STANDARDIZATION OF TECHNIQUES FOR CULTIVATION OF *TRICHOLOMA GIGANTEUM* MASSEE IN KERALA

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(2011-11-161)

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DECLARATION

I hereby declare that this thesis entitled "**Standardization of techniques for cultivation of *Tricholoma giganteum* Massee in Kerala**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other university or society.

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Dedicated to

My

Family

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LIST OF ABBREVIATIONS

%	Per cent
°C	Degree Celsius
B.E.	Biological efficiency
cm	Centimetre
dia	diameter
et al.	And others
Fig	Figure
g	Gram
h	Hours
Kg	Kilogram
l	Litre
M	Molar
mg	Milligram
ml	Millilitre
min	Minute(s)
PDA	Potato dextrose agar
PDB	Potato dextrose broth
ppm	Parts Per Million
SMS	Spent mushroom substrate
Sp	Species
Viz.,	Namely
Wt	Weight

Introduction

1. INTRODUCTION

Mushrooms have been favoured as food for mankind for a long time. Mushrooms supply a rich addition to the diet in the form of protein carbohydrate, valuable salts, minerals and vitamins. As food, the nutritive value of mushrooms deceit in between meat and vegetables. Mushrooms are also referred as "vegetarians meat".

More than 2000 species of fungi are reported to be edible throughout the world and about 283 of these are reported to be available in India. Out of these only 20 mushroom species have been cultivated for edible purpose in different parts of the world and about 10 are being produced and marketed in sizeable quantities across the world. In India at present, four varieties of mushroom *Viz., Agaricus bisporous, Pleurotus* spp., *Volvariella* spp., and *Calocybe indica* have been recommended for the year round cultivation.

Kerala with varied agroclimatic conditions favour the cultivation of variety of crops thus providing tonnes of agro waste for cultivation of mushrooms. The agrowastes consisting of cellulosic and lignolytic materials are resistant to biodegradation and ruminant digestibility. Natural plant wastes, the lignocellulosic, are the basic substrates for growth and yield of mushrooms. Other redeeming features of mushroom growing are that it requires limited space, needs no sunlight and fertile soil. The cultivation of edible mushrooms helping in recycling of agricultural wastes which also help to great extent in filling up the protein gap prevalent among large population of the country.

Tricholoma giganteum Masse commonly known as giant mushroom, a new edible mushroom pure white in colour resembling the

morphology of *Calocybe indica,* found in nature after monsoon showers has a unique taste and is best suited for the tropical climate of Kerala.

Tricholoma is rich in protein, fibre, carbohydrates and vitamins and contains an abundant amount of essential amino acids and low in fat. These qualities make it suitable for food supplement in diet.

Though *Calocybe* the milky mushroom has found a place in the daily diet its resinous taste has often created displeasure for the gourmets. This mushroom though it resembles milky does not have the resinous taste and hence more popular as a culinary item. A variety of appealing recipes including soup, cutlets, payasam and even wine can be prepared with *Tricholoma.* It has tremendous scope for export since it has good keeping quality.

It can in future be the most suited species for cultivation under Kerala conditions. Considering these facts the present study was conducted with the objective of improving the locally available strains and developing modified technologies for improving production. An attempt has thus been made to develop a domestication package for the cultivation of this trendy mushroom *Tricholoma.*

Review of Literature

2. REVIEW OF LITERATURE

2.1 COLLECTION, ISOLATION AND PURIFICATION OF NATIVE ISOLATES

Only eight species of *Tricholoma* namely *T. cremoriceps* Berk, *T. giganteum* Massee, *T. melaleucum* (Pers.) Fr., *T. subpulverulentium* (Pers.) Fr., *T. nudum* (Bull.) Fr., *T. leucocephalum* (Fr. Sensu) Lange, *T. georgii* (Clus) Fr. and *T. lobayense* Heim have been recorded from India (Sinha and Padhi, 1978; Sathe and Rahalkar 1975; Bilgrami, *et al.*,1979, 1981; Chakarvarthy and Sarkar, 1982). Dadwal and Jamaluddin, (1984) reported the occurrence of *Tricholoma giganteum* Massee from the forest of Jabalpur. A species of *Tricholoma* namely *Tricholoma lobayense* Heim was first reported from India by Chakravarthy and Sarkar in 1982. Literature survey shows that this species has not been previously reported from India (Buttler and Bisby, 1931; Sathe and Rahalkar, 1975; Sinha and Padhi, 1978; Bilgrami, *et al.,* 1979) though this has been reported from West Africa (Heim, 1970). Sarkar*et al.* (1988) reported the occurrence of nine species of edible mushrooms (*Pleurotus squarrosulus, Valvariella volvacea, V. diplasia, Termito myceseurrhizus, T. microcarpus, Marasmius* sp., *Geastrum* sp., *Calocybe indica and Tricholoma lobayense)* in the wild forest in Tripura. Survey conducted in Western Ghats by Anandh and Prakasm (2003) resulted in the collection of edible mushrooms *viz., Calocybe indica, Calocybe gambosa, Tricholoma lobayense and Tricholoma giganteum.*

2.1.1 Isolation and purification of native strains of *Tricholoma giganteum*

Kilgman (1943) reported that tissue culture isolates raised from phenotypically healthy looking mushrooms possess good fertility. Eswaran and Susan (2003) have reported isolation and purification of *Calocybe* on potato dextrose agar medium.

2.2 MORPHOLOGICAL, CULTURAL AND PHYSIOLOGICAL STUDIES

2.2.1 Identification of native isolates

Tricholoma has a very large pileus 23 cm in diameter, upper surface convex later flattens with age. Smooth appressed scales at centre, margin incurved, gills decurrent, white alternate hymenophoral trama sub regular, lack of pleurocystidia, and chielocystidia. Basidiospores are ellipsoidal, hyaline, smooth and non amyloid (Natarajan and Manjula 1983; Ganeshan 1990).

Tricholoma lobayense Heim, a related species of *Tricholoma giganteum* reported from West Bengal during summer season has a very large pileus 8-22 cm in diameter, upper surface convex at the beginning which gradually flatten with age, smooth, appressed scales being present at the centre, margin thin regular, gills decurrent, white, alternate, free towards the margin of the pileus, flesh white and fibrous, gill trama regular, consisting of parallel thin- walled hyphae, stipe 14-28 cm in length, unequal tapering towards the apex, smooth, fibrillose, solid sub-bulbous base, spore print milky white (Chakravarthy and Sarkar, 1982).

Pegler in 1982 also indicated the similarity of *T. giganteum* with *T. lobayense.* Natarajan and Manjula (1983) observed the occurrence of *Tricholoma lobayense* in Madras. Anandh (2001) reported the possibility of commercial cultivation of a new edible mushroom, *Tricholoma lobayense* closely resembling *Calocybe indica.* Cultivation exploitation of an edible tropical mushroom *Tricholoma lobayense* was done by Anandh and Praksam (2002).

2.2.2 Cultural studies of *Tricholoma giganteum*

2.2.2.1 Growth under different solid and liquid media

Jandaik and Kapoor (1975) reported that potato dextrose agar (PDA) fortified with yeast extract supported maximum growth of many mushrooms like *Pleurotus.* Zadrasil (1978) reported malt extract peptone media as a good nutrient

media for the growth of *Pleurotus* spp. Suharban (1987) suggested potato dextrose agar as a suitable medium for *Pleurotus sajor-caju, P. florida, P. flabellatus* and *P. ostreatus*.

Balakrishnan (1994) tested four different solid media *viz.*, PDA, oats agar, carrot agar, and modified oats agar medium for the growth of different *Pleurotus* spp. He found that oats agar blended with 40 per cent coconut milk (modified oats agar) supported maximum mycelia growth for all the species of *Pleurotus* tested, followed by common oats agar medium.

Cultural characterization of *Lentinus* in various solid and liquid media revealed woods extract agar as the best solid media followed by potato dextrose agar and glucose asparagines solution the best liquid medium (Kaur and Lakhanpal, 1999). Out of the eleven culture media evaluated by Rafique *et al.* (1999) potato dextrose agar (PDA) was found to be the optimum medium for the growth of *Pleurotus*.

Nasrin *et al.*, (2001) reported that the mycelia growth of *Pleurotus ostreatus, P. sajor-caju,* and *Volvariella volvacea* were maximum in medium plates containing malt extract medium. Ling *et al.*, (2005) reported potato dextrose agar as an excellent medium for the growth of *Auricularia* sp. Kinjo and Miyagi (2006) reported that the mycelial growth of *Tricholoma giganteum* was superior on the Hennerberg medium of synthetic liquid medium, and the GCMY (glucose, casamino acid, malt extract and yeast extract) medium of natural liquid medium.

Studies conducted by Garasiya *et al.* (2007) revealed that *Auricularia polytricha* grows well in malt extract agar medium. Xiao *et al.*, (2008) reported that PDA No. 4 medium composed of potato 20 %, glucose 2 %, yeast powder 0.5 %, KH_2PO_4 0.3 %, Mg So_4 0.2 % and agar 2 % at pH 7 and an incubation temperature of 28-30°C was found to be best for the mycelia growth of *Tricholoma giganteum.* Chen and Yang (2009) reported that the growth period of

Tricholoma lobayense mycelium in the mixed PDA medium was 30 days shorter than that in the conventional PDA medium.

2.2.2.2 Effect of carbon sources on the growth of Tricholoma giganteum

Litchfield *et al.* (1963) noted disaccharides as best carbon sources for the growth of morel mycelium. Carbon nutrition of *Pleurotus ostreatus* was studied by Yusef and Allam (1967). Studies revealed that maltose supported maximum sporulation of *Pleurotus ostreatus*. Similar results were reported by Bano and Srivastava (1970) in which glucose was considered as a good source of carbon and xylose as poor source for *Pleurotus flabellatus*.

Jandaik and Kapoor (1976) reported that starch was an elite source of carbon for optimum mycelial growth in *Pleurotus sajor-caju*, although glucose, sucrose, maltose and dextrin supported optimum growth of the mushroom at 3 % sugar concentration. However, in case of *Calocybe indica*, glucose, a monosaccharide supported maximum mycelial growth among 19 carbon compounds tested including starch (Chandra and Purkayastha, 1977).

Kumar and Munjal (1980) recorded maltose, glucose and fructose as better carbon sources than sucrose for *Agaricus bisporus*. Soluble starch, maltose and glucose were optimal sources for mycelial production of *Tricholoma lobayense*. Highest yield of protein was obtained on maltose and glucose in five day old submerged mycelium (Saha and Samajpati, 1987).

Kaur and Lakhanpal (1995) studied the effect of nutrient elements sources, vitamins and growth regulator on vegetative growth of *Lentinus edodes*. The study revealed maximum mycelial growth in dextrose followed by fructose and sucrose and minimum mycelial growth was recorded in starch.

Lee *et al.*, (1997) reported that glucose, starch, trehalose, maltose, sorbitol and xylitol were very good carbon sources for the mycelia production of

Tricholoma matsutake. Upadhyay (2003) had found glucose and fructose as excellent carbon sources for the growth of *Auricularia polytricha.* Sharma *et al.* (2004) observed that alanine is the best carbon source for the cultivation of *Agrocybe aegerita* followed by starch whereas citric acid proved to be the least preferred carbon source for the mycelial growth.

Results of study conducted by Thirumalvalavan *et al.* (2005 a) revealed glucose incorporation on liquid media recorded highest mycelia dry weight of *Pleurotus flabellatus* when compared to dextrose. This was followed by sorbitol, sucrose, cellulose, mannitol and starch in decreasing order of merit. Kinjo and Miyagi (2006) reported that soluble starch and mannose were the most effective carbon source for the mycelia growth of *Tricholoma giganteum.* Akata *et al.,* (2003) reported that glucose was the best carbon source for mycelia growth of *Tricholoma anatolicum* in liquid and solid media.

2.2.2.3 Effect of Nitrogen source on the growth of Tricholoma giganteum

Yusef and Allam (1967) reported that organic nitrogen was superior to inorganic nitrogen for the growth of *Pleurotus ostreatus*. Among the organic nitrogen sources, asparagine was the best. Urea was found to be the best source of nitrogen for gasteromycetes. Asparagine at 0.03 % concentration supported maximum mycelia growth of *Pleurotus sajor-caju* (Jandaik and Kapoor, 1976).

Kikon and Rao (1980) recommended organic forms of nitrogen as suitable source for the growth of *Plearotus ostreatus*. Khanna and Garcha (1983) tried various inorganic nitrogen sources namely ammonium chloride, ammonium sulphate, ammonium phosphate, ammonium tartarate, nitrate of potassium, calcium, ammonium and sodium, of which sodium nitrate produced maximum biomass of *Pleurotus sajor-caju* and *Pleurotus ostreatus.*

Complex sources of nitrogen mainly yeast extract, peptone and casein hydrolysate had more stimulatory effect on protein production of *Volvariella*

diplasia (Banerjee and Samajpati, (1989). Peptone was found to be the best for the germination and germtube growth of *Volvariella* (Banerjee *et al.*, 1990). Lee *et al.*, (1997) reported that the sources of nitrogen and vitamin in yeast extract, malt extract and soytone was very important for enhancing the growth of *Tricholoma.*

Sharma *et al.*, (2004) observed that methionine is the best nitrogen source for the cultivation of *Agrocybe aegerita* whereas sodium nitrate proved to be the least suitable for the mycelial growth. Nitrogen content of the mycelium is responsible for the skeletal development of fungi. Kinjo and Miyagi (2006) reported that the most effective nitrogen source for mycelia growth for *Tricholoma giganteum* is potassium nitrate. Studies conducted by Garasiya *et al.*, (2007) showed that the maximum dry mycelia weight of *Auricularia polytricha* is in soybean powder followed by potassium nitrate and urea. According to Chen and Yang (2009) the most suitable nitrogen source for mycelia growth of *Tricholoma lobayense is* wort peptone medium.

2.2.2.4 Effect of pH on the growth of Tricholoma giganteum

Aptly, hydrogen ion concentration is the cornerstone factor which adjudges the form and supply of various nutrients from medium to the growing mushroom mycelium under solid state and submerged conditions.

Maximum growth of *Calocybe indica* was obtained at pH 5.5 with 32 mg dry weight (Chandra and Purkayastha, 1977). Mehta and Kumar in 1985 reported that maximum mycelia growth of single spore isolates of *Agaricus brunnescens* occurred at a pH ranging from 6-7. Kurtzman and Zadrazil (1982) emphasized the use of carbonate and bicarbonate buffers to sustain the optimum hydrogen ion concentration in the medium at the same time without inhibiting the growth of *Pleurotus* mycelium.

Suharban (1987) suggested pH 5.5 was the best for the maximum dry matter production of *Pleurotus* sp. Bhattacharjee and Samajpati (1989) studied the effect

of different H ion concentration on mycelial yield of *Pleurotus sajor-caju* and suggested that pH of 5.5 was optimum for the same to increase the mycelial yield. Kaur and Lakhanpal (1999) observed the mycelial growth of *Lentinus edodes* at different pH levels ranging from 3.5 to 8.5 and concluded that acidic pH of 4.5 supported maximum growth.

The optimum pH for the growth of *Pleurotus* sp. was found to be 5.5 (Rafique *et al.*, 1999). Singh *et al.*, (2000) studied the effect of pH on different edible mushrooms like *Lentinus edodes, Agaricus bisporus, Pleurotus ostreatus, Auricularia polytricha, Morchella esculanta*etc and found suitable pH as 6.0 for the *Auricularia polytricha.* Kinjo and Miyagi (2006) reported that the optimum pH value for mycelia growth of *Tricholoma giganteum* is 5.0. Kim *et al.*, (2010) observed the mycelia growth of *Tricholoma matsutake* at different acidity (uncontrolled pH and controlled pH of 6) and concluded that pH control does not effect of mycelia growth.

2.2.2.5 Effect of temperature on the growth of Tricholoma giganteum

Jandaik and Kapoor (1975) reported that *Pleurotus sajor-caju* failed to grow at temperature 10 °C or below, or 35 °C or above and the maximum growth was recorded at 25 °C. Similar observations were made by Rangad and Jandaik (1977) with *P. cornucopiae and P. ostreatus* (Grag). Chandra and Purkayastha (1977) reported that *Calocybe indica* preferred a temperature of 30°C for the optimum growth. According to Geetha (1982) optimum temperature for the growth of *Coprinus lagopus* was 35 °C.

Lee *et al.*, (1997) reported that the mycelial growth of *Tricholoma* in Czapek-Dox medium supplemented with 0.1 % of yeast extract was excellent. Sharma *et al.*, (2004) observed that for the cultivation of *Agrocybe aegerita* maximum growth was recorded at 25 °C and there was no growth at 35 °C. Veena and Pandey (2006) observed that for the cultivation of *Ganoderma lucidum* primordial initiation was fast at 30 ± 2 °C and it is delayed by another week at 24

± 2 °C. Kinjo and Miyagi (2006) reported that *Tricholoma giganteum* prefer a temperature of 30 °C for mycelia growth. Studies conducted by Garasiya *et al.*, (2007) reported that *Auricularia polytricha* grows well in 25-30 °C.

2.2.2.6 Effect of light on growth of Tricholoma giganteum

Antonio and Fordyce (1972) observed that an appreciable quantity of light (15 minutes of sunlight) is required for the initiation of fruit bodies. The light requirement for mushroom is not photoperiodic (Munjal *et al.,* 1975). The spawn run for *Auricularia polytricha* is maximum at dark area of less than 500 lux. San Antonio (1981) reported that for the cultivation of *Lentinus edodes* as cool nights followed by warm days are essential for fruit body formation. No significant effect of light on yield of *V. diplasia* has been reported by Singh and Saxena (1983). Miles and Chang (1987) reported that for the cultivation of *Lentinus edodes* light was essential for brown-pigment formation of the mycelia coat and for fruiting-body maturation, but was not required for formation of primordia. Light is required as a trigger for fruit body production in *Volvariella volvacea* (Chang and Miles, 1989). But for pin head formation light intensity has to be increased to 2000 lux (Bhandal and Mehta, 1989). Stamets (2004) observed for the cultivation of *Auricularia polytricha* light is not required. He also noticed a light intensity of 500-1000 lux is needed for primordia and fruiting body formation.

2.3 GROWTH OF *TRICHOLOMA GIGANTEUM* ON DIFFERENT SPAWN SUBSTRATES

2.3.1 Spawn

Sinden (1934) was the first to introduce grain spawn for the cultivation of mushrooms. Different kinds of grains wheat, rye, millet etc were cooked and mixed with 1:3 per cent weight of calcium sulphate and calcium carbonate. The addition of gypsum and calcium carbonate prevents grains from clogging hours at

121 °C. The substrate after sterilization should contain 40-50 % moisture and pH of 7.5. Lemke (1971) proposed a formula for spawn preparation where the grain should contain 50 per cent moisture and the medium need to be maintained at pH of 6.5 to 6.7 for a better mycelia growth in the spawn bottle.

Kumar *et al.* (1975) suggested the use of calcium carbonate and gypsum in the proportion 1:3 respectively for better growth of grain spawn. Thape *et al.* (1978) devised a cheap and effective method of spawn production on polypropylene covers instead of glass bottles. Bhandal and Mehta (1986) observed that for the cultivation of *Auricularia polytricha* in India, sawdust spawn has better shelf life than grain spawn. Yield trials of different strains of *Agaricus bitorquis* indicated that spawn made of jowar grains supported maximum yield followed by bajra grains supplemented with shelled maize cob with 1:1 (Guleria *et al.*1989).

Studies conducted by Mathew *et al.* (1996) on the performance of different species of *Pleurotus* on spawn substrate revealed the sorghum, wheat and paddy grains were equally good for producing spawn.

Krishnamoorthy and Muthusamy (1997 a) utilized sorghum grain spawn for *Calocybe* cultivation. Spawn of oyster mushroom prepared on parboiled paddy grains were equally good as wheat for spawn preparations. Spawn prepared from parboiled paddy grains gave 7.5 % more yield than conventional cooked paddy spawns (Rathaiah and Shill, 1999). Upadhyay (1999) reported autoclaved wheat grain as excellent spawn for *Auricularia.*

According to Balakrishnan and Das (2001) sorghum, wheat or paddy grains are generally used for the preparation of spawn of *Calocybe.* Theradimani *et al.* (2001) used half cooked sorghum grains mixed with calcium carbonate at the rate of 2 % for the cultivation of *Calocybe.* Different types of cellulosic agricultural residues such as straw of paddy, wheat, barley, maize, jowar and bajra stalks, groundnut haulms, grasses and leaf fall of trees can be used as substrate for the

production of milky mushroom (Ram, 2004). *Agaricus bisporus* is generally grown in composted and pasteurized substrates where as *Pleurotus* and other species can be grown on various agricultural waste materials through suitable technologies (Yadav, 2005).

2.4 CULTIVATION OF *TRICHOLOMA GIGANTEUM* ON LOCALLY AVAILABE CHEAP SUBSTRATES

Waster materials like waste paper, sugarcane bagasse, rice straw, wood shavings, coconut waste and ragi waste were tried as bedding materials for *Pleurotus sajor-caju* cultivation (Sivaprakasam and Kandaswamy, 1981). Chang *et al.,* (1981) reported that the paddy straw served as the best substrate for production of *Pleurotus* sp. According to them paddy straw substrate increased bioefficiency, faster growth, less contamination compared to cotton waste as a substrate.

Chakravarthy *et al.,* (1981) cultivated *Calocybe indica* on paddy straw compost supplemented with inorganic substance like nitrogen, phosphorus, potassium which gave maximum milky mushroom yield when compared to paddy straw compost alone. Among the various agriculture wastes from maize, bajra, menthe, ground nut stalk, cereal straw and vegetable waste, cereal straw was found to be the most suitable substrate for the production of *Pleurotus florida* and *Pleurotus sajor-caju* (Garcha *et al.* 1983).

Experiments conducted by Dadwal and Jamaluddin (1984) revealed that barley meal and maize meal as the best substrate for the cultivation of *Tricholoma giganteum.* Combination of alfalfa hay and wheat straw significantly increased total yield and biological efficiency of *Pleurotus sajor-caju* (Royse and Bahler, 1988). Ganeshan (1990) found fresh paddy straw as a suitable substrate for cultivation of *Tricholoma lobayense.* Oil palm waste when added as a major ingredient in compost of *Agaricus* cultivation resulted in early maturation and

harvesting of fruiting bodies (Jimenez *et al.*, 1990). Hami (1990) reported that *Pleurotus ostreatus* gave maximum biological efficiency on sawdust.

Oyster mushroom cultivated on substrates *viz.*, paddy, straw, maize straw, coir dust and groundnut shells, biological efficiency varied widely with maximum in groundnut shell (Desai and Shetty, 1991). Mathew *et al.*, (1991) conducted investigation on the utilisation of solid waste from rubber processing as an alternate substrate for oyster mushroom production. Patil and Jadhav (1991) used cotton stalks as one of the best substrate for the cultivation of oyster mushroom *Pleurotus sajor-caju*. Sharma and Jandaik (1991) depicted wheat straw, and wheat straw on combination with spent straw as effective substrates in increasing yield of *Pleurotus florida* and *P. ostreatus*. Enhanced yield of *Pleurotus Ostreatus* was obtained when the mushroom was grown on tequila maguey in Mexico (Velazco *et al.*, 1991 a). Sugarcane bagasse and corns over has been utilized as substrate for the cultivation of Pleurotus in Mexico (Velazco *et al.*, 1991 b).

Trivedi *et al.*, (1991) screened various lignocellulosic substrates for the cultivation of *Calocybe indica* and reported that the mixture containing wheat straw, maize meal and dehydrated lucerne were found suitable for milky mushroom which gave increased bioefficiency. A noxious weed of tropics, *Chromolaenaodorata* when used in combination with paddy straw turned out to be a potential substrate for the cultivation of oyster mushroom *(Pleurotus flabellatus)* (Abraham and Pradeep, 1995). Lime water treated coir waste with paddy straw in 1:1 ratio proved to be a better alternative substrate to conventional substrate for *Pleurotus ostreatus* cultivation (Eyini *et al.*, 1995). Sangwan and Saini (1995) reported a method of increasing biological efficiency of *Pleurotus sajor-caju* by utilizing a combination of sugarcane bagasse, paddy straw and wheat straw as substrate.

Singh *et al.* (1995) proved that sugarcane trash in combination with wheat and paddy straw produced 74.20 per cent biological efficiency of *Pleurotus florida*. Straw of paddy, wheat, linseed, cotton, and jowar were tested for their

suitability as expensive and effective substrate (Kathe *et al.*, 1996). Mathew *et al.*, (1996) observed a water weed *Eliocharis plantogena* as a potential substrate for cultivation of *Pleurotus citrinopileatus*. Krishnamoorthy and Muthusamy (1997 b) utilised several agro wastes *viz.*, paddy straw, sorghum stalks, sugarcane bagasse, palmarosa grass, vetiver grass, groundnut haulms, soyabean hay and paddy straw compost for cultivation of *Calocybe*. Higher yield and higher biological efficiency was observed in paddy straw followed by maize stalk, sorghum stalk and vetiver grass. Paddy straw compost was not suitable for the cultivation of *Calocybe indica*.

Krishnamoorthy and Muthusamy (1997 b) utilized several agro wastes namely paddy straw, sorghum stalks, sugarcane bagasse, palmrosa grass, vetiver grass, groundnut haulms, soyabean hay and paddy straw compost for the cultivation of *Calocybe*. Higher yield and higher biological efficiency was observed in paddy straw followed by maize stalk, sorghum stalk and vetiver grass. Paddy straw compost was not suitable for the cultivation of *Calocybe indica.* Suharban *et al.* (1998) reported pseudostem of red banana as a better substrate for the oyster mushroom production when compared to pseudostem of nendran, redbanana, palayamkodan, robusta, rasakadali. A similar observation was made by Gupta *et al.* (1999) in maximizing yield of *Pleurotus sajor-caju* with paddy straw and least with betel nut husk.

Upadhyay (1999) reported that unsupplemented wheat straw after 8 weeks of cropping recorded the highest yield of 174 % biological efficiency followed by supplementation with wheat bran addition and saw dust during the cultivation of *Lentinus squarrosulus* on chemically treated wheat and paddy straw. Fermentatiobn of coir pith proved to be an effective pre-treatment to enhance the yield of milky mushroom (Bhavana and Thomas, 2002). Pandey and Tewari (2002) reported successful cultivation of *Tricholoma giganteum* with paddy straw giving biological efficiency of 92 %. Bhavana and Thomas (2003) reported cultivation of nine species of *Pleurotus* on coconut leaf stalk.

Sherin *et al.*, (2004) conducted experiment to study the suitable substrate for *Calocybe indica* cultivation among retted coir in combination with 75 % paddy straw, followed by 50 % combination of non retted coir pith and spent mushroom substrate. Pramod *et al.* (2005) observed red banana pseudostem as the most efficient substrate for the cultivation of oyster mushroom. Milky mushroom can be grown on wide range of substrates such as paddy straw, wheat straw, stalks of maize, bajra, cotton etc. Straw is chopped into small pieces (2 - 4 cm) and soaked in fresh water for 8 to 16 hours (Tewari, 2004).

Thirumalvalavan *et al.* (2005 b) reported that sorghum and sorghum plus kudhiraivali was the most suitable substrate for *Pleurotus florida.* Veena and Pandey (2006) observed that the best locally available substrate for the cultivation of *Ganoderma lucidum* was 90 % sawdust and 10 % rice bran. Kinjo and Miyagi (2006) reported that saw dust media supplemented with wheat bran and hannoki (*Alnus japonica*) gave highest yield of *Tricholoma giganteum.* Li *et al.,* (2006) conducted a study on culturing of *Tricholoma giganteum* with rape seed coat and the result indicated that fresh yield of *Tricholoma* in rapeseed cat mixed with cotton seed hull increased by 2.56 times. Amin *et al.,* (2010) reported that rice straw was the best substrate for commercial cultivation of *Calocybe indica.*

2.5 EFFECT OF DIFFERENT CASING MATERIALS

Casing is an absolute requirement for the proper fructification of *Tricholoma giganteum.*

The main function of casing layer is the production of mushrooms in quantity (Flegg, 1956).The history of edible mushroom cultivation dates back to many centuries and the use of casing to induce the development of sporophores has been practised since 17th century (Caron, 1987). An ideal casing material should be neutral in reaction, free from disease, pest, competitor organism and undecomposed vegetable matter (Phutola *et al.*, 1991). Supplementation can either be done at the time of spawning or casing but supplementation at casing allows fresh nutrients to reach the *Agaricus* and can offer strong competition of

organisms (Kurtzman, 1991). Saini and Prashar (1992) reported the higher yield of mushrooms in a casing medium consisting of farmyard manure + waste compost + soil (2:1:1). Pandey and Tewari (1994) reported the use of coir dust as a better casing material due to its high water holding capacity and porosity and clean sporophores. Doshi and Sharma (1995) utilized cow dung patties as a good casing material in *Calocybe* cultivation. Khanna *et al.* (1995) investigated on the suitability of different casing materials (farm yard manure, loam soil, clay soil burnt rice husk, two year old spent compost, and digested biogas slurry) and their combination for casing the mushroom beds of *Agaricus bisporus*. It was observed that burnt rice husk and biogas slurry can be mixed with clay, loam soil *etc.*, which can be used as casing material.

Out of the various casing materials (cow dung patties, biogas slurry, horse dung, moss grass) and their combinations tried for *Calocybe indica* biogas slurry and two year old cow dung patties were found equally suitable with biological efficiency of 98.7 per cent and 100 per cent respectively (Sharma *et al.*, 1997 a). Krishnamoorthy and Muthusamy (1997 a) reported the use of clay loam garden soil of pH 8.4 as a better casing material in *Calocybe* cultivation. Casing material consisting of sand, soil and cattle dung (1:1:1) was the best for giving higher yield of *Calocybe* (Balakrishnan and Das, 2001).

Theradimani *et al.*, (2001) concluded that optimum casing thickness for higher yield was found to be 1-2 cm. Krishnamoorthy *et al.* (2002) found that clay loam soil of pH 8.4 having 50 per cent moisture as the best casing material for *Calocybe*. Theyalso reported clay soil had moderate bulk density, more pore space, good water holding capacity and certain pseudomonads essential for the fructification of sporophores.

The combination of farmyard manure, garden soil and sand (4:2:1) was the best casing material for obtaining higher yield of *Agaricus bisporus* (Raina *et al.*, 2002). Different casing materials were evaluated for maximizing the yield of *Calocybe* by Geetha *et al.* (2002). Among the various materials coir pith supplemented with calcium carbonate gave the maximum biological efficiency,

number of sporophores as well as minimum period for sporophore production. Maria de la Fluente (2002) stated that there is a great potential for vermi compost to work as a substitute for peat moss as casing material in the production of white button mushroom (*Agaricus bisporus).*

Of the various casing materials evaluated for *Agaricus bisporus* with casing materials prepared from biogas plant slurry, burnt rice husk, farmyard manure, sandy soil and spent compost, the casing material with FYM and burnt rice husk in 2:1 proportion produced maximum yield compared with the control (Angrish *et al.*, 2003). Eswaran and Susan (2003) used casing material comprising of two year old farm yard manure and field soil in 1:1 proportion by weight and sterilised at 80°C for one hour for cultivation of *Calocybe.*

Dhar *et al.,* (2003) have revealed that the use of different agricultural wastes including vermi compost in the casing material has increased the N content and total mushroom yield of *Agaricus bisporus.* Suman and Paliyal (2004) utilised coconut coir pith, an agricultural waste available in plenty as casing material for *Agaricus bisporus*. They also reported that well rotten farm yard manure and coir pith (4:1 v/v) resulted in significant yield increase over control. Umamaheshwasri and Vijayalakshmi (2004) stated that utilization of earthworm casts as casing material helps in checking water loss by evaporation and the water holding capacity of the casts contributes to the increased yield.

Casing materials like field soil, garden soil, farm yard manure, lignite, fly ash and their combination were tried and results indicated that farm yard manure and field soil casing material was the best for giving dense mycelial growth and maximum yield of *Calocybe* (Senthilkumar *et al.*, 2005 b). Sterilization of casing material with carbendazim 100 ppm recorded maximum yield of *Calocybe* followed by 50 ppm. Lowest yield was recorded with carbendazim at 250 ppm (Senthilnambi *et al.*, 2005 a). Increased yields were observed when vermicompost was used in casing formulations in cultivation of *Agaricus bisporus* (Garcia *et al., 2005*).

Sassine *et al.,* (2005) has suggested that the casing should be very loose, otherwise the primordial cannot penetrate from the bottom to the top of the casing layer. Panna *et al.,* (2010) reported that the minimum days required from casing to primordial initiation (14.75) and the maximum number of fruiting body (5.25) was found in the bags cased with vermi compost derived from sawdust + cow dung (1:1) source and the highest yield (247/500 g) and biological efficiency (98.8%) for *Calocybe* was recorded in vermi compost derived from rice straw. Amin *et al.,*(2010) reported that the beds cased with cow dung and loamy soil gave maximum biolological efficiency for *Calocybe indica.* Yadav *et al.,* (2011) reported that casing mixture of two year old cowdung + soil (2:1) and two year old cowdung + spent compost of oyster mushroom + soil (1:1:1) were found to be the best for getting higher yield of *Tricholoma crassa.* Lakshmipathy *et al.,* (2012) reported that maximum biological efficiency for *Calocybe indica* was recorded when cased with farm-yard manure, red soil and sand in 1:3:1 proportion.

2.6 PEST AND DISEASE INCIDENCE

2.6.1 Pests

The attack of sciarid flies on cultivated mushroom was recorded by Fletcher *et al.* (1986). *Staphylinus* sp. was earlier reported to damage oyster mushroom in Thiruvananthapuram district of Kerala by Asari *et al.* (1991). Balakrishnan (1994) reported the occurrence of sciarid flies and staphylinid beetle as a pest of oyster mushroom in Kerala. Kumar and Sharma (2000) reported phorids as major pests affecting mushrooms. Kumar and Sharma (2001) studied on the seasonal abundance of mushroom pests, indicating presence of phorids and sciarids throughout the year in cropping rooms.

Pandey and Tewari (2002) suggested management of phorids and sciarids by hot water treatment at 80 °C or steam pasteurisation for one hour. They also suggested proper aeration, use of nylon nets, and use of yellow light traps as other

management strategies. Deepthi *et al.* (2003) reported for the first time another devastating pest of oyster mushroom *Scaphiosoma nigrofasciatum*. Snail was reported as a new major devastating pest of milky mushroom responsible for severe yield loss by Heera *et al.* (2006).

2.6.2 Diseases

Chakravarty *et al.* (1982) reported the inhibitory action of carbendazim at 25 ppm against the contaminants of oyster mushrooms .It was also reported by Vijay *et al.* (1986). Heavy contamination of *Trichoderma viride* in steam pasteurised straw reduced the mushroom yield which was controlled by using formalin and carbendazim (500 ppm and 75 ppm) solution for sterilisation of straw (Vijay and Sohi, 1987). Vijay and Sohi (1989) reported fungal competitors, *Trichoderma viride, Cephalosporium asperu, Cochliobolus spicifera, Drechslera bicolour* and*Phialophora*, reduced the growth of *Pleurotus* spp. by 10 to 100 %.

Doshi *et al.* (1991) observed competitor moulds like *Trichoderma viride, Sclerotium rolfsii, Aspergillus flavus*, on beds of *Calocybe* and managed them by the use of carbendazim 50 ppm and blitox 50 ppm. Of the undesirable fungi *viz.*, *Trichoderma harzianum, T. longibrachiatum, Chaetomium globosum* and *Epicoccum nigrum* on *Agaricus bisporus* bed, maximum yield loss of 50 per cent was due to *Trichoderma* sp. (Tewari and Singh 1991). Sharma *et al.* (1991) reported 45 per cent yield loss by *Trichoderma viride* and two per cent loss by *Coprinus* incidence in *Agaricus bitorquis* cultivation. Sharma and Vijay (1995) reported severe yield loss on *Agaricus bisporus* by *Coprinus*.

Sharma and Vijay (1996 a) observed high incidence of *Trichoderma viride* on steam sterilised paddy straw for *Pleurotus* cultivation resulting in 45 per cent yield loss. Sharma and Vijay (1996 b) surveyed on the incidence of brown plaster mould (90.00 %) false truffle (1-90.00 %) green mould, yellow mould and wet bubble. They also observed that competitor and parasitic

moulds on casing material and compost includes *Papulospora byssina, Trichoderma, Verticillium, Trichothecium, Coprinus, Chaetomium, Fusarium, Cladobotryium.*

Efficacy of formaldehyde fumes against competitors like *Sepedonium chrysospermum, Papulospora byssina* and mycoparasite was studied by Sharma *et al.* (1997 b). Exposure of the culture of these moulds to formaldehyde four per cent for 6-24 hours inhibited the growth of the pathogen. Dhar (1998) observed that carbendazim, benomyl, manocozeb and zineb could be best used for controlling the mould competitors of *Agaricus bitorquis*. Among the different species of *Trichoderma, Trichoderma harzianum* occurred widely than *T. viride* (Jandaik and Guleria, 1999). Anandh *et al.* (1999) studied the yield loss in *Pleurotus eous* due to the incidence by common contaminants *Trichoderma harzianum, Aspergillus flavus* and *Aspergillus nige*r. Studies revealed loss of yield ranged from 70-77 per cent.

Pani (2000) noted *Coprinus* to be a serious contaminant causing 80 per cent reduction in yield followed by *Sclerotium rolfsii* (74 %). Thakur *et al.* (2001) observed that the incidence of fungal competitors was highest during May-July and minimum during January–March. Pandey and Tewari (2002) described green mould, *Chaetomium* and *Coprinus* as the major problematic weed moulds in mushroom cultivation. Singh and Sharma (2002) reported occurrence of *Mycogone perniciosa,* causal agent of wet bubble disease in various mushroom growing units in Solan. Loss in yield to *Mycogone perniciosa* was 100 per cent in *Agaricus* cultivation which was controlled using sporogon at 0.075.

Seshagiri and Eswaran (2002) observed that inoculation of *T. harzianum,* and *A. flavus* on *Calocybe* beds after casing increased the yield of *Calocybe.* Studies were conducted by Pandey *et al.* (2003) to investigate the occurrence of competitor moulds and pathogens during the cultivation of *Calocybe indica. T. harzianum* was the most problematic weed mould during spawn run and cob web disease causing complete crop loss which could be managed by carbendazim (0.01 %).

Siddique *et al.* (2004) suggested plant derivatives especially onion had maximum inhibitory effect on *Trichoderma* followed by *Aegle marmelos.* Bhardwaj (2005) observed incidence of *T. harzianum*, *Coprinus*, *Papulospora byssina* in *Calocybe* beds which could be managed by carbendazim at 50 ppm. Krishnamoorthy *et al.* (2005 a) conducted survey on the incidence of pest and diseases of milky mushroom and observed the incidence of *Coprinus* from spawn run till harvest. Raman *et al.* (2005) reported *Aspergillus niger, Aspergillus flavus* and *Trichoderma*sp. as major contaminants of *Calocybe* spawn.

2.7 PROXIMATE CONSTITUENT ANALYSIS, SHELF LIFE AND KEEPING QUALITY OF *TRICHOLOMA GIGANTEUM*

2.7.1 Proximate constituents / Nutritive value

Purkayastha and Chandra (1976) reported protein content of *Calocybe* mycelium as 19.8 %. Among the various amino acids leucine, threonine, tyrosine and alanine were found predominant in *Calocybe*. Chandra and Purkayastha (1976) reported the gain in body weight of mice supplied with mycelial powder of *Calocybe indica.* Protein, vitamin and carbohydrate content of woods ears are reported to be higher than that of many vegetable and fruits and calorific content is relatively low (Cheng and Tu, 1978). The mushroom is known for its delicacy, flavour and aroma. Nutritionally, it is considered as a valuable vegetables, consisting of protein (10-40 %), carbohydrate (13-70 %), fat (less than 1-8 %), minerals and significant amounts of essential amino acids (Chang *et al.*,1981).

Huang *et al.*, (1989) reported six commonly cultivated mushrooms with higher per centage of saponifiable liquids. The value of saponifiable liquids range from 78.1 % in *Auricularia auriculae* to 58.8 % in *Volvariella volvaceae.* Nutritive value of *Calocybe* was accounted as 11.9 % dry matter, 2.4 % protein, 2.25 % soluble salts and 50 kcal of energy by Sivaprakasam *et al.*, (1986). Venkateshwarlu *et al.*, (1991) noted that the volatile flavour compound of

Calocybe was attributed to the presence of 1-octen-3-ol, n- octanol, and 3-octanaol.

Mineral content of the mushroom indicated as 10 % ash (dry weight basis) containing potassium, phosphorus, sodium, calcium and magnesium. Mushrooms are also rich source of vitamins like thiamine, niacin, riboflavin, folic acid, ascorbic acid and pro-vitamin D (Rajarathnam, *et al.,* 1993). Compared with other mushrooms *Calocybe* have a nutritive value of 4.1 % fat, 3.4 % crude fibre and 64 % carbohydrate (Doshi and Sharma, 1995). When compared to oyster mushroom, milky mushroom had more protein, carbohydrate and fat (Krishnamoorthy and Muthuswamy, 1997).

Nutritive content of *Agaricus* consisted of 90.10 % moisture, 3.75 % protein, 0.53 % crude fibre and 4.59 % carbohydrate (Singh *et al.*, 1999). Anandh (2001) reported nutritive value of *Calocybe indica* with 88.37 % moisture, 11.63 % dry matter, 26.5 % protein, 36.5 % fibre and 8.8 % carbohydrate. He also stated the proximate constituent composition of *Tricholoma lobayense* with 85.2 % moisture, 14.8 % dry matter, 33.2 % protein, 23.74 % fibre and 11.38 % carbohydrate. Keun Yang *et al.* (2002) reported that *Auricularia polytricha* contained 77.5 % carbohydrate and 22.5 % protein. Arumugananthan *et al.* (2003) observed total soluble salt of 5 -7 brix in *Agaricus bisporus.*

Chien *et al.*(2004) reported that an immunomodulatory protein was purified from fruiting body of an edible Jew's ear mushroom by extraction using 5 % cold acetic acid in the presence of 0.1 % 2-mercaptoethanol , followed by ammonium sulphate fractionation. Rathore and Thakore (2004) studied the effect of different substrates on nutrient composition of *Pleurotus florida* and found that the sporophores contain protein 35 %, carbohydrates 44.23 %, fat 2.20 %, fibre 9.85 %, ahs 8.72 % and moisture 89 %. Ram (2004) reported that the dried milky mushroom contain protein 32.3 %, fat 4.5 % fibre 41 % and carbohydrate 64.26 %, minerals, ash and had a good delicious flavour. Zhang *et al.* (2006) observed

fruiting bodies of *Auricularia auriculae* as rich in polysaccharides having anti oxidant properties.

Liu *et al.,* (2007) studied the nutrient content of *Tricholoma giganteum* and *Pleurotus eryngii* cultivated with cotton seed hull compost and found that contents of protein, fat, total sugar and crude fibre in *T. giganteum* and *P. eryngii* were 35.28 %, 2.91 %, 53.74 %, 8.76 % and 15.4 %, 0.55 %, 52.1 %, 5.4 % respectively. Prakasam *et al.,* (2011) reported that *Tricholoma giganteum* contain 86.20 % moisture, 32.9 % crude protein, 11.8 % carbohydrate, 0.91 % crude fat, 20.71 % crude fibre, 8.32 % ash, 5.60 % iron, 1.18 % manganese, 1.38 % zinc and 1.10 % copper.

2.7.2 Shelf life

Short term storage of white button mushrooms (*Agaricus bisporus)* in perforated and non perforated polyethylene bags at different temperature were studied by Saxena and Rai (1988). The study indicated that mushrooms could be stored in non perforated bags for 4 days at 5 °C, 2 days at 10 °C and 1 day at 15 °C without veil opening and deterioration. Mehta and Jandaik (1989) reported storage of freshly harvested fruit bodies of *Pleurotus sapidus* on non perforated polythene bags upto 72 h at room temperature and at low temperature of 0-5 °C upto 15 days. Dhar (1992) observed that *Agaricus bitorquis* had better shelf life when stored in non perforated packs with little change in quality. Storage in perforated bags resulted in weight loss, veil opening, browning and spoilage. Krishnamoorthy and Muthuswamy (1997) reported that the shelf life of milky mushroom is more than 3 days.

Post harvest washing, treatment with 125 ppm EDTA improved quality as well as shelf life of *Agaricus* (Ahlawat *et al.*, 1998). Krishnamoorthy (2004) reported that milky mushroom is having shelf life of 5-7 days at room temperature. Heera (2006) reported that *Calocybe* had better shelf life of 12.67 – 24.67 days when stored in refrigerated condition. Prakasam *et al.,* (2011) reported

that *Tricholoma giganteum* can be stored under room temperature for two days and under refrigerated storage for 6 days without any spoilage and liquefaction.

2.7.3 Preservation by dehydration

Munjal (1975) has described the air drying of mushroom either in the shade or under the sun by spreading them on a white sheet of paper. He has also stated that if the mushrooms were dried in hot air drier, the temperature during drying should not be exceeded 52°C, otherwise they lose their aroma. Singh *et al.,* (1996) has developed flow drier for the dehydration of paddy straw mushroom, and they have reported the optimum drying temperature, time and critical moisture content as 60°C, 7 h and 5 %, respectively.

2.8 ORGANOLEPTIC STUDIES

Mushroom blends well with most of the vegetables and spices to form delicious items of food (Das 1992 a, 1992 b). A study conducted by Desai *et al.* (1991) revealed that consumer acceptability of *Pleurotus sajor-caju* was poor due to the tough texture of the stipe and unattractive colour of the pileus but its flavour was found good. In a comparative study Balakrishnan (1994) showed that *Pleurotus sapidus, P.membranaceous, P.petaloides* obtained maximum consumer acceptability with respect to colour and flavour. Overall acceptability of these species was significant when compared to the standard species *Pleurotus sajor-caju* and *P. flabellatus* which were found inferior in all the qualities.

Stamets (2004) reported that *A. auricula* as superior to *A. polytricha* in culinary terms .The consumer acceptability of *A. auricula* is better when compared with that of *A. polytricha.* A variety of recipes can be prepared using fresh mushroom and dried mushroom (Das 1994, 2003; Das and Nair 2003). Das (2011) prepared different dishes using oyster mushroom, button mushroom, Jew's ear mushroom and milky mushroom and obtain maximum consumer acceptability in case of appearance, colour, flavour, taste and texture.

Materials and Methods

3. MATERIALS AND METHODS

3.1 COLLECTION, ISOLATION AND PURIFICATION OF NATIVE STRAINS

Mushrooms were collected from different places of Thiruvananthapuram district during the South West and North East monsoon season at Aryanadu, Pallichal, Thiruvallam and Nedumangad. The colours, macroscopic and microscopic characters of the collected mushrooms were noted. *Tricholoma giagnteum* obtained from three locations were isolated using standard technique. The three isolates were named as isolate 1, 2, 3 and 4. Preliminary trials were conducted and the best isolate was sent to Directorate of Mushroom Research for obtaining the coded accession number. The isolate was given accession number as DMRO - 462 from Directorate of Mushroom Research, Solan which was used for further studies.

3.1.1 Isolation and purification of native strains

3.1.1.1 Tissue culture technique

Tricholoma giganteum selected after screening were isolated using standard techniques for tissue isolation. The mushrooms were cleaned up and fresh mushrooms were surface sterilized using ethanol. In the laminar airflow chamber the mushrooms were longitudinally split into equal halves from the pileus and a small piece of tissue was removed using a sterile scalpel or an inoculation needle and then transferred to potato dextrose agar (PDA) slants under aseptic conditions in front of the flame and incubated under room temperature (28-40°C) for four days. These isolates were then purified by the hyphal tip method and maintained on PDA slants by periodic subculturing for further studies.

3.2 MORPHOLOGICAL, CULTURAL AND PHYSIOLOGICAL STUDIES

3.2.1 Identification of native isolate

The mushrooms were screened for their macroscopic details such as colour, texture, pileus, stipe, spore print, lamellae, gills and microscopic details based on data sheet as described in Appendix – I.

Spore print was made by cutting and keeping portions of the pileus on plain microscopic slides and also on black paper sheets. A medium mature mushroom sporocarp (two days after formation of initial) was taken for obtaining the spore print. The stem was removed from the joining point of the pileus and the pileus was placed on a piece of paper as gills slide towards the surface of the paper. A bell jar was placed over this to keep moist and to protect from air. After six hours the bell jar and the pileus was removed and the spore print was obtained.

3.2.1.1 *Microscopic characters were studied by the following technique*

3.2.1.1.1 *Melzer's Reagent*

One and half grams of iodine, 100 mg of chloral hydrate and 5 g of potassium iodide were mixed with 100 ml of water. The water should not be boiled or mixed with alkali. Production of blue black colour by spores on treatment with this reagent indicates amyloid nature of spores. Reddish brown indicates dextrinoid and yellow non amyloid.

3.2.1.1.2 *Cotton Blue Stain*

The stain was prepared by combining 50 ml of one per cent solution of cotton blue in lactic acid (100 g), phenol (100 g), glycerine 150 ml) and 50 ml water. The cotton blue turns spore wall of cyanophilic agarics into blue or dark blue.

3.2.2 Cultural and physiological studies

3.2.2.1 *Growth under different solid media*

The media were prepared and sterilized by autoclaving at 1.02 kg cm^{-2} for 15-20 min. The media were melted and before solidification were poured into a sterile petridish of nine cm dia and allowed to solidify, after inoculation with the culture of *Tricholoma giganteum*. The dishes were incubated at room temperature (28+-2°C). They were used to cutting discs to study the growth on different media.

Five different solid media namely Potato dextrose agar (PDA), Oat meal agar, Malt extract agar, carrot agar and Tapioca/ Jack kernel dextrose agar were used to find out the best medium for the growth of *Tricholoma giganteum.* The composition of the media used is given in Appendix II. The media were prepared and sterilized by autoclave at 15 lbs pressure for 15-20 min. After cooling it was poured into sterile petri dishes of nine centimetre diameter and allowed to solidify. The culture disc of five mm diameter cut out from seven day old culture of fungus was used for inoculation. The culture disc of the mushroom was inoculated into petri dish which was later incubated at room temperature (28 ± 2°C). Four replications were maintained for each treatment and colony diameter, nature of mycelia growth was measured at weekly intervals for 14 days.

3.3.2.3 *Growth under different liquid media*

The different media *viz*., potato dextrose, oat meal, malt extract, carrot and tapioca broths were used. The composition was same as used in the previous experiment except for the omission of agar.

The liquid media were prepared and 50 ml of each medium was dispensed in 100 ml conical flask and autoclaved at 1.02 kg cm^{-2} pressure for 20 min. The media were then inoculated with 5 mm culture disc of fungus, taken from actively growing culture under aseptic condition. The flasks were kept at room

temperature for 20 days. After 20 days the mycelia were filtered through a Whatman No: 1 filter paper and dried in an at 60 °C. The dry weights were taken until a constant weight was obtained.

3.3.2.4 Effect of different carbon source on the growth of Tricholoma giganteum

Tricholoma was grown in media with different carbon sources *viz.*, sucrose, lactose,galactose, mannitol and inositol. These were substituted for dextrose, in potato dextrose medium with agar. The media were prepared and sterilized by autoclaving at 15 lbs pressure for 15-20 min. After cooling it was poured into sterile petri dishes of nine centimetre diameter and allowed to solidify. The culture disc of five mm diameter cut out from seven day old culture of fungus was used for inoculation. The culture disc of the mushroom was inoculated into petri dish which was later incubated at room temperature (28 ± 2°C). Three replications were maintained for each treatment and colony diameter, nature of mycelia growth *etc.* were measured at weekly intervals of 14 days.

The liquid media with different carbon sources were prepared, the composition was same used in previous experiment except for the omission of agar. 50 ml of each medium was then inoculated with five mm culture disc of actively growing culture and incubated at room temperature (28 ± 2 °C). The mycelia mat was filtered after two weeks and dry weight was taken after drying at 70 °C till a constant weight was obtained.

3.3.2.5 Influence of different nitrogen sources on the growth of Tricholoma giganteum

Different forms of nitrogen as ammonium nitrate, ammonium carbonate, ammonium chloride, sodium nitrate, beef extract and peptone were substituted in Czapecks medium so as to give the same per cent of nitrogen in each case. The media were prepared and sterilized by autoclaving at 15 lbs pressure for 15-20 min. After cooling it was poured into sterile petri dishes of nine centimetre diameter and allowed to solidify. The culture disc of 5 mm diameter cut out from

seven day old culture of fungus was used for inoculation. The culture disc of the mushroom was inoculated into petri dish which was later incubated at room temperature (28 ± 2°C). Three replications were maintained for each treatment and colony diameter, nature of mycelia growth etc. were measured at weekly intervals of 14 days.

The liquid media with different nitrogen sources were prepared, the composition was same used in previous experiment except for the omission of agar. .Fifty ml of medium was taken in each 100 ml conical flask sterilized in an autoclave inoculated with five mm culture disc of actively growing culture and incubated at room temperature for 14 days. The mycelia mat was filtered through a Whatman No: 1 filter paper and dry weights were taken after drying at 70 °C until constant weight was obtained.

3.3.2.6 Effect of different hydrogen ion concentration in the media on the growth of Tricholoma giganteum

PDA medium was prepared and pH was adjusted to 4.0, 5.0, 6.0, 7.0 and 8.0 by adding 0.1 N hydrocholoricacid or 0.1 N sodium hydroxide. The media prepared were sterilized by autoclaving at 15 lbs pressure for 15-20 minutes. After cooling it was poured into sterile petri dishes of nine centimetre diameter and allowed to solidify. The culture disc of five mm diameter cut out from seven day old culture of fungus was used for inoculation. The culture disc of the mushroom was inoculated into petri dish which was later incubated at room temperature (28 ± 2°C). Three replications were maintained for each treatment and colony diameter, nature of mycelia growth etc. were measured at weekly intervals of 14 days.

Potato Dextrose broth was prepared with different pH concentration given above .Fifty millilitres of medium was taken in 100 ml conical flask and autoclaved at 1.02 kg cm^{-2} pressure for twenty minutes. The medium was then inoculated with a 5 mm disc of seven day old culture of *Tricholoma giganteum* and incubated at room temperature (28 ± 2 °C) for two weeks. The mycelia mat was filtered, dried at 70 °C until constant weights were obtained.

3.3.2.7 Effect of different temperature on the growth of Tricholoma giganteum

Twenty ml of PDA was poured into a sterile petridish on which a disc of 5mm cut with the help of cork borer was taken from the actively growing mycelium of *Tricholoma giganteum*. The disc was placed at the centre of the medium and incubated at 4, 22, 24, 26, 28, 30, and 35 ° C. The nature of the growth and radial mycelial growth was measured at weekly intervals of 14 days.

Fifty millilitres of Potato dextrose broth was taken in 100 ml conical flask and autoclaved at 1.02 kg cm^{-2} pressure for twenty minutes. The medium was then inoculated with a 5 mm disc of seven day old culture of *Tricholoma giganteum* and incubated at 4, 22, 24, 26, 28, 30 and 35 °C for two weeks. The mycelia mat was filtered dried at 70 °C until constant weights were obtained.

3.3.2.8 Effect of light and darkness on the growth of Tricholoma giganteum

Twenty ml of PDA was poured into a sterile petri dish on which a dice of 5 mm cut with the help of cork borer was taken from the actively growing mycelium of *Tricholoma giganteum*. The disc was placed at the centre of the medium and incubated at room light, intermittent light, fluorescent light and dark conditions. The nature of growth and radial mycelial growth was measured after a week.

Fifty millilitres of Potato dextrose broth was taken in 100 ml conical flask and autoclaved at 1.02 kg cm^{-2} pressure for twenty minutes. The medium was then inoculated with a 5 mm disc of seven day old culture of *Tricholom agiganteum* and incubated room light, intermittent light, fluorescent light and dark condition for two weeks. The mycelia mat was filtered dried at 70° c until constant weights were obtained.

3.3 GROWTH OF *TRICHOLOMA GIGANTEUM* ON DIFFERENT SPAWN SUBSTRATES

The mushrooms collected, isolated and maintained on PDA slants in the laboratory were inoculated in grain based spawn material for further study.

3.3.1 Preparation of grain spawn

Spawn was prepared as per the method described by Sinden (1934). Spawn was prepared using paddy grains, maize, ragi, wheat, bajra and sorghum. The grains were cooked for one hour in boiling water. The excess water was drained off and the grains were spread on a clear area. Polypropylene bags and glucose drip bottles were filled with cooked grains after mixing with calcium carbonate at the rate of fifty to sixty grams per kg of seed. The filled bags and bottles were sterilized at 1.02 kg cm^{-2} for 2 h in and autoclave. The mycelial bits from seven-day old actively growing pure culture of *Tricholoma* were inoculated aseptically and incubated at room temperature (28 ± 2°C). The mycelial growth of fungi were measured and recorded. The spawn thus obtained as mother spawn is used for further spawn production and also to raise beds.

Sawdust from tree was used for the preparation of spawn. The sawdust spawn was prepared using the formulae Sawdust 99 %, calcium carbonate 1 % and water 65 %. The substrates was thoroughly sieved to remove bigger particles and mixed with water. The substrate mixture is then filled in polypropylene bags. The filled bags were sterilized at 1.02 kg per cm^2 for two hours in an autoclave. The mycelial bits from seven day old actively growing pure culture of *Tricholoma* were inoculated aseptically and incubated at room temperature (28 ± 2 °C). The best spawn substrate was selected based on the minimum time taken for the spawn run, their nature of growth and better yield.

3.4 CULTIVATION OF *TRICHOLOMA GIGANTEUM* ON LOCALLY AVAILABLE CHEAP SUBSTRATES

The study was conducted to find out the biological efficiency of *Tricholoma giganteum* by using different locally available cheap substrates. Beds were raised following the polybag method as described by Baskaran *et al.* (1978). Modified technique instead of paddy straw bits, straw made into small twists was used for laying beds. The different substrates used for the cultivation of

mushroom included paddy straw, sugarcane bagasse, saw dust, coir pith compost, spent mushroom substrate and coir pith + paddy straw (1:1).

Paddy straw and sugarcane bagasse substrates were soaked in a solution containing carbendazim (75 ppm) and formalin (500 ppm) for 18 h. After draining excess water and air drying, they were used for mushroom bed preparation.

Saw dust, coir pith compost, spent mushroom substrate and coir pith substrates were prepared by mixing them with 1 % calcium carbonate retaining 60 % moisture content. The substrates were then sterilized at 1.02 kg per cm^2 for 2 h in an autoclave. After cooling, spawning was done.

The sterilized substrates were used for bed preparation. The beds were prepared as per the polybag method. The polythene bags of 60 x 30 cm were used for mushroom bed preparation. The paddy straw bits were placed as twist in bags and spawn laid in the centre over which paddy straw twists was laid and spawning was done this was repeated three times. This same method was applied for sugarcane bagasse sawdust, coir pith compost, spent mushroom substrate bed preparation. For coir pith + paddy straw substrate, first a twist of paddy straw was placed in a polythene bag as a layer and spawning was done at the centre and another layer of coir pith was placed and spawning was done which was repeated three times. Polythene bags were made compact tied at the top, and provided with a few holes for air circulation. The spawned bags were then transferred to an incubation chamber for spawn run. After completion of spawn run, the bag was kept for fruiting in cropping room with high relative humidity of 80-85 %. After 20 days of spawn run casing was done. Regular sprinkling of water was done. The best substrate was selected based on the criteria *viz*, time taken for case run, first harvest, second harvest, number of sporophores produced, average yield, total yield and biological efficiency.

3.5 EFFECT OF DIFFERENT CASING MATERIALS

After 20 days of spawn run, casing was done using different materials namely, red soil, red soil + sand, vermi compost, coir pith compost and red soil +

sand + cow dung (1:1:1). Casing was prepared by mixing the material with 1 % calcium carbonate retaining 60 % moisture content. The substrates were then sterilized at 1.02 kg per cm^2 for two hours in an autoclave. After cooling, spawning was done. The fully white *Tricholoma* beds were opened and casing material was spread on the top to a thickness of 3 cm and made slightly firm. After casing, standard watering procedures were used to increase casing moisture. Observations were made during the time the mycelium colonized the casing layer and during pinning stage, when the primordial developed. The best casing material was selected based on the criteria *viz*, time taken for case run, first harvest, second harvest, number of sporophores produced, average yield, total yield and biological efficiency.

3.6 PEST AND DISEASE INCIDENCE

Pest and disease incidence were noted during spawn run, case run and during sporophore formation. The beds were monitored daily for their incidence. The beds damaged by pests and diseases were noted down and nature of damage were recorded.

3.7 PROXIMATE CONSTITUENT ANALYSIS, SHELF LIFE AND KEEPING QUALITY OF *TRICHOLOMA GIGANTEUM*

3.7.1 Proximate constituent analysis

3.7.1.1 *Estimation of moisture content*

Ten grams sample was dried in an oven until constant weight obtained. The initial and final weights were noted. The difference between the two gives the result, which is converted into percent.

3.7.1.2 *Estimation of protein*

Protein content of *Tricholoma giganteum* was estimated using the method described by Bradford (1976).

One gram sample was ground in 10 ml of 0.1 M acetate buffer (pH 4.7). The materials were centrifuged at 5000 rpm for 15 min at 4 °C. The supernatant obtained was used for further analysis. The reaction of mixture consisting of 0.5 ml enzyme extract, 0.5 ml of distilled water and 5 ml of Coomassie brilliant blue G-250 was used. The reaction mixture was essayed for the absorbance of 595 nm against reagent blank. Standard graphs were prepared using the Bovine serum albumin. Using the graph, the protein content was determined as microgram albumin equivalent of soluble protein on fresh weight basis.

3.7.1.3 *Estimation of fat*

The extraction of fat was carried out using Soxhlet extraction apparatus (Moore and Stein, 1948).

Five gram of sample was weighed into an extraction thimble and placed in the extractor so that top of the thimble is over the bent siphon tube outside extractor. The extractor was connected to previous weighed extraction flask. Sufficient quantity of petroleum ether was poured into the extractor. The extractor was attached to the condenser with a constant flow of cold water. The flask was heated on a water bath. The extraction was carried out till the liquid became colourless. The flask was removed and the solvent was evaporated in an oven at 105 °C. It was dried to a constant weight. The increase in weight of flask was the fat obtained.

3.7.1.4 *Estimation of total sugars or carbohydrates (µg)*

Total carbohydrate content was estimated by anthrone method (Hedge and Hofreiter, 1962).

One hundred mg of mushroom mycelia was weighed and transferred into boiling tubes. It was hydrolyzed by keeping it in a boiling water bath for 3 hours with 5 ml of 2.5 N hydrochloric acid, cooled to room temperature and neutralized with sodium carbonate till effervescence was ceased. The tissue was ground and

volume made up to 100 ml and centrifuged at 5000 rpm for 15 min. The supernatant was collected, was used as an aliquot for analysis. From the supernatant 0.5 ml of aliquot was taken and made upto 1 ml by adding distilled water. The reaction mixture containing 0.5 ml of aliquot, 0.5 ml distilled water and 4 ml of anthrone reagent was added to the tubes and heated for 8 minutes in boiling water bath. The reaction mixture was cooled and colour read at 630 nm in a spectrophotometer (systronics UV-VIS spectrophotometer 118). The amount of carbohydrate present was calculated from the standard graph prepared using glucose and expressed in terms of milligrams of glucose equivalent per gram of sample on fresh weight basis.

3.7.1.5 *Estimation of crude fibre*

Crude fibre content was estimated by a method described by Misra *et al.* (1975).

One gram of filtered dried sample was ground with ether to remove fat. After ether extract the dried sample was boiled with 100 ml of concentrated sulphuric acid (1.25 %) for 3 minutes by adding bumping chips. The digested sample was filtered through a muslin cloth and washed with boiling water until the washings were no longer acidic. The sample again boiled with 100 ml sodium hydroxide (1.25 %) for 30 min. The digested samples were again filtered through a muslin cloth and washed with boiling water until the washings were not alkaline. The sample was washed with 25 ml of boiling 1.25 % sulphuric acid, 50 ml of water and 25 ml of alcohol. The residue was removed and transferred to preweighed ashing dish (W_1). The residue was dried at 130 °C for two hour, cooled the dish in desiccator and weighed (W_2). The residue was further ignited at 600 °C which was cooled and weighed.

$$\% \text{ of crude fibre} = \frac{\text{Loss in weight}}{\text{Weight of the sample}} \times 100$$

3.7.1.6 *Estimation of ash*

Three gram sample was transferred to a weighted silica dish. It was heated on a Bunsen burner at a low flame and when the substrate charred the dish was transferred to a muffle furnace. It was heated at 500 to 550 ° C for about 2 hours till a white ash was obtained. It was then cooled in desiccator and weighed. The difference between two gives the result, which is converted into per cent.

3.7.2 Shelf life and keeping quality

An experiment was conducted to determine the shelf life of *Tricholoma giganteum.* The mushrooms were kept at room temperature and also in refrigerated condition. Mushroom packed in polypropylene covers with and without perforation was kept in room temperature and refrigerated conditions. One sample was kept in a petri plate under room temperature and refrigerated condition. The weight of mushroom was observed each day upto one week. Characteristics such as firmness, colour, texture, days upto which no spoilage occurred were recorded.

3.7.2.1 *Preservation by dehydration*

Three different drying techniques were compared with keeping undried mushrooms as control. The drying techniques were, use of oven, hot air oven and solar energy.

3.7.2.1.1 *Use of oven*

Freshly harvested mushrooms after cleaning were kept in an oven for dehydration. The temperature was adjusted to 45 °C and the drying was done for a period of five to seven hours. the samples were weighed at regular intervals. The process was repeated until constant weight was obtained. Immediately after drying it was packed in airtight fresh polypropylene covers and stored in a

moisture and insect free condition. Periodical observations were made on changes in quality of the produce.

3.7.2.1.2 Use of hot air oven

Fresh mushrooms after cleaning were put in petri dishes and kept in a hot air oven for dehydration. The temperature was adjusted at 60 °C and the drying was done for a period of four to five hours. the samples were weighed at regular intervals. The process was repeated until almost constant weight was obtained. Immediately after drying, it was packed in polypropylene covers and periodical observations were made.

3.7.2.1.3 Drying under sun

Freshly harvested mushrooms after cleaning were spread on paper sheets under direct sun light. It was done on a bright sunny day. Exposure to full sun light for a period of seven hours. was sufficient to dehydrate the produce. The dried product was packed in polypropylene covers and stored under room temperature.

The weight loss of the dried product, percentage damage by microbes to the product in different drying methods, colour changes due to dehydration were observed and analysed.

3.8 ORGANOLEPTIC STUDIES

Tricholoma giganteum mushroom cultivated were subjected to studies of organoleptic characters like colour and appearance, texture, flavour and taste. Six different recipes *viz.*, *Tricholoma* cutlets, puffs, mushroom masala, soup, payasam and pickle were prepared and they were subjected to evaluation by ten judges based on a five point score card. The average ranking was given for each character. Score card values for each character was given in Appendix - III. The recipes are given below:

Soup

Tricholoma powder	-	100g
Shallots	-	3 g
Butter	-	30 g
Ground pepper	-	¼ tsp
Ground cardamom	-	¼ tsp
Corn flour	-	1 tsp
Milk	-	2 cups
Egg	-	1 no.
Salt	-	to taste

Melt butter, fry onions and mushroom powder mix the corn flour in two cups of milk , and boil for 10 minutes in shallow pan. Before removing from fire add beaten egg white and sprinkle pepper powder, cardamom salt and serve hot.

Cutlets

Tricholoma	-	200 g
Onion finely chopped	-	1 medium
Garlic crushed	-	3 cloves
Ginger crushed	-	a 1" inch piece
Green chilli chopped	-	1 or 2
Garam masala	-	1 tsp
Chilli powder	-	¼ tsp (optional)
Curry leaves chopped - Optional		
Salt	-	to taste
Potato boiled, peeled and mashed	-	1 medium
Egg	-	1
Plain bread crumbs	-	as needed
Oil to fry		

Finely chop mushrooms using a knife. Heat oil in a pan and add chopped onion and fry for seven to eight minutes. Add crushed ginger, garlic and green chillies and fry for three to four min. Add chopped mushrooms and sauté till the water evaporates (8-10 minutes). Add garam masala, salt to taste and curry leaves and fry till it is well sauté and switch off the flame and let it cool.

Add the mashed potato with the mushroom, mix and combine it well using hands. Make small balls, keep it in the centre of palm and give a gentle press to shape it. Dip it in beaten egg, roll it in bread crumbs and deep fry till it is golden and done.

Pickle

Tricholoma	-	300g (chopped)
Gingely oil	-	150 g
Mustard seeds	-	1 tsp
Ginger	-	50 g (chopped)
Garlic	-	50 g (chopped)
Green chillies	-	50 g (chopped)
Curry leaves	-	as required
Turmeric powder	-	1 tsp
Chilly powder	-	3 tsp
Fenugreek seeds	-	½ tsp
Vinegar	-	75 ml
Salt	-	as required

Heat the gingelly oil and fry the ingredients: mustard, ginger, garlic, green chillies and curry leaves. Add mushroom to it and fry again. Then add rest of the ingredients: turmeric powder, chilly powder, fenugreek powder, vinegar and salt. Cook in low flame until done. Let it cool and transfer into a bottle. Mushroom pickle is ready.

Payasam

Tricholoma	-	200 g
Sugar	-	½ cup
Milk	-	½ cup
Ghee	-	75 ml
Cashew nuts	-	15 nos.
Raisins	-	15 nos.
Elachi	-	5 nos.
Coconut milk	-	2 cups

Heat a pan, add ghee and fry cashews and raisins till golden yellow colour appears and keep aside. Fry the sliced mushroom in the remaining ghee. Add coconut milk cook for 10 minutes. Add sugar boil for 2 minutes and add the milk and bring to boil, let it boil in low flame. Add elachi powder, fried cashew and raisins and serve hot.

Mushroom masala

Tricholoma	-	250 g (chopped)
Green chillies	-	2 (medium size)
Onion (big)	-	2 (100 g)
Ginger shredded	-	1 tsp
Garlic	-	3 flakes
Clove	-	2
Cardamom	-	2
Cinnamon	-	2
Chilly powder	-	1 tsp
Pepper powder	-	½ tsp
Turmeric powder	-	a pinch
Salt	-	to taste
Oil	-	for frying
Curry leaves	-	a few

Heat oil in a kadai. Fry the onions, ginger, green chillies and curry leaves. Marinate the mushrooms with a paste of garlic, clove, cinnamon, cardamom, chilly powder, pepper powder, turmeric powder and salt. Add to the pan sprinkle water and close with a heavy lid. Cook for 10 minutes and serve hot.

Puffs

For the dough:

Maida	2 ½ cups
Dalda	4 tbs
Salt to taste	
Water	

For the masala filling:

Tricholoma	-	200g finely chopped
Onions	-	finely chopped
Potatoes	-	4 medium size
Cumin seeds	-	½ tsp
Turmeric powder	-	¼ tsp
Red chilli powder	-	½ tsp
Garam masala	-	½ tsp
Salt		
Oil		

Paste:

Garlic	-	2 flakes
Ginger	-	½ inch
Green chillies	-	2

Dough:

Sieve maida and salt. Add cold water and knead to a smooth dough. Cover the dough with a wet cloth and keep it aside for 30 minutes. Keep some maida for dusting.

To prepare stuffing:

Cook the potatoes. Mash the potatoes coarsely. Heat oil in a pan and add cumin seeds and ground ginger, garlic and chilli paste. Fry for few minutes. Add chopped onions and mushroom, fry until brown. Then put the mashed potatoes, turmeric poweder, red chilli powder, garam masala powder and salt. Mix well and fry until done. Remove from fire.

Puff preparation:

Melt dalda and whip it till creamy and keep it aside. Take the dough and knead once again until it becomes smooth and elastic. Roll the dough into a large thin rectangle on a flat surface by dusting with remaining maida. Apply dalda to 2/3 of the rectangle leaving ½ inch space all around.

Fold the empty portion (without dalda) first and the other side (with dalda) on top of it. Roll this lightly into a rectangle and repeat the same process twice using the remaining dalda.

Cut this into small rectangles and keep the stuffing at the centre and fold across. Seal the edges with water. Refrigerate for 30 min.

Preheat the oven at 300°C. Apply little water to a tray and arrange the puffs with enough space in between. Brush little milk on top of the puffs and bake for 45 minutes until golden brown. Serve hot.

The quality evaluation of mushroom recipes using raw as well as dehydrated mushroom was done using five point score card. The overall acceptability of cooked mushroom recipes were recorded based on evaluation done by judges.

Results

4. RESULTS

4.1 COLLECTION, ISOLATION AND PURIFICATION OF NATIVE ISOLATES

Mushrooms were collected from different parts of Trivandrum district before and after the South West and North East monsoons. The native strains of *Tricholoma* obtained from Trivandrum district had been collected from Aryanad, Pallichal, Thiruvallum and Nedumangad (Plate 1). The habitat of these native strains varied from solitary to gregarious type and they were terrestrial. Organic matter rich soil and coconut tree basins were found to be the usual spots for the occurrence of *Tricholoma* (Table 1). The period of occurrence of these mushrooms were from June – October. The selected isolate after preliminary trials was given accession number as DMRO-462 from Directorate of Mushroom Research, Solan.

4.1.1 Isolation and purification of culture

The tissue isolation of the isolates was done as per the standard method described under 3.3.1 and the cultures were maintained on PDA slants by periodical sub culturing.

4.2 MORPHOLOGICAL, CULTURAL AND PHYSIOLOGICAL STUDIES

4.2.1 Identification of native isolates

Morphological features of various isolates of *Tricholoma* obtained are given in Table 2.

Pileus: Convex, off white to creamish white in colour and fleshy in texture. The diameter of the pileus of these isolates range from 9-20 cm.

Plate 1. Isolates of *Tricholoma giganteum*

Table 1. Characteristics of isolates of *Tricholoma giganteum* under natural condition

Name of isolate	Habit	Habitat	Substrate	Place of collection
Isolate 1	Gregarious	Terrestrial	Organic matter rich soil	Aryanad
Isolate 2	Solitary	Terrestrial	Soil	Pallichal
Isolate 3	Solitary	Terrestrial	Coconut tree basin	Thiruvallum
Isolate 4	Gregarious	Terrestrial	Soil	Nedumangadu

Table 2. Morphological features of various isolates of *Tricholoma giganteum*

Name of the isolate	Pileus					Stipe					
	Shape	Colour	Diameter (cm)	Thickness (cm)	Texture	Length (cm)	Diameter (cm)	Shape	Attachment of pileus	Basal part	Surface
Isolate 1	Convex	White	11.20	2.50	Fleshy	20.20	6.20	Clavate	Central	Bulbous	Scaly
Isolate 2	Convex	White	7.10	2.00	Fleshy	12.50	5.50	Clavate	Central	Bulbous	Scaly
Isolate 3	Convex	Pale white	6.00	1.50	Fleshy	8.50	3.20	Clavate	Central	Bulbous	Scaly
Isolate 4	Convex	Pale yellow	10.60	2.20	Fleshy	17.30	5.20	Clavate	Central	Bulbous	Smooth

Stipe: Clavate shaped, smooth or hairy (scaly) surface, centrally attached to the pileus with bulbous base. The length of the stipe of each isolate of *Tricholoma* ranged from 10-22 cm with a diameter of 3-6 cm.

Spore: Ovoid to smooth ellipsoid, hyaline, inamyloid, 6.5- 7 x 4.5 – 5 µm. Spore print white (Plate 2).

The characters of gills namely arrangement, texture, margin, gills/cm, spore print, spore colour and spore shape are given in Table 3.

4.2.1.1 Melzer's Reagent

The treatment of spores taken from the spore print with Melzer's reagent showed non amyloid/ inamyloid reaction with yellow coloured spores.

4.2.1.2 Cotton blue stain

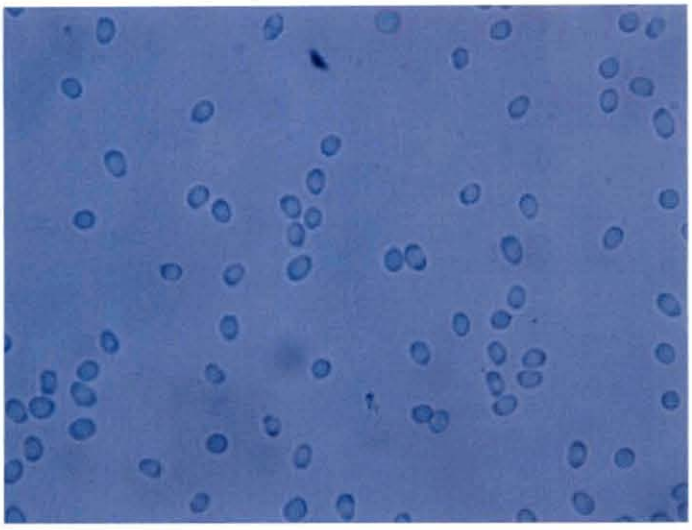
The cotton blue treatment of spores gave cyanophilic reaction by changing the spore wall colour to blue or dark blue.

4.2.2 Cultural studies and physiological studies of *Tricholoma giganteum*

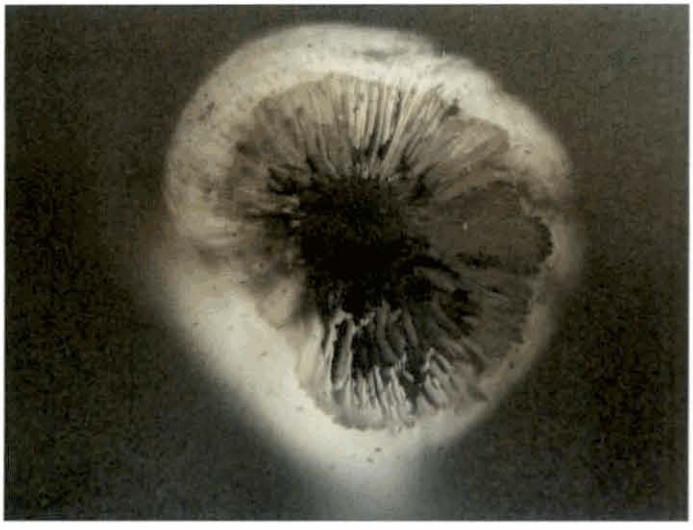
4.2.2.1 Effect of different solid media on the growth of Tricholoma giganteum

Five different solid culture media namely potato dextrose agar, malt extract agar, carrot agar, oat meal agar and tapioca dextrose agar were tested for their efficacy in supporting the radial mycelial growth of *Tricholoma giganteum*. The result showed that the media significantly differed in influencing mycelial growth of *Tricholoma* (Table 4).

The nature of mycelia growth was very much dense in oatmeal agar followed by potato dextrose agar and malt extract agar. In tapioca dextrose agar and carrot agar the nature of growth was scanty and of medium type (Plate 3).



Spore



Spore print

Plate 2. Spore and spore print of *Tricholoma giganteum*

Table 3. Characters of different native isolates

Name of the isolate	Gills				Spore				
	Arrangement	Texture	Margin	Gills/cm	Spore colour	Spore print	Shape	Cotton blue reaction	Melzers reaction
Isolate 1	Free	Brittle	Smooth	14	Hyaline	White	Ovate	Cyanophilic	Nonamyloid
Isolate 2	Free	Brittle	Smooth	12	Hyaline	White	Ovate	Cyanophilic	Nonamyloid
Isolate 3	Free	Brittle	Smooth	12	Hyaline	White	Ellipsoidal	Cyanophilic	Nonamyloid
Isolate 4	Free	Brittle	Smooth	14	Hyaline	Pale yellow	Ovate	Cyanophilic	Nonamyloid

Table 4. Growth of *Tricholoma* in different solid media

Media	Growth of *Tricholoma* (cm)*	
	7 th day	14 th day
PDA	4.42	8.80
Malt extract	3.95	8.20
Carrot agar	2.60	6.37
Oat meal agar	**5.32**	**9. 00**
Tapioca dextrose agar	3.37	7.72
CD (0.05 level)	0.400	0.363
SE	0.133	0.120

* Average of four replication

Radial growth of *Tricholoma* culture after seven days indicated that oat meal agar was superior producing 5.32 cm radial growth followed by potato dextrose agar with 4.42 cm radial growth. Malt extract agar was on par with tapioca dextrose agar having a radial growth of 3.95 and 3.37 cm. Lowest radial growth was in carrot agar with 2.6 cm.

The growth of *Tricholoma* on petridish was completed on 14th day with slight variation. The radial growth of *Tricholoma* after 14th days indicated that oat meal agar was superior with 9.0 cm of radial growth. Potato dextrose agar was found to be on par with oat meal agar in supporting the radial growth having 8.8 cm. The radial growth produced by malt extract agar and tapioca dextrose agar were 8.2 cm and 7.72 cm respectively. The lowest radial growth was found in carrot agar with 6.37 cm.

4.2.2.2 Effect of different liquid media on the growth of Tricholoma giganteum

The biomass production of *Tricholoma giganteum* was estimated in different liquid broth *viz,* potato dextrose broth, malt extract broth, carrot broth, oat meal broth and tapioca dextrose broth. The result showed that liquid media differed significantly in influencing biomass production of *Tricholoma* (Table 5).

The radial growth and biomass production of *Tricholoma* after 14 days of incubation indicated that oat meal broth was superior with 0.38 g / 50 ml of biomass production. The biomass produced by potato dextrose broth (0.21 g / 50 ml) was found to be on par with malt extract broth (0.18 g/50ml). The tapioca dextrose broth produced a biomass of 0.07 g / 50 ml. carrot extract broth was found to be least effective for growth of *Tricholoma* with biomass production of 0.03 g / 50 ml (Plate 4).

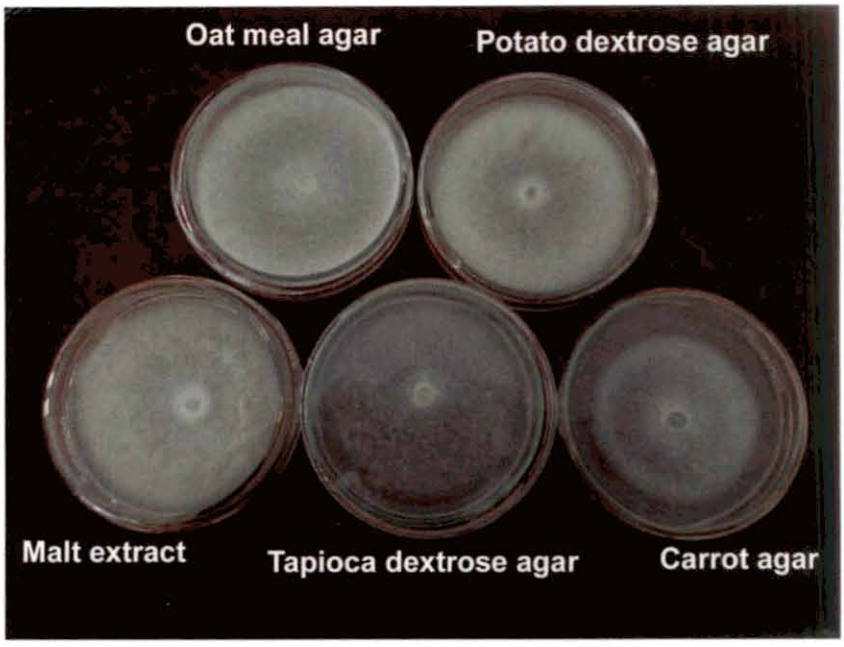


Plate 3. Growth of *Tricholoma* in different solid media

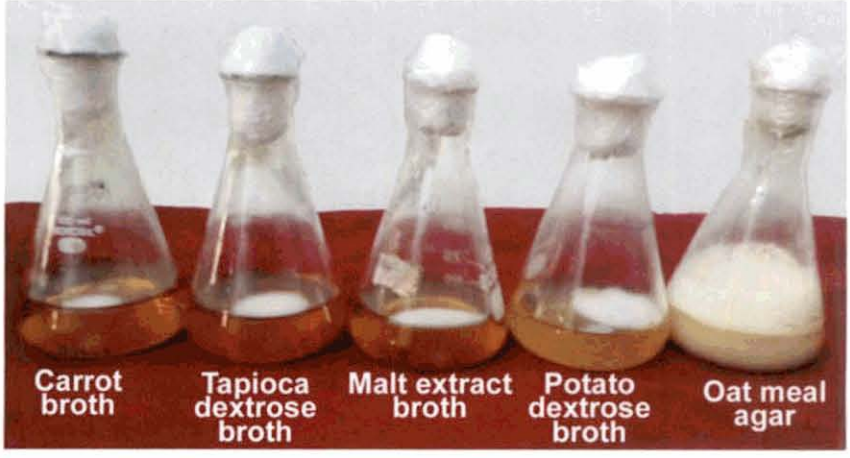


Plate 4. Growth of *Tricholoma* in different liquid media

Table 5. Growth of *Tricholoma* in different liquid media

Media	Dry weight of mycelia (g/50ml)*
PDB	0.21
Malt extract broth	0.18
Carrot broth	0.03
Oat meal broth	**0.38**
Tapioca dextrose broth	0.07
CD (0.05 level)	0.021
SE	0.007

* Average of four replication

4.2.2.3 Effect of different carbon sources on the growth of Tricholoma giganteum

Six different carbon sources namely sucrose, lactose, galactose, mannitol, inositol and dextrose were tested in solid and liquid medium for their efficacy in radial mycelia growth and biomass production of *Tricholoma.* The result showed that different carbon sources differed significantly in influencing the radial growth and biomass production of *Tricholoma giganteum* (Table 6).

Growth of *Tricholoma* in petridish was recorded seven days after inoculation with different carbon sources. The study indicated that carbon sources mannitol, dextrose and sucrose were on par, recording a radial growth of 4.32, 4.25 and 4.25 cm respectively. These were followed by inositol and lactose having 2.05 and 1.1 cm respectively. Least growth was found in galactose with 1 cm radial growth.

Growth of *Tricholoma* on different carbon sources indicated the attainment of full growth in fourteen days. The carbon sources mannitol, dextrose and sucrose were on par and completed 9.00-8.82 cm growth on petridish after 14 days (Plate 5). This was followed by inositol 4.30 cm. Least growth was observed in lactose and galactose recording a radial growth of 2.95 and 2.75 cm. The carbon sources mannitol, dextrose and sucrose are significantly superior from all others.

In liquid media, the carbon source dextrose supported maximum biomass production with 0.52 g / 50 ml followed by mannitol (0.48 g / 50 ml), sucrose (0.37 g / 50 ml) and inositol (0.22 g / 50 ml) (Plate 6). Other carbon sources *viz.*, lactose and galactose were found to the least suitable, producing 0.10 g and 0.04 g of biomass (Table 7).

Plate 5. Growth of *Tricholoma* in different carbon sources

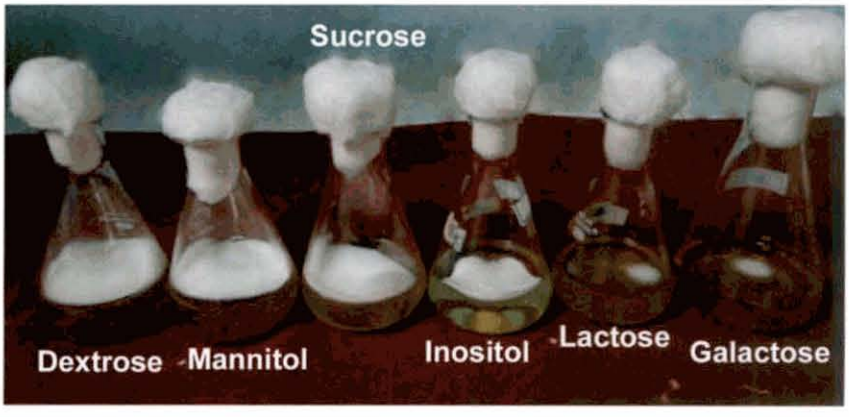


Plate 6. Growth of *Tricholoma* in liquid media using different carbon sources

Table 6. Growth of *Tricholoma* culture in different carbon source

Media	Growth of *Tricholoma* (cm)*	
	7 th day	14 th day
Sucrose	4.25	8.82
Lactose	1.10	2.95
Galactose	1.00	2.75
Manitol	**4.32**	**9.00**
Inositol	2.05	4.30
Dextrose	4.25	9.00
CD (0.05 level)	0.464	0.588
SE	0.156	0.198

* Average of four replication

Table 7. Growth of *Tricholoma* culture in liquid medium using different carbon source

Media	Dry weight of mycelia (g/50ml)*
Sucrose	0.37
Lactose	0.10
Galactose	0.04
Manitol	0.48
Inositol	0.22
Dextrose	**0.52**
CD (0.05 level)	0.030
SE	0.010

*Average of four replication

4.2.2.4 Influence of different nitrogen sources on the growth of Tricholoma giganteum

The nitrogen sources evaluated included both organic and inorganic types. Czapecks medium was used for the study. Inorganic nitrogen sources tried were sodium nitrate, potassium nitrate, ammonium nitrate, ammonium chloride and ammonium carbonate. Organic sources included beef extract and peptone.

Growth of *Tricholoma* in petridish was recorded seven days after inoculation with different nitrogen sources. The study indicated that the organic nitrogen was found to be a better source of nitrogen than the inorganic sources. Beef extract was the best source of nitrogen producing the radial growth of 4.75 cm which was followed by peptone (4.20 cm), potassium nitrate (3.40 cm), ammonium chloride (2.75 cm) and ammonium nitrate (2.15 cm). Least growth was observed in sodium nitrate and ammonium carbonate having 1.95 cm and 1.8 cm respectively.

Growth of *Tricholoma* on different nitrogen sources indicated the attainment of full growth in fourteen days. The organic nitrogen sources beef extract and peptone were on par and completed 8.55 and 9.00 cm on petridishes (Plate 7). Among the nitrate sources potassium nitrate was better compared to sodium nitrate and ammonium nitrate and they produced 8.55 cm, 6.12 cm and 4.65 cm radial growth respectively. Among the various ammonium sources, ammonium chloride was better compared to ammonium carbonate and ammonium nitrate and they produced 5.97 cm, 5.00 cm and 4.65 cm radial growth respectively (Table 8).

In liquid medium, the organic nitrogen was found to be a better source of nitrogen than the inorganic sources. Beef extract was the best nitrogen source producing the highest biomass of 0.37 g / 50 ml followed by peptone with 0.27 g (Plate 8). Among the nitrate sources potassium nitrate was better compared to sodium nitrate and ammonium nitrate and they produced 0.24 g, 0.21 g and 0.09 g of biomass respectively. Among the various ammonium sources, ammonium chloride (0.15 g) was better compared to ammonium nitrate (0.09 g) and ammonium carbonate (0.06 g) (Table 9).

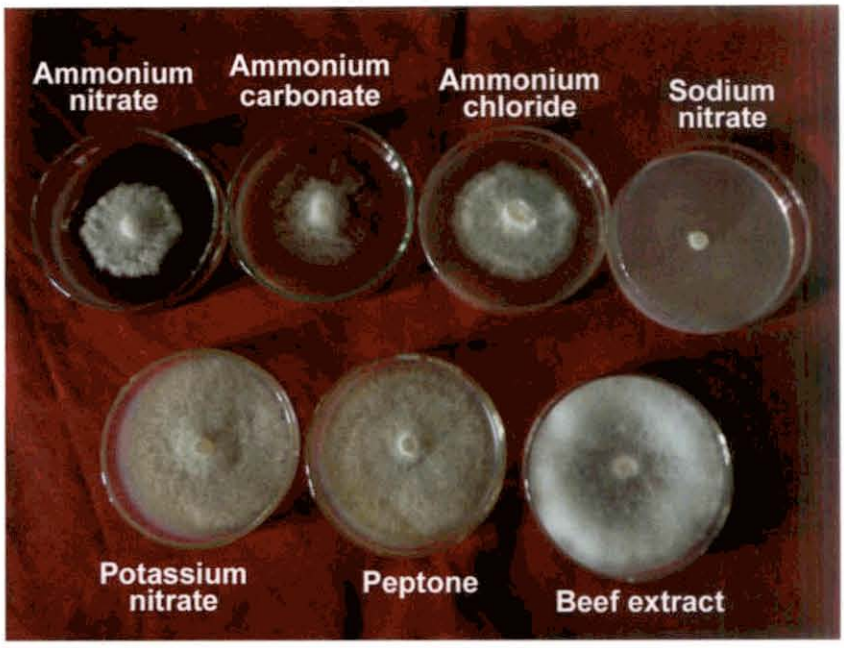


Plate 7. Growth of *Tricholoma* in different nitrogen sources

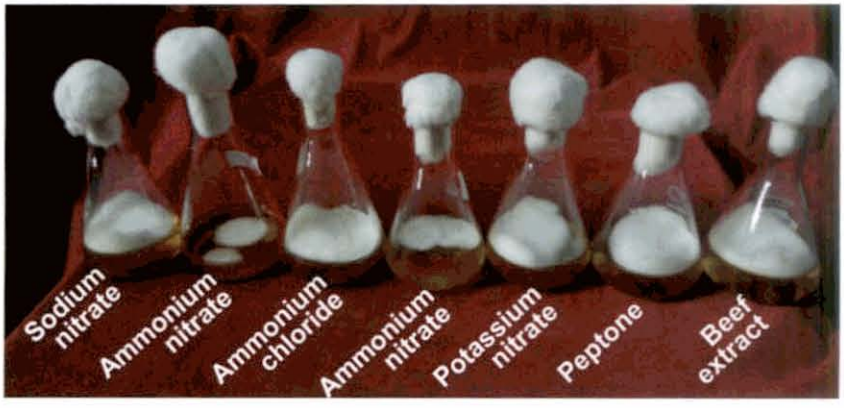


Plate 8. Growth of *Tricholoma* in liquid media using different nitrogen

Table 8. Growth of *Tricholoma* culture in different nitrogen source

Media	Growth of *Tricholoma* (cm)*	
	7 th day	14 th day
Ammonium nitrate	2.15	4.65
Ammonium carbonate	1.80	5.00
Ammonium chloride	2.75	5.97
Sodium nitrate	1.95	6.12
Potassium nitrate	3.40	7.35
Peptone	4.20	8.55
Beef extract	**4.75**	**9.00**
CD (0.05 level)	0.430	0.710
SE	0.146	0.241

* Average of four replication

Table 9. Growth of *Tricholoma* culture in liquid medium using different nitrogen source

Media	Dry weight of mycelia (g/50ml)*
Ammonium nitrate	0.09
Ammonium carbonate	0.06
Ammonium chloride	0.15
Sodium nitrate	0.21
Potassium nitrate	0.24
Peptone	0.27
Beef extract	0.37
CD (0.05 level)	0.035
SE	0.011

* Average of four replication

Of these seven nitrogen sources ammonium carbonate was the nitrogen source which contributed least to the radial growth and biomass production.

4.2.2.5 Effect of different hydrogen ion concentration in the media on the growth of Tricholoma giganteum

Five different H^+ ion concentration (pH) ranging from 4 to 8 was tested for their efficacy in the production of radial growth and biomass.

Growth of *Tricholoma* in petridish was recorded seven days after inoculation for growth in different hydrogen ion concentration. The study indicated that pH 8 was best for the growth of *Tricholoma* by producing a radial growth of 4.47 cm which was on par with pH 7 (4.22 cm) and pH 6 (4.07 cm). pH 5 and pH 4 was on par with each other by producing 2.77 cm and 2.67 cm radial growth. Least growth was found in pH 4.

Growth of *Tricholoma* on different hydrogen ion concentration indicated the attainment of full growth in fourteen days. The pH 8 and pH 7 was on par and completed 9.00-8.62 cm growth on petridish after 14 days (Plate 9). This was followed by pH 6 by producing a radial growth of 8.3 cm. Least growth was found in pH 5 (5.85 cm) and pH 4 (5.57 cm) (Table 10).

In liquid medium, it was observed that maximum biomass was obtained at pH 8 (0.38 g) which was followed pH 7 with a biomass of 0.25 g. The pH 6 and pH 5 was on par producing a biomass of 0.13 g and 0.10 g (Plate 10). Least biomass was obtained in pH 4 of 0.07 g. It was observed that *Tricholoma* prefers a pH of 6-8 rather the highly acidic environment *i.e.*, pH 4 (Table 11).

4.2.2.6 Effect of different temperature on growth of the Tricholoma giganteum

Seven different temperature conditions of 4 °C, 22 °C, 24 °C, 26 °C, 28 °C, 30°C and 35°C were tested for the efficacy in the production of maximum radial growth and biomass. The result showed that different temperature conditions differ significantly in influencing radial growth and biomass production.

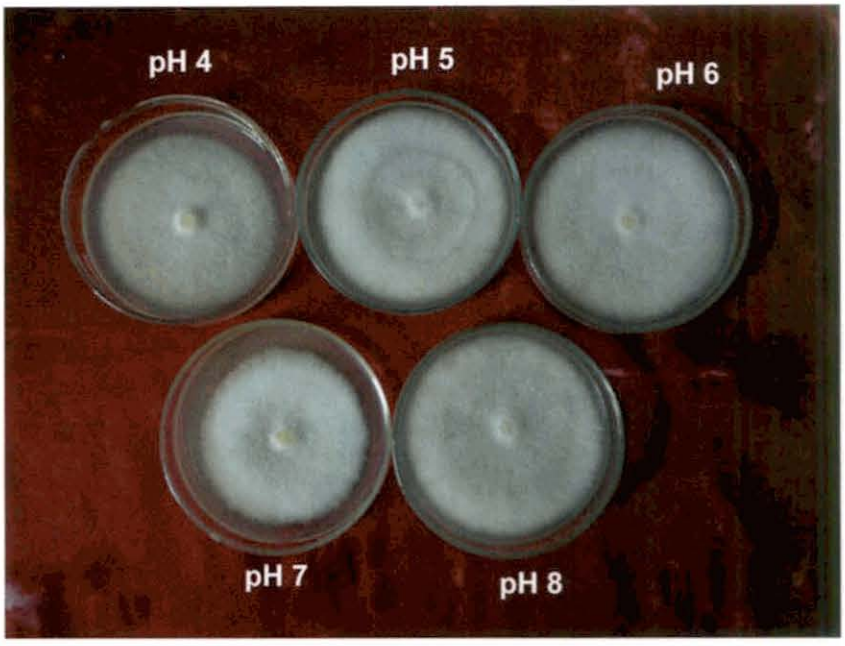


Plate 9. Growth of *Tricholoma* in different pH

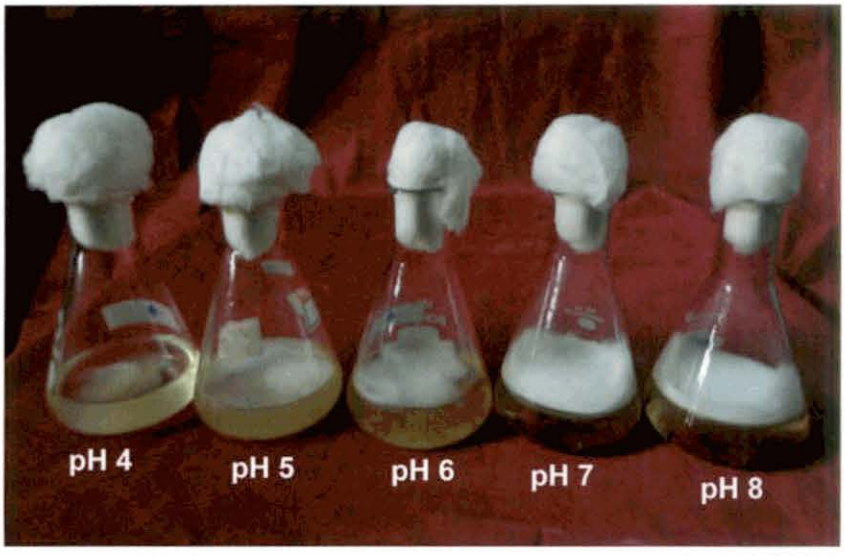


Plate 10. Growth of *Tricholoma* in liquid media of different pH

Table 10. Growth of *Tricholoma* culture in different pH level

pH	Growth of *Tricholoma* (cm)*	
	7th day	14th day
4	2.67	5.57
5	2.77	5.85
6	4.07	8.30
7	4.22	8.62
8	**4.47**	**9.00**
CD (0.05 level)	0.479	0.499
SE	0.159	0.165

* Average of four replication

Table 11. Growth of *Tricholoma* culture in liquid medium of different pH level

pH	Dry weight of mycelia (g/50ml)*
4	0.07
5	0.10
6	0.13
7	0.25
8	**0.38**
CD (0.05 level)	0.037
SE	0.012

* Average of four replication

Growth of *Tricholoma* in petridish was recorded seven days after inoculation for different temperature conditions. The maximum radial growth found in 35°C which was on par with 30 °C producing a radial growth of 4.37 cm and 4.17 cm. This was followed by 28 °C, 26 °C, 24 °C and 22 °C producing 3.97, 3.4, 3.05 and 2.8 cm radial growth. No growth was found in 4°C.

Growth of *Tricholoma* under different temperature condition indicated the attainment of full growth in fourteen days. At temperature 35 °C *Tricholoma* completed full growth (9.00 cm) on petridish after 14 days (Plate 11). Growth at temperature 30 °C and 28 °C was on par and reached a diameter of 8.55 cm and 8.32 cm growth on petridish. This was followed by temperature 24 °C and 22 °C with a growth of 6.25 cm and 6.00 cm respectively. No growth was found in 4 °C temperature (Table 12).

In liquid media, it was observed that temperature of 35 °C supported maximum mycelial growth and biomass production of 0.35 g which was followed by 30 °C , 28 °C , 26 °C, 24 °C and 22 °C temperature producing a biomass of 0.27 g, 0.21 g, 0.19 g, 0.09 g and 0.04 g respectively (Table 13). No mycelial growth was produced at 4 °C temperature (Plate12).

4.2.2.7 Effect of different light and darkness on the growth of Tricholoma giganteum

Four different ranges of light like room light, intermittent light, darkness and fluorescent light were tested for their efficacy in the production radial growth and biomass. The result showed that different light conditions differed significantly in influencing radial growth and biomass production.

Growth of *Tricholoma* in petridish was recorded seven days after inoculation for different light conditions. The maximum radial growth found in fluorescent light which was on par with intermittent light and room light 4.37 cm, 4.1 cm and 4.05 cm radial growth respectively. Least growth was observed in dark condition (2.45 cm).

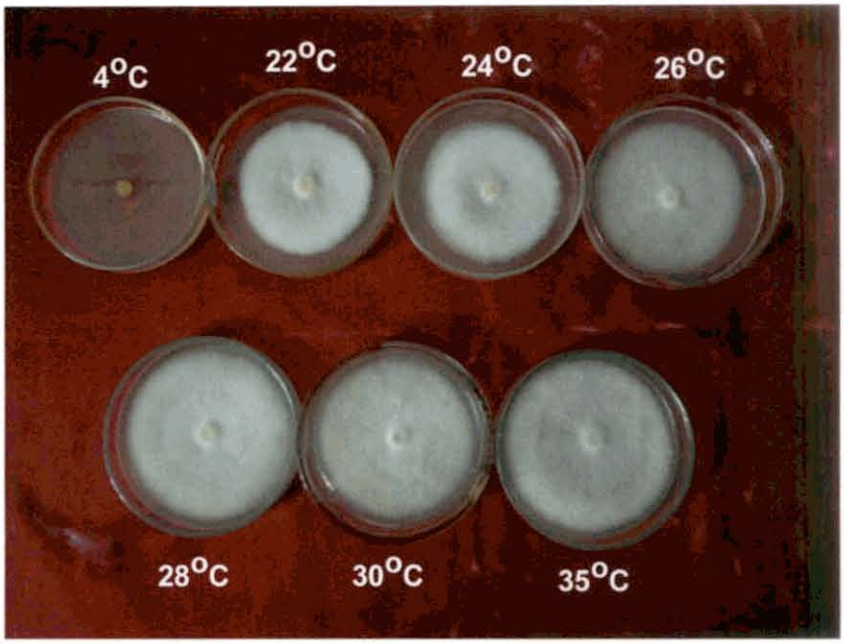


Plate 11. Growth of *Tricholoma* at different temperature

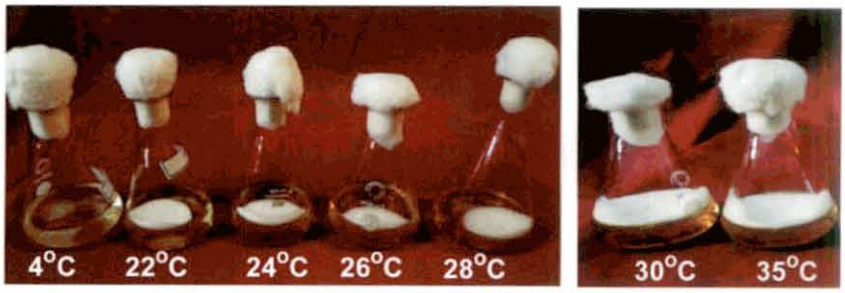


Plate 12. Growth of *Tricholoma* in liquid media at different temperature

Table 12. Growth of *Tricholoma* culture in different temperature

Temperature (°C)	Growth of *Tricholoma* (cm)*	
	7^{th} day	14^{th} day
4	0.00	0.00
22	2.80	6.0
24	3.05	6.25
26	3.40	7.22
28	3.97	8.32
30	4.17	8.55
35	**4.37**	**9.00**
CD (0.05 level)	0.255	0.204
SE	0.086	0.098

* Average of four replication

Table 13. Growth of *Tricholoma* culture in liquid medium of different temperature

Temperature (°C)	Dry weight of mycelia (g/50ml)*
4	0.00
22	0.04
24	0.09
26	0.19
28	0.21
30	0.27
35	**0.35**
CD (0.05 level)	0.016
SE	0.005

* Average of four replication

Growth of *Tricholoma* on different light condition indicated the attainment of full growth in fourteen days. The fluorescent light was on par with intermittent light and room light by producing 9.0-8.75 cm radial mycelia growth (Table 14). Least growth was found in dark condition (6.9 cm) (Plate 13).

In liquid media, it was observed that fluorescent light gave maximum mycelia growth and biomass production of *Tricholoma* having 0.34 g. This was followed by intermittent light and room light producing a biomass of 0.27 g and 0.20 g respectively (Table 15). The least biomass was produced in dark condition (0.09 g) (Plate 14) .

4.3 GROWTH OF *TRICHOLOMA GIGANTEUM* MASSEE ON DIFFERENT SPAWN SUBSTRATES

4.3.1 Time taken for spawn run on *Tricholoma giganteum* Massee

Six different substrates were evaluated on the basis of number of days taken for maximum spawn run and the nature of mycelial growth on the spawn. Spawn substrates tried were paddy, wheat, sorghum, ragi, sawdust and maize (Plate 15).

Paddy was found to be the best substrate for spawn run with a minimum of 13.66 days required for maximum spawn run with thick, white mycelial growth on the grain. It is found that the substrate paddy was on par with wheat and maize which took 14.66 and 15.66 days for maximum spawn run with a thick and fluffy growth on grains (Table 16).

The substrate ragi took 16.66 days for maximum spawn run. Partial growth on spawn was found in sorghum and it took 17.66 days for maximum spawn run.

Maximum number of days taken for spawn run was observed in saw dust with 21 days, which is significantly different from all others.

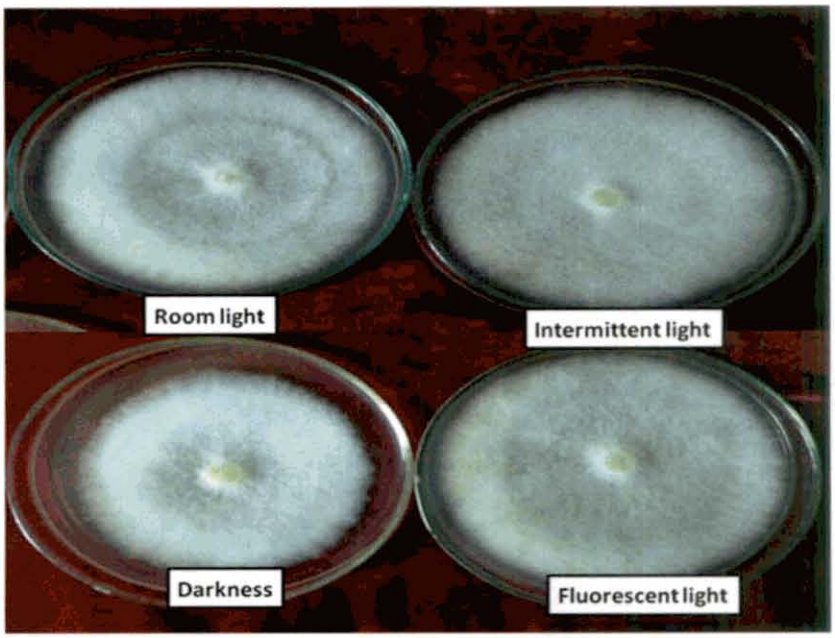


Plate 13. Growth of *Tricholoma* in different light sources

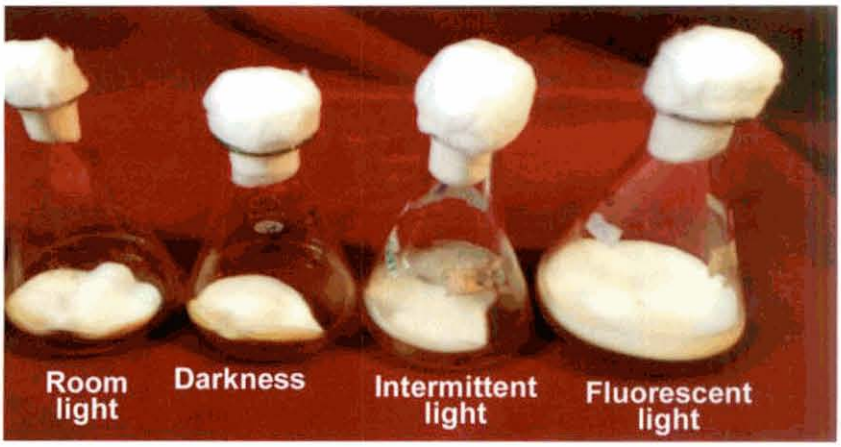


Plate 14. Growth of *Tricholoma* in liquid media using different light sources

Paddy

Wheat

Sorghum

Ragi

Maize

Saw dust

Plate 15. Different spawn substrates

Table 14. Growth of *Tricholoma* culture in different light sources

Light	Growth of *Tricholoma* (cm)*	
	7th day	14 th day
Room light	4.05	8.75
Intermittent light	4.10	8.80
Darkness	2.45	6.90
Fluorescent light	**4.37**	**9.00**
CD (0.05 level)	0.376	0.263
SE	0.124	0.087

* Average of four replication

Table 15. Growth of *Tricholoma* culture in liquid medium using different light source

Light	Dry weight of mycelia (g/50ml)*
Room light	0.20
Intermittent light	0.27
Darkness	0.09
Fluorescent light	**0.34**
CD (0.05 level)	0.025
SE	0.008

* Average of four replication

Table 16. Time taken for spawn run of *Tricholoma giganteum* on different spawn substrates

Spawn substrates	Nature of mycelial growth	Time taken for spawn run*
Paddy	+++	**13.66**
Wheat	++++	14.66
Sorghum	++	17.66
Ragi	++++	16.66
Saw dust	++	21
Maize	++++	15.66
CD (0.05 level)		1.186
SE		0.384

* Average of three replication

++++ - Thick and fluffy growth

+++ - Thick growth

++ - Poor growth

4.5.2 Yield of *Tricholoma giganteum* on different grain spawn

Six different spawn substrates were evaluated on the basis of number of days taken for maximum spawn run, days for case run after spawn run, days for first harvest, days for second harvest, total yield per bed (g / kg) and bio-efficiency. Spawn substrates tried were paddy, wheat, sorghum, ragi, maize and rubber sawdust. Grain substrates were found to better than sawdust substrate (Plate 16).

Paddy was found to be the best substrate for spawn run with a minimum of 17.33 days required for growth of mycelium in the grains which was on par with maize (18 days), ragi (19 days), sorghum (19.33 days) and wheat (19.66 days). The substrate which showed maximum days for spawn run was sawdust which was significantly different from all others.

Among the six spawn substrate the days for case run was minimum in paddy (13 days) which was on par with wheat (14.66 days), maize (15 days), ragi (15 days) and sorghum (15.66 days). The substrate sawdust took maximum time for case run (21.33 days) which was significantly different from all others.

The days for first harvest from the day of pin head formation was noted. It was observed to be between 5.33 to 6.00 days for different substrates, longest period of 6 days being in sawdust and lowest period of 5.33 days in wheat and ragi were observed. Though wheat took minimum days for first harvest but it was statistically on par with ragi, paddy, sorghum and maize.

The days for second harvest from the day of first harvest was noted. It was observed to be between 18.33 to 20.00 days for different substrates, longer period (20 days) being in wheat and lesser period (18.33 days) in paddy and ragi were observed. Though paddy took minimum days for second harvest it was statistically on par with ragi, sorghum, maize and sawdust.

Total number of sporophores harvested for different substrates ranged from 6.66 to 13.33, more number of sporophores (13.33) being in wheat which is

Maize

Paddy

Ragi

Saw dust

Sorghum

Wheat

Plate 16. Mushroom beds in different spawn substrates

on par with paddy (13.00) and less number (6.66) in sawdust was observed, which is significantly different from all others.

Total yield per bed of 1 kg for different substrates varied from 249.33 to 833.33g, highest (833.33g) being in wheat, followed by paddy (784.00 g), maize (655.00 g), ragi (655.00 g) and sorghum (645.00 g). Lowest yield (249.33g) was observed in sawdust. The statistical analysis conducted at 5 % level revealed that wheat gave maximum yield of 833.33 g which was significantly higher than rest of the substrates except paddy (784.00 g) which was on par with wheat. Also significantly higher yield was recorded in maize, ragi and sorghum which was on par with each other. The lowest yield (249.33 g) was recorded in sawdust which was significantly different from all others.

Biological efficiency for different treatments varied from 24.93 to 83.33 %, highest (83.33 %) being in wheat, followed by paddy (78.40 %), maize (65.50 %), ragi (65.50 %) and sorghum (64.50 %). Lowest bio efficiency was observed in sawdust (24.93 %). The statistical analysis conducted at 5 % level indicated that wheat gave maximum bio efficency of 83.33 % which was significantly higher than the rest of the substrates except paddy (78.4 %) which was on par with wheat. Also significantly higher bio efficiency was recorded in maize, ragi and sorghum which was on par with each other. The lowest bio efficiency was recorded in sawdust which was significantly different from all others (Table 17).

4.4 CULTIVATION OF *TRICHOLOMA GIGANTEUM* ON LOCALLY AVAILABLE CHEAP SUBSTRATES

4.4.1 Growth and yield performance of *Tricholoma giganteum* on locally available cheap substrate

Growth and yield parameters such as spawn run, case run, days for first harvest, total number of sporophores harvested, average weight, total yield and bio efficiency were studied in detail for six substrates. The results are presented in Table 18.

Table 17. Yield performance of *Tricholoma* on different spawn substrates

Treatments	Days for spawn run*	Days for case run*	Days for first harvest*	Days for second harvest*	Total no. of sporophores harvested*	Total yield per bed (gm/1kg)*	Biological efficiency (%)*
Paddy	**17.33**	**13.00**	5.66	**18.33**	13.00	784.00	78.40
Wheat	19.66	14.66	**5.33**	20.00	**13.33**	**833.33**	**83.33**
Sorghum	19.33	15.66	5.66	18.66	10.33	645.00	64.50
Ragi	19.00	15.00	5.33	18.33	11.66	655.00	65.50
Saw dust	22.33	21.33	6.00	19.33	6.66	294.33	29.43
Maize	18.00	15.00	5.66	19.00	9.33	655.00	65.50
CD (0.05 level)	2.445	3.978	0.937	3.727	1.390	66.397	6.639
SE	0.793	1.290	0.304	1.209	0.451	21.546	2.154

* Average of three replication

Table 18. Yield performance of *Tricholoma* on different bed substrates

Treatments	Days for spawn run*	Days for pinhead formation*	Days for first harvest*	Days for second harvest*	Total no. of sporophores harvested*	Average weight (gm/ button)*	Total yield per bed (gm/1kg)*	Biological efficiency (%)*
Paddy straw	18.75	13.50	6.50	17.50	**12.75**	**54.61**	**694.50**	**69.45**
Sugarcane bagasse	22.00	22.75	**6.25**	25.50	9.75	35.19	337.50	33.75
Saw dust	14.50	**10.75**	6.50	**13.50**	11.25	53.65	601.00	60.10
Coir pith compost	16.75	11.25	6.50	14.25	10.75	52.83	566.00	56.60
SMS	**16.25**	11.00	6.50	15.25	10.75	48.18	518.00	51.80
Coir pith + paddy straw	20.25	24.00	6.75	26.00	6.25	32.06	199.50	19.95
CD (0.05 level)	1.917	1.274	0.821	1.660	1.422	5.483	40.605	4.060
SE	0.645	0.428	0.276	0.559	0.478	1.845	13.666	1.366

*Average of four replication

4.4.1.1 Spawn run

The days for spawn run for different bed substrates ranged from 14.5 to 22 days, longer period (22 days) being in sugarcane bagasse followed by coir pith + paddy straw (20.25 days), paddy straw (18.75 days), coir pith compost (16.75 days), spent mushroom substrate (16.25 days) and saw dust (14.5 days).

The statistical analysis conducted at 5 % level indicated that significantly lesser period was taken by saw dust as bed substrate with 14.5 days compared to rest of the treatments except spent mushroom substrate with 16.25 days. However spent mushroom substrate and sawdust were on par with each other.

4.4.1.2 Pin head formation

The days for pinhead formation for different treatments ranged from 10.75-24 days, longer period (24 days) being in coir pith + paddy straw, followed by sugar cane bagasse (22.7 days), paddy straw (13.5 days), coir pith compost (11.25 days), spent mushroom substrate (11 days) and saw dust (10.75 days) (Plate 17).

The statistical analysis conducted at 5 % level indicated that was significantly fewer periods was taken for pinhead formation in saw dust when compared to rest of the treatments except spent mushroom substrate and coir pith compost. However saw dust, spent mushroom substrate and coir pith compost were statistically on par with each other.

4.4.1.3 Days for first harvest

The days for first harvest from the day of pinhead formation was noted. It was to be observed between 6.25 to 6.75 days for different substrates, longer period (6.75 days) being in coir pith + paddy straw and less period (6.25 days) in sugarcane bagasse and 6.50 days in paddy straw, saw dust, coir pith compost and spent mushroom substrate.

Though sugarcane bagasse took lesser days for first harvest it was statistically on par with all other substrates.

4.4.1.4 Days for second harvest

The days for second harvest from the day of first harvest were noted. It was observed between 13.5 to 26.00 days for different substrates, longer period (26.00 days) being in coir pith + paddy straw which is on par with sugarcane bagasse (25.5 days). Lesser period (13.5 days) was observed in saw dust which was on par with coir pith compost (14.25 days). Spent mushroom substrate and paddy straw took 15.25 and 17.50 days respectively.

The statistical analysis conducted at 5 % level indicated there was significantly lesser period was taken for second harvest in sawdust when compared to rest of the treatments except coir pith compost which was on par with each other.

4.4.1.5 Total number of sporophore harvested

Total number of sporophores harvested from different bed substrates ranged from 6.25 to 12.75, more number of sporophores (12.75) being in paddy straw followed by saw dust (11.25), coir pith compost (10.75) and spent mushroom substrate (10.75) and sugar cane bagasse (9.75). Lesser number of sporophores was in coir pith + paddy straw.

The statistical analysis conducted at 5 % level revealed that there was significantly higher number of sporophores in beds produced by paddy straw. Coir pith compost and spent mushroom substrate were on par with saw dust.

4.4.1.6 Average weight of sporophore

Average weight of sporophores harvested from different bed substrates ranged from 32.06 g to 54.61 g, maximum average weight (54.61 g) being in paddy followed by saw dust (53.65 g), coir pith compost (52.83 g), spent mushroom substrate (48.18 g) and sugarcane bagasse (35.19 g). Least average weight (32.06 g) was obtained in coir pith + paddy straw.

The statistical analysis conducted at 5 % level indicated there was significantly higher average yield in paddy straw which is on par with saw dust and coir pith compost (Plate 18).

Coirpith + paddy straw

Coirpith compost

Paddy straw

Sugarcane bagasse

Spent mushroom substrate

Saw dust

Plate 17. *Tricholoma* on different bed substrates

Plate 18. Pin heads of *Tricholoma giganteum*

4.4.1.7 Total yield

Total yield per bed of 1 kg for different bed substrates varied from 199.50 to 694.50 g, highest (694.50 g) being in paddy straw, followed by 601.00 g in saw dust, 566.00 g in coir pith compost, 518 g in spent mushroom substrate and 337.50 g in sugarcane bagasse. Lowest yield (199.50 g) was observed in coir pith + paddy straw.

The statistical analysis conducted at 5 % level indicated that paddy straw gave maximum yield of 694.50 g which was significantly higher than rest of the treatments. Also significantly higher yield was recorded in saw dust with 601.00 g compared to rest of the substrates except coir pith compost (566.00 g) which was on par with saw dust.

4.4.1.8 Biological efficiency

Biological efficiency for different substrates varied from 19.95 to 69.45 %, highest (69.45 %) being in paddy straw, followed by 60.10 % in saw dust, 56.6%in coir pith compost, 51.80 % in spent mushroom substrate and 33.75 % in sugarcane bagasse. Lowest (19.95 %) was obtained in coir pith + paddy straw.

The statistical analysis conducted at 5 % level indicated that paddy straw gave a maximum biological efficiency of 69.45 % which was significantly superior from all others. Also significantly higher yield was recorded in saw dust compared to rest of the substrates except coir pith compost which was on par with sawdust.

4.4.2 Effect of different substrates on yield contributing characters of *Tricholoma giganteum*

4.4.2.1 Total days of harvest

Total days for harvest was observed between 47.80 to 76.00 days for different substrates, longer period (76.00 days) being in coir pith + paddy straw which is followed by sugarcane bagasse (68.00 days). Lesser period (47.80 days)

was observed in saw dust. Coirpith compost took 54.40 days which is on par with spent mushroom substrate and paddy straw with 55.20 and 56.80 days respectively.

4.4.2.2 Length of stalk

Stipe length for different substrates ranged from 8.50 to 14.72 cm. Highest (14.72 cm) being in paddy straw and lowest was recorded in sugarcane bagasse (8.50 cm). The substrate saw dust with a stalk length of 11.18 cm was on par with coir pith compost, spent mushroom substrate and coir pith + paddy straw with 11.04 cm, 9.74 cm and 9.42 cm length of stalk respectively.

4.4.2.3 Diameter of stalk

Diameter of stalk for different substrates ranged from 1.94 to 2.62 cm. Highest (2.62 cm) being in paddy straw and lowest was recorded in sugarcane bagasse (1.94 cm). The substrate saw dust with stalk diameter of 2.40 cm was on par with coir pith compost, spent mushroom substrate and coir pith + paddy straw with 2.38 cm, 2.20 cm and 2.12 cm diameter of stalk respectively.

4.4.2.4 Diameter of pileus

Diameter of pielus for different substrates ranged from 6.58 to 8.90 cm. Highest (8.90 cm) being in paddy straw and lowest was recorded in sugarcane bagasse (6.58 cm). The substrate saw dust with pileus diameter of 8.66 cm was on par with coir pith compost (8.24 cm), spent mushroom substrate (7.62 cm) and coir pith + paddy straw (7.42 cm).

4.4.2.5 Thickness of pileus

Thickness of pileus for different substrates ranged from 0.54 to 0.76 cm. Highest (0.76 cm) being in paddy straw and saw dust and lowest (0.54 cm) was recorded in sugarcane bagasse. The substrate coir pith compost with pileus thickness of 0.68 cm was on par with spent mushroom substrate (0.64 cm). The substrate coir pith + paddy straw recorded pileus thickness of 0.58 cm (Table 19).

Table 19. Effect of different substrates on yield contributing characters of *Tricholoma giganteum*

Treatments	Total days of harvest*	Length of stalk (cm)*	Diameter of stalk (cm)*	Diameter of pileus (cm)*	Thickness of pileus (cm)*
Paddy straw	56.80	**14.72**	**2.62**	**8.90**	**0.76**
Sugarcane bagasse	**68. 00**	8.50	1.94	6.58	0.54
Saw dust	47.80	11.18	2.40	8.66	**0.76**
Coirpith compost	54.40	11.04	2.38	8.24	0.68
SMS	55.2	9.74	2.20	7.62	0.64
Coirpith + paddy straw	76. 00	9.42	2.12	7.42	0.58
CD (0.05 level)	4.895	2.745	0.665	2.596	0.156
SE	1.677	0.940	0.254	0.889	0.053

*Average of five replication

4.5 EFFECT OF DIFFERENT CASING MATERIALS

4.5.1 Growth and yield performance of *Tricholoma giganteum* on different casing materials

Growth and yield parameters such as case run, days for first harvest, total number of sporophores harvested, average weight, total yield and biological efficiency were studied. The results are presented in Table 20.

4.5.1.1 Case run

The days for case run for different substrates ranged from 12.00 to 21.70 days, lowest period (12.00 days) being in vermi compost and coir pith compost (12.70 days). Longer period was observed in red soil + sand (21.75 days).

Critical difference test conducted at 5 % level indicated the following: significantly less period was noted in vermi compost with 12.00 days compared to rest of the casing materials except coir pith compost with 12.75 days. The casing materials red soil + sand + cow dung was on par with red soil which took 18 days and 19.50 days respectively. However vermin compost and coir pith compost are on par with each other.

4.5.1.2 Days for first harvest

The days for first harvest from the day of pinhead formation was noted. It was to observed to be between 5.75 to 6.75 days for different casing materials, short period (5.75 days) being in vermi compost and more period (6.75 days) was observed in red soil + sand.

Critical difference test conducted at 5% level indicated the following: significantly less period was taken for first harvest by vermi compost (5.75 days) followed by coir pith compost (6 days) which was on par with red soil (6.5 days), red soil + sand + cow dung (6.5 days) and red soil + sand (6.75 days). The influence of vermi compost on days for first harvest was better than the rest of the casing material.

Table 20. Effect of *Tricholoma* on different casing materials

Casing material	Days for case run*	Days for first harvest*	Days for second harvest*	No. of buttons harvested*	Average weight (g/button)*	Total yield per bed (gm/1kg)*	Biological efficiency (%)*
Red soil	19.50	6.50	25. 00	9. 00	35.69	320. 00	32. 00
Red soil + sand	21.75	6.75	27.25	8.50	32.14	273.50	27.35
Vermi compost	**12. 00**	**5.75**	**16.75**	**12.50**	**55.28**	**690.75**	**69.07**
Coir pith compost	12.75	6. 00	18.75	9.75	45.23	445.50	44.55
Red soil + sand + cow dung	18. 00	6.50	22. 00	9.75	44.02	427.50	42.75
CD (0.05 level)	1.925	0.912	1.361	1.199	7.659	96.690	9.669
SE	0.639	0.302	0.451	0.397	2.541	32.083	3.208

* Average of four replication

4.5.1.3 Days for second harvest

The days for second harvest from the day of pinhead formation was noted. It was observed to be between 16.75 to 27.25 days for different casing materials, short period (16.75 days) being in vermi compost and more period (27.25 days) was observed in red soil + sand.

Critical difference test conducted at 5 % level indicated the following: significantly less period was taken for second harvest by vermi compost (16.75 days) followed by coir pith compost (18.75 days), red soil + sand + cow dung (22.00 days), red soil (25.00 days). A maximum day (27.25 days) was recorded in red soil + sand. The vermi compost influence on days for second harvest was better than the rest of the casing material.

4.5.1.4 Total number of sporophores harvested

Total number of sporophores harvested for different casing materials ranged from 8.50 to 12.50, more number of sporophores (12.50) being in vermicompost and less number of sporophores (8.50) in red soil + sand.

Critical difference test conducted at 5% level indicated the following: significantly more number of sporophores was recorded in vermi compost. Also significantly higher was recorded in coir pith compost and red soil + sand + cow dung with 9.75 numbers which was on par with red soil (9.00) and red soil + sand (8.50).

4.5.1.5 Average weight of sporophore

The average weight of sporophore harvested for different casing materials ranged from 32.14 to 55.28 g, more average weight of sporophore (55.28 g) being in vermi compost and less average weight (32.14 g) in red soil + sand.

Critical difference test conducted at 5 % level indicated the following: significantly highest average weight of sporophore was recorded in vermi compost. Also significantly higher weight was recorded in coir pith compost with 45.23 g with rest of the treatments except red soil + sand + cow dung (44.02 g)

which was on par with coir pith compost. The average weight of red soil is 35.69 g which is on par with red soil + sand (32.14 g).

4.5.1.6 Total yield

Total yield per bed of 1 kg for different casing materials varied from 273.5 to 690.75 g. Highest (690.75 g) being in vermi compost and lowest yield (273.5 g) was in red soil + sand.

Critical difference test conducted at 5 % level indicated the following: significantly highest yield was recorded in vermi compost. The casing material coir pith compost was on par with red soil + sand + cow dung with 445.5 g and 427.5 g respectively. The casing materials red soil and red soil + sand with 320 g and 273 g were found to be on par with each other. The vermi compost influence on yield was better than the rest of the casing materials.

4.5.1.7 Biological efficiency

Biological efficiency for different casing materials varied from 27.35 to 69.07 %. Highest (69.07%) being in vermi compost followed by 44.55 % in coir pith compost and 42.75 % in red soil + sand + cow dung. Lowest (27.35 %) was observed in red soil + sand.

Critical difference test conducted at 5 % level indicated the following: significantly higher yield was recorded in vermi compost. The casing material coir pith compost was on par with red soil + sand + cow dung with 44.55 % and 42.75 % respectively. Also the casing material red soil and red soil + sand with 32.0 % and 27.3 % were found to be on par with each other (Plate 19).

4.5.2 Effect of different casing materials on yield contributing characters of *Tricholoma giganteum*

4.5.2.1 Length of stalk

Stipe length of *Tricholoma* in different casing materials ranged from 13.18 to 18.36 cm. Highest stipe length of 18.36 cm was seen in vermi compost and

Red soil + sand

Red soil + sand + cow dung

Coirpith compost

Red soil

Vermi compost

Plate 19. Beds cased with different casing material

lowest was recorded in red soil + sand (13.18 cm). The casing material vermin compost was on par.with coir pith compost, and red soil with 16.9 cm and 15.12 cm stalk length respectively. The casing material red soil + sand gave a stipe length of 13.26 cm.

4.5.2.2 Diameter of stalk

Diameter of stalk for different casing materials ranged from 1.94 to 2.82 cm. Highest stalk length of 2.82 cm was in vermi compost cased beds and lowest was recorded in beds cased with red soil + sand (1.94 cm). All casing materials are on par with each other.

4.5.2.3 Diameter of pileus

Diameter of pileus for different casing materials ranged from 6.46 to 8.90 cm. Highest (8.90 cm) being in vermi compost and lowest was recorded in red soil (6.46 cm). The coir pith compost with pileus diameter of 7.92 cm was on par with red soil + sand + cow dung (7.88 cm). The casing material red soil + sand recorded pileus diameter of 6.70 cm.

4.5.2.4 Thickness of pileus

Thickness of pileus for different casing materials ranged from 0.56 to 0.74 cm, highest (0.74 cm) being in vermi compost which is on par with coir pith compost (0.72 cm), red soil + sand + cow dung (0.62 cm) and red soil (0.58 cm). Lowest (0.56 cm) was recorded in red soil + sand (Table 21).

4.5.3 Yield performance of different casing materials on *Tricholoma giganteum* on different bed substrates

Six different bed substrates *viz.,* Paddy straw, sugarcane bagasse, saw dust, coir pith compost, spent mushroom substrate and coir pith + paddy straw and five different casing materials *viz.,* red soil, red soil + sand, vermi compost, coir pith compost and red soil + sand + cow dung were evaluated on the basis of yield obtained on them.

Table 21. Effect of different casing materials on yield contributing characters of *Tricholoma giganteum*

Treatments	Length of stalk (cm)*	Diameter of stalk (cm)*	Diameter of pileus (cm)*	Thickness of pileus (cm)*
Red soil	15.12	2.36	6.46	0.58
Red soil + sand	13.26	1.94	6.70	0.56
Vermi compost	**18.36**	**2.82**	**8.90**	**0.74**
Coirpith compost	16.9	2.76	7.92	0.72
Red soil + sand + cow dung	13.18	2.40	7.88	0.62
CD (0.05 level)	3.648	0.879	1.327	0.163
SE	1.236	0.298	0.449	0.055

* Average of five replication

4.5.3.1 Yield performance of different casing materials on Tricholoma using paddy straw as bed substrate

Casing materials like red soil, red soil + sand, vermi compost, coir pith compost and red soil + sand + cow dung were used for casing paddy straw beds and their efficacy in influencing yield of *Tricholoma* on paddy straw was evaluated. The result showed that different casing materials differed significantly in influencing the yield (Table 22).

4.5.3.1.1 Number of sporophores harvested

The beds cased with vermi compost (13.00) out yielded other casing materials namely coir pith compost, red soil + sand + cow dung and red soil which were giving 11.25, 8.00 and 7.25 sporophores respectively. The least effective casing material was red soil + sand with 7.00 numbers of sporophores.

4.5.3.1.2 Total yield

The yield was highest in vermi compost (715.75 g) than other casing materials. The bed cased with coir pith compost gave 577.50 g of mushroom, followed by red soil+ sand + cow dung (447.00 g), red soil (346.00 g) and red soil + sand (313.25 g).

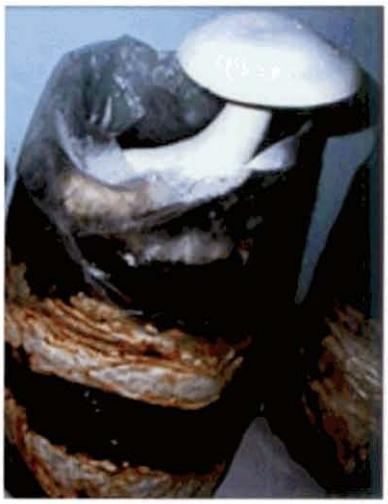
4.5.3.1.3 Biological efficiency

The biological efficiency was highest in vermi compost with 71.57 % followed by coir pith compost 57.75 %. The lowest (31.20 %) biological efficiency was recorded in beds cased with red soil + sand (Plate 20).

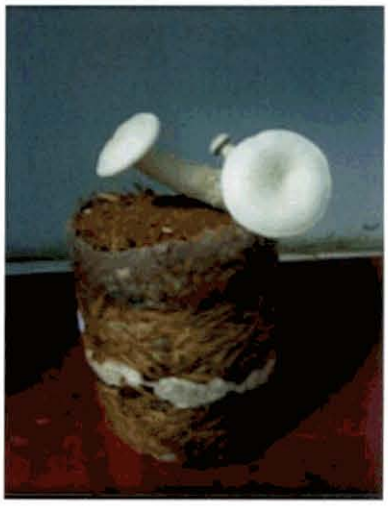
4.5.3.2 Yield performance of different casing materials on Tricholoma using sugarcane bagasse as bed substrate

Casing materials like red soil, red soil + sand, vermi compost, coir pith compost and red soil + sand + cow dung were used for casing sugar cane bagasse beds and their efficacy in influencing yield of *Tricholoma* on sugar cane bagasse was evaluated (Table 23).

Red soil + sand



Red soil + sand + cow dung



Coirpith compost

Red soil

Vermi compost

Plate 20. Paddy straw beds cased with different casing materials

Table 22. Effect of different casing materials on *Tricholoma* using paddy straw as bed substrate

Casing material	No. of buttons harvested*	Total yield per bed (gm/1kg)*	Biological efficiency (%)*
Red soil	7.25	346. 00	34.60
Red soil + sand	7. 00	313.25	31.32
Vermi compost	**13. 00**	**715.75**	**71.57**
Coirpith compost	11.25	577.50	57.75
Red soil + sand + cow dung	8. 00	447. 00	44.70
CD (0.05 level)	1.319	67.579	6.757
SE	0.437	22.424	2.242

* Average of four replication

Table 23. Effect of different casing materials on *Tricholoma* using sugarcane bagasse as bed substrate

Casing material	No. of buttons harvested*	Total yield per bed (gm/1kg)*	Biological efficiency (%)*
Red soil	**11.25**	**397.50**	**39.75**
Red soil + sand	7.75	291.25	29.12
Vermi compost	9.25	322.50	32.25
Coirpith compost	8.50	320. 00	32. 00
Red soil + sand + cow dung	7.75	321.75	32.17
CD (0.05 level)	1.347	38.581	3.858
SE	0.447	12.802	1.280

* Average of four replication

4.5.3.2.1 Number of sporophores harvested

The beds cased with red soil (11.25) out yielded other casing materials namely vermi compost and coir pith compost with 9.25 and 8.50 respectively. The casing material red soil + sand and red soil + sand + cow dung gave same number of sporophore (7.75) and were similar.

4.5.3.2.2 Total yield

The yield was highest in red soil (397.50 g) than other casing materials. The bed cased with vermi compost gave 322.50 g of mushroom which is on par with red soil+ sand + cow dung (321.75 g), coir pith compost (320.00 g) and red soil + sand (291.25 g).

4.5.3.2.3 Biological efficiency

The biological efficiency was highest in red soil with 39.75 %. The beds cased with vermi compost gave 32.25 % biological efficiency which was on par with rest of the casing materials (Plate 21).

4.5.3.3 Yield performance of different casing materials on Tricholoma using saw dust as bed substrate

Casing materials like red soil, red soil + sand, vermi compost, coir pith compost and red soil + sand + cow dung were used for casing saw dust beds and their efficacy in influencing yield of *Tricholoma* on saw dust was evaluated. The result showed that different casing materials differed significantly in influencing the yield (Table 24).

4.5.3.3.1 Number of sporophores harvested

The beds cased with vermi compost (11.00) out yielded other casing materials with red soil + sand + cow dung, coir pith compost and red soil which were giving 10.00, 9.50 and 8.75 sporophores respectively. The least effective casing material was red soil + sand with 8.50 numbers of sporophores.

Coir pith compost

Red soil + sand

Vermicompost

Red soil + sand + cow dung

Red soil

Plate 21. Sugarcane bagasse beds cased with different casing materials

Table 24. Effect of different casing materials on *Tricholoma* using saw dust as bed substrate

Casing material	No. Of buttons harvested*	Total yield per bed (gm/1kg)*	Biological efficiency (%)*
Red soil	8.75	370. 00	37. 00
Red soil + sand	8.50	340.50	34.05
Vermi compost	**11. 00**	**527.50**	**52.75**
Coirpith compost	9.50	495.75	49.57
Red soil + sand + cow dung	10. 00	437.25	43.72
CD (0.05 level)	1.150	46.217	4.621
SE	0.381	15.335	1.533

* Average of four replication

4.5.3.3.2 Total yield

The yield was higher in vermi compost (527.50 g) than other casing materials. The bed cased with coir pith compost gave 495.75 g of mushroom which was on par with vermin compost. The other casing materials red soil + sand+ caw dung, red soil and red soil + sand gave 43.72 g, 37.00 g and 34.05 g of mushrooms respectively.

4.5.3.3.3 Biological efficiency

The biological efficiency was highest in vermi compost with 52.75 % followed by coir pith compost 49.57 %. The lowest (34.05 %) biological efficiency was recorded in beds cased with red soil + sand (Plate 22).

4.5.3.4 Yield performance of different casing materials on Tricholoma using coir pith compost as bed substrate

Casing materials like red soil, red soil + sand, vermi compost, coir pith compost and red soil + sand + cow dung were used for casing coir pith compost beds and their efficacy in influencing yield of *Tricholoma* on coir pith compost was evaluated. The result showed that different casing materials differed significantly in influencing the yield (Table 25).

4.5.3.4.1 Number of sporophores harvested

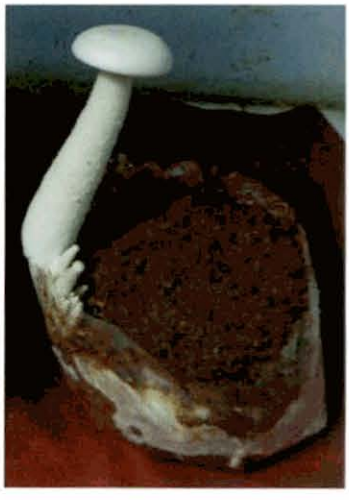
The beds cased with vermi compost (11.25) out yielded other casing materials with coir pith compost, red soil + sand + cow dung and red soil which were giving 9.00, 8.50 and 8.00 sporophores respectively. The least effective casing material was red soil + sand with 7.50 numbers of sporophores.

4.5.3.4.2 Total yield

The yield was higher in vermi compost (533.25 g) than other casing materials. The bed cased with red soil+ sand + cow dung (387.50 g), were on par with coir pith compost (38.57 g) and red soil (354.00 g). Lowest yield was obtained with red soil + sand (330.00 g).

Red soil

Vermicompost



Red soil + sand

Coir pith compost

Red soil + sand + cow dung

Plate 22. Saw dust beds cased with different casing materials

Table 25. Effect of different casing materials on *Tricholoma* using coir pith compost as bed substrate

Casing material	No. of buttons harvested*	Total yield per bed (gm/1kg)*	Biological efficiency (%)*
Red soil	8. 00	354. 00	35.40
Red soil + sand	7.50	330. 00	33. 00
Vermi compost	**11.25**	**533.25**	**53.32**
Coirpith compost	9. 00	385.75	38.57
Red soil + sand + cow dung	8.50	387.50	38.75
CD (0.05 level)	1.150	51.519	5.151
SE	0.381	17.095	1.709

* Average of four replication

4.5.3.4.3 Biological efficiency

The biological efficiency was highest in vermi compost with 53.32 % followed by red soil + sand + cow dung (38.75 %) and coir pith compost (38.57 %). The lowest (33.2 %) biological efficiency was recorded in beds cased with red soil + sand (Plate 23).

4.5.3.5 Yield performance of different casing materials on Tricholoma using spent mushroom substrate as bed substrate

Casing materials like red soil, red soil + sand, vermi compost, coir pith compost and red soil + sand + cow dung were used for casing spent mushroom substrate beds and their efficacy in influencing yield of *Tricholoma* on spent mushroom substrate was evaluated. The result showed that different casing materials differed significantly in influencing the yield (Table 26).

4.5.3.5.1 Number of sporophores harvested

The beds cased with vermi compost (11.00) out yielded other casing materials with coir pith compost, red soil + sand and red soil and red soil + sand + cow dung which were giving 8.75, 7.75, 7.50 and 7.50 respectively.

4.5.3.5.2 Total yield

The yield was higher in vermin compost (506.50 g) than other casing materials. The bed cased with coir pith compost gave 393.50 g of mushroom, followed by red soil + sand + cow dung (338.00 g), red soil (329.20 g) and red soil + sand (323.50 g).

4.5.3.5.3 Biological efficiency

The biological efficiency was highest in vermi compost with 50.65 % followed by coir pith compost 39.35 %. The lowest (32.35 %) biological efficiency was recorded in beds cased with red soil + sand (Plate 24).

Red soil

Red soil + sand

Vermi compost

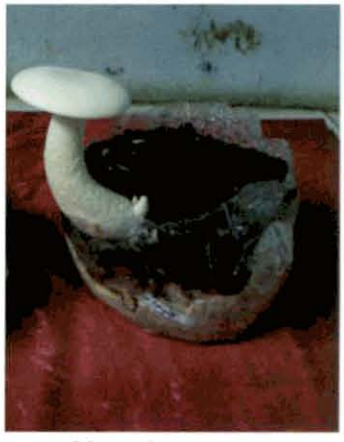
Coir pith compost

Red soil + sand + cow dung

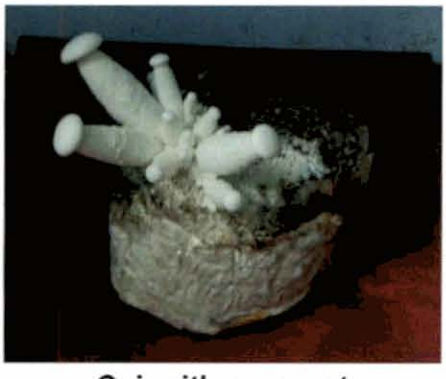
Plate 23. Coirpith compost beds cased with different casing materials

Red soil

Red soil + sand



Vermi compost



Coir pith compost

Red soil + sand + cow dung

Plate 24. Spent mushroom substrate beds cased with different casing materials

Table 26. Effect of different casing materials on *Tricholoma* using SMS as bed substrate.

Casing material	No. of buttons harvested*	Total yield per bed (gm/1kg)*	Biological efficiency (%)*
Red soil	7.50	329.25	32.92
Red soil + sand	7.75	323.50	32.35
Vermi compost	**11. 00**	**506.50**	**50.65**
Coirpith compost	8.75	393.50	39.35
Red soil + sand + cow dung	7.50	338. 00	33.80
CD (0.05 level)	1.199	57.468	5.746
SE	0.397	19.069	1.906

* Average of four replication

4.5.3.6 Yield performance of different casing materials on Tricholoma using coir pith + paddy straw as bed substrate

Casing materials like red soil, red soil + sand, vermi compost, coir pith compost and red soil + sand + cow dung were used for casing coir pith + paddy straw beds and their efficacy in influencing yield of *Tricholoma* on coir pith + paddy straw beds was evaluated. The result showed that different casing materials differed significantly in influencing the yield (Table 27).

4.5.3.6.1 Number of sporophores harvested

The beds cased with vermi compost (5.50) out yielded other casing materials with coir pith compost, red soil + sand + cow dung, red soil + sand and red soil + which were giving 4.50, 3.25, 3.00 and 2.50 respectively.

4.5.3.5.2 Total yield

The yield was highest in vermi compost (178.25 g) than other casing materials. The bed cased with coir pith compost gave 141.75 g of mushroom, followed by red soil+ sand + cow dung (95.50 g), red soil (93.50 g) and red soil + sand (88.50 g).

4.5.3.5.3 Biological efficiency

The biological efficiency was highest in vermin compost with 17.82 % followed by coir pith compost 14.17 %. The lowest (8.85 %) biological efficiency was recorded in beds cased with red soil + sand (Plate 25).

4.6 PEST AND DISEASE INCIDENCE

Pest and disease incidence were evaluated at the time of spawn run, casing and sporocarp formation.

Red soil

Red soil + sand

Vermi compost

Coir pith compost

Red soil + sand + cow dung

Plate 25. Coir pith + paddy straw beds cased with different casing materials

Table 27. Effect of different casing materials on *Tricholoma* using coir pith + paddy straw as bed substrate

Casing material	No. of buttons harvested*	Total yield per bed (gm/1kg)*	Biological efficiency (%)*
Red soil	2.50	93.50	9.35
Red soil + sand	3. 00	88.50	8.85
Vermi compost	**5.50**	**178.25**	**17.82**
Coirpith compost	4.50	141.75	14.17
Red soil + sand + cow dung	3.25	95.50	9.55
CD (0.05 level)	0.932	31.009	3.100
SE	0.309	10.289	1.028

* Average of four replication

4.6.1 Pests

The insects during the study comprised of sciarid flies and staphylinid beetles. Their incidence was found in mushrooms at the time of second harvest only (Plate 26).

4.6.1.1 Nature of damage by pests infesting Tricholoma

4.6.1.1.1 Sciarid flies

The larvae made tunnels in the stipe resulting in discolouration and decay of the fruiting bodies. Adults were also seen on the sporocarps. The larvae hide inside the pileus and gills and crawl out when the fruiting bodies were harvested. These were seen inside the plastic packets in which the mushrooms were marketed.

4.6.1.1.2 Staphylinid beetle

The young sporocarps were highly vulnerable to infestation by the insects. The grubs crawled into the surface of fruiting bodies. They were found to feed voraciously, making tunnels in the stipe as well as on the gills and lower portion of the pileus. The adults were found to make holes all over the fruiting bodies. The infestation was first seen in mature sporocarps and later seen in younger stages also. The infestation ultimately resulted in rotting of sporocarps.

4.6.1.2 Extent of damage

During spawn run and casing no incidence of pest was found. The incidence of pest was during second harvest only. The soprocarps were individually assessed to find out the presence of insects. Only two mushroom beds were found to be affected by insects.

4.6.2 Diseases

The diseases which were found during the study comprised of *Trichoderma* spp., *Cladobotryum dendroides* and *Coprinus* spp. Most of the diseases were found during second harvest and when relative humidity was high (Plate 27).

Staphylinid beetle

Sciarid flies

Plate 26. Pests of *Tricholoma*

Trichoderma

Cladobotryum dentroides

Coprinus sp.

Plate 27. Diseases of *Tricholoma*

4.6.2.1 Nature of damage

4.6.2.1.1 Trichoderma spp.

The incidence of *Trichoderma* was observed in casing material after second harvest. Infested area turned dark green once the fungus started sporulating. The growth rate of *Trichoderma* was faster than that of *Tricholoma* and spread rapidly.

4.6.2.1.2 Cladobotryum dendroids

This is also known as cob web disease. The disease starts with small, more or less circular patches of infection on casing soil. The infection spread through the pinheads and spreads the entire casing soil. Cob web disease has fine greyish white mycelium that rapidly develops. After a while, cobweb mould mycelium on the casing soil can change from a fine, cobweb like mycelium to a firmer, floury or powder like structure.

4.6.2.1.3 Coprinus spp.

Coprinus infection was seen only in areas damaged by pests and other competitor moulds where spawn run was not proper or when moisture is high in the beds.

4.6.2.2 Extent of damage

During spawn run and first harvest no incidence of diseases were found. The incidence was observed during second harvest. Majority of beds were free of infestation. Only two beds were infested with *Trichoderma* and *Coprinus* and one bed was infested with *Cladobotryum dendroides.*

4.7 PROXIMATE CONSTITUENT ANALYSIS, SHELF LIFE AND KEEPING QUALITY OF *TRICHOLOMA GIGANTEUM*

4.7.1 Proximate constituents present in *Tricholom giganteum* Massee

The proximate constituents of *Tricholoma* mushrooms were evaluated using standard technique as described in 3.7. The moisture content of *Tricholoma*

was found to be 87.46 % (fresh mushroom). Protein content present in the mushroom was estimated using Bradford technique and it was found to be 23.20 %. Fat content present in the mushroom was estimated and it was found to have an approximate value of 2.60 %. Carbohydrate content present in *Tricholoma* mushroom was estimated by anthrone method and it was found and expressed as 10.10 %. The ash content present in this mushroom was found to 11.46 %. Fibre content present in *Tricholoma* mushroom was estimated and it was found to be 19.01 % (Table 28).

4.7.2 Shelf life

The study conducted to determine the shelf life of *Tricholoma* indicated that mushrooms when stored in polypropylene covers without perforations had better keeping quality than those kept in polypropylene covers with perforations.

The mushrooms when stored in room temperature in polypropylene cover without perforation showed maximum (5.2 days) shelf life where as minimum (2.8 days) shelf life was when stored in open condition without polypropylene cover. Polypropylene cover with perforations recorded a shelf life of 3.4 days only.

Better shelf life without any microbial spoilage and liquefaction was recorded when mushroom packed in polypropylene covers without perforations in refrigerated condition (7 days). Minimum period of storage under refrigerated condition was observed in open condition without polypropylene cover. Polypropylene cover with perforations recorded 5.6 days of shelf life (Table 29).

4.7.3 Preservation by dehydration

Three different drying techniques were compared with the control, in which fresh mushrooms were kept as such. The dried products were noted for their colour change, degradation percentage, final weight of the dried product and shelf life. It was found that the mushroom dried in hot air oven got maximum shelf life of 57 days followed by oven (55 days). *Tricholoma* dried under sunlight could not be stored for a longer time and the mushroom stored as such in room conditions decayed within a short period (Table 30).

Table 28. Nutritional value of *Tricholoma giganteum*

Sl. No.	Proximate constituent	Percentage (%)
1	Moisture $	87.60
2	Protein#	23.20
3	Fat#	2.60
4	Carbohydrate#	10.10
5	Fibre#	19.01
6	Ash#	11.46

$ - Presented in fresh weight basis

- % dry weight basis

Table 29. Shelf life of *Tricholoma giganteum*

Condition	Keeping quality (days)	
	Room temperature*	Refrigerator*
Open	2.80	4.80
PP cover without perforation	**5.20**	**7. 00**
PP cover with perforation	3.40	5.60
CD (0.05 level)	0.871	0.790
SE	0.282	0.250

* Average of five replication

Table 30. Effect of methods of drying on preservation of *Tricholoma giganteum*

Dehydration methods	Initial weight (g)	Final weight (g)	Colour change	% damage by microbes	Shelf life (No. of days)
Sunlight	300	28	Light brown	12	40
Oven	300	27	Brown	-	55
Hot air oven	300	25	Dark brown	-	57
Control	300	100	Rotted	100	0

Table 31. Organoleptic studies

Products	Colour and appearance*	Texture*	Taste*	Flavour*	Overall acceptability*
Cutlets	4.7	4.6	4.3	4.3	4.6
Puffs	4.2	4.5	3.7	3.3	3.5
Payasam	4.4	3.7	4.5	4.1	3.7
Masala	3.7	3.5	4.5	3.7	3.2
Pickle	3.9	3.8	3.6	3.6	2.7
Soup	3.4	2.7	2.5	3.2	2.4
CD (0.05 level)	0.712	0.687	0.477	0.687	0.478
SE	0.249	0.240	0.236	0.240	0.167

* Average of ten replication

Masala

Payasam

Puffs

Cutlets

Pickle

Soup

Plate 28. Recipes prepared using *Tricholoma giganteum*

4.8 ORGANOLEPTIC STUDIES

Organoleptic studies were conducted by preparing recipes of both fresh and dried *Tricholoma* mushrooms and subjected to sensory evaluation. Six different recipes *viz.*, cutlet, puffs, mushroom masala, soup, payasam and pickle were prepared. The mushroom recipes for their characters like colour and appearance, texture, flavour, taste and overall acceptability were evaluated using a five point score card. The details are presented in Table 31. Among the different products, cutlets had maximum ranking for colour and appearance, texture and flavour followed by payasam which also had excellent taste. These two were mostly preferred by the judges than the others. The overall acceptability was also high for cutlets and payasam, followed by puffs, masala, pickle and soup. The least preferred product was soup with overall acceptability 2.4. The ranking of the recipes based on the colour and appearance ranged from 3.4 to 4.7, where as for texture it was 2.7 to 4.6. The ranking of recipes based on the taste and flavour were 2.5 to 4.5 and 3.2 to 4.3. The overall acceptability was high for cutlet (4.6) and least acceptability was for soup (2.4) (Plate 28).

Discussion

5. DISCUSSION

5.1 COLLECTION, ISOLATION AND PURIFICATION OF MUSHROOM

Mushrooms were collected from different parts of Trivandrum district before and after the South West and North East monsoons.. As a result four native species of *Tricholoma* were collected. They were subjected to different morphological observations and brought into pure culture. All four species were subjected to preliminary studies to explore their suitability to bring them under cultivation. Based on the preliminary observations isolate 1 was found to be the best. Hence it was sent to Directorate of Mushroom Research to obtain accession number DMRO 462. It was utilized for the further studies. Surveys conducted in Western Ghats by Anandh and Prakasam (2003) resulted in the collection of edible mushrooms like *Calocybe indica, Calocybe gambosa, Tricholoma lobayense, Tricholoma giganteum.* It was observed during our study that organic matter rich soil and coconut tree basins were the usual spots for the occurrence of *Tricholoma.* The period of occurrence was during June to October (rainy and post rainy season) which was similar to observations made by Anandh and Prakasam (2003).

Tissue isolation was done from the healthy fruiting bodies and cultures were maintained in PDA slants with periodical subculturing. Kligman (1943) suggested that the tissue cultures raised from phenotypically healthy looking mushrooms possessed good fertility.

5.2 MORPHOLOGICAL, CULTURAL AND PHYSIOLOGICAL STUDIES

Mushrooms were screened for their macroscopic characters such as shape, size and colour of basidiocarp. The shape of basidiocarp was convex, off white to creamish white in colour and diameter ranging from 9-20 cm. The stipe was clavate in shape with bulbous base and hairs were present. This is in agreement

with the observations made by Anandh and Prakasam (2003) and Pandey and Tewari (2003) on the macroscopic details of *Tricholoma giganteum.*

The reaction of spore of *Tricholoma giganteum* with melzer's reagent resulted in non-amyloid reaction which is confirmatory to the results obtained by Anandh and Prakasam (2003).Cotton blue reaction of spores resulted in cyanophilic reaction.

The isolate DMRO-462 was tested for its potentiality in supporting mycelia growth with different solid culture media *viz.*, potato dextrose agar, malt extract agar, carrot agar, oat meal agar and tapioca dextrose agar. Fluffy nature of mycelial growth was observed in oat meal agar followed by potato dextrose agar (Fig. 1). Feeble mycelial growth was observed in malt extract agar. These are in agreement with the findings of Suharban (1987) who has reported that oat meal and potato dextrose agar were effective media for the growth of *Pleurotus* sp. Mehta and Bhandal (1992) reported better mycelia growth of *Auricularia* in PDA and wheat extract agar. Heera (2006) reported that oat meal agar was the best media for the growth of *Calocybe.*

Potato dextrose has always been a suitable medium for growth of mushrooms. Results of the present study revealed that PDA was as effective as oat meal in favouring the mycelial growth of *Tricholoma.* This has been confirmatory with the results of Rafique *et al.* (1999) who concluded potato dextrose agar medium as the best for growth of different species of *Pleurotus i.e.*, 9 cm growth in 10 days time. Singh *et al.* (2000 b) also observed potato dextrose agar as the best medium for radial growth of *Pleurotus sajor-caju* and *P. sapidus* which took only seven days for full growth in petridish. Sharma and Jandaik (1984) also reported better growth of *Pleurotus eryngii* on natural media like PDA.

Malt extract and tapioca dextrose agar was equally effective as PDA in supporting mycelia growth of *Tricholoma.* Ahlawat (2002) reported that malt extract agar was the best medium for the multiplication of paddy straw

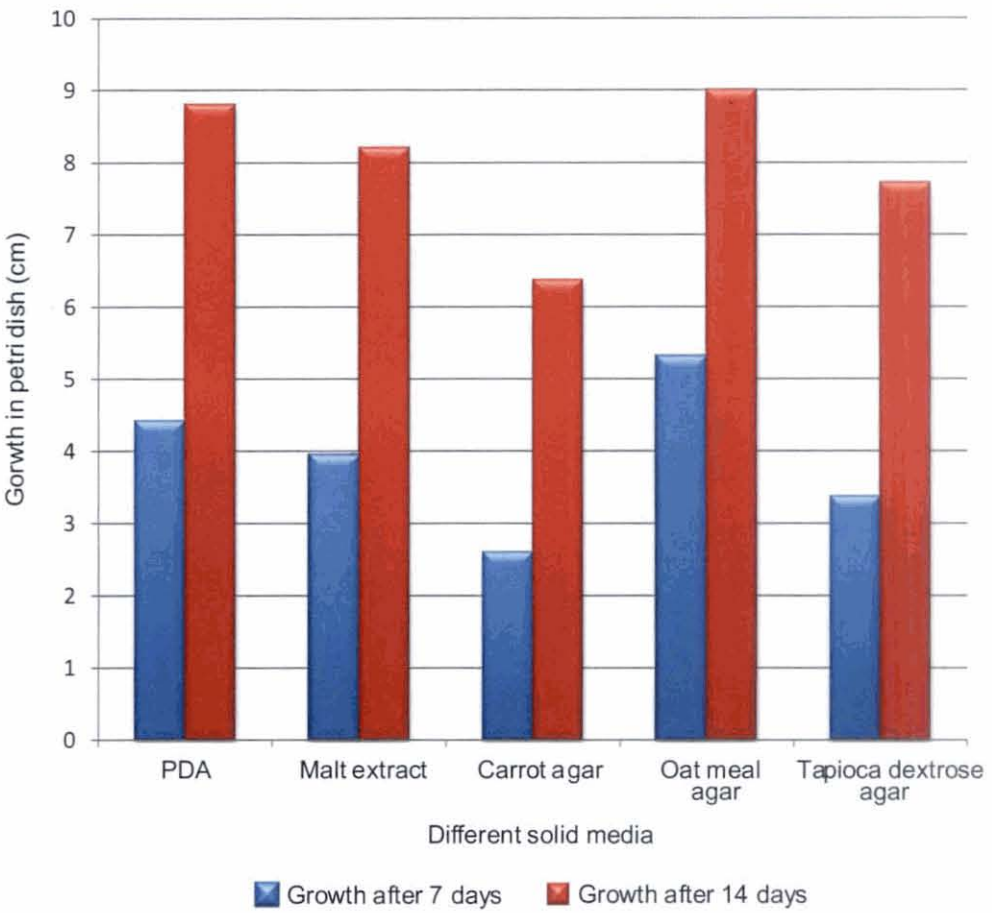


Fig. 1. Growth of *Tricholoma* in different solid media

mushroom. Pramod (2004) reported tapioca dextrose agar as the best media for *Volvariella volvacea.*

Tricholoma had excellent fluffy growth in oat meal but was scanty in malt extract media. This result is contradictory to the observations made by Sharma *et al.* (2004), who reported malt extract agar as the best for the mycelial growth of *Agrocybe aegerita.* Similar observations were made for malt extract agar for supporting mycelial growth by Das *et al.* (2000) and Yadav *et al.* (2003) on *Agaricus bisporus.*

The biomass production of *Tricholoma giganteum* was estimated in different liquid broth *viz.,* potato dextrose broth, malt extract broth, carrot broth, oat meal broth and tapioca dextrose broth.

The oat meal broth produced the highest biomass of 0.38 g / 50 ml followed by potato dextrose broth and malt extract broth. Ishikawa (1967) reported malt extract medium as best for growth of *Volvariella.* But in contradictory to this result, Suharban (1987) reported malt extract broth as unsuitable for mushroom mycelia production. Least biomass was produced in tapioca dextrose broth and carrot broth.

Six different carbon sources tested for their efficacy in radial mycelial growth and biomass production in both solid and liquid media. Mannitol, dextrose and sucrose were found to be the best (Fig. 2). This result is in accordance with the findings of Chandra and Purkayastha (1977). They reported that mannitol, glucose, dextrose and fructose supported excellent growth of *Agaricus campestris, Lentinus, Calocybe indica,* and *Volvariella.*

In the present study inositol did not produce much radial growth and mycelial dry weight in solid and liquid media. This is in accordance with the findings of Khanna and Garcha (1985) except mannitol, other sugar alcohols did not favour the growth of *Pleurotus.*

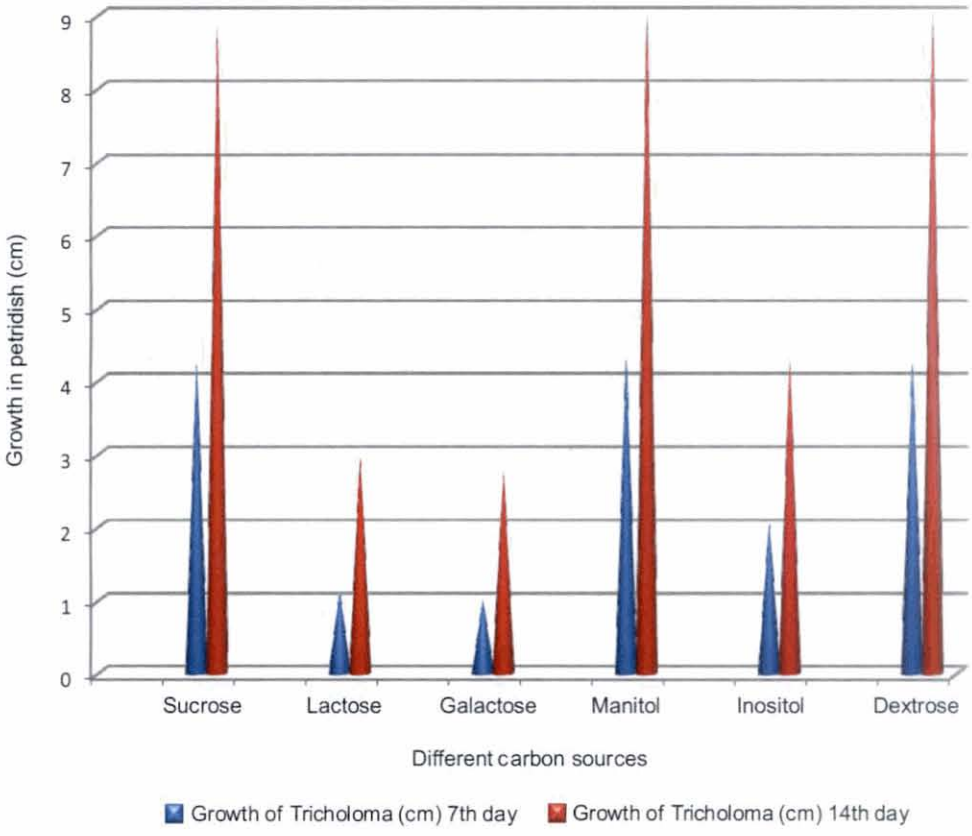


Fig. 2. Growth of *Tricholoma* in different carbon sources

Lactose and galactose was equally ineffective in supporting the mycelia growth of *Tricholoma.* Similar results have been obtained by Saha and Samajpati (1987) and Tan and Chang (1989). They observed lactose as the least suitable carbon source for biomass production of *Tricholoma*, and *Lentinus.*

When organic and inorganic sources of nitrogen were compared, organic sources were preferred by *Tricholoma* for the production of profuse mycelial growth and biomass production. Among organic sources, beef extract and peptone added media produced maximum mycelial growth and biomass (Fig. 3). This result is in accordance with the findings of Khanna and Garcha, (1983) that peptone as best source of organic nitrogen for different mushroom sp namely *Pleurotus*. Similar results have been obtained for other spp. of mushrooms namely *Pleurotus ostreatus* (Mitra and Nandi, 1989), and *Volvariella diplasia* (Banerjee *et al.*, 1990; Gupta *et al.*, 1996; Banerjee and Samajpati, 1989).

Among the inorganic sources, nitrates were preferred over ammoniacal forms. This is in accordance with the finding of Khanna and Garcha (1983). Different nitrate sources used were KNO_3 and $NaNO_3$ of which KNO_3 gave better mycelial dry weight than $NaNO_3$. This result is in agreement with finding that KNO_3 was a better source than $NaNO_3$ in *Pleurotus* as reported by Jandaik and Kapoor (1976). Similar good results were obtained in Calocybe *indica* (Chandra and Purkayastha, 1977), *Volvariella* (Ghosh and Sengupta, 1976; Mitra and Nandi, 1989) and *Agrocybe aegerita* (Sharma *et al.*, 2004).

Among the ammonium salts, nitrate, chlorides and carbonates were poor yielders of mycelial biomass of *Tricholoma.* Bano and Srivastava (1970) reported ammonium nitrate and ammonium chloride as poor N source for the growth of *P. flabellatus.* Similar observation was made by Jandaik and Kapoor (1976) on the growth of *Pleurotus sajor-caju.*

Of the five pH ranges tested both in acid and alkaline range, slightly alkaline pH of 8.0 gave maximum mycelial growth and biomass of *Tricholoma giganteum* (Fig. 4). Decrease of pH resulted in decrease of mycelial growth and biomass. This is contradictory with the results of experiments conducted by Chandra and Purkayastha (1977). They stated that pH 5.5 was optimum for the maximum mycelial biomass production of *Calocybe indica, Agaricus campestris, Lentinus, Volvariella* etc. They also found that increase or decrease in optimum pH resulted in decrease in mycelial mass. Mehta and Bhandal (1992) reported pH 6.0 for the optimum growth of mycelia biomass production in *Auricularia polytricha.*

Out of seven different ranges of temperature conditions tested it was observed that temperature of 35 °C supported maximum mycelia growth (9.00 cm) with a biomass production of 0.35 gm in 50 ml medium (Fig. 5). The other temperature conditions 30 °C, 28 °C, 24 °C and 22 °C also produced mycelial growth and supported biomass production. No growth was found in 4 °C temperature. Kurtzman (1979) reported optimum temperature for mycelial growth of *Auricularia polytricha* was 20-34 °C. Pramod (2004) reported that temperature level of 34 °C was efficient for supporting the growth of *Volvariella vovacea,* followed by 32 and 36 °C. Chandra and Parkayastha (1977) observed that *Calocybe indica* preferred a temperature of 30 °C for optimum growth. From this it can inferred that *Tricholoma* which has a close resemblance to *Calocybe* has a similar preference for high temperature of 30-35 °C.

Four different ranges of light like room light, intermittent light, fluorescent light and dark conditions were tested for their efficacy in producing radial growth and biomass of *Tricholoma* in solid and liquid media. It was observed that fluorescent light was best among the others light sources followed by intermittent light (Fig. 6). This was contradictory with the findings of Pramod (2004). He reported that fluorescent light was found ineffective in supporting radial growth of *Volvariella volvacea.*

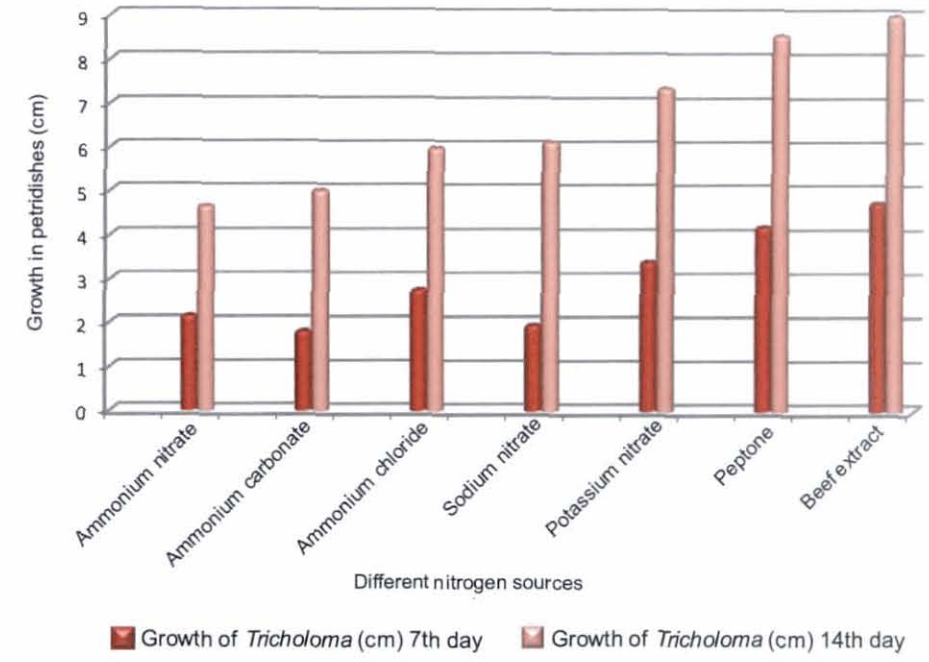


Fig. 3. Growth of *Tricholoma* in different nitrogen sources

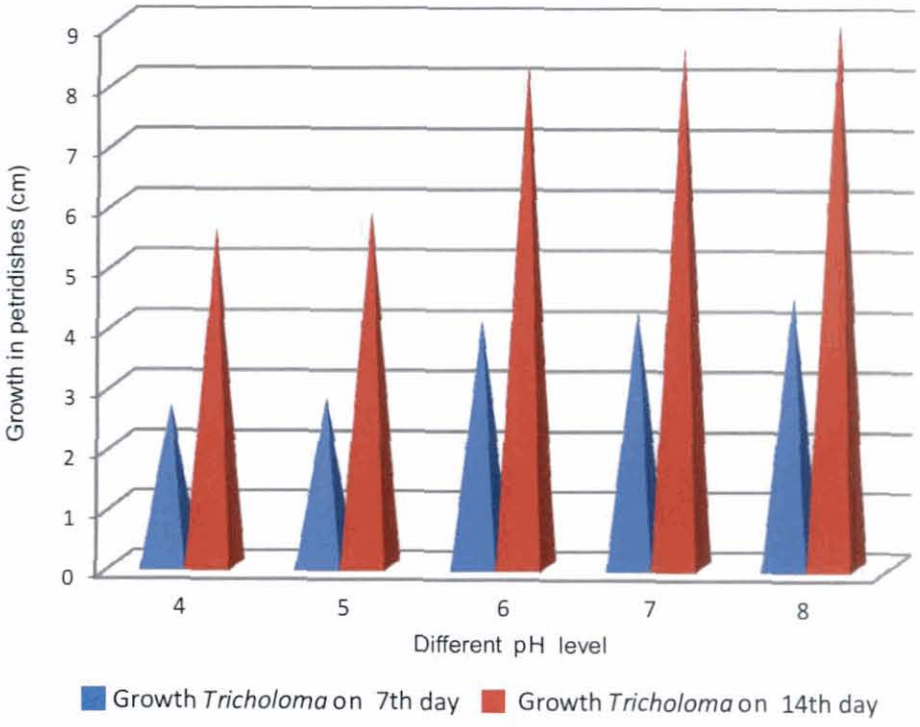


Fig. 4. Growth of *Tricholoma* in different pH level

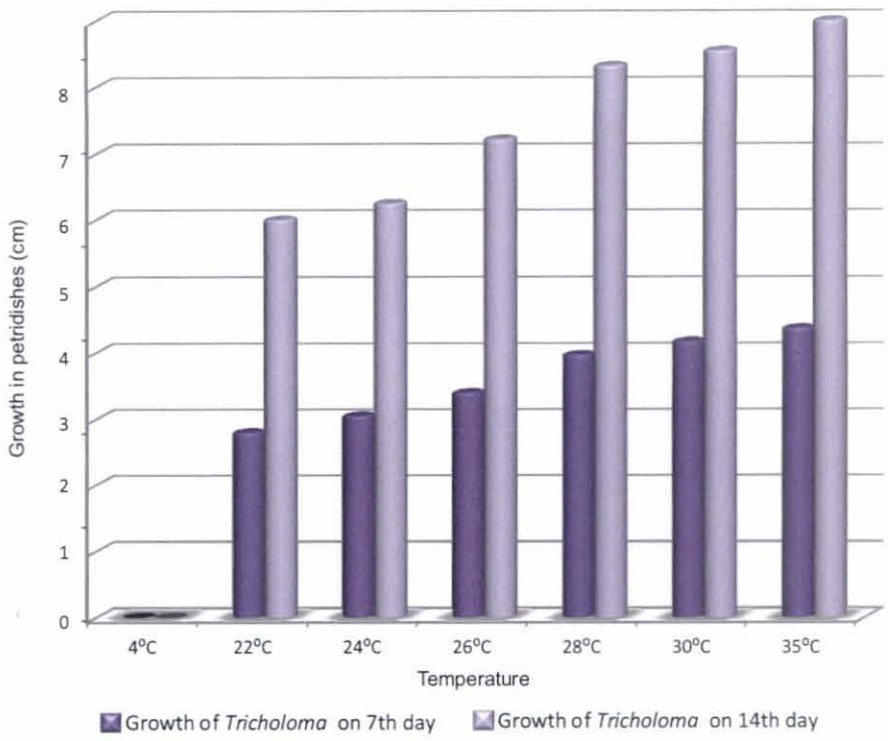


Fig. 5. Growth of *Tricholoma* at different temperature

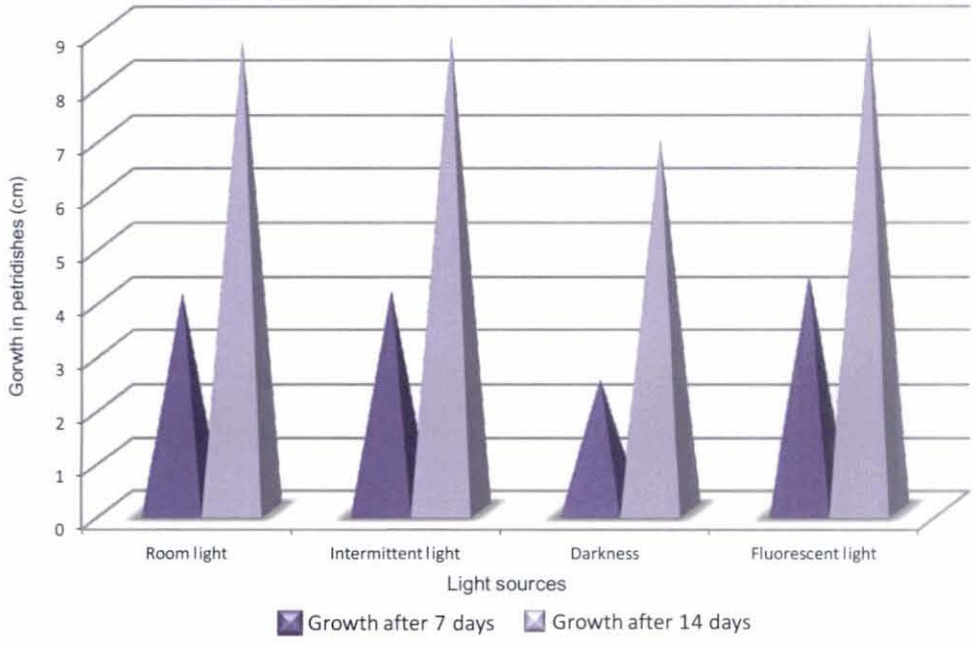


Fig. 6. Growth of *Tricholoma* in different light sources

5.3 GROWTH OF *TRICHOLOMA GIGANTEUM* ON DIFFERENT SPAWN SUBSTRATES

Sinden (1934) introduced grain spawn for the cultivation of mushrooms. Both grain substrates and saw dust substrates were tested in the present study. Grain substrates tried were paddy, wheat, ragi, sorghum, rubber saw dust and maize. Grain substrates were found to be a better spawn substrate than sawdust substrate. Paddy grain was found to be the best substrate for spawn run followed by wheat, maize, ragi and sorghum (Fig. 7). This was in accordance with the findings of Mathew *et al.*, (1996). They reported that sorghum, wheat and paddy grains were equally good for the production of *Pleurotus* spawn. Balakrishnan and Das (2001) reported that sorghum, wheat or paddy grains are generally used for the preparation of spawn of *Calocybe.*

Sawdust substrate took maximum days (21 days) for spawn run. The result is contradictory to the findings of Viela and Silverio (1982), Smith *et al.* (1987), Bhandal and Mehta (1989) who reported sawdust substrate as a better substrate than grain substrate for the cultivation of *Auricularia polytricha.*

Six different substrates were used for bed preparation. The beds spawned with wheat grains were found to be the best giving a highest yield of 833.33g followed by paddy, maize, ragi and sorghum grain spawn (Fig. 8). Lowest yield was found to be in beds spawned with sawdust spawn. Suharban *et al.*, (1987) reported that the wheat grain spawn was much better than paddy straw spawn for higher production. Purkayastha *et al.*, (1980) reported that about 65 % increase in yield was obtained with paddy grain spawn as compared to paddy straw spawn. Dominic (2012) reported that wheat grain spawn gave highest yield of 932 g for *Pleurotus eous.*

5.4 CULTIVATION OF *TRICHOLOMA GIGANTEUM* ON LOCALLY AVAILABLE CHEAP SUBSTRATES

Six different locally available cheap substrates *viz.*, paddy straw, sugarcane baggasse, sawdust, coirpith compost, spent mushroom substrates and coirpith + paddy straw were used for mushroom bed preparation.

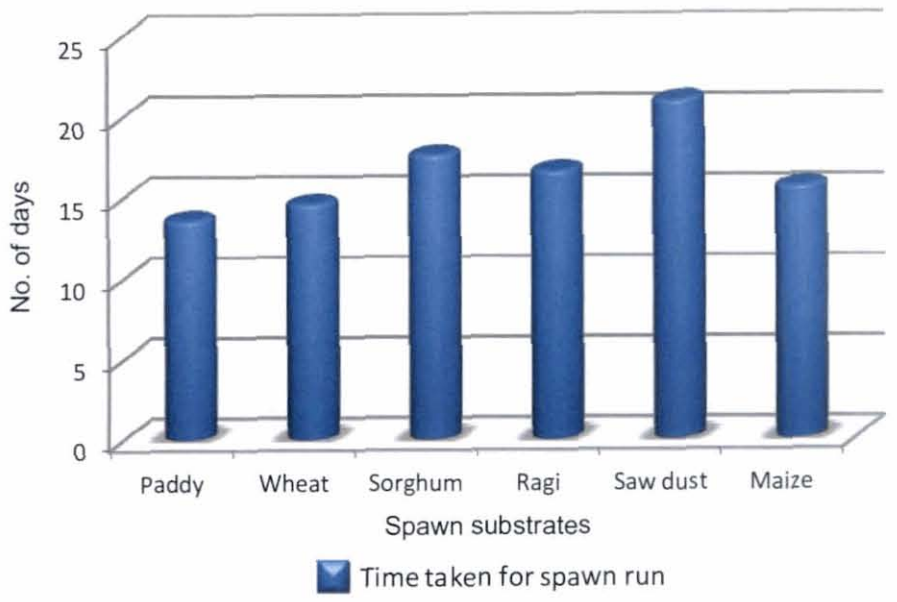


Fig. 7. Time taken for spawn run of *Tricholoma* on different spawn substrates

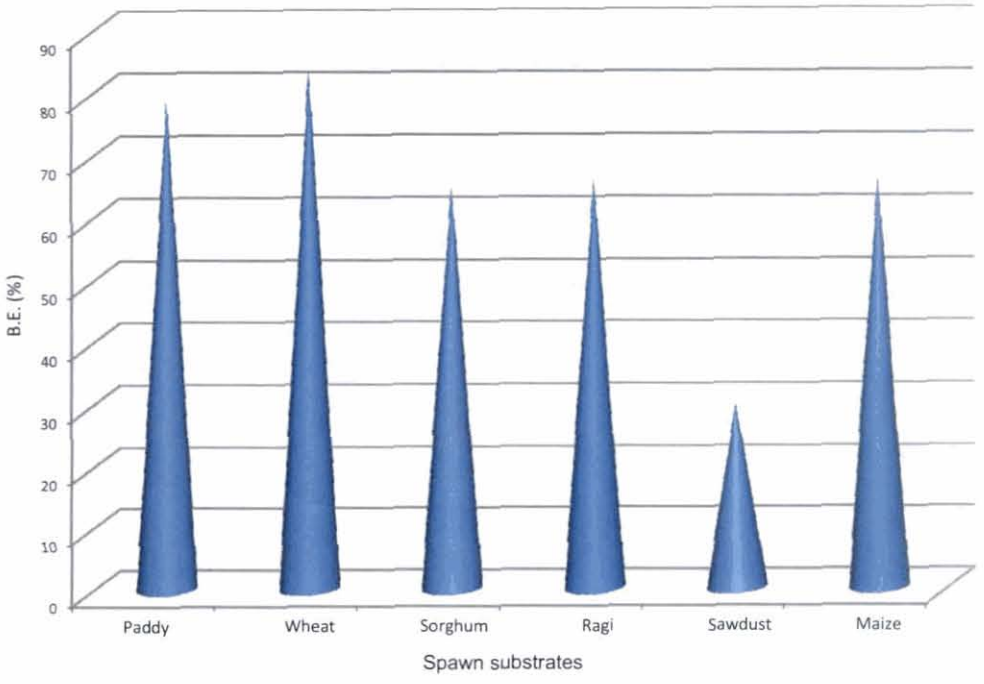


Fig. 8. Yield performance of *Tricholoma* on different spawn substrates

Highest yield of mushroom was found in paddy straw beds followed by sawdust, coirpith compost and spent mushroom substrates. Highest biological efficiency of 69.45% was recorded in paddy straw beds (Fig. 9). Similar results were observed by Pandey and Tewari (2002). They reported that cultivation of *Tricholoma giganteum* with paddy straw gave a biological efficiency of 92%. Ganeshan (1990) found fresh paddy straw as a suitable substrate for cultivation of *Tricholoma lobayense.* Wajeed and Shetty (1995) evaluated the potentialities of various locally available mushroom substrates and reported that paddy straw was the prime supporter for production of *Calocybe indica.* Chang *et al.,* (1981) reported that paddy straw served as the best substrate for production of *Pleurotus* sp. Karnawadi (2006) reported that highest biological efficiency was recorded in beds prepared with paddy straw and lowest yield was found in beds made with sugarcane bagasse.

Saw dust substrate also gave better yield of *Tricholoma* as paddy straw. This is in accordance with the findings of Kinjo and Miyagi (2006). They reported that saw dust media supplemented with wheat bran and hannoki (*Alnus japonica)* gave highest yield of *Tricholoma giganteum.*

Lowest yield was observed in sugarcane baggasse and a combination of coirpith and paddy straw. Amin *et al.,* (2010) reported that lowest biological and economic yield (259.3 and 240.3g) was obtained from sugarcane bagasse. Dayaram (2009) reported that sugarcane bagasse is least preferred substrates as *Calocybe indica* partially colonized the substrate without providing any yield.

Highest stalk length, stalk diameter, pileus diameter and pileus thickness of *Tricholoma* was found to be in paddy straw substrate. Similar observations were also made by Amin *et al.,* (2010). They reported that the highest stalk diameter, pileus diameter and pileus thickness of *Calocybe* was found in rice straw substrate.

Saw dust also give highest stalk length and diameter, pileus diameter and thickness. This is in accordance with the findings of Mondal *et al.,* (2010). They

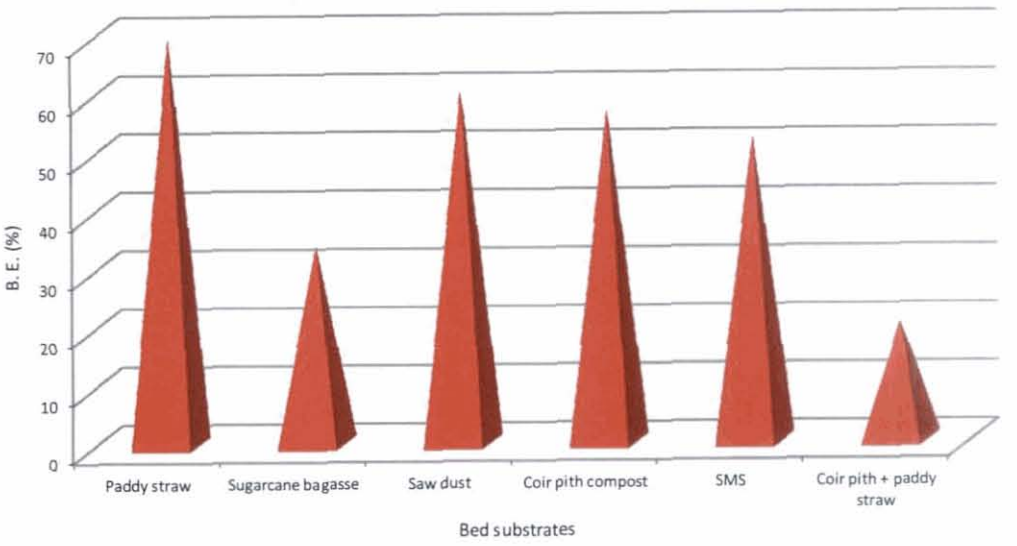


Fig. 9. Yield performance of *Tricholoma* on different bed substrates

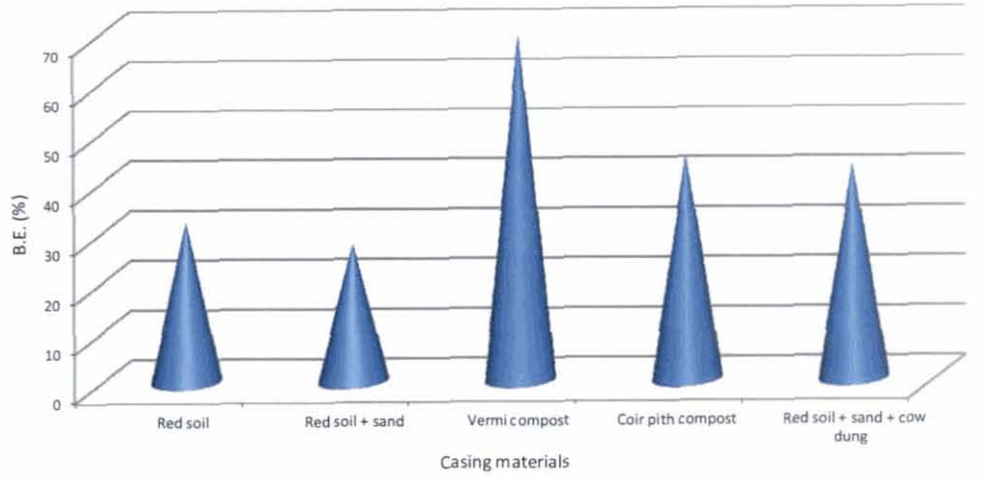


Fig. 10. Yield performance of *Tricholoma* on different casing materials

reported that saw dust substrate give highest stalk length, pileus diameter and pileus thickness.

The minimum diameter and thickness of stalk and pileus was found in sugarcane bagasse beds. Amin *et al.*,(2010) reported minimum diameter and thickness of *Calocybe* in sugarcane bagasse beds.

5.5 EFFECT OF DIFFERENT CASING MATERIALS

Unlike *Pleurotus* casing is necessary for *Tricholoma.* Casing is generally done to make a surface where uniform fruitification can take place and also to provide anchorage and essential reserves for developing sporophores of mushrooms. Five different casing materials *viz.*, red soil, red soil + sand, vermi compost, coirpith compost and red soil + sand + cow dung were used in this study.

Highest yield of mushrooms was found to be in beds cased with vermi compost (Fig. 10). This was in accordance with the findings of Umamaheshwari and Vijayalakshmi (2004). They stated that utilization of earthworm casts as casing materials help in checking water loss by evaporation and the water holding capacity of the casts contributes to the increased yield. Gracia *et al.*, (2005) reported that vericompost used as casing formulations in cultivation of *Agaricus bisporus* gave increased yield.

Coirpith compost and red soil + sand + cowdung combination were also effective for mushroom production when used as casing material for *Tricholoma* beds. Gupta (1996 and 1997) reported that decomposed coirpith either alone or in combination with farm yard manure and spent substrate can be used as casing substrate for mushrooms.

Lowest yield and biological efficiency was found in beds cased with red soil + sand.

Highest stalk length, stalk diameter, pileus diameter and pileus thickness of *Tricholoma* was found to be in beds cased with vermi compost.

These casing materials were used to case beds laid out with different bed substrates. Six different bed substrates *viz.*, paddy straw, sugarcane baggasse, sawdust, coir pith compost, spent mushroom substrates and coir pith + paddy were used in this study and casing materials such as red soil, red soil + sand, vermi compost, coir pith compost and red soil + sand + cow dung were used for testing their efficacy in producing sporophore and yield. Highest yield was found to be in beds (paddy straw, coir pith compost, spent mushroom substrates and coir pith + paddy) cased with vermi compost, this is in accordance with the findings of Yadav (2006). He reported that when casing layer was enriched with vermi compost the yield was found to be highest for *Calocybe*.

In case of sugarcane bagasse beds only red soil was found to be the best casing material.

5.6 PEST AND DISEASE INCIDENCE

Pests like sciarids and staphylinid beetle were mostly found in mushrooms. Their incidence was commonly found during high temperature. This was in accordance with the findings of Balakrishnan (1994), reported the occurrence of sciarid flies and staphylinid beetle as a pest of oyster mushroom in Kerala. The attack of sciarid flies on cultivated mushroom was also recorded by Fletcher *et al.* (1986). *Staphylinus* sp. was earlier reported to damage oyster mushroom in Thiruvananthapuram district of Kerala by Asari *et al.* (1991).

The major diseases observed during the study were the incidence of *Trichoderma* sp., *Cladobotryum dendroides* and *Coprinus* sp. on mushroom beds after casing. This is due to excess moisture present in the casing material or when relative humidity is high. Das *et al.* (1993) reported that *T. viride* was the most virulent competitor mould both in spawn bottle and in mushroom beds in

Vellayani, Thiruvananthapuram. Balakrishnan (1994) described the occurrence of *Trichoderma* sp. and *Coprinus* spp in oyster mushroom beds.

Cob web disease caused by *Cladobotryum dendroides* was also found in one bed of *Tricholoma.* Pandey *et al.,* (2003) reported the incidence of cob web disease and *Trichoderma harzianum* in *Calocybe* beds.

These incidences were found during second harvest only.

5.7 PROXIMATE CONSTITUENT ANALYSIS, SHELF LIFE AND KEEPING QUALITY OF *TRICHOLOMA GIGANTEUM*

The nutrient content of *Tricholoma giganteum* was tested to find out the proximate constituents. Nutrient composition of *Tricholoma* was found to be 87.46 % moisture (fresh weight), 23.2 % proteins, 2.6 % fat content, 10.1 % carbohydrate, 19.01 % fibre and11.46 % ash content (Fig. 11). These results were in agreement with findings of Prakasam *et al.,* (2011). They reported that *Tricholoma giganteum* contain 86.20 % moisture, 32.9 % crude protein, 11.8 % carbohydrate, 0.91 % crude fat, 20.71 % crude fibre, 8.32 % ash, 5.60 % iron, 1.18 % manganese, 1.38 % zinc and 1.10 % copper. Liu *et al.,* (2007) reported that *Tricholoma giganteum* contain 35.28 % protein, 2.91 % fat, 53.74 % total sugar and 8.76 % crude fibre.

Similar findings on the nutrient content in various mushrooms were reported by several workers. Sivaprakasam *et al.*(1986) reported nutritive value of *Calocybe* as 11.9 % dry matter, 2.4 % protein, 2.25 % soluble salts. Nutrient content of *Calocybe* was recorded with 4.1 %fat, 3.4 % crude fibre and 64 % carbohydrate (Doshi and Sharma, 1995).

Nutritive content of *Agaricus* consisted of 90.10 % moisture, 3.75 % protein, 0.53 % crude fibre and 4.59 % carbohydrate (Singh *et al.* (1999).

Anandh (2001) reported nutritive value of *Calocybe indica* as having 88.37 % moisture, 11.63 % dry matter, 26.5 % protein, 36.5 % fibre and 8.8 %

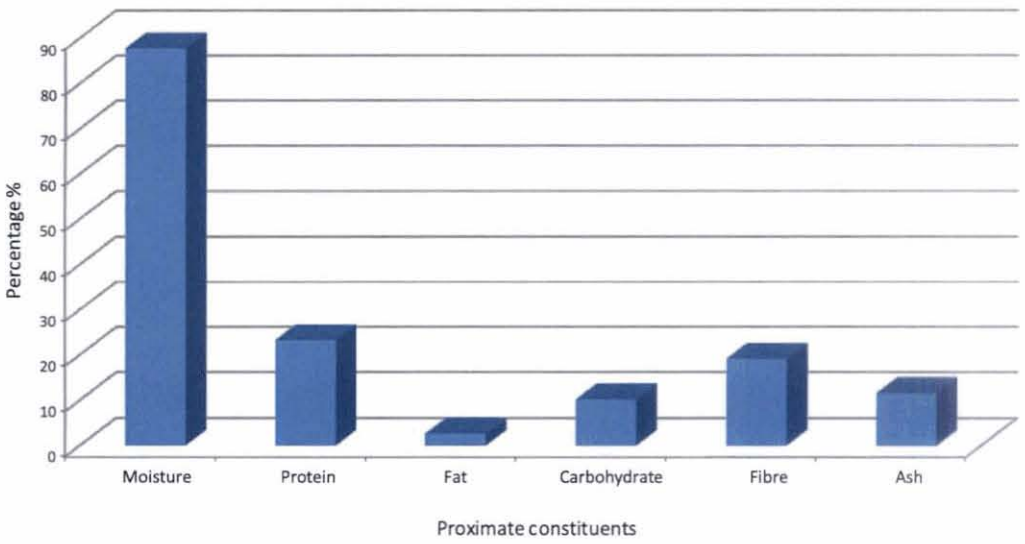


Fig. 11. Nutritional value of *Tricholoma giganteum*

carbohydrate. He also stated the proximate constituent composition of *Tricholoma lobayense* as having 85.2 % moisture, 14.8 % dry matter, 33.2 % protein, 23.74 % fibre and 11.38 % carbohydrate.

The shelf life study of *Tricholoma giganteum* showed that this can be stored in polypropylene covers without perforations for five days in room temperature and seven days in refrigerated conditions. This is in accordance with the findings of Prakasam *et al.,* (2011). They reported that *Tricholoma giganteum* can be stored under room temperature for two days and under refrigerated storage for 6 days without any spoilage and liquefaction. Krishnamoorthy (2004) reported that milky mushroom is having shelf life of five to seven days at room temperature. Heera (2006) reported that *Calocybe* had better shelf life of 12.67 – 24.67 days when stored in refrigerated condition.

Dhar (1992) stated that better shelf life of mushrooms in non perforated polyethylene packs at low temperature when compared to perforated packs at high temperature. Mehta and Jandaik (1989) also made similar observation in *Pleurotus sapidus*. Mushrooms even after harvest, under goes post harvest changes including respiration, senescence which leads to browning.

Better shelf life of *Tricholoma* is attributed to the reduced activity of enzyme at low temperature. There is a slowdown of maturity and conservation of metabolites to maintain the cell functions due to low temperature. Perforations provide greater vent area which allows more access of oxygen which directly increases the respiration rate. Respiration in a closed system (non perforated) oxygen is depleted with concomitant accumulation of carbon dioxide resulting in reduced rate of respiration until an equilibrium is reached (Saxena and Rai, 1988).

Three different dehydration techniques were compared with control (mushroom as such). It was found that the mushroom dried in hot air oven can be stored for 57 days with any damage from microbes. Mushroom dried under sunlight could not be stored for a longer time. Agarwala (1973) stated that

Volvariella could easily be dehydrated either under the sun or in hot air bath at 50-60°C, and the dried mushroom should be packed immediately after dehydration, otherwise the flavour would be lost. Singh *et al.,* (1996) developed the tough-flow drier in which the optimum drying temperature, time and critical moisture content was found to be 60 °C, seven hours and 5 % respectively.

5.8 ORGANOLEPTIC STUDIES

Mushroom blends well with most of the vegetables and spices to form delicious items of food (Das 1992 a, 1992 b). Salads, soups, snacks, main dishes, side dishes, pickles and even sweet preparations are possible with mushrooms.

Tricholoma giganteum was screened for their characters like colour and appearance, texture, taste and flavour on the basis of score card. Six different recipes were prepared and they were subjected to evaluation by 10 judges based on a five point score card. The overall acceptability was noted. Though all the recipes were delicious and tasty but the overall acceptability was high for cutlet followed by payasam, puffs, masala, pickle and soup. Balakrishan (1994) reported that *Pleurotus sapidus, Pleurotus membranaceous*, *Pleurotus petaloides* obtain maximum consumer acceptability with respect to colour appearance and flavour. Das (2011) prepared different dishes using oyster mushroom, button mushroom, jew's ear mushroom and milky mushroom and obtain maximum consumer acceptability in case of appearance, colour, flavour, taste and texture.

Summary

6. SUMMARY

Mushrooms were collected from different parts of Trivandrum district before and after the South West and North East monsoons. The native strains of *Tricholoma* obtained from Trivandrum district had been collected from Aryanad, Pallichal, Thiruvallum and Nedumangad. The collected samples were brought to the laboratory and subjected to various morphological observations including macroscopic and microscopic characters. The tissue isolation was done for obtaining the pure culture of four isolates of *Tricholoma.*

Preliminary trials were conducted which showed that among the four isolates (Isolate 1, 2, 3 and 4) Isolate 1 was the best. Isolate 1 was sent to Directorate of Mushroom Research, Solan for identification and obtaining accession number. The accession number obtained for Isolate 1 was DMRO-462.

Five different solid culture media were tested for their potentiality in supporting the radial mycelial growth of *Tricholoma.* Among these media, oat meal agar gave the maximum fluffy growth. Potato dextrose agar, malt extract agar and tapioca dextrose agar were found to be very effective in supporting the mycelial growth. Carrot agar was ineffective in supporting the mycelial growth.

Biomass production of *Tricholoma* on liquid broth revealed that oat meal broth was superior in maximum biomass production followed by potato dextrose broth and least biomass on tapioca broth and carrot broth.

Studies on the effect of carbon sources on radial mycelial growth and biomass production of *Tricholoma* in solid and liquid media revealed that mannitol and dextrose was the best in solid media and in liquid media

dextrose was found to be the best the source followed by mannitol. The isolate could not effectively utilize galactose and lactose.

Organic nitrogen sources like beef extract and peptone were preferred by *Tricholoma* over inorganic sources. Among the inorganic sources, nitrate sources were preferred to the ammoniacal ones.

Studies on the H^+ ion concentration (pH) showed that *Tricholoma* prefers an alkaline pH of 8 for maximum radial growth and biomass production than the acidic ranges.

The effect of temperature on the radial growth and biomass production of *Tricholoma* was tested and found that the temperature level of 35°C was effective in supporting the growth of *Tricholoma* followed by 30°C and 28°C.

The influence of different sources of light and darkness on the radial mycelial growth and biomass production of *Tricholoma* was evaluated and found that fluorescent light, intermittent light and room light were equally efficient in enhancing the radial growth and biomass production. Dark condition was very poor in supporting the mycelial growth.

Six different substrates were evaluated for their time taken for maximum spawn run. The spawn substrates tried were paddy, wheat, sorghum, ragi, sawdust and maize, among which grain substrates were found to be the best. Paddy grains took minimum time for spawn run followed by wheat and maize. Saw dust was found to be poor and took maximum days for spawn run.

These spawns were used for bed preparation and found that the beds spawned with wheat spawn and paddy spawn produced maximum number of fruit bodies and have the highest biological efficiency. The beds prepared with saw dust spawn was found to be poor in producing fruiting bodies.

Six different locally available cheap substrates namely paddy straw, sugarcane bagasse, saw dust, coir pith compost, spent mushroom substrate and a combination of paddy straw + coir pith were compared and found that the beds prepared with paddy straw produced maximum number of sporophores and highest biological efficiency, followed by saw dust, coir pith compost and spent mushroom substrate. In the beds prepared with coir pith and paddy straw combination minimum number of sporophores was produced.

The characters of *Tricholoma* were studied in different bed substrates. They showed wide variation in stipe length, stipe diameter, pileus diameter and pileus thickness. Among the six substrates paddy straw was the best.

Five different casing materials namely, red soil, red soil + sand, vermi compost, coir pith compost and a combination of red soil + sand + cow dung were evaluated and found that the beds cased with vermi compost produced maximum fruiting bodies and highest biological efficiency followed by coir pith compost and red soil + sand + cow dung combination. Lowest sporophore production and biological efficiency was found in beds cased with a combination of red soil + sand.

The characters of *Tricholoma* were studied in different casing materials. Highest stalk length, stalk diameter, pileus diameter and pileus thickness of *Tricholoma* was found to be in beds cased with vermi compost.

These casing materials were used to case beds laid out with different bed substrates. Six different bed substrates *viz.*, paddy straw, sugarcane baggasse, sawdust, coir pith compost, spent mushroom substrates and coir pith + paddy were used in this study and casing materials such as red soil, red soil + sand, vermi compost, coir pith compost and red soil + sand + cow dung were used for testing their efficacy in producing sporophore and yield. Highest yield was found to be in beds (paddy straw, coir pith compost, spent mushroom substrates and coir

pith + paddy) cased with vermi compost and lowest yield was found in beds cased with red soil + sand combination.

In case of sugarcane bagasse beds only red soil was found to be the best casing material.

Pest and disease incidence was very low during the study. Pests like sciarids and staphylinid beetles were mostly found in mushrooms. Their incidence was commonly found during high temperature. The major diseases observed during the study were the incidence of *Trichoderma* sp., *Cladobotryum dendroides* and *Coprinus* sp. on mushroom beds after casing. This was due to excess moisture present in the casing material or when relative humidity was high. These incidences were found during second harvest only.

The nutrient content of *Tricholoma giganteum* was tested to find out the proximate constituents. The findings revealed the nutrient composition of *Tricholoma* with 87.46 % moisture (fresh weight), 23.2 % proteins, 2.6 % fat content, 10.1 % carbohydrate, 19.01 % fibre and11.46 % ash content.

The shelf life study of *Tricholoma giganteum* showed that this can be stored in polypropylene covers without perforations for 5 days in room temperature and seven days in refrigerated conditions. Three different drying techniques namely, sun drying, oven and hot air oven for preservation were compared by keeping the mushroom as such without drying (control). It was found that mushroom dried in hot air oven at 60 ^{o}C can be stored for 57 days without any damage from microbes.

Tricholoma giganteum was screened for their characters like colour and appearance, texture, taste and flavour on the basis of score card. Six different recipes were prepared and they were subjected to evaluation by 10 judges based on a five point score card. The overall acceptability was noted. Though all the recipes were delicious and tasty the overall acceptability was high for cutlets followed by payasam, puffs, masala, pickle and soup.

The technology of cultivation of *Tricholoma* on paddy straw substrate using wheat or paddy spawn and vermi compost as casing material can be recommended as a suitable domestication package which will be transferred to the farmers along with the release of this mushroom variety.

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STANDARDIZATION OF TECHNIQUES FOR CULTIVATION OF *TRICHOLOMA GIGANTEUM* MASSEE IN KERALA

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Abstract of the
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8. ABSTRACT

The present investigation on "Standardization of techniques for cultivation of *Tricholoma giganteum* (Massee) in Kerala" was conducted at the Department of Plant Pathology, College of Agriculture, Vellayani, during 2012-2013. The aim of the study was to explore the possibility of cultivation of *Tricholoma giganteum* (Massee) on readily available cheap substrates and to develop a package for commercial cultivation in Kerala.

Mushrooms were collected from different parts of Trivandrum districts before and after the South West and North East monsoons. Preliminary trials laid out showed that isolate 1 was the best out of 4 isolates which was sent to DMR. This isolate with accession number DMRO- 462 was used for further studies.

Tricholoma has a convex pileus, off white to creamy white in colour, fleshy in texture and with a stout hairy stipe.

Cultural studies conducted showed that the isolate attained full growth in petridish in 14 days on oat meal agar and least growth was found in carrot agar medium. Out of six carbon sources dextrose was found to be best for the radial growth of *Tricholoma*, least growth was found in galactose. Among the seven nitrogen sources used to study the radial growth of *Tricholoma* in petridish beef extract was found to best and least in ammonium nitrate. Temperature of 35 ^{o}C, fluorescent light conditions and pH8 were found to be the best for the growth of *Tricholoma giganteum.*

Evaluation of six different spawn substrates showed that paddy grains was best spawn substrate since complete spawn run was attained in two weeks. Regarding yield studies beds laid out with wheat grain spawn gave highest yield of 833.33 g / bed. Saw dust took maximum time for spawn run and lowest yield was also recorded in it.

Six different locally available cheap substrates *viz.*, paddy straw, sugarcane bagasse, saw dust, coir pith compost, spent mushroom substrates and coir pith + paddy straw were used for the cultivation of *Tricholoma giganteum*. Highest yield (694.50 g) was found to be in beds prepared from paddy straw and lowest yield (199.50 g) was observed in beds laid out with coir pith + paddy straw as substrate.

Out of the casing materials tried vermi compost was found to be the best. Lowest yield was found be in beds cased with red soil + sand.

Analysis of nutrient composition of *Tricholoma giganteum* indicated that, the moisture content, protein, fat, carbohydrate, ash and fibre content was found to 87.46 %, 23.20 %, 2.60 %, 10.10 %, 11.46 % and 19.01 % respectively.

The shelf life of fresh mushroom was high (7 days) when stored in polypropylene cover without perforation in refrigerated condition. For mushroom dried in hot air oven the shelf life was found to be 60 days.

Pests like sciarid flies and staphylinid beetle were prevalent after the second harvest only. *Coprinus,* cob web (*Cladobotryum dendroides)* and *Trichoderma* causing decay of the fruiting body was observed in *Tricholoma giganteum* beds when temperature and relative humidity was high.

Results of organoleptic studies revealed that *Tricholoma* has high cooking quality and overall consumer acceptability was good. Cutlets were found to be the best when consumed by the panel of judges followed by payasam. The overall acceptability of soup made out of dried mushroom powder was comparatively poor.

Based on the results obtained during the investigation it can be concluded that *Tricholoma* is a new summer edible mushroom most suited for the Kerala conditions. The technology of cultivation of *Tricholoma* on paddy straw substrate using wheat or paddy spawn and vermi compost as casing material can be recommended as a suitable domestication package which will be transferred to the farmers along with the release of this mushroom variety.

Appendices

APPENDIX – I

DATA-SHEET

Date of collection .....

Collected by..... Locality .....

GENERAL

Substrate :

Habitat : Terrestrial / Lignicolous / Epixylose / Coprophilous

Habit : Solitary / Scattered /Gregarious

Pileus

Shape : Convex/infundibuliform/Umbonate/Petaloid/Flabelliform/Depressed

Colour :

Size : Diameter

Thickness

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

Stipe

Shape : Clavate/Cylindrical/Solid/Hollow/Slender

Size : Length :

Diameter :

Attachment to pileus : Lateral/Eccentric/Central/Resupinate

Surface : Glabrous/Scaly/Smooth/Pubescent/Fibrillose

Basal part : Globular/Bulbous/Fusoid/Cylindrical

APPENDIX – I (Continued)

Gills

Arrangement	: Remote/Free/Decurrent/Adnate/Adnexed/Sinuate
Texture	: Soft/Brittle/Waxy/Thick/Papery/Opaque
Margin	: Smooth/Wavy/Serrate/Fimbriate/Dentate
Size	: Number per cm

Veil

Type	: Present/Absent

Annulus

Type	: Present/Absent

Volva

Type	: Present/Absent

Spore print

Colour	:

Spores

Colour	:
Shape	: Ovate/Elliptical/Globose/Epiculate/Cylindrical/Fusiform/ Angular/Echinulate/Recticulate/Ovoid/Pyriform
Reaction with	
Cotton blue	: Cyanophilic/Acyanophilic
Melzer's reagent	: Amyloid/Dextrinoid/Nonamyloid

APPENDIX – II

Composition of different media

a) Potato dextrose agar (PDA)

Potato	:	200 g
Dextrose	:	20 g
Agar-agar	:	20 g
Distilled water	:	1 l

b) Malt extract agar

Malt extract	:	25 g
Agar-agar	:	20 g
Distilled water	:	1 l

c) Oat meal agar

Oats	:	40 g
Agar-agar	:	20 g
Distilled water	:	1 l

d) Tapioca dextrose agar

Jackfruit seed kernel	:	200 g
Dextrose	:	20 g
Agar-agar	:	20 g
Distilled water	:	1 l

e) Carrot agar

Carrot	:	400 ml
Dextrose	:	20 g
Agar-agar	:	20 g
Distilled water	:	1 l

APPENDIX – II (Continued)

f) Czapek – Dox Agar

$NaNO_3$	:	2 g
K_2HPO_4	:	1 g
$MgSO_4.7H_2O$	:	0.5 g
KCl	:	0.5 g
$FeSO_4$	:	0.01 g
Sucrose	:	30 g
Agar	:	20 g
Distilled water	:	1 l

APPENDIX – III

Score card

Colour and appearance	
Excellent	5
Good	4
Fair	3
Poor	2
Very poor	1
Texture	
Very good	5
Good	4
Fair	3
Poor	2
Very poor	1
Flavour	
Very soft	5
Soft	4
Slightly fibrous	3
Fibrous	2
Very fibrous	1
Taste	
Highly acceptable	5
More acceptable	4
Acceptable to certain extent	3
Less acceptable	2
Not acceptable	1
Overall acceptability	
Highly acceptable	5
More acceptable	4
Acceptable to certain extent	3
Less acceptable	2
Not acceptable	1