth of T. albuminosus on cellulose agar. Zoberi (1979) realised the importance of cellulose decomposition, in the life process of the termites as they derive their main source of metabolic substrate and energy from cellulose. The ecological role of Termitomyces in termite combs for the ligno-cellulolitic decomposition has been well documented (Zoberi, 1979 and Rohrmann, and Ross 1983).

Though many workers Purkayastha and Chandra (1975) Batra and Batra (1977), Ganguly and Sengupta (1978), Zeberi (1979), Rohrmann and Rossmann (1980) and Rebecca Thomas (1985) studied the physiological and cultural aspect of these mutualistic fungi, none has succeeded in obtaining appreciable mycelial growth in any of the media tested. In the present study also somewhat result have been obtained even after repeated experiments indicating the influence of some unknown factor for the mycelial growth and spread of the fungus. Trials were also conducted with the scanty mycelial stock culture to test the efficacy of different substrates for spawn production. Observations showed that there was complete absence of mycelial growth of the fungus in any of the substrates under study. Hence further investigations to standardise the cultivation methods remained unsuccessful indicating that more detailed studies have to be conducted in this aspect.

The true protein content of the sporophores ranged between 28.99 g/100 g and 29.49 g/100g dry matter. Mukit (1971) could obtain crude protein approximately 36g/100g dry matter in the case of Termitomyces sp. in Uganda. In the present study estimations were made for the true protein content of the sporophore. Rohrmann and Rossmann (1980) has

content
reported 35 per cent protein in T. gymnamia. Ten essential amino acids have been recorded which is in agreement with the findings of Bano et al. 1964, Makiibi, 1971 and Rohrmann and Rossmann (1980). The carbohydrate, fat and crude fibre values obtained are in agreement with previous workers (Bano et al. 1964 and Makiibi, 1975).

Various methods were attempted for the preservation of Termitomyces robustus. This included dehydration, powdering, refrigeration, preservation in brine, blanching, pickling and ketchup. All these conventional methods were found to preserve the mushrooms for prolonged or short duration storage. Dehydration and powdering and storage in air tight containers could prolong the shelf life as it prevent the microbial spoilage and arrest the cellular and extra cellular enzymatic activity. This method is less expensive and could be easily adapted under village conditions.

Refrigeration is a conventional method of storage of fruits and vegetables for shorter duration and in the case of Termitomyces sp also the shelf life was restricted to 48 h under refrigeration as in the case of many other mushrooms.

One of the common methods of preservation of mushrooms is preserving them in brine solution. Termitomyces sp could also be preserved in brine solutions of 5, 6 and 7 per cent strength. It was also found possible to preserve Termitomyces by adopting blanching or converting into pickles and ketchup as in the case of other vegetables.

SUMMARY

SUMMARY

A State wide survey was conducted during the South West and North East monsoon periods in 1984-'85 and nine species of Termitomyces were collected, identified and recorded from thirty two localities. Among the nine species of Termitomyces collected and identified T.heimii, T. clypeatus and T.microcarpus var. santalensis were the first records for Kerala.

Detailed description of the morphological and microscopical characters of the nine species were recorded in the data sheet along with the ethinomycological and gastronomic data collected from the local people. Information collected from the local people however, revealed that all the Termitomyces species were actually being consumed in the region under survey and that each species are known locally as Uppukoon, Arikoon, Pavakkoon, Milampulappan.

During the survey observations on the frequency and intensity of occurrence of the nine species showed that T.microcarpus, T.microcarpus var. santalensis and T.robustus were the most commonly and abundantly occurring mushrooms distributed throughout the State, irrespective of soil type. Observations on their habit of occurrence also revealed

that T.robustus and T.striatus were always seen solitary above the hypogeal termite combs, while T.microcarpus and T.microcarpus var santalensis occurred in widely scattered groups of more than hundred sporocarps above the scattered termite combs. T.heimii occurred in groups of more than hundred sporocarps above the partly epigeal termiaria T. clypeatus, T.globulus, T.radicatus and T.perforans also appeared in well scattered groups of 25-50 and 10-25 sporocarps above the subterranian combs.

The results relating to the periodicity of occurrences of different species of Termitomyces indicated a post monsoon (July, October) maxima for the six species belonging to subgenus Eutermitomyces and a monsoon (June, September) maxima for the two species viz. T.microcarpus and T.microcarpus var. santalensis of the subgenus pratermitomyces.

Studies conducted to observe the different stages of growth and development of T.robustus from mycelial stage till maturity revealed that different stages of development can be divided into eight stages viz. spherule, colvebud, primordial elongation, pseudorhizal stage, epigeal button, epigeal egg epigeal elongation and mature stage. The first four stages of development were hypogeal

and took 192 h to attain the 4th stage of development viz. pseudorhizal inochics pseudorhiza formed the major part of the sporocarp. The next four stages of development viz. epigeal button, epigeal egg, epigeal elongation and mature stage were epigeal and took 96 h to attain the maturity stage. Critical observations of the different stages of growth and development revealed that pseudorhiza and perforatorium played an important role in the hypogeal development of the sporocarp. It was also observed that the length of the pseudorhiza mainly depended on the depth and location of termitaria in the soil. In vitro studies on the developmental morphology of T.robustus revealed the abundant growth of the mycelia of Xylaria in all the experiments.

The data relating to the temperature and humidity of the comb of T.robustus and surrounding soil showed insignificant variations. Maximum and minimum temperature recorded inside the comb and surrounding soils were 31.2, 28.1, 29.5 and 27.0°C respectively. The humidity recorded were 100 and 99 per cent.

A comparative study of the chemical composition of the combs of T.robustus and T.heimii showed little differences in the total moisture content, cellulose, carbon, nitrogen and pH.

Isolations of other fungi from the termitaria obtained from different localities revealed the occurrence of 19 species belonging to 12 genera indicating their possible role in cellulose decomposition in the environment. Among the 19 species isolated, species of Aspergillus and Xylaria were found to be the predominant fungi in the combs.

Species belonging to Odontotermes was found to be the most common termite associated with different species of Termitomyces in Kerala. O. obesus was always found to be associated with species of Termitomyces microcarpus.

During the course of the present study the beetle Amblyrrhus cingulipennis was found to be the common pest of Termitomyces. The beetle was found to infest and feed the emerging as well as mature sporocarps and turn them unfit for consumption.

Studies on the effect of soil moisture for the production of sporophores revealed that the number of sporocarps emerged from the daily irrigated plots was more when compared to the non irrigated plots. Among the eight media tested Raheesa selective medium was found to be the only medium for the mycelial growth of T. robustus. Maximum mycelial growth of 161 mm was observed at 30°C. No growth

was noticed at temperatures above 30°C and below 25°C.

Studies on the effect of light on the mycelial growth of T.robustus showed little difference in the rate of growth cultures incubated in the light and in darkness. In vitro studies on the best source of carbon and Nitrogen for the mycelial growth of the fungus indicated that cellulose was the best source of carbon followed by maltose and lactose. Growth was poor when glucose was used as carbon source. Studies on the nutritive value of seven species of Termitomyces showed that maximum protein content of 28.99 g/100 g dry matter was observed in the case of T.heimii while minimum protein content of 19.84 g/100 g dry matter was recorded in the case of T.robustus. Ten essential amino-acids were detected from T.robustus.

Studies conducted to test the suitability of different substrates for spawn production revealed that all the six substrates under study failed to support any mycelial growth of the fungus.

Studies on the preservation of the sporocarps revealed that dehydrated sporocarps and powdered samples of the same can be preserved in closed polythene covers and airtight containers for ten months. Fresh sporocarps of Termitomyces could be stored in open polythene bags for 48 h without any

taste difference. Preservation in various concentrations of brine indicated that microbial contamination is very low at five, six and seven per cent concentrations kept for four weeks. Observations also showed that blanched specimens kept in sterilised glass jars remained fresh upto five months in room temperature. While the samples packed in polythene bags remained fresh only for four days from the same treatment kept samples under refrigeration remained fresh up to one week. Sporocarps could be pickled and stored for six months without microbial spoilage.

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* original not seen.

Appendix - I

GLOSSARY

Adnate	- gills attached to the stipe with their entire width
Agaric	- any gill fungi
Amyloid	- colour reaction with Melzer's reagent - black or slightly greyish if amyloid, brown to purplish brown when pseudoamyloid, yellowish if inamyloid (negative)
Anastamosing	- fusion between hyphae or branches to form a network
Annulus	- a ring like partial veil, or part of it round the stipe after expansion of pileus
Areolate	- divided into small segments by cracking
Attenuate	- narrowed, tapering
Basidium	- spore mother cell of basidiomycetes bearing spores on short sterigmata
Bulbous	- enlarged at the base
Buttons	- young unexpanded cap
Cheilocystidium	- cystidium in the edge of a gill
clavate	- club like
context	- the hyphal mass between the superior surface and subhymenium or trama of basidiocarps

Cuticle	- outermost layer of cap or stipe
Cystidia	- sterile, unicellular light colour large cells in the hymenium of Basidiomycetes
Epigeous	- above the ground
Fibrillose	- composed of longitudinal fibres or hairy filament
Free (gills)	- gills that do not touch the stipe
Glabrous	- smooth, not hairy
Gregarious	- growing in groups, but not in tufts
Guttulate	- spores with one or many oil droplets
Hygrophanous	- having water soaked appearance when wet
Hymenium	- a fertile layer that bears either basidia and basidiospores or asci and ascospores mixed with paraphyses etc.
Hymenophore	- the portion of the carpophore which bears the hymenium
Hypogeous	- subterranean, growing underground
Incised	- pileus margin as if cut into
Involute	- margin (of pileus) rolled inward
Lacerate	- as if roughly cut or torn
Lamellate	- having gills
Obovoid	- inversely ovate
Ochraceous	- colour of ochre, dead yellow or iron rust colour

Ovoid	- spores widest near the point of attachment
Perforatorium	- dark coloured spiny projection in the centre of pileus
Pileus	- that portion of carpophore which resembles umbrella like cap
Piriform	- pear shaped
Pleurocystidium	- cystidium on the sides of the gill
Pseudorhiza	- a root like extension of the stipe
Reflexed	- turn back (margin of pileus)
Serrate	- toothed, like edge of a saw
Sporophore	- fruit body of a mushroom
Squamose	- having scales
Stipe	- stem, stalk of fungal fruit bodies
Striate	- marked with tiny streaks
Subhymenium	- the layer of interwoven hyphae between the hymenium and trama giving rise to basidia
Subterranean	- underground habit, hypogeal
Tawny	- colour of tanned leather (dull yellowish brown)
Trama	- the tissue lying between the hymenial layer, usually consisting of densely packed or loosely interwoven hyphae

Umbo	- a central swelling like the boss of a shield
Veil	- usually membraneous structure or sometimes spider web like. It envelops the part or the entire carpophore
Viscid	- moist, sticky

Appendix - II

DATA SHEET USED FOR THE MORPHOLOGICAL DESCRIPTION OF MUSHROOM

Data Sheet

Sl.No. Date of collection:

Collected by: Locality:
(Village/Taluk/Dist.)

Final Identification:
(Confirmed by .....)

Taxonomy:

Order:

Family:

- -

GENERAL

Common name: Local name:

Soil type: Vegetation:

Substrate:

Season: Temp. R.H. Rainfall:

Any other information on climate:

Others:

- -

Habitat: Terrestrial/lignicolous/Epixylous/Coprophilous/
Humicolous

Habit: Solitary/Scattered/Caespitose/Gregarious

PILEUS

When young: Conical/spherical/Campanulate/Convex

Shape: At maturity: Infundibuliform/Umbonate/Broadly umbonate/
Campanulate/Umbilicate/Aplanate/Conical/
Convex/Petaloid/Flabelliform/Mucronate/
Depressed/Dimidiate/Resupinate/

When young :

Size:

At maturity :

Colour:

Texture : Soft/Brittle/Fleshy/Coriaceous/
Hygrophanous/Fragile/Cartilaginous/
Membraneous

Surface : Smooth/Scaly/Rugose/Rugulose/Viscid/
Striate/Dry/Squamulose/Velutinous/
Pubescent/Strigose/Sulcate/Tomentose/
Alveolate/Farinose/Floccose/Punctate/
Rivose/Rivulose

Margin : Serrate/Serrulate/Smooth/Undulate/
Reflexed/Involute/Fimbriate/Incised/
Lobed/Revolute

Before cutting:

Context : Colour:

After cutting:

Colour changes with:-

1. Melzer's reagent: Amyloid/Pseudoamyloid/Inamyloid
2. Green Vitrial : . . . .
3. Phenol : . . . .
4. Sulphovanilin : . . . . . .

GILLS

Arrangement : Remote/Free/Decurrent/Adnate/Adnexed/Sinuate

Shape : Rounded anteiely or posteriorly/lanceolate/Ventricose/Reticulate

Texture : Soft/Brittle/Cerecceus/Waxy/Thick Papery/Opaque

Margin : Smooth/Wavy/Serrate/Fimbriate/Dentate

Size : Number per Cm.

Gill trama : Regular/Irregular/Bilateral/Inverse

Cystidia

1. Pilocystidia
2. Pleurocystidia
3. Cheilocystidia
4. Caulocystidia

Size:

1.
2.
3.
4.

Shape:

a. Ventricose/ b. Clavate/
c. Filiform/ d. Napiform/
e. Lageniform/ f. Rostrate/
g. Encrusted/ h. Rostrate/
i. Lanceolate/ j. Pyriform/
k. Granulate/ l. Pointed/
m. Beaked/ n. Capitate
o. Lecythiform/ p. Cylindrical

1. 2. 3. 4.

VEIL

Type : Present/Absent Universal/Partial

Colour :

Texture : Membraneous/Fleshy/Smooth/

Position: Coriaceous

ANNULUS Present/Absent

Size :

Texture :

Colour : Fleshy/Coriaceous/Papery/

Thin

Attachment: Superior broad/Medial pendu-

lous/Inferior/Narrow fragments/

Appendiculate/Fibrillose/

Movable

STIPE

Present (Stipitate)/Absent (sessile)

Length

Size:

Diameter

Shape: Clavate/Obclavate/Cylindrical/Solid/Hollow/

Slender/Short

Attachment to Pileus: Lateral/Eccentric/Central/Resupinate

Surface: Glabrous/Scaly/Pubescent/Velutinous/

Squamose/Tomentose/Fibrillose

Before cutting:

Colour:

After cutting :

Reaction with Melzer's reagent : Amyloid/Pseudoamyloid/Inamyloid

Basal Part : Globular/Annular stripes/Fusoid/ Bulbous/Sheathing Bulbous/Marginately depressed bulb/Pseudorhizoid Rhizinous/Rhizomorphoid

VOLVA

Present/Absent : Persistent/Evanescent

Shape : Free/Lobed/Irregular/Cup like

Colour :

Texture : Soft/Fleshy/Tough/Papery

Odour: Before cutting :

After cutting :

Taste : Acrid/Mealy/Acidulous/Blunt

SPORE PRINT

Colour :

Other details :

BASIDIA

Size :

Shape :

Sterigmata : No. 1 / 2 / / 4 /

SPORES

Colour :

Reaction with:

Cotton blue : Cyanophilic/Acyanophilic

Melzer's reagent : Amyloid/Pseudoamyloid/Inamyloid

Shape : Ovate/Elliptical/Globose/Sub globose/
Apiculate/Cylindrical/Fusiform/
Angular/Echinulate/Verrucose/
Reticulate/Tuberculate/Ovoid/
Abtusely fusiform/Allantoid/Guttulate/
Pip shaped/Pyriform/Pedicilate/
Muriform/Filiform

(fig.)

Other characters of spores:

- -

ANY OTHER DETAILS:

Appendix - III

COMPOSITION OF REAGENTS, CHEMICALS AND MEDIA USED FOR THE STUDY

Reagents and Chemicals

1. Melzer's reagent. (Melzer, 1934)

Potassium iodide	-	1.5 g
Iodine	-	0.5 g
Water	-	20 ml
Chloral hydrate	-	22 g

2. Potassium hydroxide - 3 per cent aqueous solutic
3. Hydrochloric acid - 1 n solution
4. Concentrated sulphuric acid
5. Ferrous sulphate - 3 per cent aqueous solutic
6. Phenol - 2 per cent
7. Formaldehyde - 40 per cent in distilled water

Media

1. Potato dextrose agar medium

Peeled potato	-	250 g
Glucose	-	20 g
Agar	-	15 g
Distilled water	-	1000 ml
pH	-	6 - 6.5

2. Oat meal agar

Oats	-	100 g
Agar agar	-	15 g
Distilled water	-	1000 ml
pH	-	6 - 6.5

3. Malt extract medium

Malt extract	-	25.0 g
Agar agar	-	15.0 g
Distilled water	-	1000 ml

4. Czapek's agar

Sucrose	-	30 g
Sodium nitrate	-	2 g
Dipotassium phosphate	-	1 g
Magnesium sulphate	-	0.5 g
Potassium chloride	-	0.5 g
Ferrous sulphate	-	0.01 g
Agar-agar	-	15 g
Distilled water	-	1000 ml

5. Sabouraud media

Dextrose (Maltose)	-	40 g
Peptone	-	10 g
Agar	-	20 g
Distilled water	-	1 L
pH	-	5.6

6. Nutrient agar

Peptone	-	10 g
Beef extract	-	5 g
Distilled water	-	1000 ml
Agar-agar	-	20 g
pH	-	5.8 - 7.2

7. Parthasarathe's synthetic media

Glucose	-	10 g
DL - alarine	-	1 g
KH_2PO_4	-	0.2 g
$MgSO_4\ 7H_2O$	-	0.5 g
Thiamine hydrochloride	-	0.5 g
Distilled water	-	1000 ml

8. Rabeeca's selective media

Cellulose	-	10 g
$(NH_4)_2\ SO_4$	-	0.5 g
K co	-	0.5 g
KH_2PO_4	-	1.0 g
$Mg\ SO_4\ 7H_2O$	-	0.2 kg
$CaCl_2$	-	0.1 g
Yeast extract	-	0.5 g
Agar-agar	-	20.0 g
Benomyl	-	0.1 g
Karathane	-	0.000 g

Gallic acid - 0.1 g

Oxbile - 1 g

After autoclaving - streptomycin sulphate - 0.4 gl and Penicillin and Sodium salt - 0.1 g l^{-1} were added.

9. Sucker's Agar

Glycerol	-	10 g
Casein	-	3.3 g
Sodium chloride	-	2 g
Dipotassium hydrogen phosphate	-	2 g
Magnesium sulphate	-	0.25g
Calcium carbonate	-	0.02g
Iron sulphate	-	0.01g
Agar-agar	-	15 g
Distilled water	-	1000 ml

10. Martin's Rose bengal Agar

Dextrose	-	10.0 g
Peptone	-	5.0 g
Potassium dihydrogen phosphate	-	1.0 g

Rose bengal (one part in 30,000 parts of media)

Agar-agar	-	20.0 g

Streptomycin (30 mg/litre)

Distilled water	-	1000 ml
pH	-	4.5

BIOLOGY OF TERMITOMYCES SPECIES AND STANDARDISATION OF ITS CULTIVATION TECHNIQUES

By

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ABSTRACT OF A THESIS

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ABSTRACT

State wide survey was conducted during the South West and North East monsoon periods in 1984-85 and nine species of Termitomyces were collected and identified from thirty two localities of the State. Among the nine species, T. heimii, T. clypeatus and T. microcarpus var. santalensis were the first record for Kerala.

Detailed description of the morphological and microscopical characters of the nine species collected were recorded in the data sheet along with the ethno-mycological and gastronomic details. All Termitomyces species were commonly consumed by the local people during the seasons and were known by different vernacular names.

Observations on the periodicity frequency and intensity of occurrence of the nine species showed that T. microcarpus, T. microcarpus var. santalensis and T. robustus were the most commonly occurring species abundantly distributed throughout the State, irrespective of soil type. Their habit of occurrence also revealed that T. robustus and T. striatus were always seen solitary while all the other species occur gregariously consisting of ten to hundred sporocarps.

The results relating to the periodicity of occurrence of different species of Termitomyces indicated a post monsoon maxima for the seven species belonging to subgenus Eutermitomyces and a monsoon maxima for the two species viz. T. microcarpus and T. microcarpus var. santalensis of the subgenus pretermitomyces.

Studies conducted to observe the developmental morphology of T. robustus from mycelial stage till maturity revealed that different stages of development can be divided into eight stages viz. spherule, clove bud, primordial elongation, pseudorhizal stage, epigeal button, epigeal egg, epigeal elongation and mature stage. The first four stages of development were hypogeal and took 192 h to attain the 4th stage while the next four epigeal stages took only 96 h to reach the mature stage. Critical observations of the different stages of growth and development of the sporocarp revealed the significance of pseudorhiza and perforatorium in the hypogeal development of the sporocarp.

The data relating to the temperature and humidity of the comb of T. globulus and surrounding the environment showed insignificant variations.

A comparative study of the chemical composition of the comb of T. robustus and T. heimi showed little difference in the total moisture content, cellulose, carbon, nitrogen and pH.

Isolation of other fungi from the termitaria obtained from different localities revealed the occurrence of 19 species of fungi belonging to 12 genera indicating their possible role in cellulose decomposition in the environment. Among the nineteen species isolated, species of Aspergillus and Xylaria were found to be the predominent fungi in the combs.

Species belonging to Odontotermis was found to be the most common termite associated with different species of Termitomyces in Kerala. O. obesus was always found to be associated with its fungus mutualist Termitomyces microcarpus. The beetle Amblyrous cinctipinnis was found to be the common pest of Termitomyces. The beetle was found to infest and feed the emerging as well as mature sporocarps and turn them unfit for consumption.

Field trials on the effect of soil moisture for the production of sporocarps revealed that the number

of sporocarps emerged from the daily irrigated plots were more when compared to the non irrigated plots.

Among the eight media tested Rebeeca's selective medium was found to be the only medium to support scanty mycelial growth of T.robustus. Maximum mycelial growth was observed at 30°C. Experiments on the effect of light in mycelial growth of T. robustus showed no significant difference in growth.

In vitro studies indicated that cellulose was the best source of carbon followed by maltose and lactose. Maximum protein content was observed in T.heimii when compared to other species.

Trials on the suitability of different substrates for spawn production failed to support any mycelial growth of the fungus.

Dehydrated sporocarps preserved in sealed polythene covers showed maximum shelf life when compared to other methods of preservation. Though fresh sporocarps could be stored in polythene bags for only 48 h it was possible to extend their shelf life when preserved in brine solution. Blanching and pickling were the other methods of preservation of the sporocarps of Termitomyces tried.