BIOLOGY AND CULTIVATION OF *Auricularia* spp.

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DECLARATION

I here declare that this thesis entitled "**Biology and cultivation of *Auricularia* sp.**" 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, associate ship, fellowship or other similar title, of any other university or society.

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CERTIFICATE

Certified that this thesis entitled "**Biology and cultivation of A*uricularia* sp**." is a record of research work done independently by Ms. Vidya Resmi (2006-11-128) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associate ship to her.

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Dedicated to

My Achan, Amma and Balu

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LIST OF ABBREVIATIONS

^{0}C	Degree Celsius
%	Per cent
PDA	Potato Dextrose Agar
OA	Oatmeal Agar
CA	Carrot Agar
TA	Tapioca Agar
CDA	Czapeks' Dox Agar
RM	Richards' Medium
µm	Micrometre
cm	Centimetre (s)
g	Gram
mm	Millimetre
viz,	Namely
et al.	And others
kg	Kilogram
N	Normal
CD	Critical difference
@	At the rate of
sp.	Species

Introduction

1.INTRODUCTION

Mushrooms are cultivated for their nutritive and medicinal values. They are highly appreciated for their taste and flavour and are consumed both in fresh and processed forms. From the trends of past year the demand and consumption of mushroom is expected to continue increasing. India is a country with varied topography, climate and agricultural resources. A huge amount of agricultural wastes available in India coupled with climatological diversity can be successfully exploited for commercial cultivation of various edible mushrooms.

Auricularia mushrooms are the fourth most important cultivated mushrooms in the world with a unique jelly taste. Commonly known as wood ear , *Auricularia auricula* is the first recorded cultivated mushroom in the world. All the species of *Auricularia* found in nature are edible and possess medicinal properties. It is widely distributed in tropical , sub tropical and temperate conditions. Thailand is a major exporter of this mushroom for local use and 80 % of the dried produce of Thailand is exported to Hongkong , Japan and USA. *Auricularia* spp. production now represents about 11% of the total cultivated mushroom supply world wide.

The climatic conditions of Kerala are best suited for the growth of several species of *Auricularia.* It can in future be the most suited species for cultivation under Kerala conditions.

Low biological efficiency compared to other tropical mushrooms , inconsistent productivity and non appealing purplish colour of the fruiting bodies are the major drawbacks which hinder the production and acceptability of this mushroom on a commercial scale.

Based on these facts, the present study was conducted with the major objective of improving the quality isolates of *Auricularia polytricha* and developing modified technologies for improving the production with respect to utilization of substrates as well as modified cultivation techniques. Attempts were also made to study the cooking quality and overall acceptability of *Auricularia* mushrooms.

Review of literature

2. REVIEW OF LITERATURE

Mushroom cultivation is one of the most profitable and environment friendly enterprise among various horticultural crops. India is blessed with varied agro climate which makes it suitable for cultivation of different types of mushrooms. Among the 200 species of edible mushrooms widely *Auricularia* is included. *Auricularia* or black ear mushroom is widely distributed in tropical, sub-tropical and temperate climatic conditions where it grows in nature on fresh wood or decaying wooden logs.It is the oldest cultivated mushroom. Commonly known as wood ear, *Auricularia auricula* is the first recorded cultivated mushroom (Chang 1993). *Auricularia* ranks fourth among cultivated mushrooms (Chang, 1996). The earliest record of Auricularia dates back about 200-300 B.C. when it was reported that five kinds of *Auricularia* growing in China were gathered for food in the rainy season and dried in sun. It has been also known to have picked for food and used for the treatment of piles (Cheng and Tu , 1978).

Total production of *Auricularia* spp. in 1991 exceeded 465,000 t .This value is an increase of 346,000 t or 290 % over 1986 levels (Chang, 1993). *Auricularia* spp. production now represents about 11 % of the total cultivated mushroom supply world-wide. ICAR has initiated several research and development endeavours in agricultural enterprises like mushroom production in 10th Five Year Plan(AICRP Mushroom Report 2005-06) .

Auricularia or black ear mushroom belongs to Kingdom Fungi, Division Basidiomycota , Class Agaricomycetes , Order Auriculariales , Family Auriculariaceae and Genus Auricularia.

2.1 COLLECTION AND ISOLATION OF NATIVE STRAINS OF *Auricularia* spp.

It is reported that the genus *Auricularia* is known to contain more than 15 species, out of which Sohi and Upadhyay (1988) identified eight *Auricularia* spp from different parts of Himachal Pradesh.
Sharma *et al.* (1992) recorded *Auricularia delicata , Auricularia auriculae-judae , Auricularia mesenterica* . It has been reported that tissue culture isolates raised from phenotypically healthy looking mushrooms possess good fertility (Kligman, 1943) .

2.1.1 *Auricularia polytricha* (Mont.) Sacc.

Auricularia polytricha is variously called "wood ear," "tree ear," "black fungus". It is a native of Asia and some Pacific Ocean islands in humid climates. Most major Asian countries successfully cultivate *A. polytricha* today.

Purkayastha and Chandra (1985) had described the morphology of the species. Sporophores are tenaciously attached to branches of frondose trees, mainly elder but also elm, beech, walnut, willow and pines in Western North America. May be found at any part of the year. Fruiting body is 3-10 cm across, ear shaped or very irregularly cupshaped. Tough, translucent and gelatinous in wet weather. Hard and shrivelled if dry weather persists, outer surface is velvetty with greyish hairs. Hymenium is smooth, veined and reddish to purplish brown. Spores are white, oblong, curved and narrow at the base, 16-20 × 5.5-8.5 µm.

Hemmes and Desjardin (2004) described the morphological characters of *Auricularia polytricha.* The fruiting body of *Auricularia polytricha* is 50 -120 mm (2.4 - 4″) broad and 1 -2 mm (0 .023 – 0.07 ″) thick , ear shaped to convex fan shaped , texture is rubbery, upper surface is smooth to wrinkled, to velvety, with lower surface smooth to veined, glabrous , greyish brown to purplish brown, overall white to the tomentum . The spore deposit is white. The fruiting bodies of the genus *Auricularia* are waxy and resemble human ears in shape and are gelatinous to leathery and colour ranges from purplish brown to black (Schenk and Dudley , 1999)

2.2 CULTURAL STUDIES OF *Auricularia* spp.

2.2.1 Growth under different solid media

The simplest method of maintaining the strains of *Auricularia* is through subculturing of vegetative mycelium onto a suitable medium. 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.

Geetha (1982) reported that growth of *Coprinus lagopus* was maximum on PDA. Oats agar was found to be the best medium for the growth of *Pleurotus* spp. followed by PDA (Suharban ,1987). 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

mycelial 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 asparagine solution the best liquid medium (Kaur and Lakhanpal, 1999) .

Upadhyay (1999) reported that 2 %malt extract agar medium supported a good growth of *Auricularia mesenterica*. *A. mesenterica* forms white strandy mycelial growth changing to dirty creamish on malt extract medium.

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* . Similar results were obtained in the case of *Ganoderma* by Balabaskar *et al.*(2005) .

Tabata and Ogura (2003) reported the growth of *Auricularia polytricha* in potato sucrose agar (PSA). The mycelia grew well on the PSA supplemented with calcium sulfate, calcium phosphate, magnesium sulfate and magnesium chloride. Ezhilarasi *et al.*(2005) reported potato dextrose agar of pH 5.6 as the best for the growth of *Pleurotus sajor-caju* .

Ling *et al.*(2005) reported potato dextrose agar was an excellent medium for the growth of *Auricularia* sp.

Studies conducted by Garasiya *et al.*(2007) revealed that *Auricularia polytricha* grows well in malt extract agar medium.

2.2.2 Effect of Temperature on the Growth of *Auricularia* spp

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 its optimum growth. Yungchang and Yee (1977) reported that *Volvariella volvacea* and *Coprinus cinereus* grew well at a temperature range of 30˚C to 35˚C.

According to Kurtzman (1979) optimum temperature for the mycelial growth of *Auricularia polytricha* was 20 -34˚C and that of *Auricularia auricula* was 28˚C. He also reported that optimum temperature for fruiting is lower than optimum temperature for mycelial growth.

According to Geetha (1982) optimum temperature for the growth of *Coprinus lagopus* was 35˚C. Bhandal and Mehta (1989) reported that the optimum temperature for the spawn run of *Auricularia polytricha* was 25 ±2˚C. A lower temperature of 20±2˚C promotes pinhead formation of *Auricularia polytricha*.

Khan et al (1991) had reported that 25 - 30˚C as the most suitable for the growth of *Auricularia polytricha.* For *Auricularia* production, the logs must be kept at 24 -30 ˚C with 90 -100 % relative humidity and adequate air circulation. To initiate fruiting, a slight lower temperature of 12 – 20 ˚C is necessary (Stamets, 1993).

Upadhyay (1999) reported that the optimum temperature for the growth of *Auricularia mesenterica* was 30˚C (90 mm radial growth in 10 days). Mycelial growth is completely inhibited at 10 and 40˚C. Singh *et al*.(2000) had also reported that 25 - 30˚C was the most suitable for the growth of *Auricularia polytricha* .Zervakis *et al.* (2001) observed optimum temperature for growth of *Auricularia auriculae* lies between 20 – 25 ˚C .Keun Yang *et al*.(2002) reported optimum temperature for biomass of *Auricularia polytricha* was 20˚C.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 *et al.* (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. Studies conducted by Garasiya *et al.* (2007) reported that *Auricularia polytricha* grows well in 25 - 30˚C.

2.2.3 Effect of light on the growth of of *Auricularia* spp

San Antonio (1981) reported that for the cultivation of *Lentinus edodes* as cool nights followed by warm days are essential for fruit body formation. Miles *et al*.(1987) reported that for the cultivation of *Lentinus edodes* light was essential for brown-pigment formation of the mycelial 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) and the light requirement is not photoperiodic (Munjal *et al*.,1975) , but no significant effect of light on yield of *V. diplasia* has been reported by Singh and Saxena (1983). Antonio and Fordyce (1972) observed that an appreciable quantity of light (15 minutes full sunlight) is required for the initiation of fruit bodies.

The spawn run for *Auricularia polytricha* is maximum at dark area of less than 500 lux. 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 primordial and fruiting body formation.

2.2.3 Effect of pH on the Growth of *Auricularia* spp

Hiroe and Kamyoshi (1937) reported that pH 5.0 – 6.0 was the best for the mycelial growth of *Cortinellus shiitake*. Suharban (1987) reported that pH 5.5 was the best for the dry matter production of *Pleurotus* spp. Best pH for the

mycelial growth of Jews ear mushroom was found to be between 5-6 (Guiling and Fuwen, 1988). The optimum pH for the growth of *Pleurotus* sp was found to be 5.5 (Rafique *et al.*, 1999). Kaur and Lakhanpal (1999) observed that 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.

Singh *et al.* (2000) studied the effect of pH on different edible mushrooms like *Lentinula edodes, Agaricus bisporus, Pleurotus ostreatus, Auricularia polytricha, Morchella esculenta* etc and found suitable pH as 6.0 for *Auricularia polytricha* .Keun Yang *et al*.,(2002) reported optimum pH for biomass of *Auricularia polytricha* was at pH 4 .Sharma *et al*.,2004 observed that for the cultivation of *Agrocybe aegerita* 9 is the optimum pH for the mycelial growth followed by 8 and 7 in the decreasing order .

A suitable medium with pH is very important. Studies conducted by Garasiya *et al.* (2007) reported that *Auricularia polytricha* cannot sustain in the alkaline substrate. In the cultivation, pH of the substrate as well as water should be in the range of 6-6.5.

2.2.5 Effect of carbon sources on the growth of *Auricularia* spp.

Madelin (1956) observed starch and glucose as good carbon sources for many edible mushrooms like *Pleurotus flabellatus* and xylose as poor source. Studies revealed that maltose supported maximum sporulation of *Pleurotus ostreatus*. Similar results were obtained by Bano ansd Srivastava (1970) in which glucose was considered as a good source of carbon and xylose as poor source for *Pleurotus flabellatus.* Hashimoto and Takahashi (1974) observed xylose and arabinose as poor sources of carbon for *Pleurotus ostreatus.* Banerjee *et al.* (1990) observed starch as the best exogenous carbon source for the germination and germ tube growth of *Volvariella volvacea* followed by maltose, glucose and fructose.

Kaur and Lakhanpal (1995) studied the effect of nutrient element sources, vitamins and growth regulator on the 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.

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.* (2005a) revealed glucose incorporation on liquid media recorded highest mycelial dry weight of *Pleurotus flabellatus* when compared to dextrose. This was followed by sorbitol, sucrose, cellulose, mannitol and starch in decreasing order of merit.

Carbon plays a vital role in the growth of all microorganisms including mycelial growth of *Auricularia polytricha.* Studies conducted by Garasiya *et al.*(2007) reported that the highest dry mycelial weight of *Auricularia polytricha* was in starch. In 2003, Upadhyay had found glucose and fructose as excellent carbon sources for the growth of *Auricularia* spp.

2.2.6 Effect of nitrogen sources on the growth of *Auricularia* spp.

Organic nitrogen was superior to inorganic nitrogen for the growth of *Pleurotus ostreatus.* Among the organic nitrogen sources asparagine was the best. (Yusef and Allam , 1967). Nitrates,chloride and tartarate of ammonium supported the mycelial growth of *Pleurotus ostreatus* and *Pleurotus sajor-caju* (Bano and Srivastava ,1970; Hashimoto and Takahashi ,1974) .Kikon and Rao (1980) recommended organic forms of nitrogen as suitable source for the growth of *Pleurotus 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 ostreatus* and *Pleurotus sajor-caju.*

Urea was found to be the best source of nitrogen for gasteromycetes. Mitra and Nandi (1989) observed inorganic nitrogen source ammonium sulphate was the best producing maximum mycelial biomass on rice straw dust and peptone as the best organic nitrogen source. 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 germ tube growth of *Volvariella* (Banerjee *et al.*, 1990).

Upadhyay (2003) had found calcium nitrate, urea, asparagine and alanine as the best nitrogen sources for the good mycelial growth of *Auricularia polytricha.*

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 medium is responsible for skeletal development of fungi. Studies conducted by Garasiya *et al.* (2007) showed that the maximum dry mycelial weight of *Auricularia polytricha* is in soybean powder followed by potassium nitrate and urea. Similar results were reported by Khan *et al.* (1991).

2.3 COMPARATIVE EFFICIENCY OF 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 (Stoller, 1962). Spawn bottles were filled with the substrate and sterilized for 2-3 hours at 121˚C.The substrate after sterilization should contain 40-50% moisture and pH of 7.5. Thapa et *al.* (1978) devised a cheap and effective method of spawn production on polypropylene covers instead of glass bottles.

For the cultivation of *Auricularia* there are mainly two types of spawn *viz.* grain or saw dust spawn.

a. Grain spawn: grain spawn is prepared on sorghum or wheat grains in glucose bottles or in polypropylene bags and incubated at 25 ˚C for 15- 20 days.

b. Saw dust spawn: Sawdust spawn is prepared by using any of the following formulae.

1. Saw dust (65%), wheat bran (15%), used tea leaves (20%), water (65%)

2. Saw dust (78%), sucrose (1%), wheat bran (20%), calcium carbonate (1%), and water (65%)

3. Saw dust(880g) , rice bran(320g) , sucrose (30g) , potassium nitrate(4g) , calcium carbonate(6g) , water (2 litres)

Bhandal and Mehta (1989) 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 that sorghum ,wheat and paddy grains were equally good for producing spawn .

Krishnamoorthy and Muthusamy (1997a) utilized sorghum grain spawn for *Calocybe* cultivation. Spawn of oyster mushroom prepared on parboiled paddy grains was 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.*

2.3.2 SPAWN SUBSTRATES

Cultivation of *Auricularia* spp. in China is almost 2000 years old (Lou, 1980). Wooden logs which were inoculated with spores from basidiocarps were used for growing of *Auricularia* spp. Inoculated logs were kept in shade and covered with straw to maintain moisture content of the straw. The method was very primitive and inconsistent results were obtained. The same method was followed by Reinking (1921) in Philippines to grow *Auricularia polytricha.*

Purkayastha and Nayak (1981) reported increased yield when nitrogenous substrates were used for the cultivation of *Calocybe. Calocybe indica* could be cultivated on both composted as well as non composted rice straw (Chakravarty *et al*., 1981).

Auricularia polytricha and *Auricularia auriculae* are commercially cultivated using a mixture of saw dust and rice bran. Viela and Silverio (1982) gave composting technique using sawdust and calcium carbonate (1%) and reported 25 to 65% biological efficiency.

Among the various agricultural wastes from maize, bajra, mentha, groundnut stalk, cereal straw and vegetable waste, cereal straw was found to be the best substrate for the production of *Pleurotus florida* and *Pleurotus sajor-caju* (Garcha *et al*.,1983). Experiments conducted by Dadwal and Jamaluddin (1985) revealed barley meal and maize meal as the best substrate for the cultivation of *Tricholoma giganteum* .

Smith *et al*., 1987 reported that cultivation of *Auricularia* spp. on wood logs without or little pretreatment gave a yield of 21 g dry/ kg dry substrate. The cultivation of *Auricularia* spp. in sterilized saw dust and rice bran mixture in polythene bags by axenic culture method gives a yield of approximately 70-75 g dry/ kg dry substrate.

Combination of alfalfa hay and wheat straw significantly increased total yield and biological efficiency of *Pleurotus sajor-caju* (Royse and Bahler, 1988). Cultivation method of wood ear mushroom *Auricularia* spp. was done successfully on wheat straw as substrate by Bhandal and Mehta (1989).

Ganeshan (1990) found fresh paddy straw as suitable substrate for the 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). Oyster mushroom cultivated on substrates viz., paddy straw, maize straw, and coir dust and groundnut shells, biological efficiency varied widely with maximum in groundnut shell (Desai and Shetty, 1991). Patil and Jadhav (1991) used cotton stalks as one of the best substrates for the cultivation of oyster mushroom (*Pleurotus sajor-caju).*

Thakur and Bhandal (1993) reported wheat straw, paddy straw and saw dust as the most suitable substrates for the cultivation of *Auricularia polytricha.* Lin *et al*., 1993 reported that dead branches, fallen leaves and pruning wastes from tea plants were suitable for the cultivation of *Auricularia polytricha.*

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). Straw of paddy, wheat, cotton and jowar were tested for their suitability as substrate for the cultivation of *Pleurotus sajor-caju,* of which cotton stalk was the inexpensive and effective substrate (Kathe *et al*., 1996).

Krishnamoorthy and Muthusamy (1997b) utilized several agro wastes namely paddy straw , sorghum stalks , sugarcane bagasse , palmrosa grass , vetiver grass , groundnut haulms , soybean 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.

R.C. 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 *Auricularia mesenterica.* Upadhyay and Rai (1999) reported the successful cultivation of Lentinus *squarrosulus* on chemically treated wheat and paddy straw.

Fermentation 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.

In the cultivation of *Agrocybe aegerita* wheat straw supported the maximum and fastest growth (Sharma *et al*., 2004).Among the six different substrates viz cotton stalk , groundnut haulms , groundnut shell , paddy straw , sorghum straw and sugarcane bagasse , paddy straw was found to be the best substrate for *Calocybe indica* producing maximum sporophore yield (Eswaran and Susan , 2003) . Sherin *et al*. (2004) conducted experiment to study the suitable substrate for *Calocybe indica* cultivation among retted, non-retted and composted coir pith and also paddy straw. The study revealed that maximum yield and sporophore production were obtained in non-retted coir in combination with 75% paddy straw, followed by 50% combination of non retted coir pith and spent mushroom substrate. Pramod *et al*. (2004) reported that the highest biological efficiency of *Volvariella* was reported on oil palm waste when compared to other locally available substrates dried water hyacinth, banana leaves and sugarcane baggasse (Pramod *et al*. (2005) observed red banana pseudostem as the most efficient substrate for the cultivation of oyster mushroom. Sudhakar *et al*.(2005) reported that combination of horse gram and capsicum waste gave higher yield of *Pleurotus sajor-caju* and lesser time for spawn run and first harvest .

Thirumalvalavan *et al.* (2005b) reported that sorghum and sorghum plus kudhiraivali was the most suitable substrate for *Pleurotus florida* .Veena *et al*.(2006) observed that the best locally available substrate for the cultivation of *Ganoderma lucidum* was 90% saw dust and 10% rice bran .

Studies conducted by Garasiya *et al.* (2007) showed that for the cultivation of *Auricularia polytricha* the substrate should contain lignin and cellulose in the available form and sawdust and wheat straw gave a good mycelial yield .

2.3.3 SUBSTRATE SUPPLEMENTATION

Supplementation of compost with cotton seed meal enhanced the yield of *Agaricus* (Beck and Rasmussen, 1968; McCanna, 1968). Seth (1976) stated wheat bran as a suitable organic supplement to compost for *Agaricus* cultivation.Zadrasil (1980) reported the effect of supplementary straw substrate with ammonium nitrate, soybean meal or alfalfa meal on the fructification and yield coefficient. Organic supplements affected not only yield rise, but also raise in protein content and higher yields coefficient. Addition of organic supplements in the form of horse gram powder, cotton seed powder, yeast mud, groundnut cake or rice bran to rice straw beds of *Pleurotus sajor-caju* showed increase in yield and increased protein content.

Highest yield of *Calocybe* was obtained when paddy straw was supplemented with 5 % maize meal (Purkayastha *et al*., 1981). Bano and Rajarathnam (1982) suggested highest yield of *Calocybe* was obtained by supplementing straw substrate with horse gram powder and yeast mud at 4.4% and 2.2% respectively with 100% B.E. Purkayastha (1985) observed combination rice straw and wheat straw along with maize meal as suitable substrate for the cultivation of *Calocybe* .

Bhandal and Mehta (1986) have cultivated Indian strain of *Auricularia polytricha* on fresh as well as composted wheat straw supplemented with rice bran with 60 to 80 % biological efficiency .Studies conducted by Gunasegaran and Graham (1987) indicated that among the organic additives like rice bran, corn meal, coconut cake and tobacco dust, rice bran was the suitable additive for increasing the yield of *Pleurotus sajor-caju* . Increasing concentration of rice bran from 5 to 10% resulted an increase of biological efficiency from 50 to 59%.

Studies conducted by Smith *et al*. (1987) indicated that *Auricularia polytricha* can be cultivated by axenic culture method using sterilized sawdust supplemented with rice bran. Li *et al*.(1988) reported enhanced yield of paddy straw mushroom by supplementing with 5 % wheat bran followed by 10 % cotton

hull. Bhandal. and Mehta (1989) reported the cultivation of *Auricularia polytricha* on wheat straw supplemented with 4% rice bran.

Enhancement of rice straw with soybean flour gave the highest yield (79% BE) of *Pleurotus* (Mahmoud and El – Kattan, 1989).

Tan and Chang (1989) reported addition of 10 per cent wheat flour to sawdust media resulted in doubling of biological efficiency of *Lentinus edodes*. Similarly addition of tea leaves to saw dust enhanced yield upto 6–7 folds.Azizi *et al*. (1990) suggested cultivation of *Pleurotus sajor-caju* on sugarcane bagasse fortified with 1% ammonium sulphate and 0.5% KH_2PO_4. Four agricultural wastes namely oil palm fruit pericarp fibre, rice husk, melon husk and coconut fruit fibre were supplemented with NPK fertilizer and used as substrate to cultivate *Pleurotus tuber regium*. Oil palm fruits pericarp fibre at 1% level gave the maximum yield (Isikhuemhen and Okhuoya, 1990).

Supplementing mushroom beds at spawning with extra organic nitrogen 0.3 -0.6% of dry substrate, increases yield in both *Pleurotus sajor-caju* and *Pleurotus florida* (El- Kattan *et al*., 1991). Paddy straw supplemented with wheat bran recorded high yield of *Pleurotus eous* and lower yield with paddy straw, sugarcane bagasse mixture (Gupta *et al.*, 1991).

Supplementation of neemcake @ 5% reduced number of days of spawn run and maturity in all *Pleurotus* sp. Concentration above 5% had detrimental effect on yield (Nallathambi , 1991) . Savalgi and Savalgi (1991) demonstrated higher mushroom yield of *Pleurotus sajor-caju* ,*Pleurotus florida , Pleurotus ostreatus* when cotton waste substrates were supplemented with 4% rice bran and 2% soya dal powder .

According to Trivedi *et al*. (1991) the best substrate for the cultivation of *Calocybe* was chemically sterilized wheat straw supplemented with maize meal. Supplementation with cotton seed meal @ 1 and 2% on compost on dry weight basis gave higher yield by 20 -30% of *Agaricus* (Gupta and Vijay, 1992).

Thakur and Bhandal (1993) reported wheat straw supplemented with wheat bran is the most suitable substrate for the cultivation of *Auricularia polytricha* and it gave good biological efficiency.

Geetha and Sivaprakasam (1994) reported neem cake and cotton seed as amendment for enhanced yield of *Pleurotus* sp. They also stated increase in concentration of organic amendments above 4% was detrimental to yield.

Sharma *et al*.(1994) investigated on the supplements suitable for cultivation of *Calocybe* and found that maize meal , rice husk , coconut husk were suitable . Addition of salts of ferrous sulphate and zinc sulphate also increased the biological efficiency. Kathe *et al*. (1996) observed improved yield of *Pleurotus sajor-caju* with 3% soyabean supplementation. Neem cake at 2% rate of supplementation on paddy straw reduced the time taken for spawn run and hastened the maturity of sporophores of *Pleurotus sajor-caju* (Hazarika, 1998).

Among the different supplements viz wheat bran , maize grain powder , rice bran , mahua cake and neem cake (5%) , neem cake supplemented substrate gave maximum yield of *Pleurotus citrinopileatus* and reduced the number of days taken for spawn run (Srivastava and Singh , 1999) .

Upadhyay (1999) reported higher yields of *Auricularia mesenterica* with unsupplemented wheat straw as substrate followed by supplementation with wheat bran addition. He also observed the supplementation *Auricularia mesenterica* wih rice husk inhibited the growth and development of basidiocarps.

Balakrishanan and Das (2001) reported high biological efficiency of *Calocybe* by cultivation on spent mushroom substrate supplemented with 20%rice bran. Supplementation of paddy straw with pigeon pea dal at 5% on dry weight basis during spawning gave the highest yield and maximum biological efficiency followed by rice bran in cultivating *Pleurotus* sp. (Dubey , 2001) . Wheat straw compost supplemented with soybean cake resulted in maximum yield of *Agaricus* over control (Saharan and Guleria, 2001).

Chemically treated coir pith supplemented with neem cake (10%) or rice bran (10%) increased the yield of *Pleurotus florida* and reduced the time taken for mushroom production (Geetha *et al*., 2002). Deoiled soybean meal @ 2 % dry weight of substrate resulted in significantly higher yield of *Pleurotus* (Wange *et al*., 2002). Eswaran and Susan (2003) reported addition of maize flour (5%) to the paddystraw hastened the vegetative growth and sporophore yield of *Calocybe indica* .

Tabata and Ogura (2003) reported the growth of *Auricularia polytricha* in a sawdust medium supplemented with calcium or magnesium salts. On sawdust medium, the supplementation with calcium phosphate, calcium carbonate, magnesium sulfate or magnesium carbonate resulted in good mycelial growth. The calcium content of fruit body grown on the sawdust medium was increased 1.1–1.5 times by supplementation of 1–5% of calcium phosphate or calcium carbonate. The magnesium content was increased 1.7–2.2 times by 0.5% of magnesium carbonate, magnesium hydroxide, magnesium sulfate and magnesium chloride.

Zacharia and Doshi (2004) evaluated effect of different supplements (cotton linter, tremitorium soil, coconut husk, dehydrated lucrene, maize meal, rice bran and wheat bran at different concentration on the yield of *Tricholoma crassa*. Maximum fruiting bodies were obtained when supplemented with cotton linter (10%). Sporophores failed to develop in lucrene, maize meal, rice bran, and cotton seeds banana pseudostem and wheat bran due to weakened vegetative growth prior to casing.

Senthil Kumar *et al*., (2005) reported groundnut oil cake as a suitable supplement compared to neem cake in maximizing the biological efficiency of *Pleurotus sajor-caju ,Pleurotus djamor , Pleurotus eous* . Additives like chicken manure, farmyard manure, horsegram, soyabean and their combinations were tried for the cultivation of oyster mushroom. Paddy straw supplemented with

horse gram and chicken manure produced maximum yield of *Pleurotus sajor-caju* (447 g),*Pleurotus djamor*(462 g) , *Pleurotus eous*(480 g) .

Sharma *et al*., (2006) reported addition of 10% wheat bran in sawdust resulted in quickest spawn run and highest biological efficiency in *Flammulina velutipes* . Other supplements (soybean meal, cotton seeds cake and deoiled soybean) resulted in reduced linear growth.

Garasiya *et al*., (2007) reported the addition of 5% wheat bran to wheat straw substrate recorded the maximum yield of *Auricularia polytricha* when compared with other agro –waste substrates like sawdust, wheat straw, 2%wheat bran etc.

2 .4 NUTRITIVE VALUE

Chang and Chan (1973) observed more protein content in pileus than the volva in *Volvariella volvaceae*. Stipe and volva exhibit a more or less similar pattern in protein content and electrophoretic spectra while the pileus possessed a unique pattern. Certain protein bands exist only in pileus at specific developmental stage. 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 vegetables and fruits and calorific content is relatively low (Cheng and Tu, 1978).

Kurtzman (1979) reported that *Auricularia* sp. was rich in protein. Each 100 g contained 10.6 g protein, 357 mg Ca, 201 mg P, 185 mg Fe. It was known

to contain carotene, vitamin B1, vitamin B2, mannan, glucuronic acids, lecithin, cephalin.

According to Chan (1981) the young fruiting body of *Auricularia polytricha* contained 4.8 % total solublesugar, 4.9 %lipid, 3.4 % crude fibre, 4.7 % ash and 9.3 % protein. On fresh weight basis it had moisture content 97.1 %.The mature fruiting body of *Auricularia polytricha* contained 5.9 % total solublesugar,5.2 % lipid, 3.5 % crude fibre , 5.2 % ash and 8.5 % protein . On fresh weight basis it had moisture content 95.6 %.

Nutritive value of *Auricularia* sp.was accounted as 89.18 % moisture (fresh weight basis), 8.3 % crude protein, 82.8 % crude fat, total carbohydrate 63.0 %, nitrogen free carbohydrate 19.8% crude fibre 4.7 % ash 3.51 %. (Chang and Hayes, 1978., Chang and Miles, 1991).

On dry weight basis *Auricularia polytricha* has high protein (7.59 %), followed by fibre (3. 69 %) and lowest in fat (1. 12 %). Appreciable amount of calcium and zinc are also present in the fruiting body of *Auricularia polytricha* (Lu and Tang, 1986).

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

Compared with other mushrooms *Calocybe* have a nutritive value of 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* 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 *Tricholomma 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 immuno modulatory protein was purified from fruiting body of an edible Jew's ear mushroom by extracting using 5 % cold acetic acid in the presence of 0.1 % 2-mercaptoethanol , followed by ammonium sulphate fractionation.

Zhang *et al*. (2006) observed fruiting bodies of *Auricularia auriculae* are rich in polysaccharides having anti oxidant properties.

2.5 ORGANOLEPTIC STUDIES

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* .

Materials and Methods

3. MATERIALS AND METHODS

3. COLLECTION AND ISOLATION OF *Auricularia* spp.

3.1 COLLECTION OF MUSHROOM FLORA

A survey was conducted in different parts of Thiruvananthapuram district during June-November 2007 to collect different strains of *Auricularia* spp under natural conditions during rainy and post rainy seasons. The forest ranges, meadows, fallen wooden logs were surveyed regularly during rainy and post rainy days for the collection of mushrooms. The collected specimens were brought to the laboratory, packed in polythene covers and subjected to various studies.

Isolation and maintenance of these cultures were carried out by adopting tissue culture method. Healthy, medium sized mushroom sporocarp was taken and its surface was wiped with cotton dipped in 70 per cent alcohol. The mushroom was split longitudinally and a small tissue from the newly exposed split surface was scooped out with the help of a sterile forceps 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 ± 4 oc) for four days. These isolates were then purified by the hyphal tip method and maintained on PDA slants by periodic subculturing.

Identification of native strains of native *Auricularia* spp was carried out following the procedure outlined by Nair (1990). Comparison of the morphological characters was done following the published works in literature (Natarajan and Manjula , 1978; Purkayastha and Chandra , 1985; Suharban ,1987; Balakrishnan , 1994)

Specimens were collected at different stages of development and general observations like locality , type of substrate , date of collection etc were recorded

in the field itself. The specimens were then taken to the laboratory after wrapping in waxed paper sheet for further studies. The collections were serially numbered. The detailed characters were recorded following the techniques and proforma developed by Nair (1990). The proforma is given in Appendix I.

Spore prints were prepared by taking the fruiting body and pileus was placed on a piece of white / black paper with lower surface facing downwards and kept under a bell-jar for two hours at room temperature. After two hours the pileus was removed carefully, the sporeprint dried and sealed within plastic cover and characters studied.

The micro characters were studied with free hand sections mounted in lactophenol and by tissue maceration.

Macrochemical and metachromatic reactions of various parts of the basidiocarps were studied following the methods perfected by Walting (1971). The test was carried out on the surface context of pileus and base. For this fresh tissue (one cm square) was dissected out and placed in the depression of porcelain plate. A few drops of Melzer's reagent were applied and the reaction indicated by colour changes was recorded. Melzer's reaction of sporemass was detected following the methods of Waltling (1971). Small portion of spore was transferred to a clean microscopic slide and mounted in Melzer's solution and colour change noted under microscope. The reaction was graded as amyloid if the spores were coloured blue black to dark violet and non-amyloid if the colour change was yellow brown. All the microscopic characters were recorded by the drawings using a camera lucida attached to the microscope.

3.2 ISOLATION AND PURIFICATION OF NATIVE STRAINS OF *Auricularia*

Tissue Culture Technique

The jew's ear mushrooms obtained from different parts of Trivandrum were isolated using standard technique for tissue isolation. The mushrooms were cleaned up to remove adhering soil particles. A fresh mushroom was surface sterilized using ethanol. In the laminar flow chamber, the mushroom was longitudinally split into equal halves from the pileus and a small piece of tissue was removed using a sterile scalpel. The tissue was placed in petridish containing potato dextrose agar medium (PDA) under aseptic conditions .Eight to ten such pieces were placed in different petridishes . The dishes were incubated at room temperature 28 ± 2°C for seven days. The initial growth was transferred into PDA slants, and purified by hyphal tip method.

3.3 IDENTIFICATION AND SCREENING OF ISOLATES

3.3.1 Growth of *Auricularia* spp. on solid media under laboratory condition

Six different solid media namely potato dextrose agar (PDA) ,oat meal agar (OA), carrot agar (CA), tapioca agar medium (TA), czapek's dox agar (CDA) and richards' medium (RM) were used to find out the best medium for the growth of *Auricularia* spp. The composition of the media used is given in Appendix II. The media were prepared and sterilized by autoclaving at 1.02 kg cm^2 for 15-20 minutes. The cooled media before solidification were poured into sterile petridishes of 9 cm diameter and allowed to solidify. Culture disc of 5 mm

diameter cut out from seven day old culture was placed in the centre of the medium and incubated at room temperatures. Colony diameter was measured when the growth was completed in any of the petridishes for each fungus. The experiment was replicated thrice.

3.3.2 Effect of different temperature on the growth of *Auricularia* spp.

In order to assess the optimum temperature for the maximum growth of *Auricularia* spp , five millimeter culture discs of actively growing seven day old culture of this fungus was inoculated in 50 ml PDA broth and incubated at 25 , 30, 35 and 40 ˚C. After 10 days of incubation the mycelial mat was filtered, dried at 70˚C and dry weight was taken till two consecutive weights were equal. Three replications were kept for each treatment.

3.3.3 Effect of different pH on the growth of *Auricularia* spp.

The pH of PDA broth was adjusted to 4,5,6,7 and 8 by using 0.1 N NaOH. Fifty ml of each medium was taken in 100 ml conical flask and autoclaved at 1.02 kg cm² for 15-20 minutes. The medium was inoculated by a five millimeter culture disc of seven day old culture of the *Auricularia* spp. and incubated at room temperature for 10 days. The mycelial mat was filtered, dried at 70˚C and dry weight was taken till constant weight was obtained. Three replications were maintained.

3.3.4 Effect of different carbon sources on the growth of *Auricularia* spp.

Auricularia was grown in media with different carbon sources *viz,* sucrose, lactose, glucose and mannitol were substituted for dextrose in potato dextrose medium without agar . Fifty ml of each medium was then inoculated by 5 mm culture discs of actively growing culture, incubated at room temperature (28 ± 2˚C). The mycelial mat was filtered after two weeks and dry weight was taken after drying at 70˚C till a constant weight was obtained. Three replications were maintained in each case.

3.3.5 Effect of different nitrogen sources on the growth of *Auricularia* spp.

Auricularia was grown in media with different nitrogen sources *viz,* asparagine, ammonium nitrate , ammonium carbonate , ammonium chloride were substituted in Czapeks medium as to give the same percentage of nitrogen in each case . Fifty ml of sterilized medium was inoculated at room temperature for 14 days . The mycelial mat was filtered through a Whatman No: 1 filter paper and dry weight was taken after drying at 70˚C till a constant weight was obtained . Three replications were maintained in each case.

3.3.6 Effect of light and darkness on the growth of *Auricularia* spp.

In order to assess the requirement of light for the maximum growth of *Auricularia* spp , five millimeter culture discs of actively growing seven day old culture of this fungus was inoculated in 50 ml PDA broth and incubated at normal day light , under fluorescent lamp and dark conditions . After 10 days of incubation the mycelial mat was filtered, dried at 70˚C and dry weight was taken till two consecutive weights were equal. Three replications were kept for each treatment.

3 .4. CULTIVATION OF *Auricularia*

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

3 .4 .1 Preparation of grain spawn

Spawn was prepared using paddy grains and maize grains. 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 50 -60 g /kg of seeds. The filled bags and bottles were sterilized at 1.02 kg cm $^{-2}$ for two hours in an autoclave. The mycelial bits from seven day old actively growing pure culture of *Auricularia* were inoculated aseptically and incubated at room temperature (29 ± 2˚C). Three replications were maintained in each case and 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.

3 .4 .2 Preparation of sawdust spawn

Sawdust of various trees like rubber, mango, anjili, jack , coconut and rubber were used for the preparation of spawn .The sawdust spawn was prepared using the formulae of Sawdust 79 %, wheat bran 20 %, calcium carbonate 1 %, and water 65 %.

All the substrates were 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 cm $^{-2}$ for two hours in an autoclave. The mycelial bits from seven day old actively growing pure culture of *Auricularia* were inoculated aseptically and incubated at room temperature (29 ± 2˚C). Three replications were maintained in each case and mycelial growth of fungi were measured and recorded.

3 .4 .3 Time taken for maximum spawn run on different spawn substrates

Grains like half boiled paddy grains and maize grains , rice bran ,wheat bran , coir pith compost and sawdust of various trees like rubber , mango , anjili , jack and rubber were used as substrates for the preparation of *Auricularia* spawn . The spawn substrate is prepared by following the above method. The best spawn substrate was determined based on criteria like time taken for spawn run and incidence of pests and competitor moulds.

3 . 5 . YIELD ON LOCALLY AVAILABLE CHEAP SUBSTRATES

The study was conducted to find out the biological efficiency of *A. polytricha* by using different locally available substrates. Beds were raised following the poly bag 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, rubber sawdust, coconut sawdust, mango sawdust, jack sawdust and anjili sawdust.

Paddy straw substrate was soaked in water overnight, taken out, excess water removed and the substrate was sterilized by boiling for about one hour. They were then air dried and used for the layout of beds.

Sawdust substrate was prepared by mixing sawdust with 2 % calcium carbonate retaining 60 % moisture content. The substrate was then sterilized at 1.02 kg cm $^{-2}$ for two hours in an autoclave. After cooling spawning was done.

The sterilized substrates were used for bed preparation. Polybag method of cultivation was adopted. The substrate paddy straw made into a twist was placed in polybag as a layer. Layer spawning was done along with the periphery which was repeated four times. Thorough mixing of spawning was done in the case of sawdust substrate .Polythene bags were made compact tied at the top, and also provided 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 % and by opening the bag from top to get a good yield . The best substrate was selected based on criteria *viz,* time taken for spawn run , mushroom production , number of sporophores produced, total yield and incidence of pests and competitor moulds.

3 .6 . EFFECT OF FOOD SUPPLEMENTS ON THE YIELD

The food supplements used for the experiment included rice bran and wheat bran. The supplements rice bran and wheat bran were mixed @ 20 g / kg of substrate and 40 g / kg of substrate for laying out the beds. The beds were laid in polythene bags of 60 × 45 cm using the substrate. Spawning was done. Polythene bags were made compact tied at the top, and also provided 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 % and by opening the bag

from top to get a good yield . The best substrate was selected based on criteria like time taken for spawn run , mushroom production , number of sporophores produced , total yield and incidence of pests and competitor moulds.

3.7. ESTIMATION OF NUTRIENTS / PROXIMATE CONSTITUENTS

3.7.1 Estimation of Moisture content (%)

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

3.7.2 Estimation of ash

Three gram sample was transferred to a weighed silica dish. It was heated on a bunsen burner at a low flame and when the substance charred the dish was transferred to a muffle furnace. It was heated at 500 -550 ºC for about two hours till a white ash was obtained. It was then cooled in a dessicator and weighed. The difference between these two gives the result which is converted into %.

3.7.3 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 30 minutes, by adding bumping chips. The digested sample was filtered through a muslin cloth and washed with boiling waste until the washings were no longer acidic. The sample again boiled with 100 ml sodium hydroxide (1.25 %) for 30 minutes. 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 pre weighed ashing dish (W1). The residue was dried at 130˚C for two hours, cooled the dish in dessicators and weighed (W2). The residue was further ignited at 600 ˚C, which was cooled and weighed.

% Crude fibre = Loss in weight / Weight of the sample X 100

3.7.4 Estimation of Proteins (µg)

Protein content was estimated using method described by Bradford (1976). One gram sample was ground in 10 ml of 0.1 M acetate buffer (pH 4.7). The material was centrifuged at 5000 G for 15 minutes at 4 ˚C temperature. The supernatant obtained was used for further analysis. The reaction mixture consisting of 0.5 ml extract , 0.5 ml of distilled water and 5 ml of coomassie brilliant blue G -250 . The reaction mixture was assayed for the absorbance at 595 nm against reagent blank standard graphs was 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.

3.7.5 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 three 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 hundred ml and centrifuged at 5000 rpm for 15 minutes. The supernatant was collected, was used as an aliquot for analysis. From the supernatant 0.5 ml aliquot was taken and made up to one 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 specrophometer 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.

3.7.6 Estimation of total free aminoacids

Total free aminoacid content was estimated by a method described by Balasubramanian and Sadasivam (1987).

500 mg of the sample was weighed and ground in a pestle and mortar with small quantity of acid – washed sand. To this homogenate 5 to 10 ml of 80 % ethanol was added and centrifuged. Suppernatant was saved. To .1ml of this extract 1 ml of ninhydrin solution was added and the volume was made up to 2 ml with distilled water. The tube was heated in a water bath for twenty minutes. 5 ml of diluent was added and the content was mixed after 15 minutes the intensity of purple colour was read against the reagent blank in a colorimeter at 570 nm. The standard was prepared by dissolving 50 mg leucine in 50 ml of distilled water. The standard curve was drawn using absorbance Vs concentration. The concentration of total free aminoacids was found and expressed as percentage equivalent of leucine.

3.7. 7 Estimation of Fat

The extraction of fat was carried out using Soxhlet extraction apparatus(Moore and Stein, 1948). 5 g of sample was weighed in to an extraction thimble and placed it in the extractor so that top of the thimble is over the bent siphon tube out side extractor. The extractor was connected to previously weighed extraction flask. Sufficient quanity of petroleum ether was poured in to the extractor. The extractor was attached to the condensor with a constant flow of cold water. The flask was heated on a water bath. The extraction was carried out till the liquid became colour less. The flask was removed and the solvent was evaporated in an oven at 105^0c. It was dried to a constant weight. The increase in weight of flask was the fat obtained.

3 .8 .ORGANOLEPTIC CHARACTERS

Auricularia mushroom cultivated were subjected to studies of organoleptic characters like colour and appearance, texture, flavour and taste. Four different recipes viz ., *Auricularia* soup , *Auricularia* fritters , *Auricularia* scramble , *Auricularia* masala were prepared and they were subjected to evaluation by 10 judges based on a score card and subjected to Kruskal Wallis test for analysis . The average ranking was given for each character. Score card values for each character was given in Table 19.

The comparative quality test of both raw and cooked mushrooms was done following Kruskal Wallis test using the same scoring test. The overall

acceptability of cooked mushroom was noticed based on the evaluation done by judges.

Results

4.RESULTS

4.1 COLLECTION AND ISOLATION OF NATIVE STRAINS OF *Auricularia* spp.

A survey was conducted in different parts of Thiruvanthapuram district during June – November 2007 and several native strains of *Auricularia* spp. were collected . The native strains of *Auricularia* spp. were obtained from different locations of Thiruvananthapuram district *viz,* Vellayani , Balaramapuram , Kalliyoor , Palappur and Vanchiyoor. The habitat of these native strains varied from solitary to gregarious types and they were terrestrial . It was also observed that coconut tree basin, fallen wooden logs were usual spots for the occurrence of *Auricularia* spp. The collected native strains were described and identified as per the procedure outlined by Nair (1990) and brought them into pure culture (Table 1).

4.1.1 Morphological Characters of Newly Collected Native Isolates

Macro characters and micro characters.of the newly collected native species of *Auricularia polytricha* (Mont.) Sacc. were studied in detail as explained under materials and methods .The explanation of scientific terms were given in Appendix III .

4.1.2 *Auricularia polytricha* (Mont.) Sacc.

The native strains of *Auricularia polytricha* were collected from Vellayani, Balaramapuram, Kalliyoor Palappur and Vanchiyoor panchayats of Thiruvanathapuram district during June- November 2007.

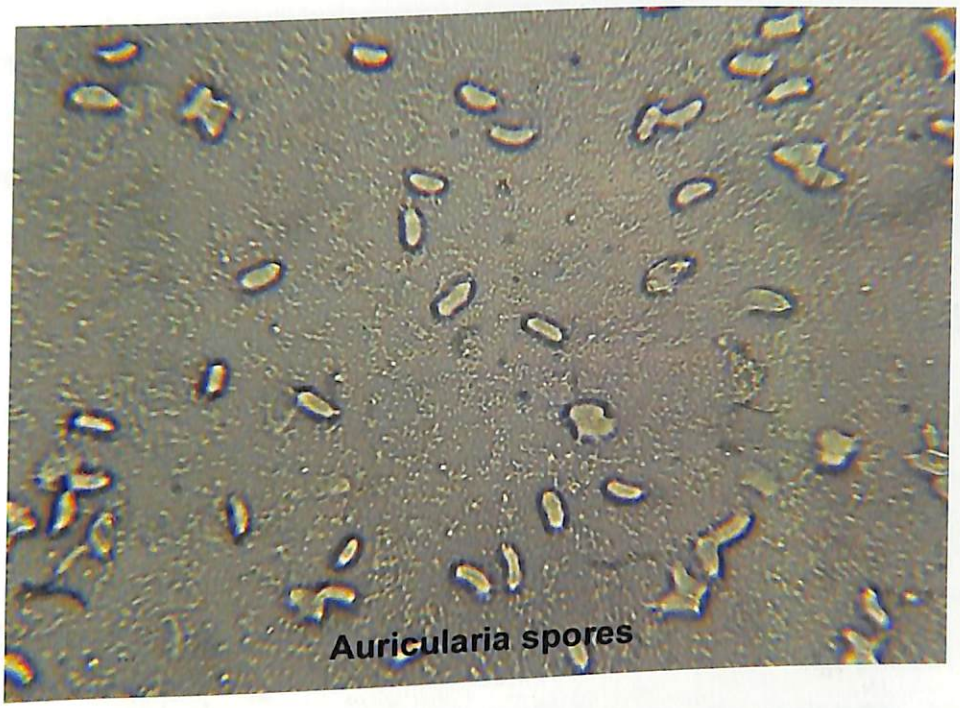
Habitat : Sporophores in groups on mango tree,anjili tree,coconut tree basin and dead wooden logs , caespitose , imbricate

Table 1 Native strains of *Auricularia* spp. collected

Sl.no.	Name of *Auricularia* spp. collected	Substrate	Location and period of collection
1	*Auricularia polytricha* (Mont.) Sacc.	Dead stumps of mango tree	Kalliyoor and Palappur , June 2007
2	*Auricularia polytricha* (Mont.) Sacc.	Coconut tree basin	Balaramapuram, June 2007
3	*Auricularia polytricha* (Mont.) Sacc.	Dead stumps of mango tree	Vellayani and Balaramapuram , July 2007
4	*Auricularia polytricha* (Mont.) Sacc.	Dead stumps of ajinli tree	Vellayani , Palappur and Balaramapuram , July 2007
5	*Auricularia polytricha* (Mont.) Sacc.	Dead stumps of mango tree	Kalliyoor , Balaramapuram , Vanchiyoor and Palappur, October 2007
6	*Auricularia polytricha* (Mont.) Sacc.	Dead woods	Vanchiyoor, Vellayani and Palappur , October 2007
7	*Auricularia polytricha* (Mont.) Sacc.	Fallen wooden logs	Vanchiyoor , November 2007

Plate 1 Native strain of Auricularia spp.collected

Plate 2 Morphological characters of Auricularia spp.collected



Pileus : 3- 10 cm diameter , generally concolorous , initially spathulate , ear shaped to flabelliform when old , coriaceous , glabrous , margin incurved , non squamous and non squarrose . hygrophanous ,rubbery textured ,uppersurface is smooth to wrinkled , to velvety , with lower surface smooth to veined , glabrous , greyish brown to purplish brown , overall white to the tomentum .

Stipe : absent , even if present very small, 0.5-1 cm length and 1-1.5 cm diameter. Volva and annulus absent .

Spores : basidia elongated , cylindrical with three tranverse septae . spores are white , sausage shaped and quite large . 16 -18 × 6 -8 μ m . spore print is white .

4.1.3 Microchemical reagents

These reagents enabled to identify the mushrooms

4.1.4 Melzers reagent

The treatment of spores taken from sporeprint with melzers reagent showed dextrinoid reaction by changing the spore wall colour to pink to purple.

4.1.5 Cotton blue stain

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

4.2 ISOLATION AND PURIFICATION OF CULTURE

The tissue isolation of the isolates was done as per the standard method of tissue culture technique described earlier (3.2) and the cultures were maintained on PDA slants by periodical subculturing .

4.3 Cultural studies of *Auricularia* spp.

4.3.1 Effect of different solid media on the growth of *Auricularia* spp.

In general , *Auricularia* spp. prefer natural media for their growth and growth in synthetic media is very poor . Six different solid culture media namely potato dextrose agar (PDA) medium , oat meal agar(OA) medium , carrot agar medium , tapioca agar medium , Richards' medium and Czapeks dox medium were tested for their efficacy in supporting the radial mycelial growth of *Auricularia* spp. The result showed that the media significantly differed in influencing mycelial growth of *Auricularia* spp. (Table 3).

The nature of mycelial growth of *Auricularia* spp. was very much fluffy, white with a purplish tinch in PDA followed by oat meal medium . The growth pattern in PDA and OA was very thick with no fruiting body production in both cases . In Richards' medium and Czapeks dox medium the growth pattern was sparse . They were found to be inferior in supporting the growth as it was feeble in nature . In carrot agar medium , tapioca agar medium the nature of growth was thin (Plate --) .

The radial growth of *Auricularia* spp. after seven days indicated that PDA was superior with 9.0 cm of radial growth . The OA was found to be on par with PDA in supporting the radial growth having 8.93 cm . Tapioca agar medium and carrot agar medium were found to be on par with OA on radial growth having 8.86 and 8.83 cms of radial growth respectively .

4.3.2. Effect of different liquid media on the growth of *Auricularia* spp.

The biomass production of *Auricularia* spp. was estimated in different liquid broths *viz,* potato dextrose broth , oat meal broth , carrot broth , tapioca broth , Richards' broth and Czapeks dox broth (Plate ___) . The result showed that liquid media differed significantly in influencing biomass production of *Auricularia* spp. (Table 4) .

The radial growth and biomass production of *Auricularia* spp. after seven days of incubation indicated that oat meal broth was superior with 0.88 g / 100 ml of biomass production . The biomass production with potato dextrose broth was found to be on par with oat meal broth with 0.856 g / 100 ml . The biomass production on carrot was found to be 0.826 g and it was on par

with tapioca . The rest of the treatments ie , growth on carrot broth , tapioca broth , Richards broth and czapeks dox broth differed significantly .

4.3.3 Effect of different carbon sources on the growth of *Auricularia* spp.

Five different carbon sources namely lactose , glucose , sucrose , mannitol , dextrose and a control medium were tested for their efficacy in biomass production of *Auricularia* spp. The result showed that different carbon sources differed significantly in influencing biomass production of *Auricularia* spp . (Plate --- and Table 5) .

The carbon source sucrose supported maximum biomass production with 1 g /100 ml. Glucose supported a biomass production of 0.966 g /100 ml and it was found to be on par with lactose that supported biomass production of 0.976 g /100 ml . The other carbon sources *viz,* mannitol and dextrose produced 0.893 g and 0.903 g respectively . The medium with carbon source as control produced 0.833 g of biomass .

4.3.4 Effect of different nitrogen sources on the growth of *Auricularia* spp.

The nitrogen sources evaluated included different inorganic sources like asparagine , sodium nitrate ($NaNO_3$),ammonium carbonate (NH_4CO_3) and ammonium chloride (NH_4Cl) .A medium was also maintained with nitrogen source as control . The result showed that different nitrogen sources differed significantly in supporting biomass production of *Auricularia* spp. (Plate ---- and Table 6) .

Among the various nitrogen sources asparagine was found to be the best nitrogen source producing the highest biomass of 0.99 g /100 ml . Sodium nitrate produced a biomass of 0.89 g and it was found to be on par with ammonium chloride . Ammonium carbonate produced a biomass of 0.87 g . The medium with nitrogen source as control recorded the least biomass production having 0.84 g /100 ml .

Table 2 Growth characters of *Auricularia* spp. on different solid media after seven days of incubation .

Sl. no	character	Potato dextrose agar (PDA)	Oat meal agar (OA)	Richards medium (RM)	Czapeks dox medium (CDA)	Carrot agar (CA)	Tapioca agar (TA)
1	a	White fluffy with a purplish tinch	White fluffy with a purplish tinch	White fluffy with a purplish tinch	White fluffy with a purplish tinch	White fluffy with a purplish tinch	White fluffy with a purplish tinch
2	b	Thick	Thick	Sparse	Sparse	Thin	Thin
3	c	No	No	No	No	No	No

a) colour of mycelium
b) growth pattern
c) fruit body production

Plate 3 Growth of *Auricularia* in different solid media after seven days of incubation

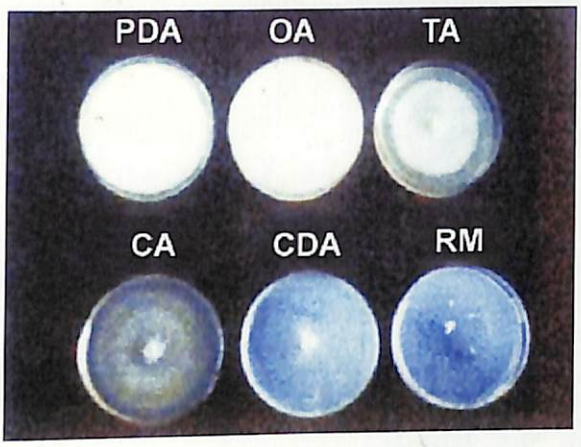


Plate 4 Growth of *Auricularia* in different liquid media after seven days of incubation

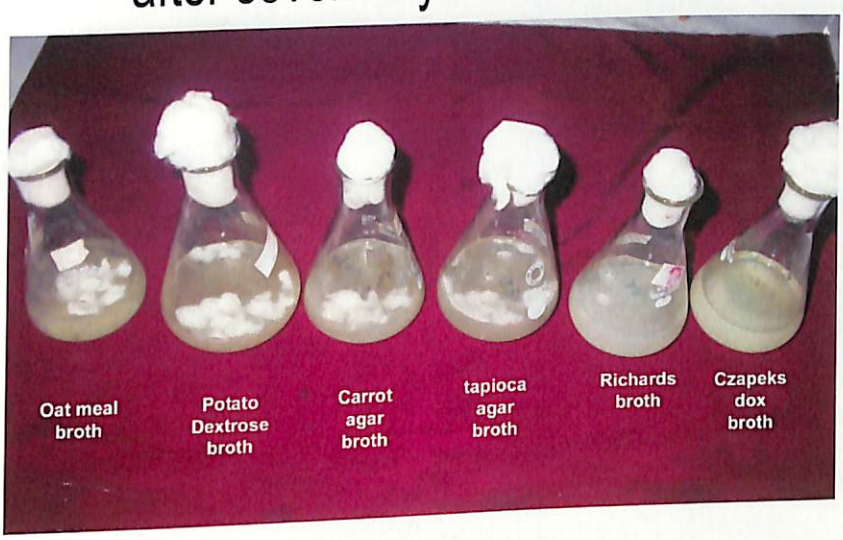


Plate 5 Effect of different carbon sources on the growth of *Auricularia* spp.

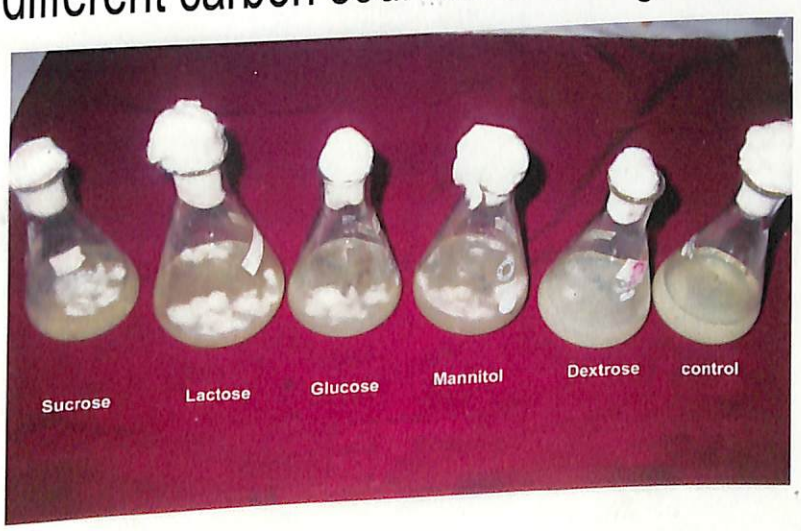


Table 3 Growth of *Auricularia* in different solid media after seven days of incubation (diameter in cm)

Media	Diameter (cm)
Potato dextrose agar medium (PDA)	9.0
Oat meal agar medium (OA)	8.93
Richards' medium (RM)	7.93
Czapeks dox medium (CDA)	7.93
Carrot agar medium (CA)	8.83
Tapioca agar medium (TA)	8.86
CD	0.138

Table 4 Growth of *Auricularia* in different liquid broth after seven days of incubation (dry weight in grams)

Sl.no.	Media	dry weight of mycelium (grams)
1.	Potato dextrose broth(PDB)	0.856
2.	Oat meal broth	0.876
3.	Richards' broth	0.543
4.	Czapeks dox broth	0.566
5.	Carrot agar broth	0.826
6.	Tapioca agar broth	0.823
	CD	0.0397

Table 5 Growth of *Auricularia* in different carbon sources after seven days of incubation (dry weight in grams)

Sl.no.	carbon sources	dry weight of mycelium (grams)
1.	Lactose	0.976
2.	Glucose	0.966
3.	Sucrose	1.0
4.	Mannitol	0.893
5.	Dextrose	0.903
6.	Control	0.833
	CD	0.134

Table 6 Growth of *Auricularia* in different nitrogen sources after seven days of incubation (dry weight in grams)

Sl.no.	Nitrogen sources	dry weight of mycelium (grams)
1.	Asparagine	1.02
2.	Sodium nitrate	0.893
3.	Ammonium carbonate	0.886
4.	Ammonium chloride	0.893
5.	Control	0.846
	CD	0.015

4.3.5 Effect of different pH conditions on the growth of *Auricularia* spp.

Five different H+ion concentration (pH) ranging from 5 to 9 was tested for their efficacy in the production of maximum biomass as given in Table 7 and Plate ---

It was observed that there was an increase in biomass production from pH 5 to 9 . Least biomass production was observed in pH 5 (0.87 g). pH 6 recorded a biomass production of 0.91 g . pH 7 gave a biomass production of 0.95 g .

Maximum biomass production was shown with pH 9 (0.99g) and pH 8 showed a biomass production of 0.986 g which was on par with pH 9 .

4.3.6 Effect of different temperature conditions on the growth of *Auricularia* spp.

Five different temperature conditions of 10 °c , 15 °c,20 °c , 25 ° c and 30 ° c were tested for their efficacy in the production of maximum biomass as given in Plate --- and Table 8 . The result showed that different temperature conditions differed significantly in influencing biomass production .

It was observed that temperature of 30 ° c supported maximum mycelial growth and biomass production of 0.89 g . the other temperature conditions of 25 ° c , 20 °c and 15 °c supported biomass production of 0.85 g , 0.82 g and 0.56 g respectively . The lowest biomass production was recorded with a temperature of 10 ° c having 0.54 g .

4.3.7 Effect of different light conditions on the growth of *Auricularia* spp.

Four different ranges of light like sunlight , roomlight , intermittent light and dark conditions were tested for their efficacy in the production of biomass as given in Plate – and Table 9 . The result showed that different light conditions differed significantly in influencing biomass production .

It was observed that room light gave maximum mycelial growth and biomass production of *Auricularia* spp. having 0.95 g . The dark conditions with no light provided recorded a biomass of 0.89 g . The intermittent light conditions of alternate light and dark recorded a biomass of 0.85 g . The direct sunlight provided recorded the least biomass production of 0.82 g .

Table 7 Growth of *Auricularia* in different pH conditions after seven days of incubation (dry weight in grams)

Sl.no.	pH	dry weight of mycelium (grams)
1.	5.0	0.873
2.	6.0	0.906
3.	7.0	0.95
4.	8.0	0.986
5.	9.0	0.993
	CD	0.022

Table 8 Growth of *Auricularia* in different temperature conditions after seven days of incubation (dry weight in grams)

Sl.no.	Temperature (ºC)	dry weight of mycelium (grams)
1.	10	0.543
2.	15	0.566
3.	20	0.823
4.	25	0.856
5.	30	0.893
	CD	.012

Table 9 Growth of *Auricularia* in different light conditions after seven days of incubation (dry weight in grams)

Sl.no.	light conditions	dry weight of mycelium (grams)
1.	sunlight	0.793
2.	Room light	0.95
3.	Intermittent light	0.856
4.	Dark ness	0.821
	CD	0.016

Plate 6 Effect of different nitrogen sources on the growth of Auricularia spp.

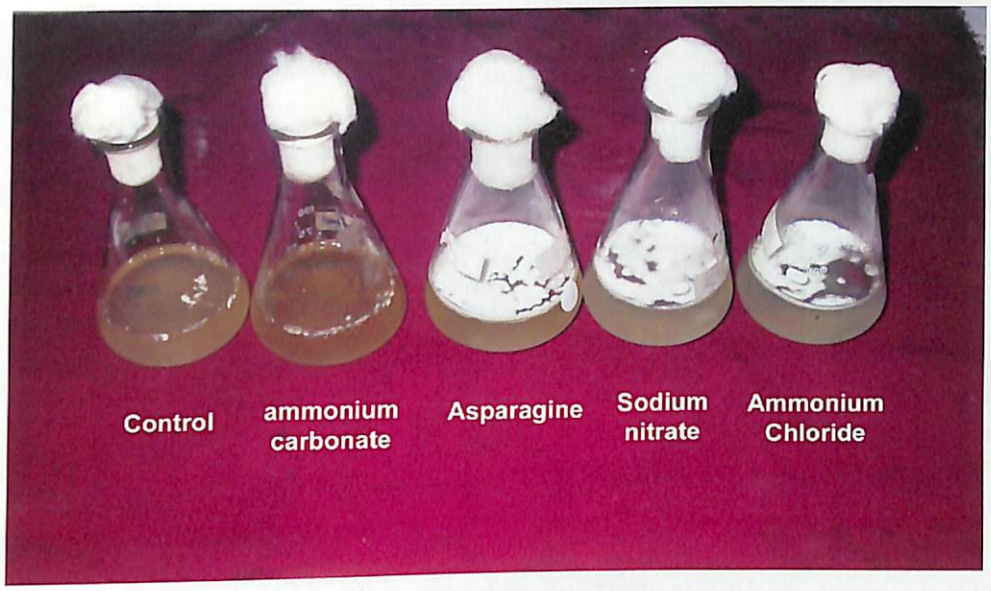


Plate 7 Effect of different pH on the growth of Auricularia spp.

Plate 8 Effect of different Temperature on the growth of Auricularia spp.

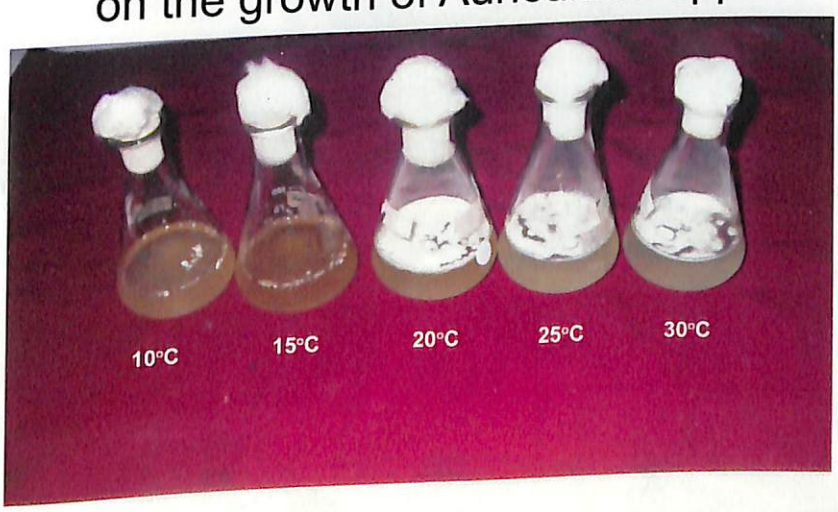


Plate 9 Effect of different light on the growth of Auricularia spp.

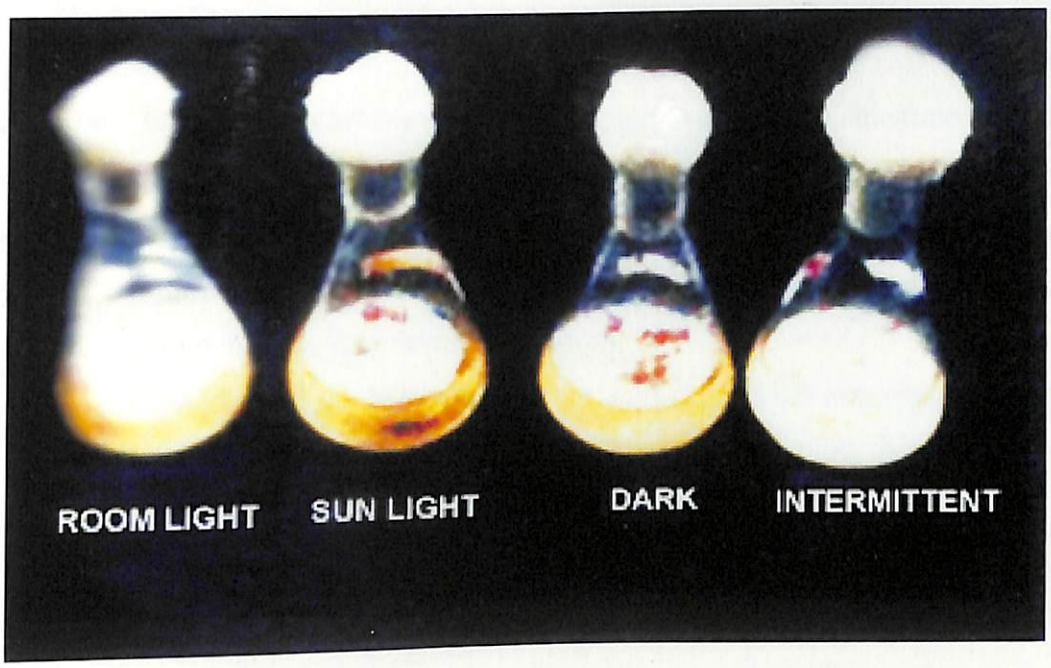


Plate 10 Yield of *Auricularia* on different spawn substrates

4.4 Time taken for maximum spawn run on different spawn substrates

Ten different spawn substrates were evaluated on the basis of number of days taken for maximum spawn run. The spawn substrates evaluated included both grain substrate and sawdust substrate. Grain substrates tried were maize grain, paddy grain, rice bran and wheat bran. Sawdust substrates tried were rubber sawdust, anjili sawdust, jack sawdust, mango sawdust and coconut sawdust .Coir pith compost was also tried as a spawn substrate. Grain substrate was found to be a better spawn substrate than sawdust substrate.

Maize grain was found to be the best substrate for spawn run with a minimum of 16 days required for fluffy growth of mycelium in the grains. The results showed that various spawn substrates differed significantly in number of days taken for maximum spawn growth (Table 10 and Plate 10). Other grain substrates like wheat bran, paddy grain and rice bran took a minimum of 17 days, 18 days and 22 days respectively for the maximum spawn run. Paddy grain was found to be on par with wheat bran.

Among sawdust substrates, rubber sawdust was found to be the best substrate for spawn run with a minimum of 18 days required for fluffy growth of mycelium covering the substrate. Coconut sawdust required a minimum of 19 days for the complete spawn run. Anjili sawdust and mango sawdust required a minimum of 20days for the complete spawn run. Coir pith was proved to be an inferior substrate as it provided no mycelial growth and there was no spawn run.

Table 10 Yield of *Auricularia* on different spawn substrates based on number of days

Sl.no.	Spawn substrates	Nature of mycelial growth	Time taken for spawn run (days)
1.	Rubber sawdust	++++	18
2.	Ajinli sawdust	++	20
3.	Jack sawdust	+++	23
4.	Mango sawdust	+++	20
5.	Rice bran	++	22
6.	Wheat bran	++++	17
7.	Paddy grain	++++	18
8.	Maize grain	++++	16
9.	Coir pith	-	-
10.	Coconut sawdust	+++	19
	CD		0.0135

++++ - thicker and fluffy growth

+++ - thick growth

++ - poor growth

4.5 Yield of *Auricularia* sp.on locally available cheap substrates

Six different locally available cheap substrates were evaluated on the basis of yield obtained on them. The substrates evaluated were paddy straw , rubber saw dust , anjili sawdust , mango sawdust , jack saw dust and coconut sawdust . The result showed that various substrates differed significantly in influencing the yield of *Auricularia* sp.(Table 11) .

It was observed that rubber sawdust when used as a substrate for the growth of *Auricularia* gave maximum yield of 180.67 g in three harvests . Mango sawdust , jack sawdust coconut sawdust and anjili sawdust gave yield of 173.93 g ,168.1 g , 158.3 g and 152 .36 g of *Auricularia* respectively in three harvests . The lowest yield of *Auricularia* (131.73 g) was recorded when paddy straw alone was used as a substrate .

4.6 Yield of *Auricularia* sp. on different food supplements along with paddy straw

Food supplements like wheat bran and rice bran at different levels of concentration like wheat bran @ 2.5% , wheat bran @ 5 % , rice bran @ 2.5 % , rice bran @ 5 %were supplemented with the substrate paddy straw and their efficacy in influencing yield of *Auricularia* on paddy straw was evaluated .Five different treatments like wheat bran @ 2.5% , wheat bran @ 5 % , rice bran @ 2.5 % , rice bran @ 5 % and a control treatment with no food supplement added were tested . The result showed that different treatments differed significantly in influencing the yield (Table 12) .

Maximum yield of *Auricularia* (192.86 g) was obtained when wheat bran @ 5 % was used as supplement to paddy straw substrate. Wheat bran @ 2.5 % gave a yield of 170 .2 g of *Auricularia* which was found to be on par with rice bran @ 5 % that gave an average yield of 172.63 g. The supplement rice bran @ 2.5 % gave a yield of 155.5 g . The lowest yield of 131.73 g of *Auricularia* was recorded when paddy straw was used alone with no supplements.

Plate 11 Yield of *Auricularia* on different locally available cheap substrates

Plate 12 Yield of *Auricularia* on different food supplements along with locally available cheap substrates

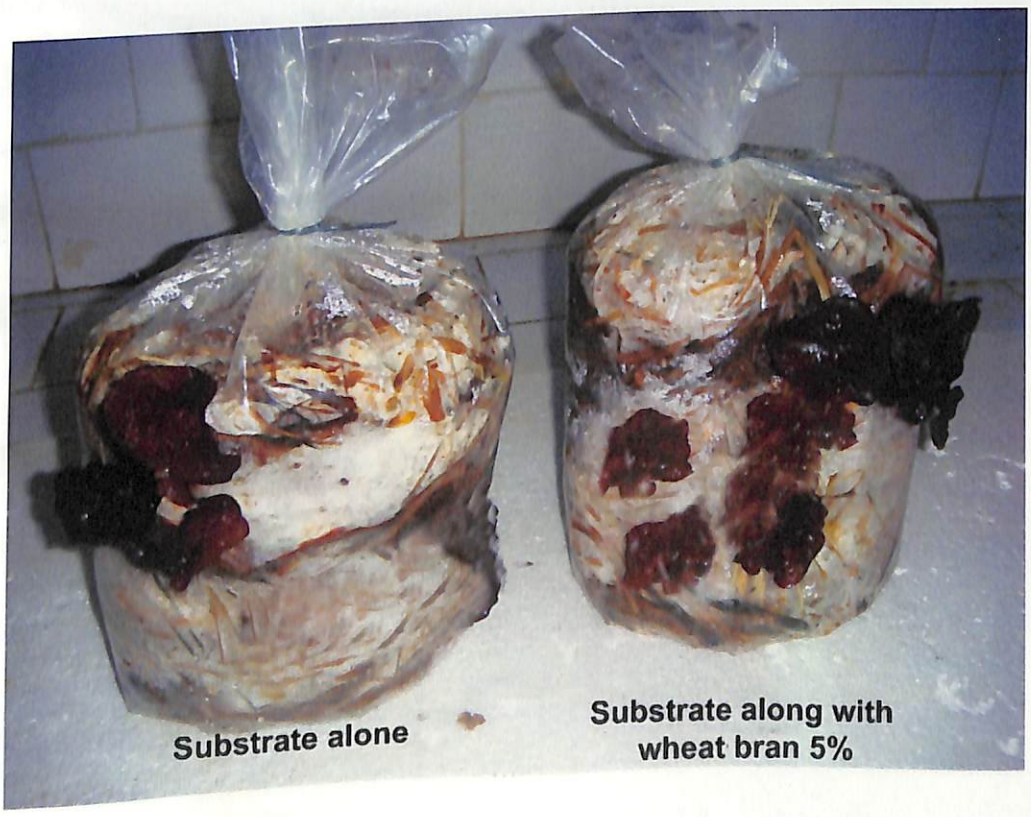


Table 11 .Yield of *Auricularia* on different locally available cheap substrates

Sl.no.	Substrates	Mean yield (grams) / 3 kg substrate
1.	Paddy straw	131.73
2.	Rubber sawdust	180.67
3.	Anjili sawdust	152.36
4.	Mango sawdust	173.93
5.	Jack sawdust	168.1
6.	Coconut sawdust	158.3
	CD	3.397

4.7 Yield of *Auricularia* sp.on different food supplements along with rubber sawdust

Food supplements like wheat bran and rice bran at different levels of concentration like wheat bran @ 2.5%, wheat bran @ 5 % , rice bran @ 2.5 % , rice bran @ 5 %were supplemented with the substrate rubber sawdust and their efficacy in influencing yield of *Auricularia* on rubber sawdust was evaluated .Five different treatments like wheat bran @ 2.5% , wheat bran @ 5 % , rice bran @ 2.5 % , rice bran @ 5 % and a control treatment with no food supplement added were tested . The result showed that different treatments differed significantly in influencing the yield (Table 13).

Maximum yield of *Auricularia* (274 g) was obtained when wheat bran @ 5 % was used as supplement to rubber sawdust substrate. Wheat bran @ 2.5 % gave a yield of 253.8g of *Auricularia* which was found to be on par with rice bran @ 5 % that gave an average yield of 253.43 g . The supplement rice bran @ 2.5 % gave a yield of 215.9 g . The lowest yield of 180.67 g of *Auricularia* was recorded when rubber sawdust was used alone with no supplements.

4.8 Yield of *Auricularia* sp.on different food supplements along with mango sawdust

Food supplements like wheat bran and rice bran at different levels of concentration like wheat bran @ 2.5%, wheat bran @ 5 %, rice bran @ 2.5 %, rice bran @ 5 %were supplemented with the substrate mango sawdust and their efficacy in influencing yield of *Auricularia* on mango sawdust was evaluated .Five different treatments like wheat bran @ 2.5% , wheat bran @ 5 % , rice bran @ 2.5 % , rice bran @ 5 % and a control treatment with no food supplement added were tested . The result showed that different treatments differed significantly in influencing the yield (Table 14) .

Maximum yield of *Auricularia* (263.43 g) was obtained when wheat bran @ 5 % was used as supplement to mango sawdust substrate. Wheat bran @ 2.5 % gave a yield of 243.63 g of *Auricularia* which was found to be on par with rice bran @ 5 % that gave an average yield of 245.1 g . The supplement rice bran @ 2.5 % gave a yield of 198.23 g . The lowest yield of 173.93 g of *Auricularia* was recorded when mango sawdust was used alone with no supplements.

Table 12 . Yield of *Auricularia* on different food supplements along with paddy straw substrate

Sl.no.	Food supplements	Mean yield (grams) / 3 kg substrate
1.	Paddy straw + wheat bran (2.5%)	170.2
2.	Paddy straw + wheat bran (5%)	192.86
3.	Paddy straw + rice bran (2.5%)	155.5
4.	Paddy straw + rice bran (5%)	172.63
5.	Paddy straw alone	131.73
	CD	3.265

Table 13 .Yield of *Auricularia* on different food supplements along with rubber sawdust substrate

Sl.no.	Food supplements	Mean yield (grams) / 3 kg substrate
1.	Rubber sawdust + wheat bran (2.5%)	253.8
2.	Rubber sawdust + wheat bran (5%)	274
3.	Rubber sawdust + rice bran (2.5%)	215.9
4.	Rubber sawdust + rice bran (5%)	253.43
5.	Rubber sawdust alone	180.67
	CD	7.693

Table 14 Yield of *Auricularia* on different food supplements along with mango sawdust substrate

Sl.no.	Food supplements	Mean yield (grams) / 3 kg substrate
1.	Mango sawdust + wheat bran (2.5%)	243.63
2.	Mango sawdust + wheat bran (5%)	263.43
3.	Mango sawdust + rice bran (2.5%)	198.23
4.	Mango sawdust + ricebran (5%)	245.1
5.	Mango sawdust alone	173.93
	CD	3.357

4.9 Yield of *Auricularia* sp.on different food supplements along with jack sawdust

Food supplements like wheat bran and rice bran at different levels of concentration like wheat bran @ 2.5% , wheat bran @ 5 % , rice bran @ 2.5 % , rice bran @ 5 %were supplemented with the substrate jack sawdust and their efficacy in influencing yield of *Auricularia* on jack sawdust was evaluated .Five different treatments like wheat bran @ 2.5% , wheat bran @ 5 % , rice bran @ 2.5 % , rice bran @ 5 % and a control treatment with no food supplement added were tested . The result showed that different treatments differed significantly in influencing the yield (Table 15) .

Maximum yield of *Auricularia* (249.1 g)was obtained when wheat bran @ 5 % was used as supplement to jack sawdust substrate . Wheat bran @ 2.5 % gave a yield of 232.36 g of *Auricularia* which was found to be on par with rice bran @ 5 % that gave an average yield of 231.5 g . The supplement rice bran @ 2.5 % gave a yield of 188.66 g . The lowest yield of 168.1 g of *Auricularia* was recorded when jack sawdust was used alone with no supplements.

4.10 Yield of *Auricularia* sp.on different food supplements along with coconut sawdust

Food supplements like wheat bran and rice bran at different levels of concentration like wheat bran @ 2.5% , wheat bran @ 5 % , rice bran @ 2.5 % , rice bran @ 5 %were supplemented with the substrate coconut sawdust and their efficacy in influencing yield of *Auricularia* on coconut sawdust was evaluated .Five different treatments like wheat bran @ 2.5% , wheat bran @ 5 % , rice bran @ 2.5 % , rice bran @ 5 % and a control treatment with no food supplement added were tested . The result showed that different treatments differed significantly in influencing the yield (Table 16) .

Maximum yield of *Auricularia* (240.66 g) was obtained when wheat bran @ 5 % was used as supplement to coconut sawdust substrate. Wheat bran @ 2.5 % gave a yield of 223.43 g of *Auricularia* and rice bran @ 5 % gave an average yield of 218.53g. The supplement rice bran @ 2.5 % gave a yield of 178.6g. The lowest yield of 158.3 g of *Auricularia* was recorded when coconut sawdust was used alone with no supplements.

Table 15 Yield of *Auricularia* on different food supplements along with jack sawdust substrate

Sl.no.	Food supplements	Mean yield (grams) / 3 kg substrate
1.	Jack sawdust + wheat bran (2.5%)	232.36
2.	Jack sawdust + wheat bran (5%)	249.1
3.	Jack sawdust + rice bran (2.5%)	188.66
4.	Jack sawdust + rice bran (5%)	231.5
5.	Jack sawdust alone	168.1
	CD	4.143

Table 16 Yield of *Auricularia* on different food supplements along with coconut sawdust substrate

Sl.no.	Food supplements	Mean yield (grams) / 3 kg substrate
1.	Coconut sawdust + wheat bran (2.5%)	223.43
2.	Coconut sawdust + wheat bran (5%)	240.66
3.	Coconut sawdust + rice bran (2.5%)	178.6
4.	Coconut sawdust + rice bran (5%)	218.53
5.	Coconut sawdust alone	158.3
	CD	3.714

4.11 Yield of *Auricularia* sp.on different food supplements along with anjili sawdust

Food supplements like wheat bran and rice bran at different levels of concentration like wheat bran @ 2.5% , wheat bran @ 5 % , rice bran @ 2.5 % , rice bran @ 5 %were supplemented with the substrate anjili sawdust and their efficacy in influencing yield of *Auricularia* on anjili sawdust was evaluated .Five different treatments like wheat bran @ 2.5% , wheat bran @ 5 % , rice bran @ 2.5 % , rice bran @ 5 % and a control treatment with no food supplement added were tested . The result showed that different treatments differed significantly in influencing the yield (Table 17) .

Maximum yield of *Auricularia* (231.26 g)was obtained when wheat bran @ 5 % was used as supplement to anjili sawdust substrate . Wheat bran @ 2.5 % gave a yield of 214.23 g of *Auricularia* and rice bran @ 5 % gave an average yield of 207.26 g . The supplement rice bran @ 2.5 % gave a yield of 166.1g. The lowest yield of 152.36 g of *Auricularia* was recorded when anjili sawdust was used alone with no supplements.

4.12 Nutritional value

The proximate constituents of *Auricularia* mushroom were evaluated by using standard techniques as described in 3.7 .The moisture content of *Auricularia* was found to be 89.18 %. Protein content present in the mushroom was estimated by using Bradford technique and it was found to be 8.2 %. Fat content present in the mushroom was estimated and it was found to have an approximate value of 1.22 %. Carbohydrate content present in *Auricularia* mushroom was estimated by anthrone method and it was found to have an approximate value of 63 %(fresh weight). The concentration of total free aminoacids was found and expressed as 21 %. Fibre content present in *Auricularia* mushroom was estimated and it was found to have an approximate value of 4.5 %. Ash content of *Auricularia* mushroom was estimated and it was found to have an approximate value of 3.41 %.

Table 17 Yield of *Auricularia* on different food supplements along with ajinli sawdust substrate

Sl.no.	Food supplements	Mean yield (grams) / 3 kg substrate
1.	Ajinli sawdust + wheat bran (2.5%)	214.23
2.	Ajinli sawdust + wheat bran (5%)	231.26
3.	Ajinli sawdust + rice bran (2.5%)	166.1
4.	Ajinli sawdust + ricebran (5%)	207.26
5.	Ajinli sawdust alone	152.36
	CD	5.064

Table 18 Nutritive constituents of *Auricularia*

Sl.no	Proximate constituent	Percentage (%)
1.	Moisture	89.18
2.	Protein	8.2
3.	Fat	1.22
4.	Carbohydrate (fresh)	63
5.	Total free amino acids	21
6.	Fibre	4.5
7.	Ash	3.41

4.13 ORGANOLEPTIC CHARACTERS

Auricularia mushroom cultivated were subjected to studies of organoleptic characters like colour and appearance, texture, flavour and taste. Four different recipes viz ., *Auricularia* soup , *Auricularia* fritters , *Auricularia* scramble , *Auricularia* masala were prepared and they were subjected to evaluation by 10 judges based on a score card and subjected to Kruskal Wallis test for analysis . The average ranking was given for each character. The native isolates screened for their characters like colour, appearance, texture and flavour on the basis of score card subjected to Kruskal Wallis test were ranked. On different organoleptic characters tested *Auricularia* fritters was found to have maximum flavour. *Auricularia* soup has appealing colour and appearance and it was found to be the most tasty recipe. *Auricularia* masala was found to have maximum texture with an average value of 3.4. The details are given in Table 19.

Plate 13 Organoleptic studies on *Auricularia*

Table 19 Organoleptic characters

Sl. No	Recipe	Colour and appearance*	Texture*	Taste*	Flavour*
1.	Fritters	3.1	3.1	3.1	3.3
2.	Soup	3.6	2.9	3.5	3.2
3.	Masala	3.2	3.4	3.2	2.9
4.	Scramble	3.2	3.1	3.0	3.0
		3.275	3.125	3.2	3.1

* Average of ten rankings

Discussion

5. DISCUSSION

India is blessed with varied agro climate which makes it suitable for cultivation of different types of mushroom. *Auricularia* or black ear mushroom is widely distributed in tropical, sub-tropical and temperate climatic conditions where it grows in nature on fresh wood or decaying wooden logs. Commonly known as wood ear, *Auricularia auricula* is the first recorded cultivated mushroom (Chang, 1993). *Auricularia* ranks 4th among cultivated mushrooms (Chang, 1996). It was reported that five kinds of *Auricularia* growing in China were gathered for food in the rainy season and dried in sun. It has been also known to have picked for food and used for the treatment of piles (Cheng and Tu, 1978).

In the present investigation cultivation of local species of *Auricularia* was attempted in different substrates. Invitro studies on nutritional requirements, studies on comparative efficiency of different substrates, supplements on spawn production, studies on nutritional value and organoleptic tests were conducted.

Attempts were made to collect native strains of *Auricularia* sp. by conducting intensive survey in different locations of Thiruvananthapuram district *viz,* Vellayani , Balaramapuram , Kalliyoor , Palappur and Vanchiyoor during monsoon and post monsoon periods . Mushrooms were screened for macroscopic details such as shape, size and colour of basidiocarp .The pileus was generally concolorous, initially spathulate, ear shaped to flabelliform when old, coriaceous, glabrous, margin incurved, hygrophanous, rubbery textured and uppersurface was smooth to wrinkled, to velvety, with lower surface smooth to veined, glabrous, greyish brown to purplish brown, overall white to the tomentum. This is in agreement with observations made by Hemmes and Desjardin (2004) and (Schenk and Dudley, 1999) on the macroscopic details of *Auricularia polytricha.* The

sporophores of this mushroom was found growing in groups on dead stumps of mango tree, anjili tree, coconut tree basins and fallen wooden logs. They were coriaceous and attached laterally to the substratum

Earlier workers have reported the occurrence of different strains of *Auricularia* sp. from India including those collected in the present investigation (Sohi and Upadhyay ,1988 ; Sharma *et al.*,1992) .

Auricularia polytricha which was collected from dead stumps, fallen wooden logs during monsoon and post monsoon period in the present study was collected earlier by Hemmes and Desjardin (2004). The detailed observations made in the present study are found to be in full agreement with the description of the species made by Purkayastha and Chandra (1985); Schenk and Dudley, (1999) and Hemmes and Desjardin (2004).

. The tissue isolation of the isolates was done as per the standard method of tissue culture technique and the cultures were maintained on PDA slants by periodical subculturing. The isolation of *Auricularia polytricha* on PDA medium from the sporophore was reported by Ling *et al.* (2005).

In general, *Auricularia* spp. prefers natural media for their growth and growth in synthetic media is very poor. Among the natural media tested, *Auricularia* spp. grew best in Potato Dextrose Agar (PDA) and, Oat Meal (OA) and their growth in Tapioca Agar medium and Carrot Agar medium was thin and superficial. Many of the workers suggested potato-sucrose-agar (PSA) (Tabata and Ogura;2003) , Potato Dextrose Agar (PDA) (Ling *et al* .,2005) , malt extract agar medium (Garasiya *et al.*,2007) for the growth of *Auricularia* spp. Of the different liquid media tested OA was found to be the best in supporting radial growth and biomass production of *Auricularia* spp .Potato Dextrose broth, Carrot broth and Tapioca broth also supported the growth of *Auricularia* spp. None of the workers suggested the use of synthetic media for the growth of *Auricularia*

spp. The results of the present investigation also clearly indicate that for growth and fruit body production *Auricularia* spp. prefer natural media.

Out of five different ranges of temperature conditions tested it was observed that temperature of 30°C supported maximum mycelial growth and biomass production of 0.89 g. The other temperature conditions of 25°C, 20°C and 15°C also supported biomass production. The lowest biomass production was recorded with a temperature of 10°C. The results of the present investigation is similar to the findings of various other workers who reported the optimum temperature conditions for the growth of *Auricularia* spp. Kurtzman (1979) reported optimum temperature for the mycelial growth of *Auricularia polytricha* was 20 -34˚C and that of *Auricularia auricula* was 28˚C . Singh *et al.*(2000) had also reported that 25 - 30˚C as the most suitable for the growth of *Auricularia polytricha.* None of the workers reported the growth of *Auricularia polytricha* above 40˚C. Inability /poor growth of fungi at higher temperature were attributed to failure of methionine biosynthesis to keep pace with other processes. Observations of certain workers that *Auricularia polytricha* preferred a temperature of 25 – 30 ˚C is again confirmed by this study.

Four different ranges of light like sunlight, roomlight, intermittent light and dark conditions were tested for their efficacy in the production of biomass of *Auricularia* spp. It was observed that room light gave maximum mycelial growth and biomass production of *Auricularia* spp.followed by the dark conditions with no light provided. The direct sunlight provided recorded the least biomass production of *Auricularia* spp. The results of the present investigation is similar to the findings of various other workers who reported the optimum light conditions for the growth of *Auricularia* spp. The spawn run is maximum at dark area of less than 500 lux. 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 primordial and fruiting body formation.

Under given conditions, growth of a fungus will be maximum over a certain range of initial pH values of the medium and will fail to grow at high and low extremes. In this study *Auricularia polytricha* preferred an alkaline pH of 8 and 9 for maximum biomass production. However, it was contradictory to the findings of Keun Yang *et al*., (2002) who reported optimum pH for biomass of *Auricularia polytricha as* pH 4. Singh *et al.* (2000) found suitable pH as 6.0 for *Auricularia polytricha.*

Carbon plays a vital role in the growth of all microorganisms including mycelial growth of *Auricularia polytricha.* Out of different carbon sources namely lactose , glucose , sucrose , mannitol , dextrose and a carbon control medium were tested for their efficacy in biomass production of *Auricularia* spp. It was observed that sucrose supported maximum biomass production. Glucose and lactose also supported good biomass production .The findings of various other workers also confirmed similar results. Upadhyay (2003) had found glucose and fructose as excellent carbon sources for the growth of *Auricularia polytricha*. Garasiya *et al.* (2007) reported that the highest dry mycelial weight of *Auricularia polytricha* was in starch.

The inorganic nitrogen sources like asparagine, sodium nitrate ($NaNO_3$), ammonium carbonate (NH_4CO_3) and ammonium chloride (NH_4Cl) were tested to find the efficacy for the production of mycelial biomass .Among the various nitrogen sources asparagine was found to be the best nitrogen source producing the highest biomass. Sodium nitrate and ammonium chloride added media produced high biomass. This result was in accordance with the findings of calcium nitrate, urea, asparagine and alanine as the best nitrogen sources for the good mycelial growth of *Auricularia polytricha* (Upadhyay, 2003). Studies

conducted by Garasiya *et al.*(2007) showed that the maximum dry mycelial weight of *Auricularia polytricha* is in soybean powder followed by potassium nitrate and urea. Similar results were obtained by Khan *et al.* (1991).

Sinden (1934) introduced grain spawn for the cultivation of mushrooms. Both grain substrates and sawdust substrates were tested in the present study. Grain substrates tried were maize grain substrate, paddy grain substrate, rice bran and wheat bran. Sawdust substrates tried were rubber sawdust, anjili sawdust, jack sawdust, mango sawdust and coconut sawdust .Coir pith compost was also tried as a spawn substrate. Grain substrate was found to be a better spawn substrate than sawdust substrate. Maize grain was found to be the best substrate 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 that sawdust substrate as a better substrate than grain substrate for the cultivation of *Auricularia polytricha* In the present study it is observed that cultivation of *Auricularia polytricha* is also possible in sawdust substrate .Among sawdust substrates, rubber sawdust was found to be the best substrate for the cultivation of *Auricularia.*

Thakur and Bhandal (1993) reported wheat straw, paddy straw and saw dust as the most suitable substrates for the cultivation of *Auricularia polytricha.* Lin *et al*. (1993) reported that dead branches, fallen leaves and pruning wastes from tea plants were suitable for the cultivation of *Auricularia polytricha* .

Upadhyay (1999) reported autoclaved wheat grain as excellent spawn for *Auricularia.* Studies conducted by Garasiya *et al.* (2007) showed that for the cultivation of *Auricularia polytricha* the substrate should contain lignin and cellulose in the available form and sawdust and wheat straw gave a good mycelial yield. 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 *Auricularia.*

Food supplements like wheat bran and rice bran at different levels of concentration like wheat bran @ 2.5 %, wheat bran @ 5 %, rice bran @ 2.5 %, rice bran @ 5 %were supplemented with both grain substrate and saw dust substrate to test their efficacy in influencing yield of *Auricularia* spp. It is confirmed from the study that addition of food supplements to the substrate give better results. The result obtained is in accordance with findings of wheat bran supplemented substrate give higher yield with higher biological efficiency (Thakur and Bhandal ,1993).However results obtained in the present study is contradictory to the findings of various other workers . Upadhyay (1999) reported higher yields of *Auricularia mesenterica* with unsupplemented wheat straw as substrate followed by supplementation with wheat bran addition. He also observed the supplementation of *Auricularia mesenterica* with rice husk inhibited the growth and development of basidiocarps.

Bhandal and Mehta (1986) have cultivated Indian strain of *Auricularia polytricha* on fresh as well as composted wheat straw supplemented with rice bran with 60 to 80 % biological efficiency. Tabata and Ogura (2003) reported the growth of *Auricularia polytricha* in a sawdust medium supplemented with Ca or Mg salts. Garasiya *et al*.(2007) reported the addition of 5 % wheat bran to wheat straw substrate recorded the maximum yield of *Auricularia polytricha* when compared with other agro –waste substrates like sawdust, wheat straw, 2 %wheat bran etc.

The nutrient content of *Auricularia* spp . is tested to find out the proximate constituents . The findings revealed the nutrient compostion of *Auricularia* spp. with 89.18 % moisture, 10.82 % dry matter, 8 .2 % proteins, 82 % fat content, 63% CHO(fresh weight) content, 4 .7 % fibre and 3.51% ash content. These results were in agreemnt with findings of other workers like Chang and Hayes

(1978), Chang and Miles (1991). Kurtzman (1979) reported that *Auricularia* sp. has 10.6 % protein. On dry weight basis *Auricularia polytricha* has high protein (7.59 %), followed by fibre (3. 69 %) and lowest in fat (1. 12 %). Appreciable amount of calcium and zinc are also present in the fruiting body of *Auricularia polytricha* (Lu and Tang, 1986). Keun Yang *et al*. (2002) reported *Auricularia polytricha* contained 77.5 %(fresh weight) carbohydrate and 22.5 %protein. Zhang *et al*. (2006) observed fruiting bodies of *Auricularia auriculae* are rich in polysaccharides having anti oxidant properties.

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* 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.

Arumugananthan *et al*.(2003) observed total soluble salt of 5 -7 brix in *Agaricus bisporus* .

The native *Auricularia* spp . is screened for their characters like colour and appearance , texture , taste and flavour on the basis of score card . Four different recipes were prepared and they were subjected to evaluation by 10 judges based on a score card and subjected to Kruskal Wallis test for analysis. The average ranking was given for each character. The results showed that native *Auricularia* spp. obtained consumer acceptability with respect to colour and

appearance, texture, taste and flavour . Stamets (2004) revealed that *A. auricula* is superior to *A. polytricha* in culinary terms .The consumer acceptability of *A. auricula* is better when compared with that of *A. polytricha* .

Summary

6. SUMMARY

The study entitled "Biology and cultivation of *Auricularia* sp."was conducted in Thiruvananthapuram district of Kerala State . The native strains of *Auricularia* sp. collected by conducting intensive survey in different locations of Trivandrum district *viz,*Vellayani , Balaramapuram , Kalliyoor , Palappur and Vanchiyoor during monsoon and post monsoon periods were included in the study .

Macroscopic studies conducted on the morphological characters of the the collected species was confirmed as *Auricularia polytricha* . Sporophores were found in groups on mango tree, anjili tree,coconut tree basin and dead wooden logs. The pileus was found to be rubbery textured ,uppersurface is smooth to wrinkled , to velvety , with lower surface smooth to veined , glabrous , greyish brown to purplish brown , overall white to the tomentum .

Microscopic studies conducted on *Auricularia* revealed that basidia were elongated , spores were white , sausage shaped and spore print was white .

In vitro studies on the growth of this mushroom on different media, at different temperature and pH levels revealed that *Auricularia* preferred natural media like PDA and OA. Their growth on synthetic substrates was very poor. The fungi studied preferred a temperature range of 25 -30°C for their optimum growth. The fungi were able to grow at pH 7, 8 and pH 9.

In vitro studies on nutritional requirements of *Auricularia* revealed that it preferred sucrose and asparagine as the best carbon and nitrogen sources respectively.

In vitro studies on light requirements of *Auricularia* revealed that it preferred room light for maximum mycelial growth and biomass production .It was revealed that direct sunlight recorded minimum biomass production .

Studies on time taken for maximum spawn run on different spawn substrates revealed that *Auricularia* preferred grain substrate to saw dust .It was proved that maize grain required an approximate of 17 days for maximum spawn run.

Studies on yield of *Auricularia* sp.on locally available cheap substrates revealed that the yield was maximum in rubber saw dust. Mango sawdust, jack sawdust coconut sawdust and anjili sawdust also gave an appreciable yield .The paddystraw substrate gave minimum yield .

It was revealed that the addition of various food supplements to the substrates increased the yield of *Auricularia.* The yield was found to be the highest when 5 % wheat bran was used as a supplement along all substrates. 5 % rice bran and 2.5 % wheat bran were also proved to be good supplements .

The studies on the proximate constituents of *Auricularia* mushroom revealed that it had high nutritional value with high percentage of carbohydrate and protein. The study revealed that *Auricularia* had a high percentage of moisture content of nearly 91 % .

The organoleptic studies were conducted on *Auricularia* mushroom based on culinary terms .The studies revealed that it had high consumer acceptability despite its rubbery texture .

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Abstract

BIOLOGY AND CULTIVATION OF *Auricularia* spp.

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Abstract of the
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8.ABSTRACT

The local strain of *Auricularia polytricha* collected from various parts of Thiruvanthapuram district as a part of study entitled "Biology and cultivation of *Auricularia* sp." were used to study the modified cultivation techniques and overall acceptability of *Auricularia* mushrooms.

This fungi preferred natural media such as potato dextrose agar and oatmeal agar , a temperature range of 30C and alkaline pH for their optimum growth.

In vitro studies were conducted to find out the best carbon source and nitrogen source for its optimum growth. Sucrose was found to be the best carbon source and asparagines as the best nitrogen source.

Time taken for maximum spawn run on different spawn substrates were tested and grain substrate was found to be a better spawn substrate than sawdust substrtae.

Maize grain was found to be the best substrate for spawn run with a minimum of 16 days required for the fluffy growth of mycelium covering the substrate.

Among sawdust substrates ,rubber sawdust was found to be the best substrate for spawn run with a minimum of 18 days required for the fluffy growth of mycelium covering the substrate.

Yield of *Auricularia* sp. on different locally available cheap substrates were evaluated and rubber sawdust was found to be the best substrate giving maximum yield.

Yield of *Auricularia* sp. on different food supplements along with available cheap substrates were evaluated and it was observed that addition of food supplements increased the yield of *Auricularia* sp.

The proximate constituents of *Auricularia* mushroom were tested and it was observed that *Auricularia* sp. has high percentage of moisture,protein and carbohydrate content.

The results of the organoleptic studies showed that *Auricularia* sp. has high cooking quality and good overall consumer acceptability.

Appendices

APPENDIX -1

Collection No……. Date of collection ……..

Collected by……

1. General
   - Locally
   - Habitat
   - Any other details

2. Pileus

   Colour…….. Diameter ……..

   Shape : immature mature…….

   Texture : soft, brittle, fleshy, coriaceous, membranous

   Surface : dry, moist, greasy, smooth, downy, velvety, shaggy, peeling out easily or not.

   Margin : Regular, wavy, smooth, rough, furrowed, incurved or not/striate or not

   Veil : Present/absent Colour
   Abundant, scarce,
   Appendiculate/membranous

   Chemical
   Reaction : amyloid/non-amyloid/dextrnoid

3. Gills/Popres/ Teeth
   - Colour
   - Arrangement : remote/free/adnate/adnexed sinuate crowded or distant, easily separable from pilear tissue or not.
   - Consistency : Pliable/brittle/ waxy/fleshy
   - Size : ni/cm
   - Gill edge : Special features if any

4. Stipe
   - Position : Central/eccentric/sessile

Colour :

Size : Length……..diameter……
Consistency : fleshy/leathery/woody
Surface

Characters : fibrillose/dry/viscid
Pubscent/squamose/glabrous

Annualus : present/absent size…….single/double
Membranous/fiamentous

Position : apical,medical,basal

Volva : present/absent
Shape
Colour

Texture : fleshy/tough/papery

5. Flesh

When wet When dry

Colour in pileus
Colour in stipe
Change in colour when exposed to air

6. Others

Presence of abnormal liquid/milky fluid/others
Before cutting/after cutting
Any other character

APPENDIX – I I
Composition of the media and reagents used for the study

1. Potato Destrose Agar

Potato	-	200g
Dextrose	-	20g
Agar	-	15g
Distilled water	-	1000ml
pH	-	6 to 6.5

2. Carrot Agar

Carrot	-	200g
Dextrose	-	20g
Agar	-	15g
Distilled water	-	1000ml
pH	-	6 to 6.5

3. Oat Meal Agar

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

4. Tapioca Agar

Tapioca extract	-	100 g
Agar	-	15g
Distilled water	-	1000ml
pH	-	6 to 6.5

5. Czapek's Dox Agar

Sucrose	-	30g
Sodim nitrate	-	2g
Dipotassium Phosphate	-	1g
Magnesium Sulphte	-	0.5g
Potassium Chloride	-	0.5g

Ferrous suiphate	-	0.01g
Agar	-	15g
Distilled water	-	1000ml

6. Richards' medium

Potassium nitrate	-	10G
Potassium dihydrogen phosphate	-	5g
Magnesium sulphate	-	2.5g
Ferric chloride	-	50g
Agar	-	15g
Distilled water	-	1000ml
pH	-	6 to 6.5

Reagents and stains

1. Melzer's reagent (Melzer, 1934)

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

2. Lactphenol cotton blue

Anhydrous lactophenol	-	67ml
Distilled water	-	20ml
Cotton blue	-	0.1g

APPENDIX –I I I

Caespitose	-	In groups or tufts like grass
Imbricate	-	Partly covering one another like the tiles on a roof
Spathulate	-	Like a spoon in form
Flabelliform	-	Like a fan; in the form of a half circle
Coriaceous	-	Like leather in texture
Decurrent	-	Running down the stipe
Infundibuliform	-	Funnel like
Tomentose	-	Having a covering of soft, matted hairs, downy
Concolorous	-	Of one colour
Campanulate	-	Bell like in form
Hygrophanous	-	Having a water soaked appearance when wet
Clavate	-	Club like
Lamellulae	-	A small lamella which runs from the edge of the pileus towards the stipe
Trama	-	The tissue lying between two hymenial layer, usually consisting of densely packed or loosely interwoven hyphae
Appressed	-	Closely flattened down
Amyloid	-	Colour reaction with Melzer's reagent – black or slightly greyish if amyloid, brown to purplish brown when pseudoamyloid, yellowish if non amyloid