STANDARDIZATION OF TECHNIQUES FOR CULTIVATION OF BUTTON MUSHROOM (*Agaricus* spp.) IN KERALA.

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(2013-11-151)

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DECLARATION

I, hereby declare that this thesis entitled **"Standardization of techniques for cultivation of Button mushroom (*Agaricus* spp.) in Kerala"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Beloved Parents

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
lbs	Pounds per square inch
PDA	Potato dextrose agar
mm	Milli meter
°C	Degree Celsius
CD	Critical difference
cm	Centimeter
et al.	And other co workers
g	Gram
hrs	Hours
i.e.	That is
ml	Milli litre
Kg	Kilo gram
min.	Minutes
mg	Milli gram
Sl. No.	Serial number
sp. or spp.	Species (Singular and plural)
viz.	Namely
pH	Negative logaritham of hydrogen ions
SMS	Spent mushroom substrate
µ	Micron meter

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Introduction

1. INTRODUCTION

Mushrooms are cultivated for their nutritive and therapeutic values. They are highly valued for their taste and flavor and are consumed both in fresh and processed forms. From the trends of previous year, the demand and consumption of mushroom is expected to increase in future. India is blessed with a varied agro-climate, abundance of agricultural residues and manpower, making it appropriate for cultivation of all types of temperate, tropical and sub-tropical mushrooms. A huge amount of agricultural wastes available in India together with climatological diversity can be successfully exploited for commercial cultivation of various edible mushrooms.

Mushrooms offer an array of nutrients, making them a suitable food for vegetarians. Cooked mushrooms are an amazing protein source, which, even though incomplete, is easily supplemented by grains. They also provide iron, riboflavin, and niacin; offer good amounts of potassium, selenium, copper, and zinc; and they are full of fiber. Besides giving delightful flavor to foods, mushrooms contribute more nutrition to daily food.

In India at present, four varieties of mushroom *viz.*, *Agaricus bisporus*, *Pleurotus* spp., *Volvariella* spp., and *Calocybe indica* have been suggested for the year round cultivation. *Agaricus* spp. is commonly known as button mushroom. White button mushroom is a non-traditional agricultural cash crop grown in India. It is cultivated all over the world and contributes about 40 per cent of the total world production of mushrooms. This is still the most popular variety for domestic as well as export market and is cultivated on composted substrate both as seasonal as well as under controlled environmental conditions, around year. Presently, India is producing about 1.2 lakh tonnes mushrooms in which temperate button mushroom contributes the major part (85%).

Problems of unemployment, poverty and malnutrition are inextricably connected and are acute in the vulnerable sectors of the society. Obviously, these cannot be solved by conventional land based cultivation. Enhancement of income of small farmers, landless labourers and unemployed youths by diversification of opportunities of income generation through subsidiary occupations are the new challenges. Mushroom cultivation thus will go a long way in solving the twin problem of poverty and malnutrition in the country.

The climatic conditions of hilly places in Kerala like Ponmudi, Munnar, Wayanad, etc. are best suited for the growth of *Agaricus.* Button mushrooms can also be cultivated under controlled conditions in plains though makes the production expensive. Even then it can in future be the most suited species for cultivation under Kerala conditions. Therefore the present investigation was conducted to study the feasibility of button mushroom cultivation in plains of Kerala.

Based on these facts, the present study was conducted with the major objective of exploring the possibility of cultivation of *Agaricus* species and develop a modified technique for the production of quality compost with alternative agricultural waste. Attempts were also made to study the shelf life, cooking quality and overall acceptability of button mushroom in Kerala.

Review of Literature

2. REVIEW OF LITERATURE

2.1 COLLECTION, IDENTIFICATION, ISOLATION, AND PURIFICATION OF ISOLATES

Two species of *Agaricus viz, Agaricusarvensis* Schaeff and *A. campestris* Fr. were collected by Bose and Bose (1940) during a study from meadows and pastures of North West Himalayas, Bihar and West Bengal. An edible species of the genus *Agaricus* ie; *A. basianuloses* was collected and described by Paracer and Chahal (1962) from Ludhiana.

A. trisulphuratus Berk from the genus *Agaricus* was reported and studied by Natarajan and Manjula (1981) from South India while doing a survey. Bhavani Devi (1982) identified the occurrence of three species of *Agaricusviz. A. arvensis, A. campestris* and *A. placomyces.* in a survey conducted in Kerala. Roy and Sampati (1986) reported *Agaricus trisulphuratus* Berk for the first time from West Bengal.

A. comtulus Fr., *A. cylindriceps* var. *cylindriceps* Murr., and *A. xanthodermus* were identified and described by Atri*et al.* (1992) in a survey conducted at Patiala, Punjab. Bhavani Devi (1995) identified two species of the genus *Agaricusviz, A. diminutives* Peck and *A. iodolens* Heim for the first time from India while doing a survey. Vrinda *et al*. (1997) studied the agaric flora of Western Ghats of Kerala state. They collected and described *A. johnstonii* from Tropical Botanical Garden and Research Institute campus.

Collections of *Agaricus* spp. were done by Thara (2001) during and after the south-west and north-east monsoon periods of 1998-2000 in twenty agro climatic zones of Kerala and forty two species of *Agaricus* were collected. Melghat forest

region from Central India was surveyed by Karwa and Rai (2009) for occurrence of medicinal and culinary mushrooms during the years 2005-2008. Out of the 153 species identified during this survey, ten species of *Agaricus* were recorded from different localities. Of these, seven species namely *A. bitorquis, A. subrufescens, A. augustus, A. placomyces, A. essettei*, *A.basioanolosus* and *Agaricus* sp. *Nov* (a new species) were reported for the first time from the region.

A survey was conducted by Thiribhuvanamala *et al.* (2011) in the Anaikatti, Attapadi, Palghat, Siruvani, Nilgiris and Kallar regions of the Western Ghats of India during 2008 -10.About 68 mushroom flora belonging to 19 genera were recorded in this survey and seasonal occurrence of *Agaricus* from October-January was observed. Kaur*et al.* (2013) reported fourteen species of edible *Agaricus* from Punjab.

Senthilarasu (2014) collected and identified a total of 13 species of *Agaricus* belonging to 10 genera in 9 families including *A. abruptibulbus, A. arvensis, A. bisporus, A. bitorquis, A. brunnescens, A. campestris, A. micromegethus, A. pattersoniae, A. placomyces, A. scitulus, A. semotus, A. subedulis, A. sylvaticus, A. sylvicola, A. vaporarius, A. woodrowii, A. xanthodermus* and *A. xantholepis*. Owaid *et al.* (2014) collected and isolated different species of wild *Agaricus* from Western Iraq.

2.2 MORPHOLOGICALSTUDIES

Carrera *et al*. (2001) isolated and identified different strains of wild *Agaricus* and they conducted a study on fruiting body morphology of different strains of these wild *Agaricus* species. Collected species include *A. abruptibulbus* Peck, *A. albolutescens* Zeller, *A. augustus* Fries, *A. bisporus* var. *bisporus* (Lange) Imbach, *A. bitorquis* (Quél.) Sacc., *A. campestris* Link : Fries, *A. hortensis* (Cooke) Pilat, *A. osecanus* Pilát, *A. robustissimus* Panizzi, and *A. subrufescens* Peck. Thara (2001) identified more than fourty species of *Agaricus* and studied the morphological

characters of these species. She reported that most of the collected species had white to brownish pileus with scaly surface and bulbous shaped, centrally attached stipe.

2.3 CULTURAL AND PHYSIOLOGICAL STUDIES

2.3.1 Growth of Agaricus bisporus under different media

Fritsche (1968) evaluated different media for the growth of *A. bitorquis* and reported that malt extract agar and compost agar as the best media for culture maintenance. Iqbal *et al*. (1988) tested different media namely complete medium, malt extract medium and potato dextrose medium for the growth of *Agaricus* and found that complete medium gave maximum radial colony diameter of this mushroom followed by the malt extract and potato dextrose agar. An experiment was conducted by Khan *et al*. (1991) to study the factors affecting the growth of *A. brunnescens* Peck on various culture media, *viz*., malt extract agar, potato dextrose agar and wheat extract agar and the maximum radial growth of mycelium was observed on malt extract agar.

In the investigation on radial growth of *A.bitorquis* on various culture media under different cultural conditions, Furlan *et al*. (1997) observed that mycelia growth was maximum on potato dextrose agar and malt soya peptone agar medium.

Thara (2001) studied the effect of different media on the growth of *Agaricus* and found that malt extract gave good growth in both solid and liquid media. *A. bisporus* produced maximum mycelia growth in malt extract medium Yadav *et al*. (2003). An experiment was conducted by Uddin *et al*. (2012) to investigate the mycelial colony proliferation of *A. biporus* in different media namely potato dextrose agar, yeast potato dextrose agar and malt extract agar and the best mycelia growth was observed in YPDA (Yeast Potato Dextrose Agar).

2.3.2. Effect of carbon sources on the growth of A.bisporus

Chandra and Purkayastha (1977) conducted an experiment to study the effect of different carbon sources on the growth of *A. campestris* and reported that glucose and fructose supported maximum biomass production of this mushroom. Different carbon sources like hexose, namely fructose, glucose and mannose can give sufficient amount of carbon for the growth of *A. bisporus* (Dirtsch, 1978). An experiment was conducted by Kumar and Munjal (1980) on influence of different carbon sources on the growth of *A. bisporus* and reported that maltose, glucose and fructose as superior carbon sources when compared to sucrose. *A. bisporus* is able to degrade dead bacteria and to exploit them as a better source of carbon, nitrogen and phosphorus (Fermor & Wood, 1981).

Kalisz *et al.* (1986) tested the growth of *A. bisporus* on defined liquid media in presence of protein as a sole source of carbon, nitrogen and sulphur and revealed that protein was used as the best source for glucose when provided as a sole source of carbon. Baurs *et al.* (1994) reported that *A. bisporus* exhibited good growth when glucose was used as the carbon source in a defined buffered medium.

Vahidi and Hamedi (2006)studied the effects of different carbon sources including glucose, lactose, sucrose, manitol, starch, galactose, maltose and fructose in two different media (Complex and synthetic) on production of polysaccharides by *A. blazei*. They reported that the highest growth and polysaccharide production were obtained when galactose and starch were used as carbon source. Liu and Wang (2009) revealed that the carbon sources like glucose and corn flour extract were found to be the best for the growth of *Agaricus*. They selected a practical medium for reducing cost and enhancing both biomass and intra cellular polysaccharide (IPS) production.

2.3.3 *Effect of nitrogen sources on the growth of A. bisporus*

Kalisz *et al*. (1986) evaluated various nitrogen sources for the growth of *A. bisporus* and stated that it can utilize protein as the best source of nitrogen where catabolite repression was observed when media were added with ammonium chloride and protein. Khan *et al.* (1991) tried various nitrogen sources for the growth of *A. bisporus* and observed that peptone as the best source. Baurs*et al*., (1994) stated that *A. bisporus* cannot utilize organic nitrogen containing substance as only source of nitrogen. Mantovani *et al*. (2007) added nitrogen sources namely urea and ammonium sulfate to the medium supplemented with cassava extract for the in vitro mycelial growth of *A. brasiliensis* in different C:N ratios in the dark at 28°C. They measured the radial mycelia growth after 8 days and reported that nitrogen from urea boosted growth of this mushroom better than ammonium sulfate or any mixture of nitrogen.

Liu and Wang (2009) conducted a study to find out the best nitrogen source for *A. blazei* for the production of biomass and intra cellular polysaccharide production. The results showed that intra cellular production was growth associated and supported maximum when wheat bran was used as the nitrogen source.

2.3.4. Effect of pH on the growth of A. bisporus

Hayes (1972) studied the effect of different pH on vegetative growth of *A. bisporus* and observed that growth was possible over an extended range of pH from 3.5 – 9.0, for an optimum growth most media require a pH of 6.8 – 7.0. The results of study conducted by Song (1976) revealed that minimum and maximum pH for the growth of *A. bitorquis* was found to be 4 and 10. Chandra and Purkayastha (1977) observed that pH of 5.5 was best for the higher biomass production of *A. campestris.* Dirtsch (1978) reported that the optimum pH for maximum growth of *A. bisporus* was observed to be 5.3.

Mehta and Kumar (1985) evaluated different pH for the mycelial growth of single spore isolates of *A. brunnescens* and concluded that maximum growth was found at pH ranging from 6-7. The growth of *A. bitorquis* was found to be maximum at a pH of 5.0 as reported by Furlan in 1997. Optimum pH for the growth of *A. bisporus* was found to be 6 (Singh *et al*., 2000). Thara (2001) reported that *Agaricus* showed increased growth up to pH 6 and then decreased as pH increased.

2.3.5 Effect of temperature on the growth of A. bisporus

The germination and growth of *A. bisporus* was found to be highest at a temperature of 25^0C (Losel, 1964). Fritsche in 1968 evaluated the temperature necessity of *A. bisporus* and *A. bitorquis* and found that they grow best at 24^0C and 20-30^0C. Hayes (1972) conducted a detailed study on temperature requirement of *A. bisporus* and revealed that minimum growth was obtained at 3^0C but the maximum limit for most of the strains was found to be 30^0C with an optimum at 24 - 25^0C. Dirtsch (1978) revealed that *A. bisporus* produced filamentous growth in liquid medium at a temperature of 25^0C.

Temperature has direct bearing on crop productivity, the bed temperature in the cropping room is directly influenced by air temperature, so it's the air temperature that has to be managed (Arkenbout, 1988). *A. bitorquis* showed best mycelial growth in all media when incubated at 25^0C (Iqbal, 1988).

Khan *et al.* (1991) reported that the optimum temperature for maximum radial growth of *Agaricus* on malt extract media was 25^0C. Smith and Love (1995) reported that the maximum growth of brown capped *Agaricus* strain obtained from *Cupressus* leaf litter was observed at 24^0C.

A study was conducted by Furlan *et al*. (1997) to find out the temperature requisite of *A. bitorquis* and concluded that these have higher growth rates at 30^0C than 20 or 25^0C.

Growth of the *A. bisporus* was found to be highest at 25ºC (Thara, 2001). Largeteau *et al*., (2011) studied the diversity in the ability of *A. bisporus* wild isolates to grow at high temperature and reported that *A. bisporus* var. *burnettii* required 25 °C for fruiting. Kaur *et al* (2014) evaluated the effect of different temperature on various strains of *A. bisporus* (Lange) Sing. They screened various strains for their ability to grow at different temperatures and also for their yield potential with climatic change. They studied twenty four strains of *A. bisporus* (Lange) Sing. (SSI 01/12- SSI 15/12, AVT 01- AVT 06, P1, U3 and S11) were evaluated for linear growth and biomass production at five different temperatures (15° to 27°C) on potato dextrose medium and revealed that biomass production was maximum at 15 °C to 18 °C and showed consistently maximum growth at 24 °C to 27 °C

2.3.6. Effect of light on the growth of A. bisporus

Gramss (1984) reported that growth of certain mushrooms like *A. campestris* and *A. edulis* was uninfluenced by change in light conditions. He conducted the experiment by keeping mushroom beds in moderate day light and in the dark. Bhandal and Mehta (1989) conducted an experiment on effect of light for the cultivation *Agaricus* sp. and reported that light intensity of 2000 lux was needed for fruiting body formation of *A. bisporus*.

A. bitorquis exhibited rapid mycelial growth in the darkness when compared to the presence of light (Furlan *et al*., 1997). For the fruiting body initiation and development, light (especially blue light) is considered to be one of the most important and essential environmental factors (Moore, 1998).

2.4 GROWTH OF *A. bisporus* ON DIFFERENT SPAWN SUBSTRATES

2.4.1 Spawn production and spawning

Chang and Miles (2004) stated that spawn is a medium through which the mycelium of a fruiting culture has grown and which serves as the inoculum or "seed" for the substrate in mushroom cultivation.

Spawn production in *Agaricus* mushroom is same as that of in other mushrooms (Stroller, 1962). Guleria *et al.* (1989) reported that spawn prepared from sorghum grains showed maximum yield of *A. bitorquis* followed by bajra grains added with shelled maize cob with 1:1. The success of mushroom cultivation and its yield performance depends to a large extent on the quality of the spawn used (Bahl, 1998).

Mushroom spawn can be prepared on any kind of cereal grain like wheat, rice, bajra, sorghum, ragi, etc. (Rai *et al*. 2005).

2.5 YIELD PERFORMANCE STUDY

2.5.1 *Effect of different compost materials on the growth of A. bisporus*

Mantel *et al*. (1972) suggested long method of composting for the first time for the cultivation of button mushroom. Seth and Shandilya (1975) reported that compost can be supplemented with muriate of potash, super phosphate and other trace elements in addition to suitable carbon and nitrogen source. An effort for the cultivation of *A. bitorquis* was taken by Tewari and Sohi (1976) at Banglore. Kachroo *et al.* (1979) stated that paddy straw compost can be used for cultivation of button mushroom in Kashmir condition.

A study was conducted by Patil and Shinde (1983) to evaluate different methods of spawning and reported that spawning in which spawn was mixed thoroughly in the compost while filling the compost in polythene cover was found to be the best.

A study was conducted by Upadhyay and Vijay (1988) for the standardization compost thickness for the cultivation of *Agaricus* and reported that a thickness of 30-37.5 cm gave maximum yield and concluded that compost thickness plays an important role in the production of button mushroom.

Jimenez *et al*. (1990) reported that addition of oil palm waste in composting could cause early development of fruiting bodies of *Agaricus*. Cormican and Staunton (1991) reported that the influence of different nitrogen levels in the compost was undependable in the case of productivity of *A. bisporus*. Vijay and Gupta (1992) stated that a mixture of wheat and paddy straw can be used for compost preparation.

Beyer and Beelman (1995) reported that the amount of gypsum added in compost has no influence on the productivity of *A. bisporus*. Compost prepared using long method besides taking more time for preparation gives low yield as it is prone to attack of many pests and diseases (Vijay and Gupta, 1995).

Vijay and Sharma (1996) conducted an experiment on chemical sterilization of compost prepared by long method and the facts obtained indicated that the compost prepared with 3 quintals of base materials and treated with 1.5 litres of formalin and 50g bavistin two days before spawning removes almost all undesirable organisms and considerably upturns the yield.

Sanjez and Royse (2001) used a sterilized, non-composted substrate (basal mixture) comprising of oak sawdust (28%), millet (29%), rye (8%), peat (8%), alfalfa meal (4%), soybean flour (4%), wheat bran (9%), and CaCO3 (10%) to produce the common cultivated mushroom *A. bisporus*. Sugar- cane bagasse and wheat straw are

the potential substrates for composting for the cultivation of *Agaricus* as reported by Have *et al*. (2003).

A. bisporus is generally grown in composted and disinfected substrates of various agricultural waste materials using suitable technologies (Yadav, 2005). Baysal *et al.* (2007) cultivated *A. bisporus* on wheat straw based compost materials mainly wheat bran, chicken manure and pigeon manure and locally available casing materials. They reported that the highest mushroom yield was obtained from compost prepared from wheat straw mixed with pigeon manure with peat and perlite mixture as casing materials.

Mamiro *et al*. (2007) studied the use of spent mushroom substrate compost mixed with non – composted substrate and supplementation period of compost in the cultivation of *A. bisporus.* They reported that mushroom yield was greatest on a 50/50 mixture of NCS/SMC. Mamiro and Royse (2008) used a combination of spent mushroom compost and non - composted substrate in different ratios for the cultivation of *A. bisporus* and evaluated its effect on yield performance and biological efficiency of mushrooms obtained. The values obtained in this experiment were 10.9 kg/m² for yield and 61.5% for biological efficiency when they used in 50/50 ratio. When the ingredients were mixed in 75/25 ratio the yield was 11.9 kg/m² and biological efficiency was 67.3%.

Andrade *et al.* (2008) stated that several components can be used for compost preparation and the composting materials can be changed according to the seasonal availability of ingredients. *A. bisporus* grew faster on compost pre-colonized for 1, 2 or 3 days by *Scytalidium thermophilum* compared to non-colonized compost and overlaid with protein - rich supplements as casing material (Castillo *et al*., 2009).

Silva *et al*. (2009) stated that the method of composting and composting ingredients have an important role in yield production of mushrooms. They suggested the use of agro industrial waste for composting to reduce the cost of cultivation of *Agaricus*.

Commercial production of white button mushroom was started in the hilly regions of the country (17- 18°C) like Chail (Himachal Pradesh) Kashmir and Ooty (Tamil Nadu) (Mehta *et al*., 2011). Matute *et al*. (2011) produced *A. blazei* on non-composted substrates prepared from sunflower seed hulls and spent oyster mushroom substrate. Mycelia growth rate and yield performance were evaluated both in the absence and in the presence of different supplements such as peat, vermi compost or brewery residues.

An experiment was conducted by Uddin *et al*. (2012) to investigate the effect of various composts namely wheat: paddy (1:1) straw compost, paddy straw compost and decomposed cow dung on growth and yield performance of white button mushroom (*A. biporus*) and obtained maximum yield in wheat: paddy straw compost and the lowest in decomposed cow dung.

Button mushroom is commercially cultivated on well prepared compost which is a product of fermentation process brought about by different thermophilic microorganisms and the ingredients needed for composting comprises of wheat straw, chicken manure, brewer's grain, gypsum and urea in a proportionate manner (Maheswari, 2013). Kumar and Singh (2013) evaluated different composts for the cultivation of *A. bisporus* and revealed that maximum yield obtained when the wheat straw based compost was used for cultivation. Dehariya et. al. (2013) reported that compost prepared from saw dust was a poor substrate for the cultivation of A. bisporus.

Use of poultry manure and wheat bran in rapid composting enhances the substrate quality and yield performance of *A. bisporus* (Wakchaure *et al*., 2013). Production of quality compost for the cultivation of white button mushroom *A. bisporus* using alternative and agricultural waste beyond the exploration of good quality strains are the main factors related to improve yield (Jesus *et al.*, 2013).

Spent mushroom substrate comprises 1.4 per cent nitrogen, which alone or in combination with poultry manure can be used for compost preparation by short method of composting (Vijay *et al.*, 2013). Usage of paddy straw as composting base material for the cultivation of *A. bisporus* was found to be practicable, economically viable and ecologically desirable (Dahiya *et al.*, 2013). Favara *et al*., (2014) reported that the spent compost is a good option to be used as an ingredient in the compost formulation for *A. blazei* cultivation.

2.5.2 Effect of different casing materials on the growth of Agaricus

Dhar and Kapoor (1990) reported that the addition of casing materials with protein rich organic supplements could increase the yield up to 20-30% if the quality of compost is good. Kurtzman (1991) recommended supplementation of casing materials with protein rich substances and stated that it provide fresh nutrients for the development pinheads.

Sharma and Vijay (1992) revealed that the casing materials without any treatment were found to be better when compared to the casing soil treated in various ways including sterilization, steam pasteurization and bavistin treatments. Different casing materials such as farm yard manure, loam soil, clay soil, burnt rice husk, two year old spent mushroom compost and digested biogas slurry and their mixture for casing the mushroom beds of *A. bisporus* were suggested by Khanna *et al*. (1995).

Noble and Gaze (1995) reported that the mushroom yield was independent on casing porosity, water retention and bulk density. Gupta (1997) suggested coir pith

compost either alone or a mixture of farm yard manure and spent mushroom substrate can be used as casing material for mushrooms.

Raina *et al*. (2002) reported that combination of farm yard manure, soil and sand in the ratio 4:2:1 gave maximum yield of *A. bisporus*. Vermi compost can be used as a substitute for sphagnum peat moss as casing material in the cultivation of white button mushroom, *A. bisporus* as reported by Fluente (2002).

Dhar *et al*. (2003) recommended several casing materials such as cattle manure, forest litter, tree bark, sugarcane bagasse, rice husk ash, biogas slurry, coirpith, vermi compost, municipal garbage compost, etc. for the cultivation of *A. bisporus*. Among the various casing materials evaluated for *A. bisporus*, casing materials prepared from biogas plant slurry, burnt rice husk, farmyard manure, sandy soil and spent compost, the casing material with FYM and burnt rice husk in 2:1 proportion gave maximum yield compared to the control (Angrish *et al*., 2003).

A. bisporus has a necessity for a "casing layer" that has specific physical, chemical and microbiological properties, which enhances the initiation of pinheads. These pinheads then may develop further into sporophores by differentiating the tissue (Noble *et al*., 2003).

According to Gulser and Peksen (2003) different types of casing materials can be used are tea waste, peat and composted coir pith. Coir pith compost is an agricultural waste which can be used for casing in beds of *A. bisporus* as reported by Suman and Paliyal (2004).Casing layer is the nutritionally deficient medium which helps in the initiation and development of sporophores (Jarial *et al.,* 2005).Use of vermi compost as casing material in the cultivation of *A. bisporus* could give better yield (Garcia *et al.,* 2005). Ratnoo and Doshi (2012) used Farm Yard Manure (FYM), Spent Mushroom Compost (SMC), vermi compost as casing materials for *Agaricus* successfully.

2.6 INCIDENCE OF PESTS AND DISEASES

2.6.1 *Pests*

Sciarids are small fungal gnats, which feed on compost, mushroom mycelium and fruiting bodies of *A. bisporus* (Shandilya *et al*., 1975). The important ones are *Bradysia paupera, B. tritici and Lycoriella auripila*. After fruiting body formation larva enters into the stipe, starts feeding and forms tunnels inside the stipe.

The sciarid and phorid flies can be managed by screening of doors and ventilators with nylon net of 35 meshes and also by keeping light traps (Sandhu and Arora, 1990).The nematodes found in mushroom beds have been classified into myceliophages and saprophages nematodes (Nagesh and Reddy, 2000), the myceliophges are the ones who suck the mycelia sap and leave it devitalized, while the saprophages nematodes are commonly associated with low quality compost and unsterilized casing materials.

White *et al.* (2000) reported that mushroom sciarid flies are pests of *A. bisporus* and their increased population can lead to fruitless mushroom cultivation. Fletcher and Gaze (2008) stated that *L.auripila* is one of the most destructive pests of cultivated mushrooms, *A. bisporus* (Lange) Imbach.

2.6.2 *Diseases*

Mantal (1973) recommended chemical sterilization of casing soil with formalin whereas Shandilya *et al.* (1976) advocated integrated sterilization of casing mixture that includes, steam sterilization at 60ºC for 1 hour for the management of dry bubble disease. Thapa and Seth (1989) reported the occurrence of brown spot disease of white button mushroom (*A. bisporus* (Lange) Imbach) due to *Gliocladium deliquescens* Sopp. seriously affected the pinhead development and the yield was much reduced as mushroom production declined.

The organism causing mummy disease of button mushroom is *Pseudomonas aeruginosae* and the infected fruiting bodies have tilted caps, the veil will be easily broken and they have a mummified appearance (Wuest and Zarkower, 1991.) *Trichoderma viride* and *Coprinus* were reported to cause the yield loss of *A. bitorquis* up to 45 and 2 percent respectively (Sharma, 1991).

The natural incidence of wet bubble disease of white button mushroom, *A. bisporus* was recorded between 1 to 100% in different farms in and around Solan and Bilaspur in Himachal Pradesh as well as the Morni Hills in Haryana, India (Sharma and Kumar, 2000).

Jandaik and Gularia (2002) reported *Lecanicillium fungicola* from 12 months old spent mushroom compost and suggested proper sterilization of spent compost if used for cultivation. Sources of contamination and vectors of the fungal diseases in button mushroom cultivation include insects (members of the Sciaridae and Phoridae), dust containing fungal spores, employees in the production unit and multiple-use packaging which is shared by producers (Damiecka and Maszkiewicz, 2006).

Sharma *et al.* (2007) stated that the main source of bacterial blotch in the mushroom cultivation is the casing soil and the disease is favoured by high temperature as well as humidity and poor ventilation in the cropping houses. (Zare and Gams (2008) stated that dry bubble, caused by the fungus *L. fungicola* is a serious and common disease of white button mushroom *A. bisporus* (Lange) Imbach. Grogan (2008) reported that this pathogen can be spread by means of dust, flies, mites and debris.

The button mushrooms infected by cobweb disease become soft and later the mushroom will be covered by the cottony mycelium of the pathogen (Gea *et al.*, 2012).

Green mould is one of the most destructive diseases in mushroom cultivation which is mainly caused by different species of *Trichoderma* like *T. viridae, T. harzianum, T. hamatum,* etc. and in this case a dense pure white growth of mycelium may appear on casing surface which resembles mushroom mycelium, later on this mycelia mat turns to green colour due to heavy sporulation of causal agent (Hatvani *et al*., 2012).

Berendsen *et al.* (2012) reported the germination of *L. fungicola* causes dry bubble disease in the cultivated mushroom *A. bisporus*. The pathogen of wet bubble disease in *A. bisporus* is *Mycogone perniciosa*, which results either in partial or total failure of the crop (Kouser *et al*., 2013).

Gea *et al*., (2014) stated that dry bubble disease induced by *L. fungicola* has been observed as an important disease of white button mushroom (*A. bisporus*) in India. They reported that the use of compost tea made from spent mushroom substrate can be used as an effective method for the biological control of dry bubble disease in button mushroom. The symptoms of dry bubble disease produced on well differentiated fruiting body were localized light brown depressed spots, the adjacent spots coalesce together to form irregular blotches (Kumar *et al*., 2014). In India, it has been reported to cause serious yield losses and is a common contaminant, occurring in button mushroom cultivation in the Kashmir valley (Kouser *et al*., 2014).

Brown blotch disease of button mushroom caused by *Pseudomonas tolaasii* could control effectively using antagonistic bacteria like *P. putida, P. reactants, P. fluorescens* and *Bacillus subtilis* (Tajalipour *et al*., 2014).

2.7. SHELF LIFE, COOKING QUALITY AND PROXIMATE CONSTITUENTS ANALYSIS

2.7.1 *Shelf life*

Agaricus mushroom is highly perishable in nature as it contains about 90% of water (Hammond and Nicholas, 1975). Saxena and Rai (1988) conducted a study on short duration storage of white button mushrooms (*A. bisporus)* in polyethylene packets with and without perforations at various temperatures. This experiment concluded that the mushrooms can be kept in non-perforated polythene packets for 4 days at lower temperature without decay. Texture is an important factor which contributing to mushroom quality (Mc Garry and Burton, 1994). Colour changes have been observed in *Agaricus* after harvest as well as with age and deteriorating condition of fruiting bodies (Richard, 2005).

One effective method to prolong shelf life of mushrooms during postharvest storage and commercialization is modified atmosphere packaging (Kim *et al*., 2006). Mushrooms are usually packed in plastic trays over-wrapped with perforated PVC films and stored under refrigeration temperature. They were wrapped in PVC over-wraps which were efficient in reducing weight loss, in retaining the vitamin C content and in reducing browning during storage (Mota *et al*,. 2006).

The short shelf life of mushrooms is mainly because of their increased respiration, loss of water and fast metabolic activity (Ares *et al*., 2007). The shelf life of mushrooms is less due to its very high respiration rate-of about 28.2-43.6 mg CO_2 per kg fresh weight per hour at 0°C and 280 mg CO_2 per kg fresh weight per hour at 19°C (Rai and Arumuganathan, 2008).

The main processes which contribute to mushroom deterioration after harvest are (i) discoloration, (ii) browning, (iii) loss of closeness, (iv) weight loss and (v)

texture changes (Aguirre *et al*., 2009).Suitable packaging can delay postharvest deterioration and senescence of mushrooms (Taghizadeh *et al*., 2010).

Studies on shelf life of mushrooms indicated that storage in 1 or 2% O_2 + 0 or 4% CO_2 was best in keeping the quality and firmness of *A. bisporus*. High CO_2 prevented cap opening at 12°C. However it was also found that atmospheres with high CO_2 concentration resulted in more cap browning, although O_2 concentration did not have any effect on colour (Thompson, 2010).

Button mushrooms develop brown colour on the surface of the pileus due to the action of enzyme phenol oxidase, which results in shorter shelf life (Mehta *et al*., 2011). It was observed that application of nitrous oxide in combination with modified atmosphere packaging (MAP) can extend the storage life of button mushroom up to 12 days (Jiang *et al*., 2011).

Packaging, coating, refrigeration and dipping in sorbitol and calcium chloride are the most common techniques used for prolonging the shelf life of mushrooms (Koushki *et al*., 2011). To extend the shelf life of button mushrooms, different treatments like precooling, packing, irradiation and processing practices have been effectively exploited (Surabhi and Devina, 2012).

Post-harvest dipping treatment of *A. bisporus* in 0.25% citric acid showed maximum keeping quality as it maintains excellent whiteness and no veil opening up to 96 hours of storage at 5°C. Good whiteness and no veil opening up to 96 hours of storage at 12°C (Ratnoo and Doshi, 2013). Modified atmospheric packaging offers a reasonable packaging system that partly avoids enzymatic browning, fermentation and other biochemical processes by maintaining a controlled gas atmosphere (Akbarirad *et al*., 2013).

2.7.2 Proximate constituents / Nutritive value

Mushrooms are becoming more important in our diet due to their nutritional value, related to high protein and low fat / energy contents (Agahar-Murugkar and Subbulakshmi, 2005).

Singh *et al.* (1999) reported that *Agaricus* contains 90.10 % moisture, 3.75 % protein, 0.53 % crude fibre and 4.59 % carbohydrate. Manzi *et. al*. (2001) studied the nutritional content of *A. bisporus* and reported that it consists of dietary fibres, chitin and beta glucans in variable amounts. Several studies have analyzed proximate constituents in the white button mushroom *A. bisporus* with change in mineral contents (Mattila *et al*., 2001; Vetter, 2003). Ekiz *et al*. (2005) stated that *A. bisporus* contains Molybdenum in the range of 0-2.7 µg / 100 g fresh weight.

Masamba and Mwale (2010) estimated the nutrient and mineral contents of *A. bisporus* and they concluded that the *A. bisporus* was found to contain 3.0 % for protein, 0.8 % for fat and 2.2%, 8.4%, 0.2% for calcium, iron and magnesium, respectively on wet basis.

Mushrooms are highly nutritive, low-calorie food with good amount of proteins, vitamins and mineral contents (Khatun *et al*., 2012). Koyyalamudi *et al*. (2013) analyzed the *A. bisporus* obtained from two farms for minerals; white button mushroom was seen to contain Copper at level of more than 30%, Selenium at level of more than 13% and Molybdenum at level of 6.4 – 10% of daily Required Dietary Intake as defined by Australian National Health and Medical Research Council.

2.7.3 Cooking quality

Das (2011) reported that recipes prepared from mushroom *Agaricus* scored good consumer acceptability in case of colour, flavour, taste and texture. Kaur et al. (2013) carried out works to add value to the bakery products utilizing mushroom

along with tomato and curry leaves. They reported that bread prepared after the addition of 2 per cent mushroom powder was found acceptable. Singh et al. (2013) assessed the effect of value addition of *A. bisporus* powder to various baked products like buns and cakes. It was observed that the use of powdered mushroom up to a concentration level of 15 per cent in both the products was found to be in acceptable limits. Vaidya et. al (2013) reported that different indigenous products available in the market can fortified with nutritionally enriched mushrooms to overcome the deficiency diseases.

Materials and Methods

3. MATERIALS AND METHODS

3.1 COLLECTION, ISOLATION AND PURIFICATION OF ISOLATES

Mushrooms were collected from different locations of Thiruvananthapuram district during the south west and north east monsoon seasons. Fruiting bodies of tentatively identified *Agaricus* spp. obtained from the surveyed locations along with those isolates obtained from AICRP (All India Co-ordinated Research Project) centre were transferred to the Department of Plant Pathology, College of Agriculture Vellayani, for further isolation using standard tissue culture technique.

3.1.1 Isolation and Purification of Collected Specimens

3.1.1.1 Tissue Culture Technique

Fresh fruiting bodies of the collected mushrooms were cleaned and were surface sterilized using ethanol. The mushrooms were longitudinally split into equal halves and a small piece of tissue from the junction of the pileus and stipe was detached using a sterile inoculation needle. The detached piece of mushroom tissue was transferred to petri dishes containing solidified potato dextrose agar (PDA) under aseptic conditions and incubated at room temperature for 14 days. These isolates were then purified by the hyphal tip method and maintained on PDA slants for further studies.

3.2 MORPHOLOGICAL, CULTURAL AND PHYSIOLOGICAL STUDIES

3.2.1 Morphological Studies and Identification of Isolates

Macroscopic and microscopic characters including colour and texture of fruiting bodies and spores of the collected mushrooms were observed in detail based on the data sheet as described in Appendix I.

Spore print was made by cutting and transferring portions of the pileus on white paper sheets. A partially mature mushroom sporocarp (two days after formation of initial) was taken for obtaining the spore print. The pileus was detached from the stipe and was placed inverted, on a piece of white paper with gills facing the surface of the paper. A bell jar was placed over this piece of paper to maintain humidity. After six hours the bell jar and the pileus were removed and the spore print obtained on the paper was observed under the microscope.

Preliminary trials for evaluating the performance of the different isolates of collected *Agaricus* specimens were conducted by observing the growth of each isolate on PDA as well as nature and development of their spawns in paddy grains. All the isolates were identified at the Directorate of Mushroom Research, Solan and given coded accession numbers. The isolate that performed best in the preliminary trials was used for the subsequent studies.

3.2.2 Cultural and Physiological Studies

3.2.2.1 Growth of Agaricus in Different Solid Media

Growth of selected isolate of *Agaricus* which performed best in preliminary trials was cultured in different solid media namely potato dextrose agar (PDA), oat meal agar, malt extract agar, carrot agar and Czapek's Dox agar to find out the best medium for its growth. The compositions of the media used are given in Appendix II. Each of the cooled and molten sterilized medium was poured into sterile petri dishes of nine cm diameter and allowed to solidify. A five mm diameter culture disc of the isolate was transferred from seven day old culture of the fungus inoculated at the centre of each solidified media contained in the petri dishes. Four replications were maintained for each treatment and the petri dishes were incubated at 25±2°C for 14 days. Observation of colony diameter was measured at weekly intervals for a period of 14 days.

3.2.2.2 Growth of Agaricus in Different Liquid Media

Broths of five different media *viz*., potato dextrose, oat meal, malt extract, carrot and Czapek's Dox were used. The composition was same as used in the previous experiment except for the exclusion of agar.

Fifty ml of each broth was prepared and dispensed in 100 ml conical flask and then sterilized. The broths were then inoculated with five mm culture disc of fungus transferred from actively growing culture, under aseptic condition. The flasks were incubated at 25±2°C for 20 days. After 20 days the mycelial mat was filtered through a Whatman No: 1 filter paper and dry weights were taken after drying at 70 ºC until constant weight was obtained.

3.2.2.3. Effect of Different Carbon Sources on the Growth of Agaricus

Selected isolate of *Agaricus* was grown in media containing different carbon sources *viz.,* glucose, fructose, xylose, mannose and sucrose. These carbon sources were substituted for dextrose, in potato dextrose agar medium. The media were prepared and sterilized by autoclaving at 15 lbs pressure for 20 min. After cooling each medium was poured separately into sterile petri dishes of nine cm diameter and allowed to solidify. A culture disc of five mm diameter, cut out from seven day old culture of the isolate, was inoculated separately in each medium and incubated at 25 ± 2 ºC. Four replications were maintained for each treatment. Colony diameter was measured at weekly intervals up to14 days.

The above experiment was repeated in liquid media with above mentioned carbon sources. The liquid media with different carbon sources were prepared; the composition was same as that used in previous experiment with the exclusion of agar. Fifty ml of each sterilized medium was separately then inoculated with five mm disc of actively growing culture and incubated at 25 ± 2 ºC for 20 days. The mycelila mat was filtered through a Whatman No: 1 filter paper and dried at 70 ºC and weighed until constant weight was obtained.

3.2.2.4. Influence of Different Nitrogen Sources on the Growth of Agaricus

Different forms of nitrogen such as potassium nitrate, ammonium carbonate, ammonium chloride, beef extract and peptone were substituted for sodium nitrate in Czapek's Dox medium so as to give the same per cent of nitrogen in each case. The media were prepared and sterilized by autoclaving at 15 lbs pressure for 20 min. After cooling it was poured into sterile petri dishes of nine cm diameter and allowed to solidify. Five mm diameter disc cut out from seven day old culture of fungus was used for inoculation. The culture disc of the mushroom was inoculated into petri dish which was later incubated at 25±2 ºC. Three replications were maintained for each treatment. Colony diameter was measured at weekly intervals up to14 days.

The liquid media with different nitrogen sources were prepared with the same composition as used in previous experiment except for the exclusion of agar. Fifty ml of each sterilized medium was separately then inoculated with five mm disc of actively growing culture and incubated at 25 ± 2 ºC for 20 days. The mycelial mat was filtered through a Whatman No: 1 filter paper and dry weights were taken after drying at 70 ºC until constant weight was obtained.

3.2.2.5. Effect of Different Hydrogen Ion Concentrations on the Growth of Agaricus

Potato dextrose agar medium was prepared by separately adjusting the pH to 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 by adding 0.1 N hydrocholoric acid or 0.1 N sodium hydroxide. The media prepared were sterilized by autoclaving at 15 lbs pressure for 20 min. After cooling it was poured into sterile petri dishes of nine centimetre diameter and allowed to solidify. Discs of five mm diameter cut out from seven day old culture of fungus were used for inoculation. The culture disc of the mushroom was inoculated into petri dish which was later incubated at 25±2 ºC. Four replications were maintained for each treatment. Colony diameter was measured at weekly intervals up to14 days.

Potato dextrose broths were prepared with different pH concentrations as mentioned above. Fifty ml of medium was taken in 100 ml conical flask and autoclaved at 15 lbs pressure for 20 min. The medium was then inoculated with a five mm disc of seven day old culture of the selected isolate and incubated at 25 ± 2 ºC for 20 days. The mycelial mat was filtered through a Whatman No: 1 filter paper and dry weights were taken after drying at 70 ºC until constant weight was obtained.

3.2.2.6. Effect of Different Temperature on the Growth of Agaricus

Sterile petri dishes plated with PDA was inoculated with five mm culture disc of actively growing mycelium of selected isolate. The inoculated petri dishes were incubated at 5 ºC, 10 ºC, 15 ºC, 20 ºC, 25 ºC, and 30 ºC. The radial mycelial growth was measured at weekly intervals up to 14 days.

Fifty ml of potato dextrose broth was taken in 100 ml conical flask and autoclaved at 15 lbs pressure for 20 minutes. The medium was then inoculated with a 5 mm disc of seven day old culture of isolate and incubated at 5 ºC, 10 ºC, 15 º C, 20 ºC, 25 ºC, and 30 ºC for twenty days. The mycelial mat was filtered through a Whatman No: 1 filter paper and dry weights were taken after drying at 70 ºC until constant weight was obtained.

3.2.2.7. Effect of Light and Darkness on the Growth of Agaricus

Sterile petri dishes plated with PDA was inoculated with five mm culture disc of actively growing mycelium of the selected isolate. The inoculated plates were incubated in ambient light, fluorescent light and dark conditions. Colony diameter was measured at weekly intervals up to 14 days.

The experiment was repeated in potato dextrose broth medium. The broths were then inoculated with a five mm disc of seven day old culture of *Agaricus* and incubated under ambient light, fluorescent light and dark condition for 14 days.

The mycelial mat was filtered through a Whatman No: 1 filter paper and dry weights were taken after drying at 70 ºC until constant weight was obtained.

3.3 GROWTH OF *Agaricus* ON DIFFERENT SPAWN SUBSTRATES

The selected isolate was evaluated for growth on different spawn substrates.

Spawn was prepared using different substrates namely grains of paddy, maize, ragi, wheat and sorghum according to the method described by Sinden (1934).

The above grains were cooked separately for one hour in boiling water. The excess water was drained off and the grains were spread on a clean area for drying. The cooked grains of each substrate were mixed with calcium carbonate at the rate of 50 g per kg of grains. Calcium carbonate amended grains were then packed separately in polypropylene bags at the rate of 300 g per bag and sterilized at 15 lbs pressure for 2 hrs in an autoclave. Mycelium bits from seven days old actively growing pure culture of *Agaricus* isolate were inoculated aseptically into sterilized grains of the different substrates packed in polypropylene bags. Inoculated bags were incubated at a temperature of 25 ± 2ºC for 15 days. Time taken for spawn run and nature of mycelium growth of the isolate in each substrate were observed and recorded. The best spawn obtained was maintained as mother spawn for subsequent spawn production as well as for raising mushroom beds.

3.4 YIELD PERMORMANCE STUDIES

This study was conducted to find out the yield performance of selected isolate of *Agaricus* using different composts and different casing materials. Cultivation of *Agaricus* was done according to the method described by Rai *et al*. (2005). Growth and yield parameters such as days taken for complete mycelia run, number of sporocarps produced, average weight of sporocarp, total yield per bed

after three harvests and average yield on different substrate combinations were studied in detail for six substrates. Different steps of cultivation include composting, spawning and casing.

3.4.1 Composting

The following base materials were used for composting.

1. paddy straw
2. sugarcane bagasse
3. spent mushroom substrate
4. saw dust
5. wheat bran
6. pearl millet straw

Composting using each of the selected substrate was done as follows.

Five kilograms of each of the above base material was placed in a plastic basin of diameter sixty centimetres. The base material was then added with mixture of poultry manure, gypsum and urea in the ratio 10 : 4 : 0.3 : 0.15. The compost mixture in the container was sprinkled with water and frequently turned for 24 - 48 hrs until it absorbed sufficient moisture (around75 per cent). The compost mixture was subsequently turned at every 3 days interval for a period of 25 days. On the 28^{th} day the pile was crushed and checked for the emission of ammonia. The composted treatments in which smell of ammonia persisted were treated with formalin and left in the open till the smell of ammonia could not be detected. The fully composted substrates prepared as above were next used for the bed preparation.

3.4.2 Bed preparation

Beds of the selected isolate of *Agaricus* were prepared using spawned compost raised in various substrates as mentioned in 3.4.1 overlaid with different casing materials as follows. Different materials were separately used for casing over the spawned substrate in the polythene bags in order to determine the best treatment combination of compost substrate and casing materials suitable for better yield performance of the selected isolate.

3.4.2.1 Preparation of Spawned Compost

Polythene bags of size 60 x 30 cm, open at one end and pin pricked for aeration were used for the mushroom bed preparation. The sterilized compost was thoroughly mixed with spawn produced in the best spawn substrate observed in 3.3 at the rate of 500g per ten kg of compost. The spawned compost thus prepared was filled in polythene bags up to a height of 20 cm. There after the open end of polythene bag was tied and the prepared beds containing the spawned beds were stored at 23±1°C and 90 per cent humidity, for completion of spawn run. After complete mycelia run, bags were opened and casing was done as follows.

3.4.3 Casing

After the completion of mycelia run, casing was done over the spawned compost in the polythene bags using different materials, as follows.

1. Vermi compost

2. Tea waste

3. Coir pith compost

4. Red soil + sand + cow dung (1:1:1).

Each of the above casing material was mixed separately with one per cent calcium carbonate to retain 60 per cent moisture content. The calcium carbonate amended casing material was packed in polypropylene bags and were then sterilized at 15 lbs pressure for two hrs. After cooling, the materials were used for casing over spawned compost of the prepared mushroom beds.

The fully white *Agaricus* beds were opened and casing material was packed firmly on the top up to a thickness of 3 cm. After casing, the beds were sprayed with water to give sufficient moisture. Observations of the mycelia run and emergence of pinheads were taken from the time of mycelia colonization on the casing material up to the harvest of the fruiting bodies.

3.5 INCIDENCE OF PESTS AND DISEASES

Incidence of pests and diseases was observed from the period of spawn run, case run up to the harvest of the fruiting bodies. The beds were monitored daily for the occurrence of pests and diseases. Nature and intensity of damages were recorded.

3.6. SHELF LIFE, PROXIMATE CONSTITUENT ANALYSIS AND COOKING QUALITY

3.6.1. Shelf Life

An experiment was conducted to determine the shelf life of *A. bisporus* isolate DMRO 707. The mushrooms harvested from beds were stored at room temperature as well as in refrigerated condition after packing in polypropylene covers with and without perforation.

a) Polypropylene cover without perforation

b) Polypropylene cover with perforation

c) Paper box without perforation

d) Paper box with perforation

The weight of mushroom was observed each day up to one week. Characteristics such as firmness, colour, texture, days up to which no spoilage occurred were recorded.

3.6.2 Proximate Constituent Analysis

3.6.2.1 Estimation of Moisture Content

Ten grams of mushroom sample was dried in an oven until constant weight was obtained. The initial and final weights were noted. The difference between the two weights gives the result, which can be converted into per cent.

3.6.2.2 *Estimation of Protein*

Protein content of *A. bisporus* isolate DMRO 707 was estimated using the standard method described by Bradford (1976).

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

3.6.2.3 *Estimation of Fat*

The extraction of fat was carried out using Soxhlet extraction apparatus (Sadasivam and Manikam, 1992).

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

3.6.2.4 *Estimation of Total Sugars or Carbohydrates*

Total carbohydrate content was estimated by anthrone method (Sadasivam and Manikam, 1992).

One hundred mg of *Agaricus* mushroom powder was weighed and transferred into boiling tubes. It was hydrolyzed by keeping it in a boiling water bath for 3 hours with 5 ml of 2.5 N hydrochloric acid, cooled to room temperature and neutralized with sodium carbonate till the effervescence was ceased. The tissue was ground and volume was made up to 100 ml and centrifuged at 5000 rpm for 15 min. The supernatant was collected and was used as an aliquot for analysis. From the supernatant, 0.5 ml of aliquot was taken and made up to 1 ml by adding distilled water. The reaction mixture containing 0.5 ml of aliquot, 0.5 ml distilled water and 4 ml of anthrone reagent was added to the tubes and heated for 8 minutes in boiling water bath. The reaction mixture was cooled and colour read at 630 nm in a spectrophotometer (Systronics UV-VIS spectrophotometer 118). The amount of carbohydrate present was calculated from the standard graph prepared using glucose and expressed in terms of milligrams of glucose equivalent per gram of sample on dry weight basis.

3.6.2.5 Estimation of Crude Fibre

Crude fibre content was estimated by a standard method described by Sadasivam and Manikam(1992).

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

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

3.6.2.6 ***Estimation of Ash***

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

3.6.2.7. Estimation of Nitrogen, Phosphorus and Potassium

Digestion of sample

Digestion of the sample was carried out using kjeldhal's digestion assembly. Weighed 0.5 gm of dried finally powdered mushroom sample was placed in to the long tube of kjeldhal's digestion assembly and put a pinch of digestion mixture (Na_2So_4 + $CuSo_4$ + selenium powder (100:10:1)). Then added 10 ml con.H_2So_4 and kept it overnight. On the next day, tubes were placed on the bent of the digestion unit and connected the flames tube. Then connected the instrument to water supply. Set the temperature at 50 ^{0}C initially and then increased the temperature up to 350^{0}C. On completion of digestion, solution in the tube became clean. After cooling, transferred the contents in to 100 ml volumetric flask and made up the volume up to 100 ml.

3.6.2.7.1. Estimation of Nitrogen

Nitrogen content in the *A. bisporus* was estimated using Kjeldhal's micro distillation unit. Took 10 ml of digested sample in micro kjeldhal flask and then it was fixed in to the distillation unit. Then automatically added 10 ml of 40% Na OH. Simultaneously, took a conical flask and added 3 drops of mixed indicator. Four per cent boric acid was poured into this flask by pressing the boric acid button. Set the time of distillation as 8 minutes and started the operation. At the end of distillation, the solution in the conical flask left became blue in colour.

Collected ammonia, in the conical flask was then titrated with 0.02N H_2So_4, till the appearance of a faint pink colour.

$$\text{Percentage of nitrogen} = \frac{(y-x) \times 0.02 \times 0.014 \times 100 \times 100}{0.5 \times 10}$$

Weight of sample taken = 0.5gram

Normality of H_2So_4 = 0.02N

Aliquot taken = 10ml

1ml of 1N H_2So_4 = 0.014 of nitrogen

Blank value = x

Titre value = y

3.6.2.7.2. Estimation of Phosphorus

For the preparation of standards took 2,4,6,8 and 10 ml of 50 ppm standard phosphorus solution in a volumetric flask to get 2,4,6,8 and 10 ppm phosphorus. Then took 10 ml of digest in a 50 ml standard flask. Added 10 ml of Barton's reagent and made up to 50 ml. Then allowed 30 minute for full colour development. It will be stable for two months, if phosphorus concentration is less it will be stable for only two weeks. Prepared a blank and read the intensity of colour in the spectrophotometer at 470nm

Percentage of total phosphorus = x×50/10×100/0.5×1/10000

x = Concentration of total phosphorus.

3.6.2.7.3. Estimation of Potassium

Set up the flame photometer and aspirated working standards. Pipetted out 5ml aliquot from sample extract to 50 ml volumetric flask and made up the volume. After aspiration of working standards, aspirated the sample and noted the readings.

Percentage of K = x×50/5×100/0.5×1/10000

3.6.3 Cooking Quality of A. bisporus

Three different recipes *viz*., *Agaricus* cutlets, mushroom masala and soup were prepared and they were subjected to evaluation by ten judges based on a five point score card. Parameters like colour and appearance, texture, flavour, taste and overall acceptability were rated. Score card values for each character is given in Appendix - III. The recipes are given in Appendix – IV.

Results

4. RESULTS

4.1 COLLECTION, ISOLATION AND PURIFICATION OF ISOLATES

Agaricus spp. was collected from south Kerala after the South West and North East monsoons. (Plate 1). The habit of these strains varied from solitary to gregarious type and they were terrestrial. Organic matter rich soil was found to be the usual spots for the occurrence of *Agaricus* (Table 1). The period of occurrence of these mushrooms were from June – October. Collected isolates along with two strains already present in the AICRP centre were sent to Directorate of Mushrooms for getting accession numbers. The selected isolate after preliminary trials was given accession number as DMRO-707 and identified as *Agaricus bisporus* from Directorate of Mushroom Research, Solan.

4.1.1 Isolation and purification of culture

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

4.2 MORPHOLOGICAL, CULTURAL AND PHYSIOLOGICAL STUDIES

4.2.1 Identification of isolates

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

Pileus: Convex, white to pale yellow in colour and fleshy in texture. The diameter of the pileus of these isolates range from 4-8 cm.

Table 1. Characteristics of isolates of *Agaricus* under natural condition

Name of isolate	**Habit**	**Habitat**	**Substrate**	**Place of collection**
Isolate 1	Solitary	Terrestrial	Meadow	Vellayani
Isolate 2	Gregarious	Terrestrial	Organic matter rich soil	Vellayani
Isolate 3	Gregarious	Terrestrial	Soil	Vellayani
Isolate 4	Solitary	Terrestrial	Soil	Ponmudi
Isolate 5	Gregarious	Terrestrial	Compost	Solan
Isolate 6	Gregarious	Terrestrial	Compost	Patna
Isolate 7	Gregarious	Terrestrial	Compost	Munnar

(A) Isolate 1

(B) Isolate 2

(C) Isolate 3

(D) Isolate 4

(E) Isolate 5

(F) Isolate 6

(G) Isolate 7

Plate 1. Collected isolates of *Agaricus* spp.

Table 2. Morphological features of various isolates of *Agaricus* spp.

Name of isolate	Colour	Texture	Pileus			Stipe				
			Shape	Diameter (cm)	Thickness (cm)	Length (cm)	Diameter (cm)	Shape	Attachment of pileus	Surface
Isolate 1	Pale yellow	Fleshy	Convex	6.30	2.30	3.20	2.20	Bulbous	Central	Scaly
Isolate 2	Pale yellow	Fleshy	Convex	7.10	1.70	4.50	2.90	Bulbous	Central	Scaly
Isolate 3	Pale yellow	Fleshy	Convex	4.70	1.50	3.50	2.20	Bulbous	Central	Scaly
Isolate 4	White	Fleshy	Convex	5.60	2.50	4.30	2.10	Bulbous	Central	Scaly
Isolate 5	White	Fleshy	Convex	5.10	2.20	4.10	2.60	Bulbous	Central	Smooth
Isolate 6	White	Fleshy	Convex	5.80	2.60	3.70	2.50	Bulbous	Central	Scaly
Isolate 7	White	Fleshy	Convex	5.40	2.90	4.90	2.30	Bulbous	Central	Scaly

Table 2. continued.

Name of the isolate	Gills				Spore		
	Arrangement	Texture	Margin	Gills/cm	Spore colour	Spore print	Shape
Isolate 1	Free	Brittle	Smooth	24	Chocolate brown	Brown	Ovoid
Isolate 2	Free	Brittle	Smooth	32	Chocolate brown	Brown	Ovoid
Isolate 3	Free	Brittle	Smooth	21	Chocolate brown	Brown	Ovoid
Isolate 4	Free	Brittle	Smooth	28	Chocolate brown	Brown	Ovoid
Isolate 5	Free	Brittle	Smooth	39	Chocolate brown	Brown	Ovoid
Isolate 6	Free	Brittle	Smooth	37	Chocolate brown	Brown	Ovoid
Isolate 7	Free	Brittle	Smooth	42	Chocolate brown	Brown	Ovoid

Stipe: Bulbous shaped, scaly surface, centrally attached to the pileus. The length of the stipe of each isolate of *Agaricus* ranged from 3 - 5 cm with a diameter of 2-3 cm.

Spore: The Spores (Plate 2 A) were ovoid in shape and chocolate brown in colour, spore print was brown coloured (Plate 2 B).
Microscopic characters of different isolates are given in table 3. The gills of all ioslates were freely arranged with smooth margin.

Among the different isolates tested preliminarily for their growth on PDA (Table 4 and Plate 3) and paddy grains (table 5 and Plate 4), radial growth and spawn development of isolate seven was found to be best. The isolates were identified from Directorate of Mushroom Research, Solan. The isolate seven which was accorded the accession number DMRO 707 and identified as A. bisporus (Lange) Imbach, according to the identification of the different isolates made at DMR, was used for further studies.

4.2.2 Cultural studies and physiological studies of A. bisporus

4.2.2.1 Influence of different solid media on the growth of A. bisporus

In the case of media, five different solid media namely Potato dextrose agar (PDA), Oat meal agar, Malt extract agar, carrot agar and Czapeck's Dox agar were tested for their efficacy in supporting the radial mycelial growth of A. bisporus. The result showed that the media significantly differed in influencing mycelial growth of A. bisporus (Table 6).

The nature of mycelia growth was very much dense in potato dextrose agar followed by malt extract agar. In oat meal agar and Czapeck's Dox agar the nature of growth was sparse and of medium type and there was no growth in carrot agar (Plate 5).

Table 3. Microscopic characters of different isolates.

Name of the isolate	Hyphae		Spore shape	Spore size (µ)	Spore print
	Colour	Septation			
Isolate 1	Hyaline	Septate	Ovoid	5.5-8.5 x 4-6.5	Brown
Isolate 2	Hyaline	Septate	Ovoid	5.1-7.9x3.9-5.1	Brown
Isolate 3	Hyaline	Septate	Ovoid	4.8-7x4.3-6.7	Brown
Isolate 4	Hyaline	Septate	Ovoid	5.2-6.4x4.2-4.9	Brown
Isolate 5	Hyaline	Septate	Ovoid	5.3-8.1x3.8-6.3	Brown
Isolate 6	Hyaline	Septate	Ovoid	5-7.8x4.3-7.5	Brown
Isolate 7	Hyaline	Septate	Ovoid	4.7-5.5x4.1-5	Brown

Table 4. Growth of different isolates of *Agaricus* on potato dextrose agar medium

Isolate number	Radial growth in cm on 14th day*
Isolate 1	2.30^{g}
Isolate 2	3.30^{f}
Isolate 3	4.47^{d}
Isolate 4	6.73^{c}
Isolate 5	8.10^{b}
Isolate 6	4.03^{e}
Isolate 7	**9.00^{a}**
CD(0.05)	0.217

* Average of three replications

Means followed by similar superscripts are not significantly different at 5% level.

Table 5. Number of days taken for spawn run on paddy grains by different isolates of *Agaricus*

Isolate number	No. of days taken for spawn run*
Isolate 1	20.67[a]
Isolate 2	17.33[b]
Isolate 3	14.67[d]
Isolate 4	13.00[e]
Isolate 5	12.67[e]
Isolate 6	16.33[c]
Isolate 7	**11.67[f]**
CD(0.05)	0.941

* Average of three replications

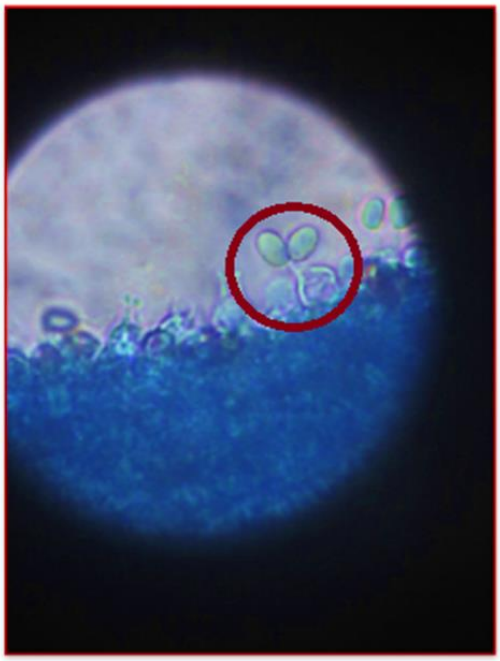
Means followed by similar superscripts are not significantly different at 5% level.

Table 6. Growth of A. bisporus in different solid media

Media	**Radial growth (cm)**	
	7 th day	14 th day
Carrot agar	0.00 **e**	0.00 **e**
Czapeck's Dox agar	2.35 **d**	4.90 **d**
Malt extract agar	3.20 **b**	6.40 **b**
Oat meal agar	2.65 **c**	5.30 **c**
Potato Dextrose Agar	**4.48** **a**	**9.00** **a**
CD (0.05)	0.158	0.176

* Average of four replications

Means followed by similar superscripts are not significantly different at 5% level.



(A) Microscopic view of spores of *A. bisporus*

(B) Spore print of *A. bisporus*

Plate 2. Spores and spore print of *A. bisporus*

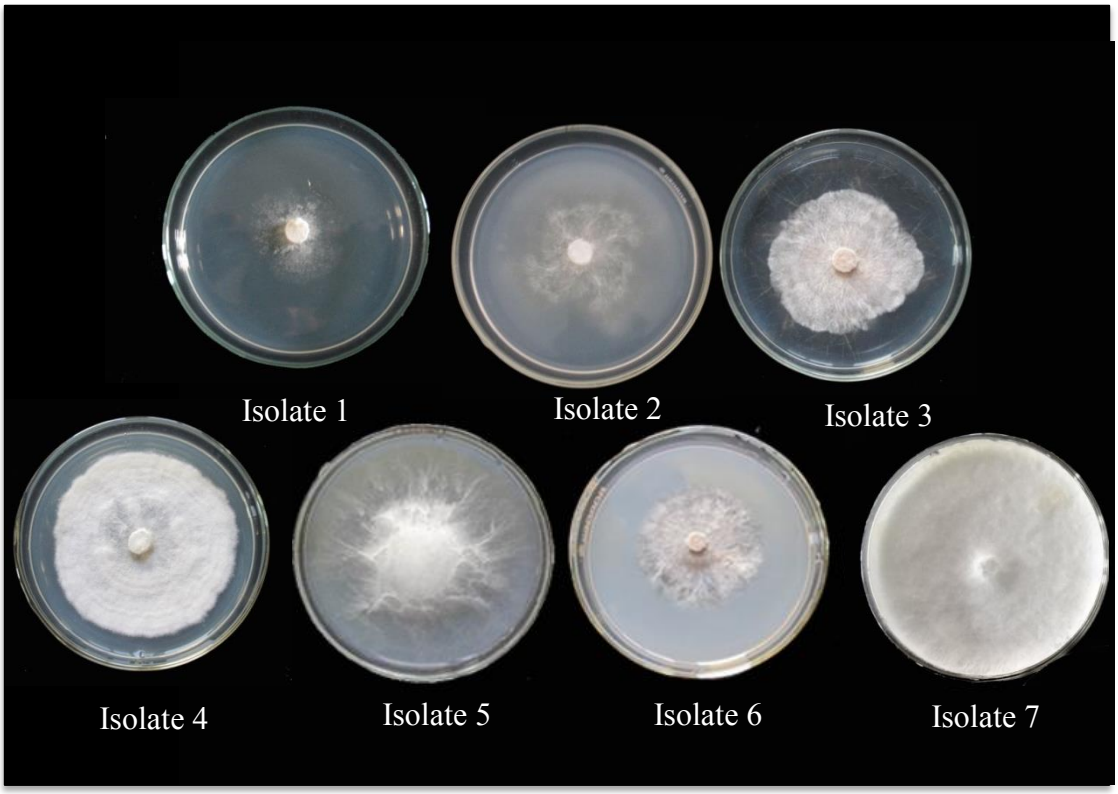


Plate 3. Growth of different isolates of *Agaricus* on potato dextrose agar medium

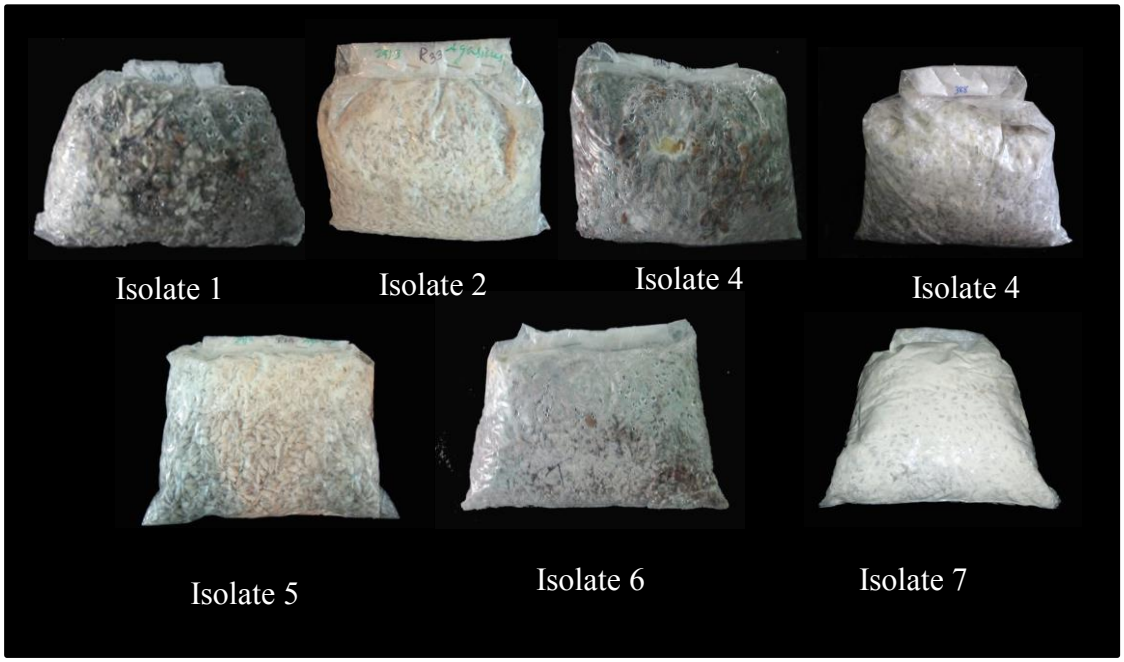


Plate 4. Growth of different isolates of *Agaricus* on paddy grains

Radial growth of *Agaricus* isolate DMRO 707 after seven days was highest on potato dextrose agar in producing 4.46 cm radial growth followed malt extract agar with 3.20 cm, oat meal agar with 2.65 cm and Czapeck's Dox with 2.35 cm radial growth. No growth was found in carrot agar media. Radial growth of the isolate was significantly different in each medium tested.

Radial growth of *Agaricus* isolate DMRO 707 was highest on PDA (9.00 cm) on seventh day as well as 14th day after inoculation. Malt extract agar (6.40 cm) was found to be next best media which was followed by oat meal agar (5.30 cm) and Czapeck's Dox agar (4.90 cm). No growth was found in Carrot agar media.

4.2.2.2 Influence of different liquid media on the growth of A. bisporus

The biomass production of *A. bisporus* isolate DMRO 707 was estimated in different liquid media *viz*, potato dextrose broth, oat meal broth, malt extract broth, carrot broth and Czapeck's Dox broth. The result showed that liquid media differed significantly in influencing biomass production of *A. bisporus* (Table 7).

The biomass production of *Agaricus* after 20 days of incubation indicated that potato dextrose broth was found to be the most promising treatment with 124.33 mg / 50 ml of biomass production. This was followed by malt extract broth (102.66 mg/50ml), oat meal broth (77.99 mg/50ml) and Czapeck's Dox broth (16.33 mg/50ml). No growth was found in carrot broth. (Plate 6). All these treatments were found to be significantly different from each other.

Table 7. Growth of *A. bisporus* in different liquid media

Media	**Dry weight of mycelium (mg/50 ml)**
Carrot broth	0.000
Czapeck's Dox broth	16.33^{d} (4.16)
Malt extract broth	102.66^{b} (10.18)
Oat meal broth	77.99^{c} (8.89)
Potato Dextrose broth	**124.33^{a} (11.19)**
CD (0.05)	(0.001)

* Average of three replications

Values shown in parenthesis are √x+1 transformed values

Means followed by similar superscripts are not significantly different at 5% level.

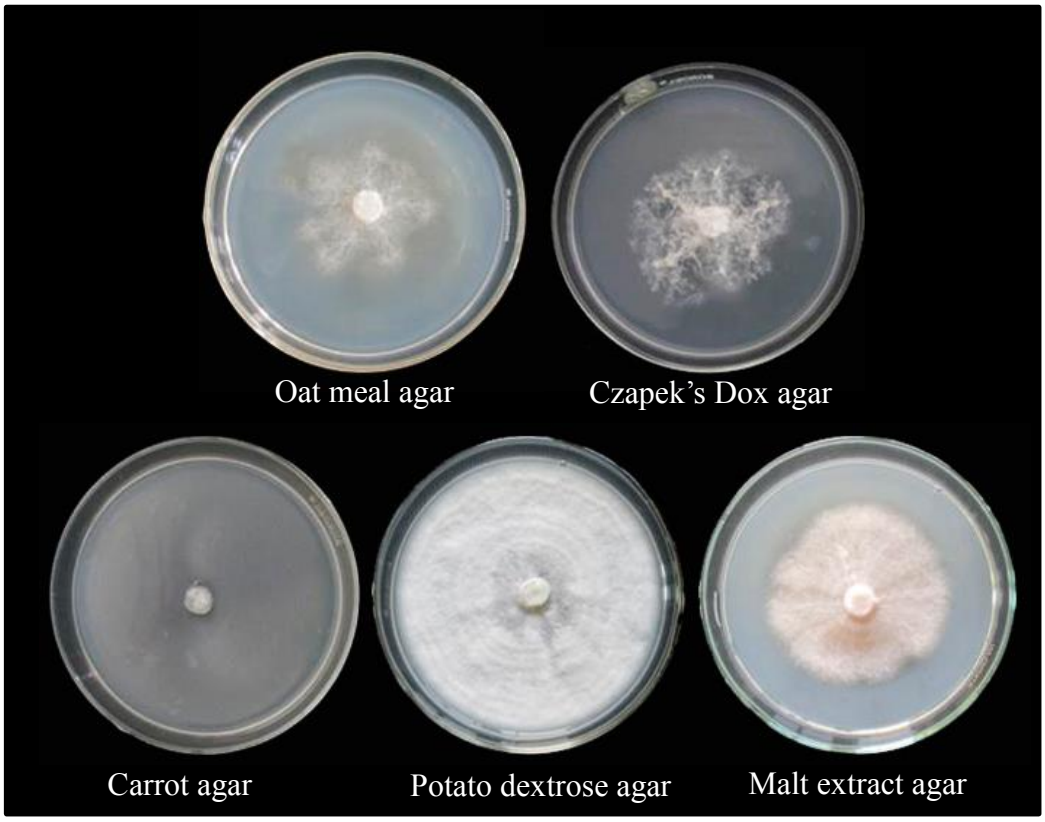


Plate 5. Growth of *A. bisporus* in different solid media

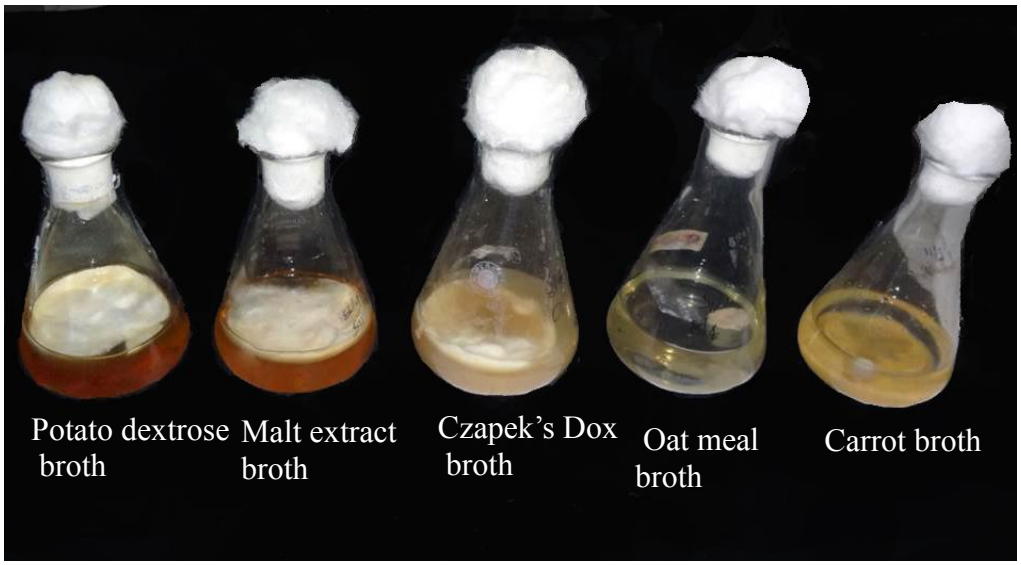


Plate 6. Growth of *A. bisporus* in different liquid media

4.2.2.3 Effect of different carbon sources on the growth of A. bisporus

Five different carbon sources namely glucose, sucrose, fructose, xylose and mannose were tested in solid and liquid medium for their efficacy in radial mycelia growth and biomass production of *A. bisporus isolate DMRO 707.* The different carbon sources differed significantly in influencing the radial growth (Table 8 ; Plate 7) and biomass production of the isolate used.

Among the carbon sources substituted in PDA, highest radial mycelia growth of the isolate DMRO 707 after seven days of incubation (4.53 cm) was recorded in glucose substituted medium. On the same medium full growth of the isolate was attained fourteen days after incubation. This was followed by fructose substituted medium in which radial growth of 3.30 and 6.55 cm observed seven days and fourteen days after incubation respectively. Seven days after incubation the radial mycelia growth of the isolate on sucrose and xylose substituted PDA media were 2.63 and 2.60 cm respectively and were on par. On the same media observations of the radial mycelia growth fourteen days of incubation were 5.53 and 5.45 cm respectively which were on par. Least growth was observed in PDA substituted with mannose on which the isolate DMRO 707 produced 1.03 cm radial growth seven days after incubation and 1.93 cm after fourteen days of incubation.

In potato dextrose broth substituted with different carbon sources, glucose supported maximum biomass production the isolate DMRO 707 with 128.60 mg / 50 ml followed by fructose (57.17 mg / 50 ml), sucrose (44.15 mg / 50 ml) and xylose (35.71 mg / 50 ml) (Plate 8). The carbon source, mannose was least suitable in inducing mycelia growth producing 15.88 mg/50ml of biomass (Table 9).

Table 8. Growth of *A. bisporus in solid media* in different carbon sources

Media	**Radial growth of (cm)***	
	7th day	14th day
Glucose	**4.53[a]**	**9.00[a]**
Fructose	3.30[b]	6.55[b]
Xylose	2.60[c]	5.45[c]
Mannose	1.03[d]	1.93[d]
Sucrose	2.63[c]	5.53[c]
CD (0.05)	0.265	0.174

* Average of four replications

Means followed by similar superscripts are not significantly different at 5%level.

Table 9. Growth of *A. bisporus* in liquid media using different carbon sources

Media	Dry weight of mycelia (mg/50ml)*
Glucose	128.60^{a} (11.38)
Fructose	57.16^{b} (7.63)
Xylose	35.71^{d} (6.06)
Mannose	15.88^{e} (4.11)
Sucrose	44.15^{c} (6.72)
CD (0.05)	0.325

*Average of four replications

Values shown in parenthesis are $\sqrt{x+1}$ transformed values

Means followed by similar superscripts are not significantly different at 5% level.

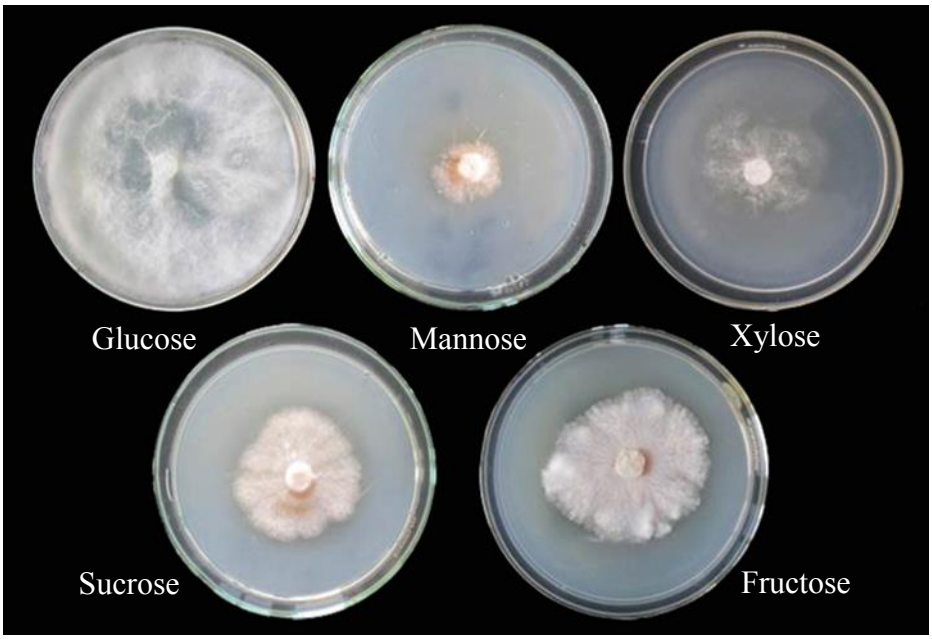


Plate 7. Growth of *A. bisporus* in solid media using different carbon sources

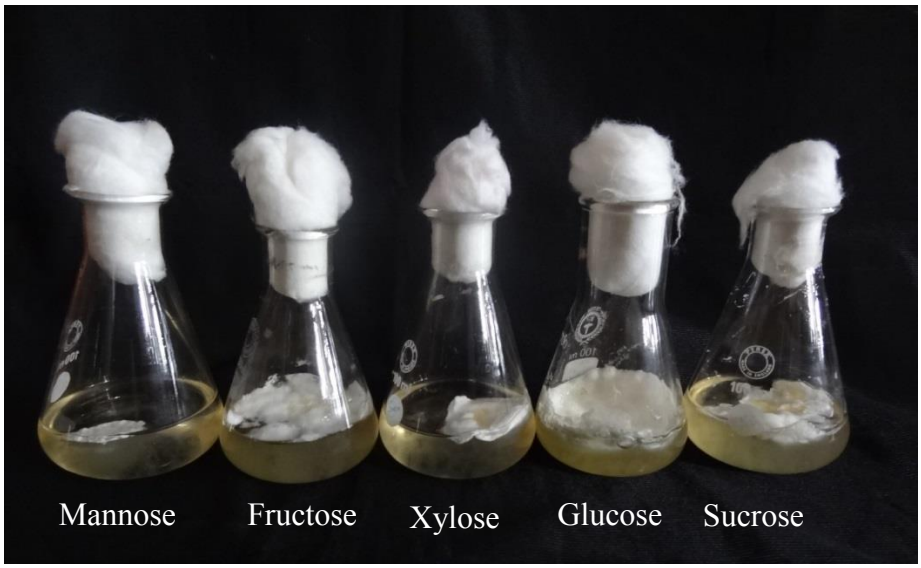


Plate 8. Growth of *A. bisporus* in liquid media using different carbon sources

4.2.2.4 Effect of different nitrogen sources on the growth of A. bisporus

Influence of both organic and inorganic nitrogen sources substituted in solid media was evaluated after seven days and fourteen days after incubation (table 10; Plate 9). Beef extract and peptone were the organic nitrogen sources while inorganic sources were potassium nitrate, sodium nitrate, ammonium chloride and ammonium carbonate. Czapeck's Dox medium was used for the study.

Among the different nitrogen sources substituted in Czapeck's Dox medium highest radial mycelia growth of the isolate DMRO 707 after seven days of incubation (4.53 cm) was recorded in beef extract substituted medium. On the same medium full growth of the isolate was attained fourteen days after incubation. This was followed by peptone substituted medium in which radial growth of 3.83 cm and 7.63 cm observed seven days and fourteen days after incubation respectively. Seven days after incubation the radial mycelia growth of the isolate on ammonium chloride and potassium nitrate substituted Czapeck's Dox media were 2.67 and 2.33 cm respectively. On the same media observations of the radial mycelia growth after fourteen days of incubation were 5.33 cm and 4.63 cm respectively. Least growth was observed in Czapeck's Dox medium substituted with sodium nitrate on which the isolate DMRO 707 produced 2.13 cm radial growth seven days of incubation and 4.20 cm after fourteen days of incubation. No growth of the isolate was observed in medium substituted with ammonium carbonate.

In Czapeck's Dox broth substituted with different carbon sources, beef extract supported maximum biomass production the isolate DMRO 707 with 122.76 mg / 50 ml followed by peptone with a biomass 63.49 mg/ml. The medium substituted with ammonium chloride, potassium nitrate and sodium nitrate induced a biomass production of 53.43, 25.70 and 12.83 mg respectively.

Table 10. Growth of *A. bisporus in solid media* in different nitrogen sources

Media	Growth of *Agaricus* (cm)*	
	7 th day	14 th day
Peptone	3.83[b]	7.60[b]
Ammonium carbonate	0.00[f]	0.00[f]
Beef extract	4.53[a]	9.00[a]
Potassium nitrate	2.33[d]	4.60[d]
Sodium nitrate	2.13[e]	4.20[e]
Ammonium chloride	2.67[c]	5.30[c]
CD (0.05)	0.173	0.230

* Average of three replications

Means followed by similar superscripts are not significantly different at 5% level.

Plate 9. Growth of *A. bisporus* in solid media using different nitrogen sources

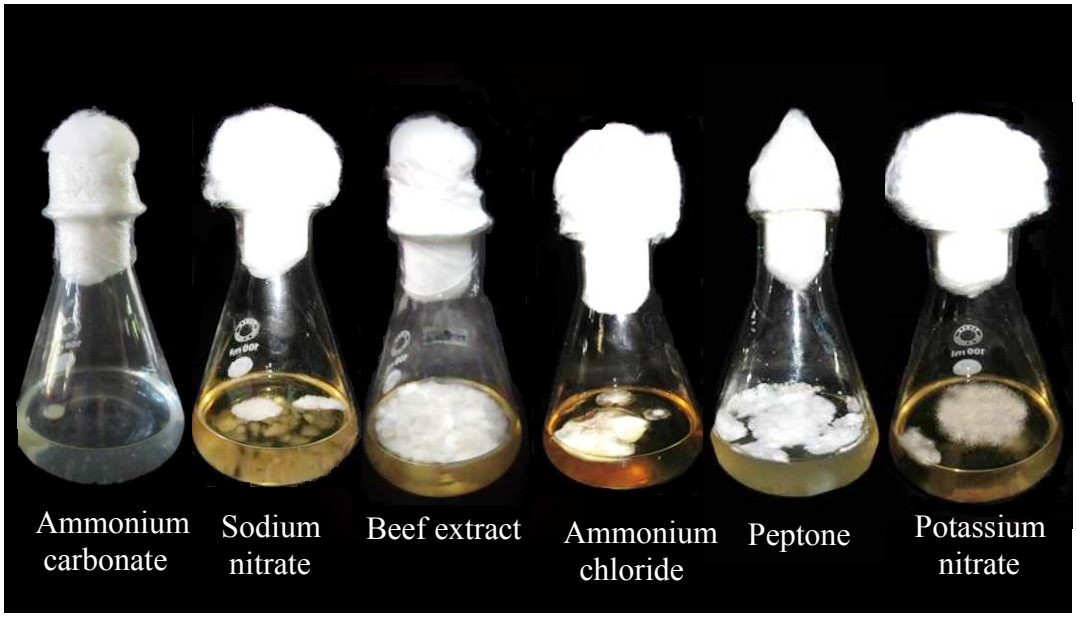


Plate 10. Growth of *A. bisporus* in liquid media using different nitrogen sources

Growth of the isolate DMRO 707 was not observed in ammonium carbonate substituted medium. (Table 11; Plate 10).

4.2.2.5 Effect of Different Hydrogen Ion Concentrations in the Media on the Growth of A. bisporus

Six different H^+ ion concentrations (pH) ranging from 4 to 9 were tested in solid and liquid potato dextrose medium for their efficacy in inducing mycelia growth and biomass production of *A. bisporus* isolate DMRO 707. The different H^+ ion concentrations differed significantly in influencing the radial growth (Table 12; Plate 11) and biomass production of the isolate used.

Among the PDA media with different H^+ ion concentrations, highest radial mycelia growth of the isolate DMRO 707 after seven days of incubation (4.43·cm) was recorded in PDA medium with pH 6. On the same medium full growth of the isolate was attained fourteen days after incubation. Seven days after incubation the radial mycelia growth of the isolate on PDA medium with pH 7 and 8 were 4.23 and 4.20 cm respectively and were on par. On the same media observations of the radial mycelia growth after fourteen days of incubation were 8.70 cm and 8.60 cm respectively which were on par. Seven days after incubation the radial mycelia growth of the isolate on PDA medium with a pH of 9, 5 and 4 were 3.65cm, 3.33 cm and 3.13 cm respectively. On the same media observations of the radial mycelia growth after fourteen days of incubation were 7.30 cm, 6.60 cm and 6.40 cm respectively.

In potato dextrose broth with different pH, pH 6.0 supported maximum biomass production the isolate DMRO 707 with 125.88 mg/50 ml followed by pH 7.0 (118.94 mg), pH 8.0 (112.67 mg), pH 9.0 (95.02) and pH 5.0 (79.83 mg) and being on par (Table13; Plate 12).The pH 4.0 was least suitable in inducing mycelia growth producing 70.33 mg/50ml of biomass.

Table 11. Growth of *A. bisporus* in liquid media using different nitrogen sources

Media	**Dry weight of mycelia (mg/50ml)***
Peptone	63.49 [b] (8.03)
Ammonium carbonate	0.00 [f]
Beef extract	**122.76 [a] (11.17)**
Potassium nitrate	25.69 [d] (5.17)
Sodium nitrate	12.83 [e] (3.72)
Ammonium chloride	53.43 [c] (7.38)
CD (0.05)	0.121

* Average of three replications

Values shown in parentheses are $\sqrt{x+1}$ transformed values

Means followed by similar superscripts are not significantly different at 5 % level

Table 12. Growth of *A. bisporus* in solid media using different pH levels.

pH	Radial growth (cm)*	
	7th day	14th day
4.0	3.13 e	6.35 e
5.0	3.33 d	6.65 d
6.0	**4.43** a	**9.00** a
7.0	4.23 b	8.68 b
8.0	4.20 b	8.55 b
9.0	3.70 c	7.32 c
CD (0.05)	0.115	0.214

* Average of four replications

Means followed by similar superscripts are not significantly different at 5 % level

Table 13. Growth of *A. bisporus* in liquid media using different pH levels

pH	Dry weight of mycelia (mg/50ml)*
4	^{f} 70.33 (8.45)
5	^{e} 79.83 (8.99)
6	^{a} **125.88 (11.26)**
7	^{b} 118.94 (10.95)
8	^{c} 112.67 (10.66)
9	^{d} 95.02 (99.79)
CD (0.05)	2.763

* Average of four replications

Values shown in parentheses are $\sqrt{x+1}$ transformed values

Means followed by similar superscripts are not significantly different at 5 % level

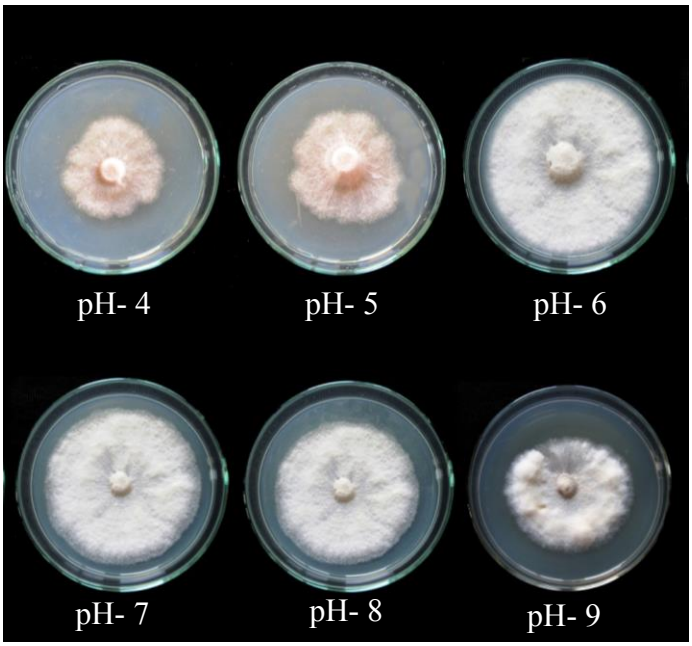


Plate 11. Growth of *A. bisporus* in solid media using different pH levels

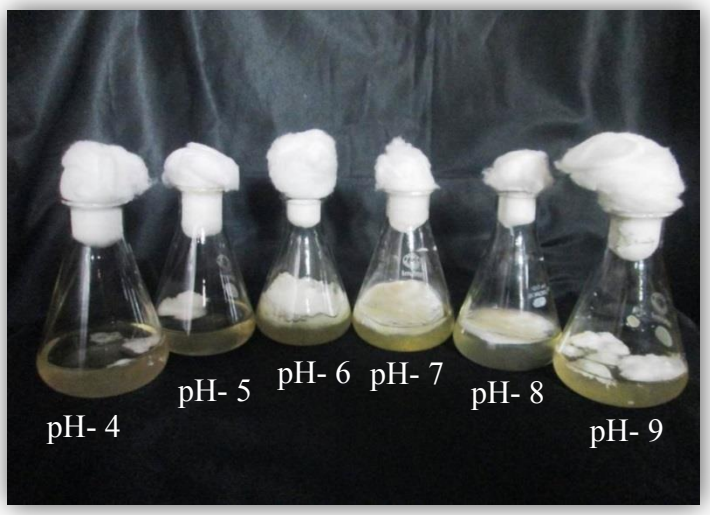


Plate 12. Growth of *A. bisporus* in liquid media using different pH levels

4.2.2.6 Effect of Different Temperature on Growth of the A. bisporus

Six different temperature conditions of 5 °C, 10 °C, 15 °C, 20 °C, 25 °C and 30°C were tested in solid and liquid potato dextrose medium for their efficacy in inducing mycelia growth and biomass production of *A. bisporus* isolate DMRO 707. The different temperatures differed significantly in influencing the radial growth (Table 14; Plate 13) and biomass production of the isolate used.

Among the PDA media incubated at different temperature conditions, highest radial mycelia growth of the isolate DMRO 707 after seven days of incubation (4.47 cm) was recorded at 25°C. At the same temperature full growth of the isolate was attained fourteen days after incubation. Seven days after incubation the radial mycelia growth of the isolate on PDA medium incubated at 20 °C, 30 °C and 15 °C were 4.23cm, 3.93 cm and 1.63 cm respectively. At the same temperatures observations of the radial mycelia growth after fourteen days of incubation were 8.53 cm, 8.00 cm and 3.30 cm respectively. No radial mycelia growth of the isolate was observed in medium incubated at 5 °C and 10^0C.

In potato dextrose broth incubated at different temperature, 25 °C supported maximum biomass production the isolate DMRO 707 with 125.87 mg /50 ml followed by 20 °C, 30 °C and 15 °C temperature producing a biomass of 116.37 mg, 105.37 mg and 43.23 mg respectively No biomass production of the isolate was observed in medium incubated at 5 °C and 10^0C (Table 15; Plate 14).

Table 14. Growth of *A. bisporus* in solid media at different temperature

Temperature (°C)	Radial growth (cm)*	
	7th day	14th day
5	0.00 e	0.00 e
10	0.00 e	0.00 e
15	1.63 d	3.30 d
20	4.23 b	8.50 b
25	**4.47** a	**9.00** a
30	3.93 c	8.00 c
CD (0.05)	0.137	0.183

* Average of three replications

Means followed by similar superscripts are not significantly different at 5 % level

Table 15. Growth of *A. bisporus* in liquid media at different temperature

Temperature (°C)	**Dry weight of mycelia (mg/50ml)***
5	0.00
10	0.00
15	43.23 [d]
20	116.37 [b]
25	**125.87** [a]
30	105.37 [c]
CD (0.05)	2.151

* Average of three replications

Means followed by similar superscripts are not significantly different at 5 % level

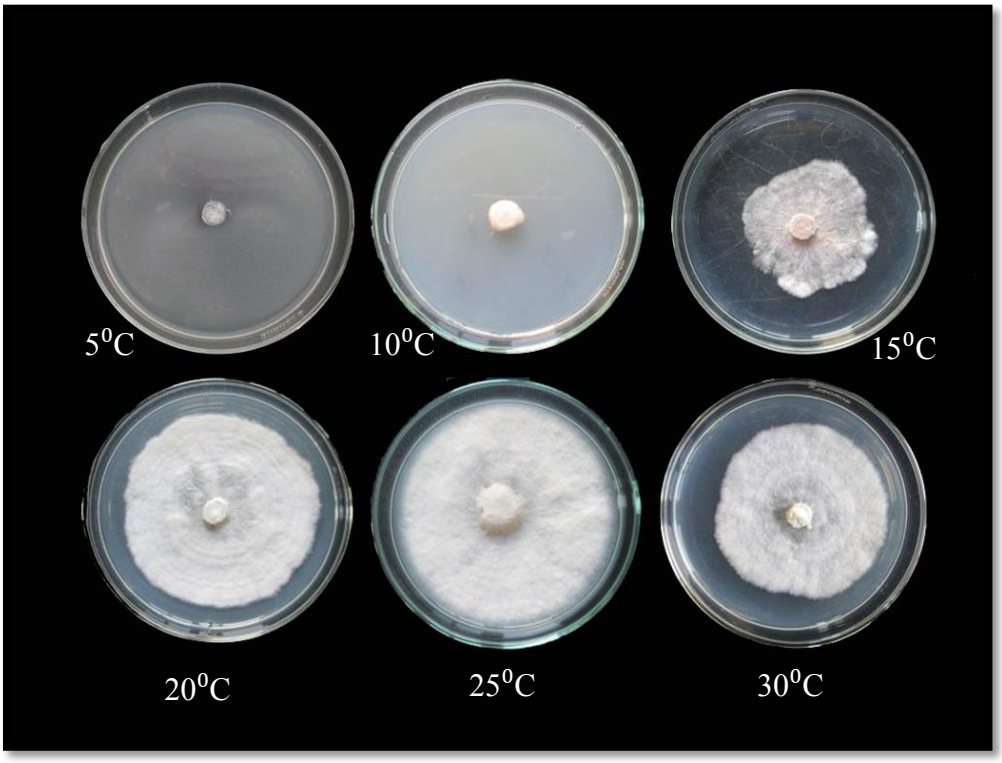


Plate 13. Growth of *A. bisporus* in solid media at different temperature

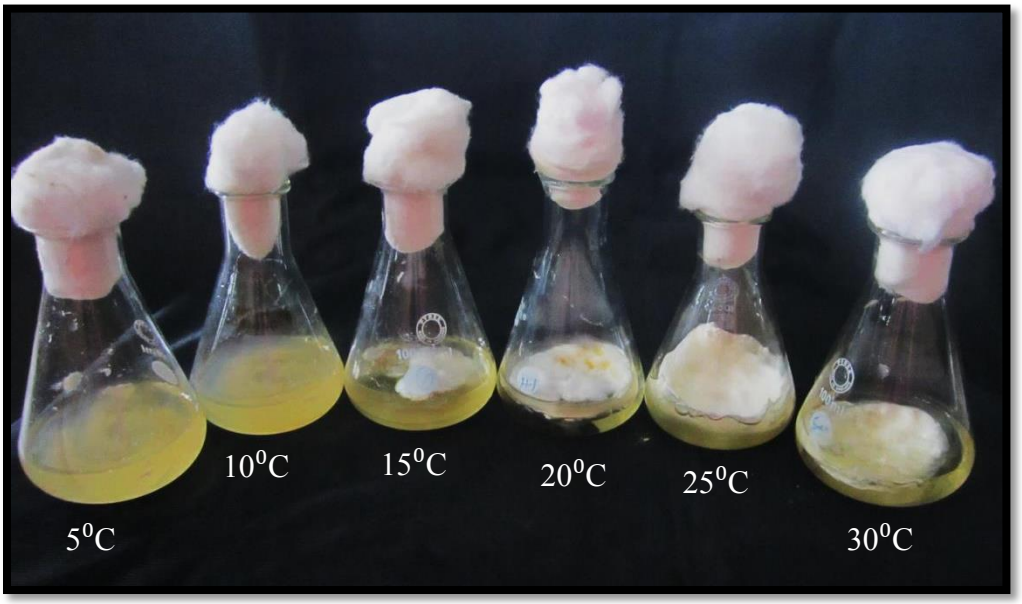


Plate 14. Growth of *A. bisporus* in liquid media at different temperature

4.2.2.7 Effect of Different Light Conditions on the Growth of A. bisporus

Three different ranges of light like ambient light, darkness and fluorescent light were tested in solid and liquid potato dextrose medium for their efficacy in influencing radial growth (Table 16; Plate 15) and biomass production of the isolate used.

Among the PDA media incubated at different light conditions, highest radial mycelia growth of the isolate DMRO 707 after seven days of incubation (4.50 cm) was recorded in the darkness. At the same condition full growth of the isolate was attained fourteen days after incubation. Seven days after incubation the radial mycelia growth of the isolate on PDA medium incubated at ambient condition and fluorescent light were 4.16 cm and 3.02 cm respectively. At the same conditions observations of the radial mycelia growth after fourteen days of incubation were 8.30 cm and 6.04 cm respectively.

In potato dextrose broth incubated at different light conditions, darkness supported maximum biomass production the isolate DMRO 707 with 0.13 g /50 ml followed by ambient condition producing a biomass of 0.12 g/50 ml. Least biomass was produced in potato dextrose broth incubated at fluorescent light in which the isolate DMRO 707 produced 0.11g/50 ml after twenty days of incubation. (Table 17; Plate 16).

4.3 GROWTH OF *A. bisporus* ON DIFFERENT SPAWN SUBSTRATES

Five different grain substrates were evaluated on the basis of number of days taken for maximum spawn run and the nature of mycelial growth on the spawn. Spawn substrates tried were grains of paddy, wheat, sorghum, ragi, and maize (Plate 17).

Table 16. Growth of *A. bisporus* in solid media under different light sources

Light	**Radial growth (cm)***	
	7th day	14th day
Fluorescent light	3.02[c]	6.04[c]
Ambient condition	4.16[b]	8.30[b]
Darkness	**4.50**[a]	**9.00**[a]
CD (0.05)	0.113	0.218

* Average of five replications

Means followed by similar superscripts are not significantly different at 5 % level

Table 17. Growth of *A. bisporus* in liquid media under different light sources

Light	**Dry weight of mycelia (g/50ml)***
Normal	0.12[a]
Darkness	**0.13**[a]
Fluorescent light	0.11[a]
CD (0.05)	1.033

* Average of five replications

Means followed by similar superscripts are not significantly different at 5 % level

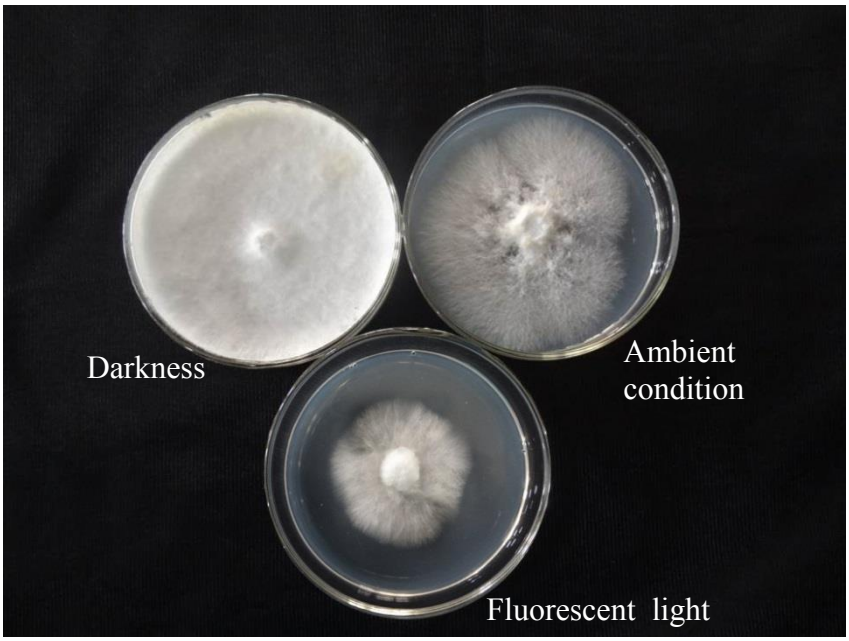


Plate 15. Growth of *A. bisporus* in solid media under different light sources

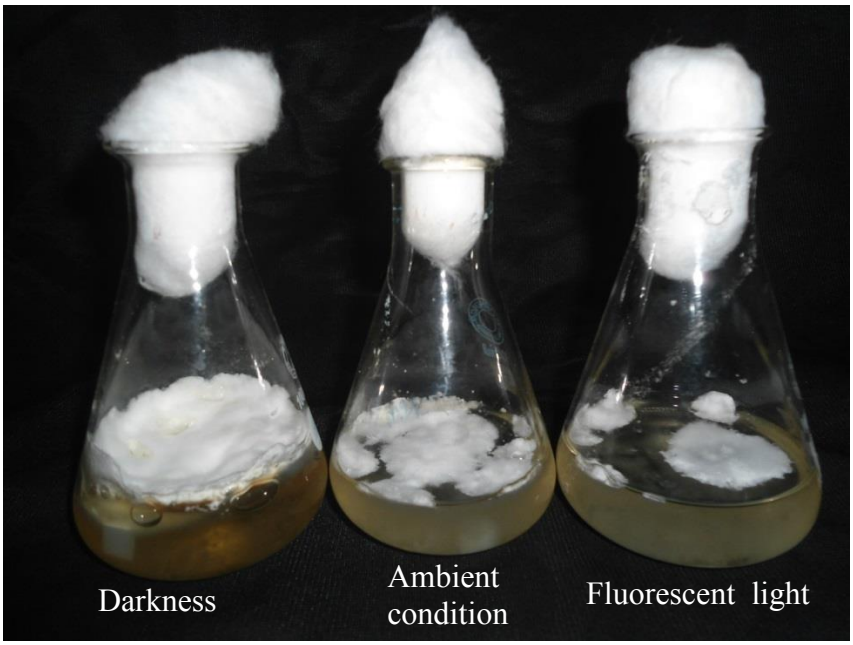


Plate 16. Growth of *A. bisporus* in liquid media under different light sources

Plate 17. Mycelial growth of *A. bisporus* on different spawn substrates

Maximum spawn run of the isolate was produced in paddy grain within a period of 12 days. White, thick and cottony mycelia growth was observed in this spawn substrate. Maximum spawn run was observed on maize and wheat substrates within a period of 13 and 15days respectively. White, thick and fluffy growth was produced on these two substrates (Table 18). Maximum number of days for spawn run was recorded on substrates sorghum (17 days) and ragi (20 days). All treatments were significantly different from each other.

4.4 YIELD PERFORMANCE STUDIES

Growth and yield parameters, such as days taken for complete mycelia run in the spawned compost, number of sporocarps produced, average weight of sporocarp per bed, total and average yield per bed after three harvests, were studied in detail for six different combinations of spawned compost substrates overlaid with four casing materials. Results of the study are presented in Table 19 (Plate 18).

4.4.1 Evaluation of Combinations of Composting Substrates and Casing Materials

Effects of the beds prepared using different substrates (composts prepared from pearl millet straw, paddy straw, sugarcane bagasse, saw dust, wheat bran and spent mushroom substrate) of the spawned composts, that were overlaid with casing materials (vermi compost, coir pith compost, tea waste and red soil+ sand+ cow dung in the ratio 1: 1: 1, on the growth of *A. bisporus* isolate DMRO 707, were observed as follows:

4.4.1.1 Number of Days Taken for Complete Mycelial Run

The number of days taken for completion of mycelia run of the isolate DMRO 707 in beds prepared from combinations of different composts and casing

Table 18. Time taken for spawn run and nature of mycelial growth of *A. bisporus* on different spawn substrates

Spawn substrates	**Nature of mycelia growth**	**Time taken for spawn run***
Paddy	++++	**12.00**[a]
Wheat	+++	15.00[c]
Maize	++++	13.00[b]
Ragi	++	20.00[e]
Sorghum	++	17.00[d]
CD (0.05)		0.905

* Average of four replications

++++ - Thick and fluffy growth

+++ - Thick growth

++ - Poor growth

Means followed by similar superscripts are not significantly different at 5 % level

Table 19. Yield performance of *Agaricus* on different composts with different casing materials

Treatment combinations	Days taken for complete mycelial run*	No. of sporocarps*	Average weight of sporocarp* (g)	Total yield per bed after three harvests* (g)	Average yield* (g)
T1 (Paddy straw+ poultry manure+ urea +gypsum **)x C1 (**Vermi compost)	14.00^{b}	10.00^{cde}	10.24^{cde}	119.45 (10.97)^{h}	39.82^{i}
T1 (Paddy straw+ poultry manure+ urea +gypsum **)x C2** (Coir pith compost)	13.33^{b}	16.33^{a}	20.99^{a}	271.96 (16.52)^{d}	90.65^{de}
TI (Paddy straw+ poultry manure+ urea +gypsum) **x C3** (Red soil + sand + cow dung (1:1:1))	13.00^{b}	14.33^{c}	12.22^{c}	155.65 (12.52)^{g}	52.33^{h}
T1 (Paddy straw+ poultry manure+ urea +gypsum) **x C4** (Tea waste)	13.00^{b}	19.33^{b}	18.75^{b}	253.68 (15.96)^{e}	84.56^{f}
T2 (Sugarcane bagasse+ poultry manure+ urea +gypsum **) x C1 (**Vermi compost)	13.00^{bc}	17.67^{b}	17.92^{b}	216.62 (14.75)^{f}	72.21^{g}
T2 (Sugarcane bagasse+ poultry manure+ urea +gypsum **) x C2** (Coir pith compost)	12.33^{cde}	19.00^{a}	21.94^{a}	290.41 17.07)^{bc}	96.8^{bc}
T2 (Sugarcane bagasse+ poultry manure+ urea +gypsum **) x C3** (Red soil + sand + cow dung (1:1:1))	12.00^{cde}	18.67^{b}	17.53^{b}	252.27 (15.91)^{e}	84.09^{f}
T2 (Sugarcane bagasse+ poultry manure+ urea +gypsum **) x C4** (Tea waste)	11.67^{de}	16.33^{b}	17.25^{b}	284.09 (16.88)^{c}	94.7^{cd}
T3 (SMS+ poultry+ manure+ urea +gypsum) **x C1 (**Vermi compost)	16.33^{a}	6.33^{e}	8.88^{e}	56.92 (7.61)^{k}	18.98^{k}
T3 (SMS+ poultry+ manure+ urea +gypsum **) x C2** (Coir pith compost)	16.33^{a}	10.00^{cd}	11.7^{cd}	115.18 (10.78)^{h}	38.39^{i}
T3 (SMS+ poultry+ manure+ urea +gypsum **) x C3** (Red soil + sand + cow dung (1:1:1))	16.33^{a}	7.67^{e}	8.59^{e}	66.73 (8.23)^{j}	22.24^{k}

Continued.

Table 19. Yield performance of *Agaricus* on different composts with different casing materials. Continued.

T3 (SMS+ poultry+ manure+ urea +gypsum **)X C4** (Tea waste)	16.00[a]	10.00[de]	9.75[de]	96.95 (9.9)[i]	32.32[j]
T4 (Sawdust + poultry manure+ urea +gypsum **) x C1 (**Vermi compost)	-	-	-	-	-
T4 (Sawdust + poultry manure+ urea +gypsum **) x C2** (Coir pith compost)	-	-	-	-	-
T4 (Sawdust + poultry manure+ urea +gypsum **) x C3** (Red soil + sand + cow dung (1:1:1))	-	-	-	-	-
T4 (Sawdust + poultry manure+ urea +gypsum **) x C4** (Tea waste)	-	-	-	-	-
T5 (Wheat bran+ poultry manure+ urea +gypsum **) x C1 (**Vermi compost)	-	-	-	-	-
T5 (Wheat bran+ poultry manure+ urea +gypsum **) x C2** (Coir pith compost)	-	-	-	-	-
T5 (Wheat bran+ poultry manure+ urea +gypsum **) x C3** (Red soil + sand + cow dung (1:1:1))	-	-	-	-	-
T5 (Wheat bran+ poultry manure+ urea +gypsum **) x C4** (Tea waste)	-	-	-	-	-
T6 (Pearl millet straw+ poultry manure+ urea +gypsum **) x C1 (**Vermi compost)	11.33[ef]	15.00[b]	18.51[b]	261.18 (16.19)[de]	87.06[ef]
T6 (Pearl millet straw+ poultry manure+ urea +gypsum **) x C2** (Coir pith compost)	11.00[ef]	23.33[a]	22.66[a]	325.97 (18.08)[a]	108.66[a]
T6 (Pearl millet straw+ poultry manure+ urea +gypsum **) x C3** (Red soil + sand + cow dung (1:1:1))	11.33[ef]	12.33[b]	17.69[b]	249.97 (15.84)[e]	83.33[f]
T6 (Pearl millet straw+ poultry manure+ urea +gypsum **) x C4** (Tea waste)	10.00[f]	21.33[a]	21.52[a]	301.76 (17.4)[b]	100.59[a]
CD (0.05)	1.609	1.976	2.116	0.353	4.068

*Average of four replications, T - Compost used, C – Casing material used, SMS – Spent mushroom substrate

Means followed by similar superscripts are not significantly different at 5 % level

(A) Paddy straw + poultry manure+ urea +gypsum x vermi compost

(B) Paddy straw+ poultry manure+ urea +gypsum x coir pith compost

(C) Paddy straw+ poultry manure+ urea +gypsum x red soil + sand + cow dung (1:1:1)

(D) Paddy straw+ poultry manure + urea +gypsum x tea waste

Plate 18. Performance of *A. bisporus* on different compost substrates with different casing materials

(E) Sugarcane bagasse+ poultry manure + urea +gypsum x vermi compost

(F) Sugarcane bagasse + poultry manure +urea +gypsum x coir pith compost

(G) Sugarcane bagasse + poultry manure+ urea +gypsum x red soil + sand + cow dung (1:1:1)

(H) Sugarcane bagasse + poultry manure+ urea +gypsum x tea waste

Plate 18. Continued. Performance of *A. bisporus* on different compost substrates with different casing materials

(I) Spent mushroom substrate + poultry manure+ urea +gypsum x vermi compost

(J) Spent mushroom substrate + poultry manure+ urea +gypsum x coir pith compost

(K) Spent mushroom substrate + poultry manure+ urea +gypsum x red soil + sand + cow dung (1:1:1)

(L) Spent mushroom substrate+ poultry manure+ urea +gypsum x tea waste

Plate 18. Continued. Performance of *A. bisporus* on different compost substrates with different casing materials

(M) Pearl millet straw + poultry manure + urea +gypsum x vermi compost

(N) Pearl millet straw+ poultry manure + urea +gypsum x coir pith compost

(O) Pearl millet straw+ poultry manure + urea +gypsum x red soil + sand + cow dung (1:1:1)

(P) Pearl millet straw+ poultry manure + urea +gypsum x tea waste

Plate 18. Continued. Performance of *A. bisporus* on different compost substrates with different casing materials

materials, ranged from 10.00 to 16.33 days. Spawn run on compost prepared from pearl millet straw was completed within a period of 10.00 days which was significantly superior to other substrates used for composting. This was followed by compost prepared from sugarcane bagasse on which the spawn run was completed in 12 days. On the composts prepared from paddy straw and spent mushroom substrate, spawn run of DMRO 707 was completed within 13 and 16 days respectively. Mycelia growth was not observed in compost prepared from sawdust and wheat bran.

4.4.1.2 Number of Sporocarps Harvested

Total number of sporocarps harvested from beds prepared using different composts with various casing materials ranged from 6.33 to 23.33. Maximum number of sporophores (23.33) was harvested from the bed prepared from spawned pearl millet compost overlaid with the casing material, coir pith compost. Beds prepared using spawned pearl millet compost and cased with tea waste (21.33) were on par with beds prepared from spawned sugarcane bagasse compost cased with coir pith compost (19.00) and spawned paddy straw compost cased with tea waste (19.33). Pinheads were not formed in beds prepared from combination of either saw dust compost or wheat bran compost with any of the casing materials.

4.4.1.3 Average Weight of Sporocarps

Sporophores from different beds were harvested and average weight was calculated. Average weight of sporophores ranged from 8.88 g to 22.66 g. Maximum average weight of 22.66 g was obtained in beds prepared from spawned pearl millet compost cased with coir pith compost which were on par with the same compost cased with tea waste (21.52 g), spawned paddy straw compost overlaid with coir pith compost as casing material and spawned paddy straw compost cased with tea waste. Lowest average weight of sporocarps was

recorded in beds prepared from spawned spent mushroom substrate cased with vermi compost which was on par with the average weight of sporocarps obtained in bed prepared from same spawned compost that was cased with a mixture red soil, cow dung and sand.

4.4.1.4 Total Yield per Bed After Three Harvests

Total yield per bed using combinations of different spawned compost substrates and casing materials varied from 56.92 to 325.97 g. Highest yield of 325.97 g was recorded for three harvests in spawned pearl millet compost cased with coir pith and was significantly superior to the remaining treatments. This was followed by combination of spawned pearl millet compost and casing material tea waste which produced 301.76 g. Lowest yield of 56.92 g was observed in beds prepared from spawned spent mushroom substrate compost overlaid with vermi compost.

4.4.1.5 Average Yield per Bed After Three Harvests

Average yield from beds using combinations of different spawned compost substrates and casing materials varied from 18.98 g to 108. 66 g. The average yield was maximum in beds prepared from combination of spawned pearl millet compost cased with coir pith which was on par with the same spawned compost cased with tea waste as casing material (100.59 g). Average yield was minimum (18.98 g) in beds prepared from spawned spent mushroom substrate cased with vermi compost.

4.5 INCIDENCE OF PESTS AND DISEASES

A few incidences of pests and diseases were observed in the mushroom beds during the cultivation of *A. bisporus* isolate DMRO 707.

4.5.1 Pests

The insects sciarid (*Lycoriella* sp.) flies and staphylinid beetles were observed in the beds of *A. bisporus*.

4.5.1.1 Nature of Damage by Pests Infesting A. bisporus

4.5.1.1.1 Sciarid flies

The larvae of sciarid flies were observed feeding the tissues inside the stipe there by causing tunnel formation, discolouration and decay of buttons. Besides the stipe larvae and adults of sciarid flies were also seen on the pileus and gills of the mushroom *A. bisporus* isolate DMRO 707 (Plate 19).

4.5.1.1.2 Staphylinid beetles

These beetles infested young sporocarps and fed on the superficial tissues of fruiting bodies there by producing holes all over the fruiting bodies. The infestation of this beetle was observed in both young and mature fruiting bodies leading to the rotting of buttons (Plate 20).

4.5.1.2 Extent of Damage

Incidences of sciarids and staphylinid beetles occurred during sporophores formation of the mushroom. Four per cent of the mushrooms beds were infested with sciarids (Table 20) whereas thirteen per cent of beds were infested with staphylinid beetles (Table 21). Incidence of pests was negatively correlated with temperature but was not influenced by relative humidity.

Table 20. Incidence of sciarid flies during the cultivation of *A. bisporus*

Time	No. of beds infested (out of 24)	Temperature	Relative humidity
Spawn run	-	24	90
Casing	-	23	90
Sporocarp formation	1	18	90

Table 21. Incidence of staphylinid beetles during the cultivation of *A. bisporus*

Time	No. of beds infested (out of 24)	Temperature	Relative humidity
Spawn run	-	24	90
Casing	-	23	90
Sporocarp formation	3	18	90

Plate 19. Fruiting body infested with sciarid flies

Plate 20. Fruiting body infested with staphylinid beetles

4.5.2 Diseases

The fungi like *Trichoderma* sp. and *Coprinus* sp. were some of the competitors that occurred in the beds during the cultivation of *A. bisporus*. Incidence of these occurred during spawn run, case run and fruiting body formation.

4.5.2.1 Nature of Damage

4.5.2.1.1 Trichoderma sp.

Initially the infested area was covered with a dense white growth of mycelium whch later turned to dark green due to heavy sporulation of fungus (Plate 21).

4.5.2.1.2 Coprinus sp.

This fungus which was found singly or in clusters on beds of *Agaricus*, was at first cream coloured and later turned dark blue in colour (Plate 22).

4.5.2.2 Extent of Damage

Incidence of these fungi was found at the time of spawn run, case run and sporocarp formation. During the cultivation of *Agaricus,* occurrence of *Trichoderma* sp. (Table 22) and *Coprinus* sp. (Table 23) was found in 41 and 79 per cent of total number of beds respectively. Incidence of *Coprinus* sp. was negatively correlated

Table 22. Infestation of *Trichoderma* sp. during the cultivation of *A. bisporus.*

Time	No. of beds infested (out of 24)	Temperature	Relative humidity
Spawn run	5	24	90
Casing	3	23	90
Sporocarp formation	2	18	90

Table 23. Infestation of *Coprinus* sp. during the cultivation of *A. bisporus.*

Time	No. of beds infested (out of 24)	Temperature	Relative humidity
Spawn run	4	24	90
Casing	6	23	90
Sporocarp formation	9	18	90

Plate 21. Bed infested with *Trichoderma* sp.

Plate 22. Beds infested with *Coprinus* sp.

with temperature whereas *Trichoderma* sp. was positively correlated but was not influenced by relative humidity.

4.6 SHELF LIFE, PROXIMATE CONSTITUENT ANALYSIS AND COOKING QUALITY OF *A. bisporus*

4.6.1 Shelf Life

The study showed that the mushrooms stored in refrigerated condition had better shelf life as compared to room temperature. Maximum shelf life (7.50 days) was recorded when mushrooms were kept in polypropylene covers without perforation in refrigerator. This was followed by polypropylene cover with perforations (6.25 days), paper box without perforations (5.75 days) and paper box with perforations (5.50 days). Polypropylene cover with perforations was on par with paper box without perforations (Plate 23).

In the case of mushrooms kept in room temperature, maximum shelf life was recorded in polypropylene cover without perforations (5.75 days). Polypropylene cover with perforations, paper box without perforation and paper box with perforations had a shelf life of 3.75, 3.50 and 3.25 days respectively and were found to be on par. (Table 24).

4.6.2 Proximate Constituents Present in *A. bisporus*

The proximate constituents present in *Agaricus bisporus* were analyzed. The moisture content of *Agaricus* was found to be 90.03% (fresh mushroom), 29.1% protein, 9.37% ash, 22.63% fibre, and 2.10% fat, 4.58% carbohydrate, 3.76% nitrogen, 0.58% phosphorus and 1.54% potassium. (Table 25).

Table 24. Shelf life of *A. bisporus*

Condition	Keeping quality (in days)		Signs of spoilage	
	Room temperature	Refrigerator (4^0C)	Room temperature	Refrigerator (4^0C)
Polypropylene cover without perforation	**5.75**[a]	**7.50**[a]	Slimy coating, bad odour	Wrinkling, drying
Polypropylene cover with perforation	3.75[b]	6.25[b]	Brown patches	Brown patches
Paper box without perforation	3.50[b]	5.75[b]	Slimy coating, bad odour	Browning
Paper box with perforation	3.25[b]	5.50[c]	Mold growth	Mold growth
CD (0.05)	0.656	0.681		

* Average of four replications

Means followed by similar superscripts are not significantly different at 5 % level

With perforation Without perforation

In paper box at room temperature

With perforation Without perforation

In polypropylene cover at room temperature

With perforation Without perforation

In paper box at refrigeration

With perforation Without perforation

In polypropylene cover at refrigeration

Plate 23. Signs of spoilage of *A. bisporus* (after 1 week)

Table 25. Proximate constituents present in *A. bisporus*

Sl. No.	Proximate constituent	Percentage (%)
1	Moisture $	90.03
2	Protein#	29.10
3	Carbohydrate#	4.58
4	Fibre#	22.63
5	Ash#	9.37
6	Fat#	2.10
7	Nitrogen#	3.76
8	Phosphorus#	0.58
9	Potassium#	1.54

$ - Presented in fresh weight basis

- % dry weight basis

4.6.3 Cooking Quality

Organoleptic studies were conducted by preparing recipes of both fresh and dried *Agaricus* mushrooms and subjected to sensory evaluation. Three different recipes *viz.,* cutlet, mushroom masala and soup were prepared. The mushroom recipes were tested for their characters like colour, texture, flavour, taste and overall acceptability which were evaluated using a five point score card. The details are presented in Table 26 and Plate 24.

Among various products, soup obtained an overall acceptability score of 4.70. It got a score of 4.40 for colour and appearance, 4.30 for texture, 4.20 for taste and 4.60 for flavour.

Cutlets got a comparative lower score for overall acceptability. All the other parameters scored above four with respect to colour and appearance, texture, taste and flavour.

Mushroom masala obtained a low score for colour compared to the other two products whereas ali the other parameters scored on par with cutlet and soup.

Table 26. Scores obtained for different recipes

Products	Colour and appearance*	Texture*	Taste*	Flavour*	Overall acceptability*
Soup	4.40	4.30	4.20	4.60	4.70
Cutlet	4.20	4.00	4.50	4.10	4.40
Masala	3.80	4.10	4.70	4.40	4.30
CD (0.05)	0.346	0.327	0.419	0.349	0.376

* Average of ten replications

(C) Cutlets

(B) Soup

(A) Masala

Plate 24. Various recipes tested for cooking quality

Discussion

5. DISCUSSION

Agaricus spp. were collected from south Kerala during and after the south west and north east monsoons. Tissue isolation of mushrooms collected from different locations along with those obtained from AICRP centre, College of Agriculture, Vellayani was done. Total seven isolates thus obtained were sent to Directorate of Mushroom Research, Solan for further identification and also for according their accession numbers. These isolates that were maintained on potato dextrose agar (PDA) slants were used for further studies.

Morphological and microscopic characters of all the isolates were studied. Isolate seven with accession number DMRO 707 which performed best among the different isolates tested for their growth on PDA (Figure 1) and paddy grain (Figure 2) was further identified as *Agaricus bisporus* (Lange.) Imbach. Bhavani Devi (1982) identified the occurrence of three species of *Agaricus viz*. *A. arvensis, A. campestris* and *A. placomyces.* in a survey conducted in Kerala. Thara (2001) described the morphological characters of more than fourty isolates of *Agaricus* which were collected during and after south west monsoon in twenty agro climatic zones of Kerala. Thiribhuvanamala *et al.* (2011) observed the seasonal occurrence of *Agaricus* from Western Ghats of India during 2008 -10 from October-January and recorded about 68 mushroom flora belonging to 19 genera, from these locations. Senthilarasu (2014) conducted a survey in Maharashtra and collected a total of 13 species of *Agaricus* belonging to 10 genera of 9 families including *A. abruptibulbus*, *A. arvensis, A. bisporus, A. bitorquis, A. brunnescens, A. campestris, A. micromegethus, A. pattersoniae, A. placomyces, A. scitulus, A. semotus, A. subedulis, A. sylvaticus, A. sylvicola, A. vaporarius, A. woodrowii, A. xanthodermus* and *A. xantholepis.*

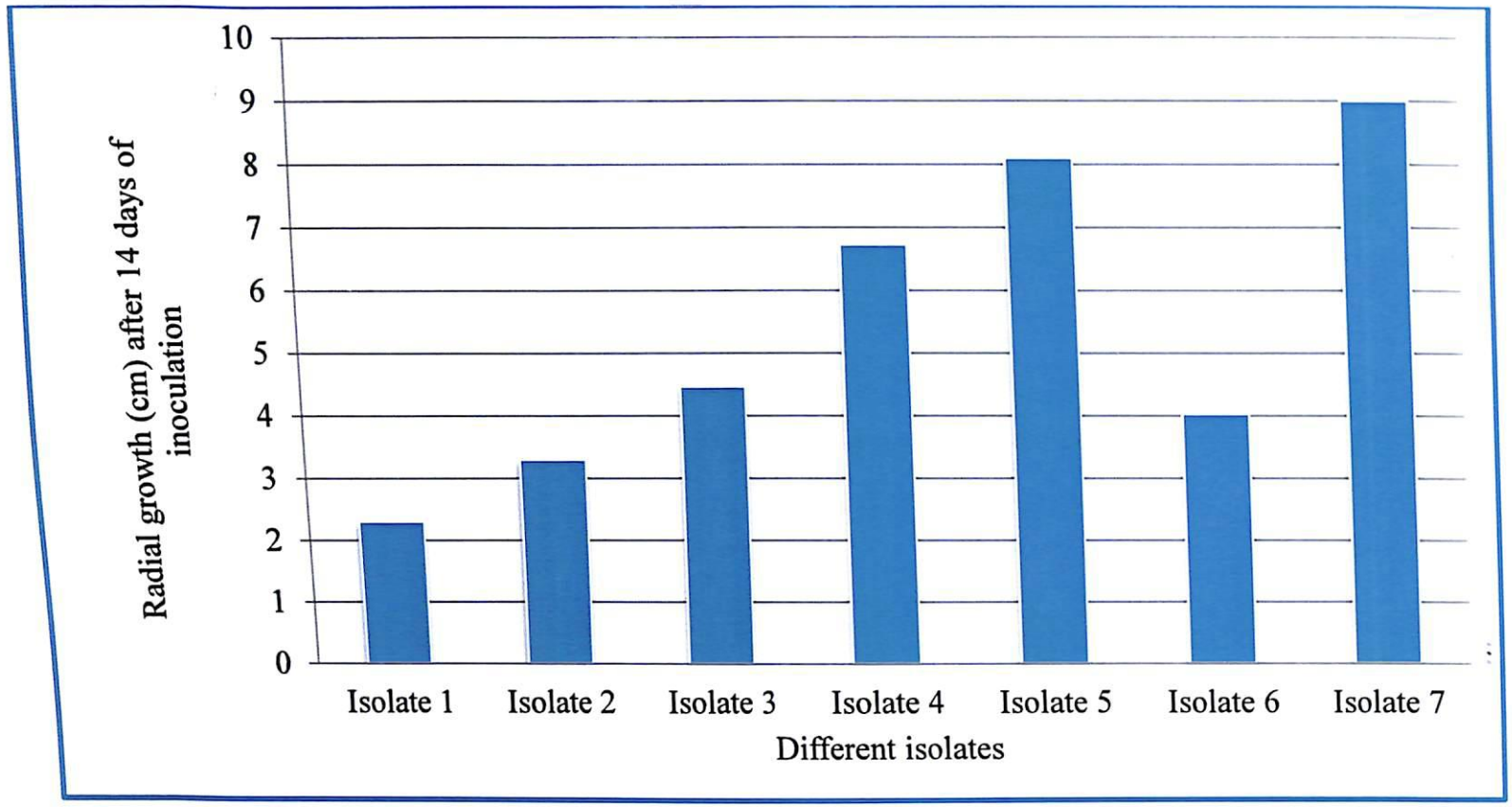


Figure 1. Growth of different isolates of *Agaricus* on potato dextrose agar medium

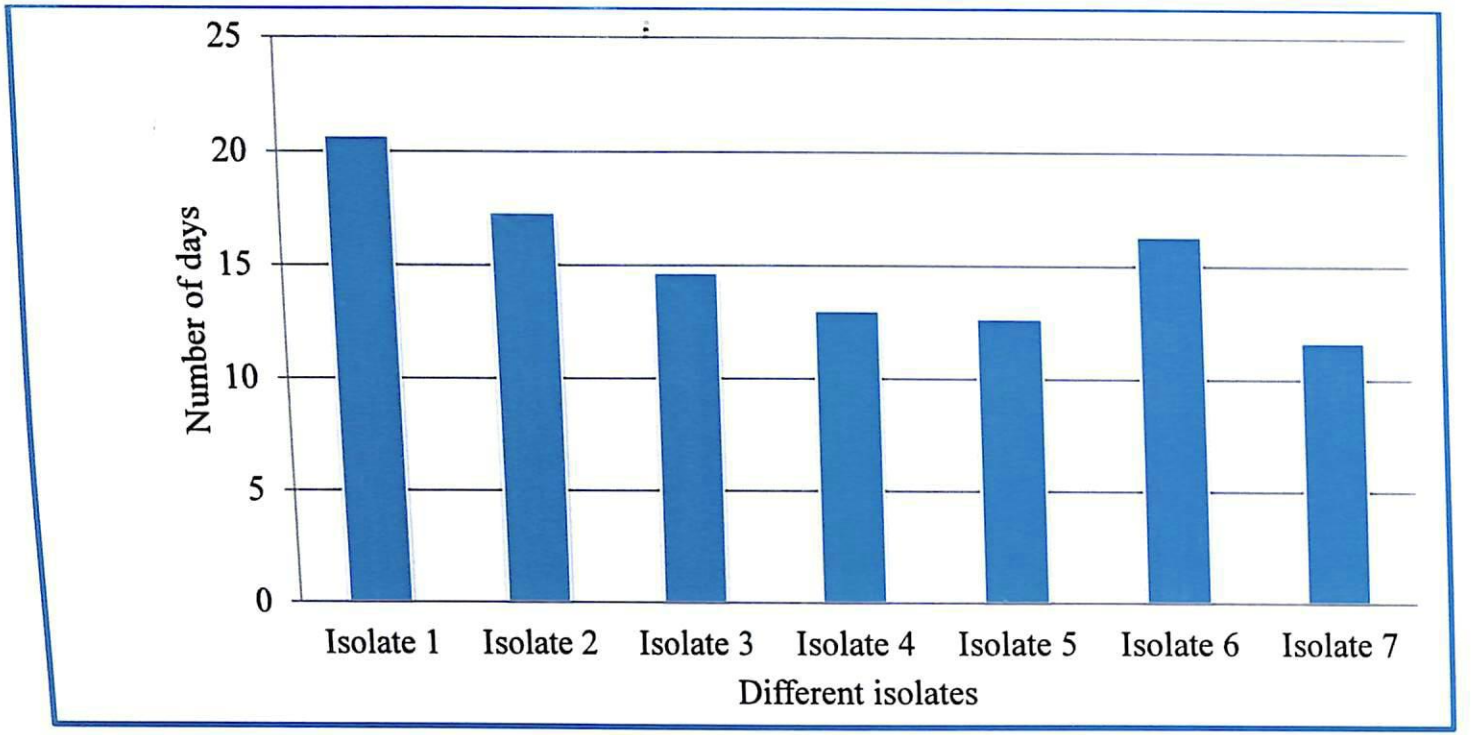


Figure 2. Number of days taken for spawn run on paddy grains by different isolates of *Agaricus*

In studies on morphological and microscopic features of pileus, stipe, gills and spores of various isolates of *Agaricus* obtained, the colour of fruiting body ranged from white to pale yellow and texture of every isolate was fleshy. In each of the isolates collected, the pileus was convex with scaly surface, centrally attached stipe and bulbous shaped base. The gills of each isolate were freely arranged, brown coloured and brittle textured. Studies of the spore print and spore of the isolate DMRO 707 revealed brown colour of the spore as well as the bispored nature of basidium. Carrera *et al*. (2001) conducted a study on fruiting body morphology of different strains of wild *Agaricus* species and observed that *Agaricus* had brown coloured gills and bisporous basidium. Thara (2001) collected more than fourty species of *Agaricus* and described the morphological characters of these species. She reported that most of the collected species had white to brownish pileus with scaly surface and bulbous shaped, centrally attached stipe.

A. bisporus isolate DMRO 707 was tested on different solid (Figure 3) and liquid (Figure 4) media *viz.,* potato dextrose, malt extract, carrot, oat meal and Czapek's Dox to test comparative growth of mycelia in the different media. Radial growth of the isolate was highest on potato dextrose agar followed by malt extract agar with thick and fluffy growth on both media. Sparse growth was observed in oat meal and Czapek's Dox agar and no growth was observed in carrot agar. This study was in accordance with the observation of Furlan *et al*. (1997) who recorded higher radial growth of mycelia of *A. bitorquis* on potato dextrose agar. Khan *et al*. (1991) recorded fluffy growth of *Agaricus* on malt extract. They studied the factors affecting the growth of *A. brunnescens* and reported that maximum growth was observed in malt extract medium. In similar studies conducted by Yadav *et al*. (2003), *A. bisporus* produced maximum mycelial growth in malt extract medium. Thara (2001) studied the effect of different media on the growth of *Agaricus* and found that malt extract gave good growth in both solid and liquid media. Uddin *et al*. (2012) recorded the best mycelia growth in YPDA (Yeast Potato Dextrose Agar), in an

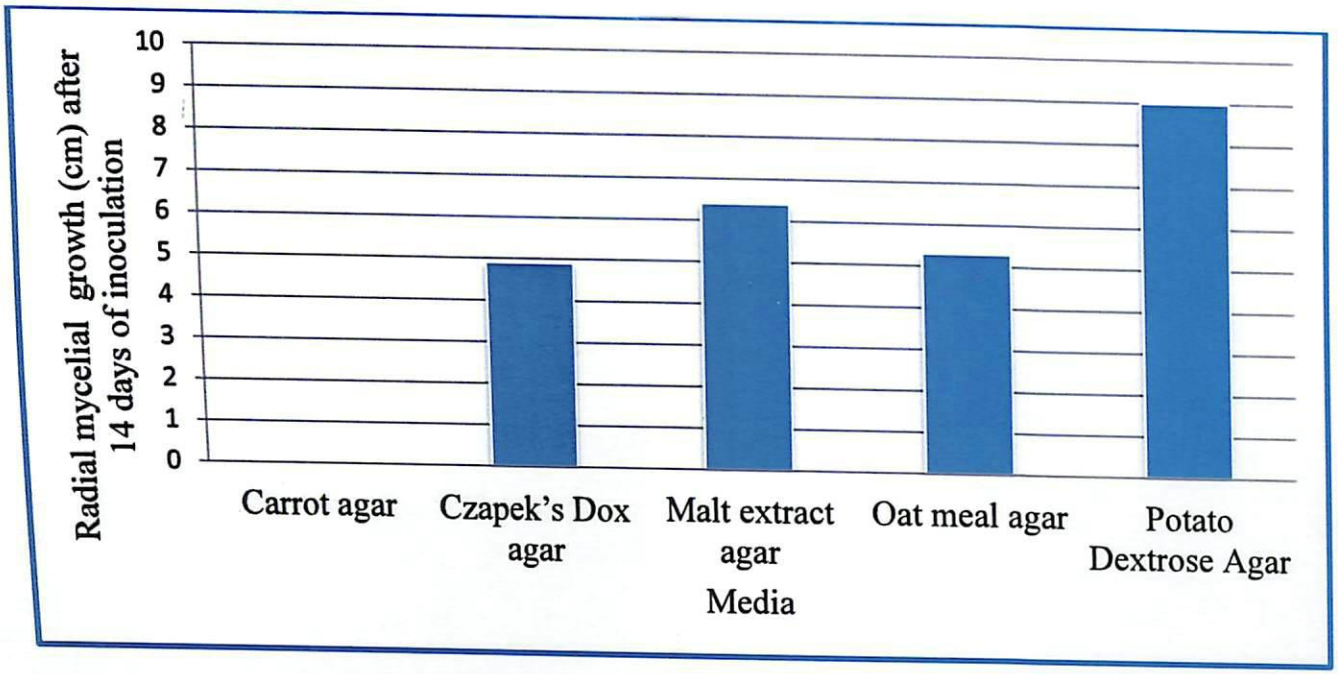


Figure 3. Growth of *A. bisporus* in different solid media

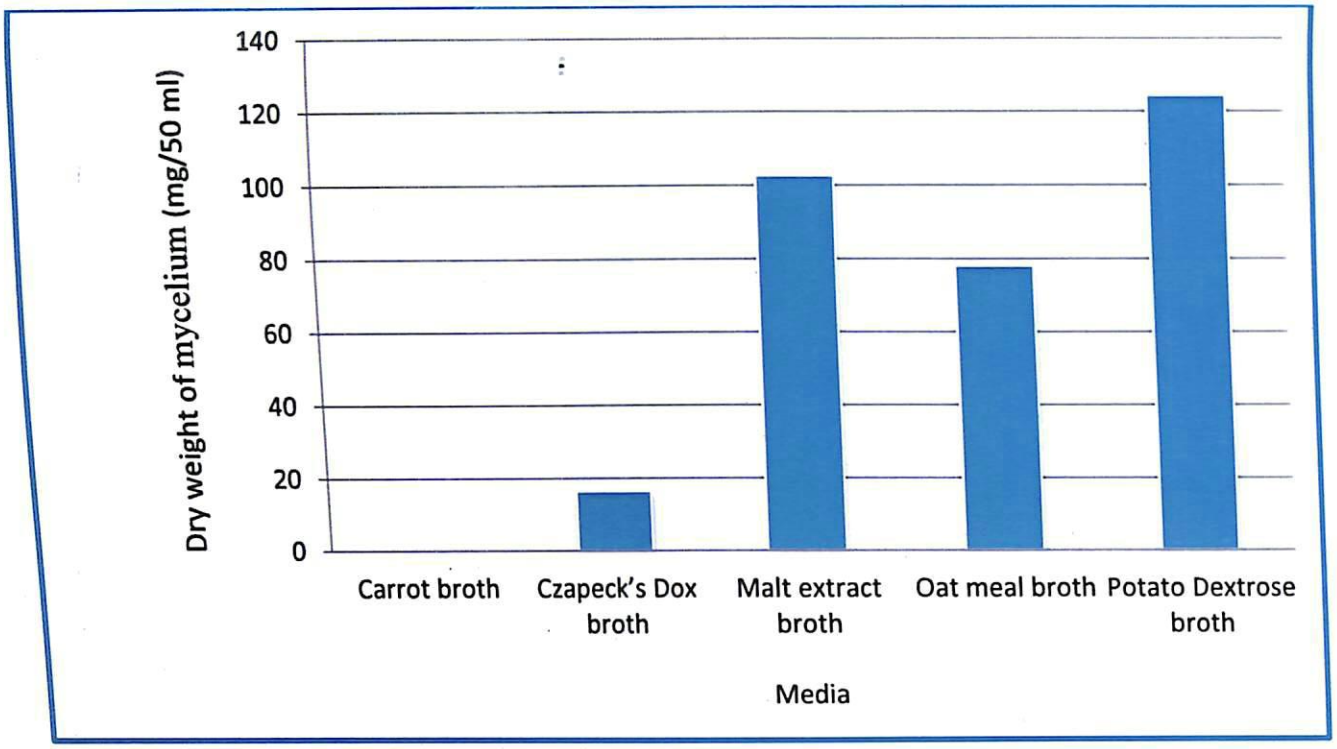


Figure 4. Growth of *A. bisporus* in different liquid media

experiment conducted to investigate the mycelial colony proliferation of *A. biporus* on different media (*viz*. PDA, YPDA and MEA). Thus in the present investigation both potato dextrose and malt extract medium have been identified as the best media for producing maximum mycelial growth of *A. bisporus.*

Five different carbon sources namely glucose, sucrose, fructose, xylose and mannose were tested in solid (Figure 5) and liquid (Figure 6) medium for their efficacy in radial mycelial growth and biomass production of *Agaricus*. The study indicated that glucose was the best carbon source followed by fructose. Kumar and Munjal (1980) recorded glucose and fructose as better carbon sources than sucrose, for *A. bisporus.* Similar results were observed by Kalisz *et al* (1986) who evaluated *A. bisporus* on defined liquid media and found that glucose was effectively utilized when provided as a sole source of carbon. *A. bisporus* exhibited good growth in a defined buffered medium with glucose as a carbon source (Baurs *et al.*, 1994). Study conducted by Liu and Wang (2009) also supported the present result and revealed that the carbon source like glucose was found to be the best for the growth of *Agaricus*. Vahidi and Hamedi (2006) studied the effects of different carbon sources including glucose, lactose, sucrose, manitol, starch, galactose, maltose and fructose in two different media (complex and synthetic) on production of polysaccharides by *A. blazei* and observed highest growth and polysaccharide production when galactose and starch were used as carbon source which did not conform to the result of present study.

Beef extract was found to be better source of organic nitrogen than peptone in the experiment conducted to evaluate the effects of both organic and inorganic nitrogen sources on the growth of *A. bisporus* isolate DMRO 707 in both solid (Figure 7) and liquid (Figure 8) media. The inorganic sources of nitrogen *viz,* potassium nitrate, sodium nitrate, ammonium chloride and ammonium carbonate induced only sparse growth of isolate in both solid and liquid media. Kalisz *et al*.

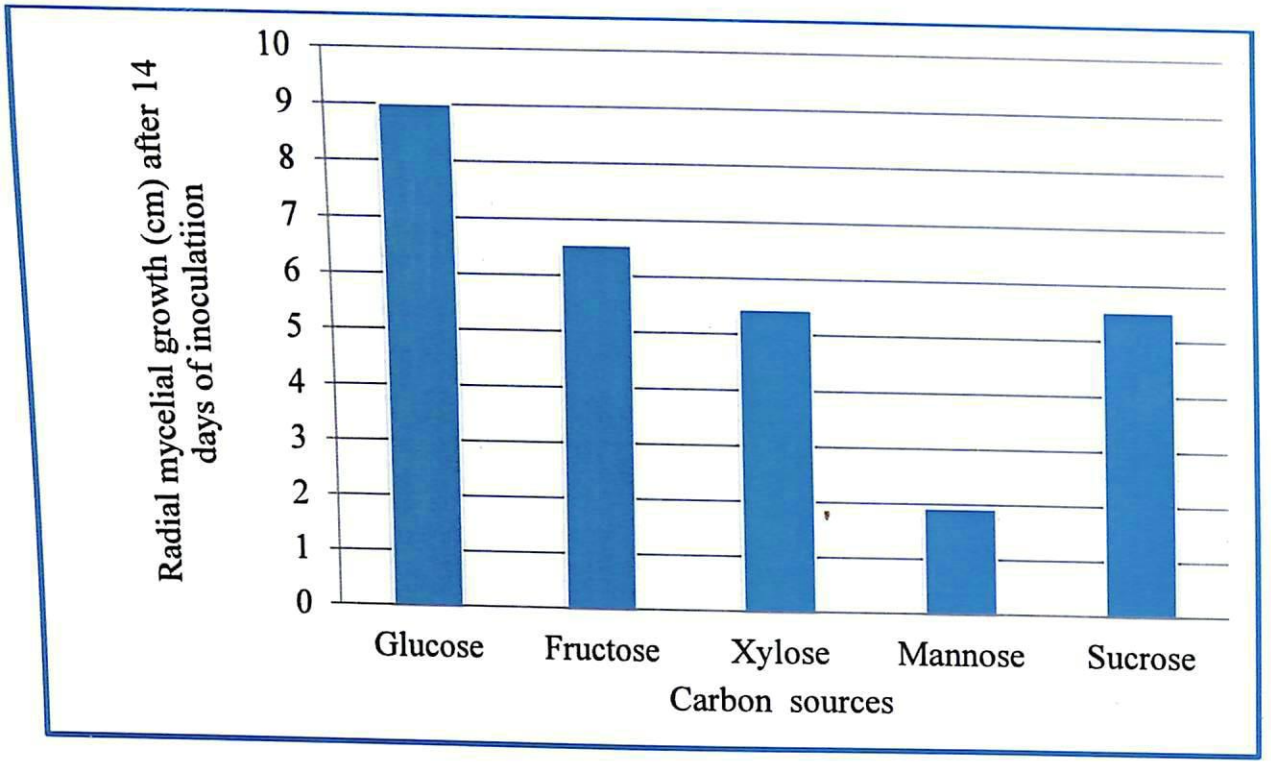


Figure 5. Growth of *A. bisporus* in solid media using different carbon sources

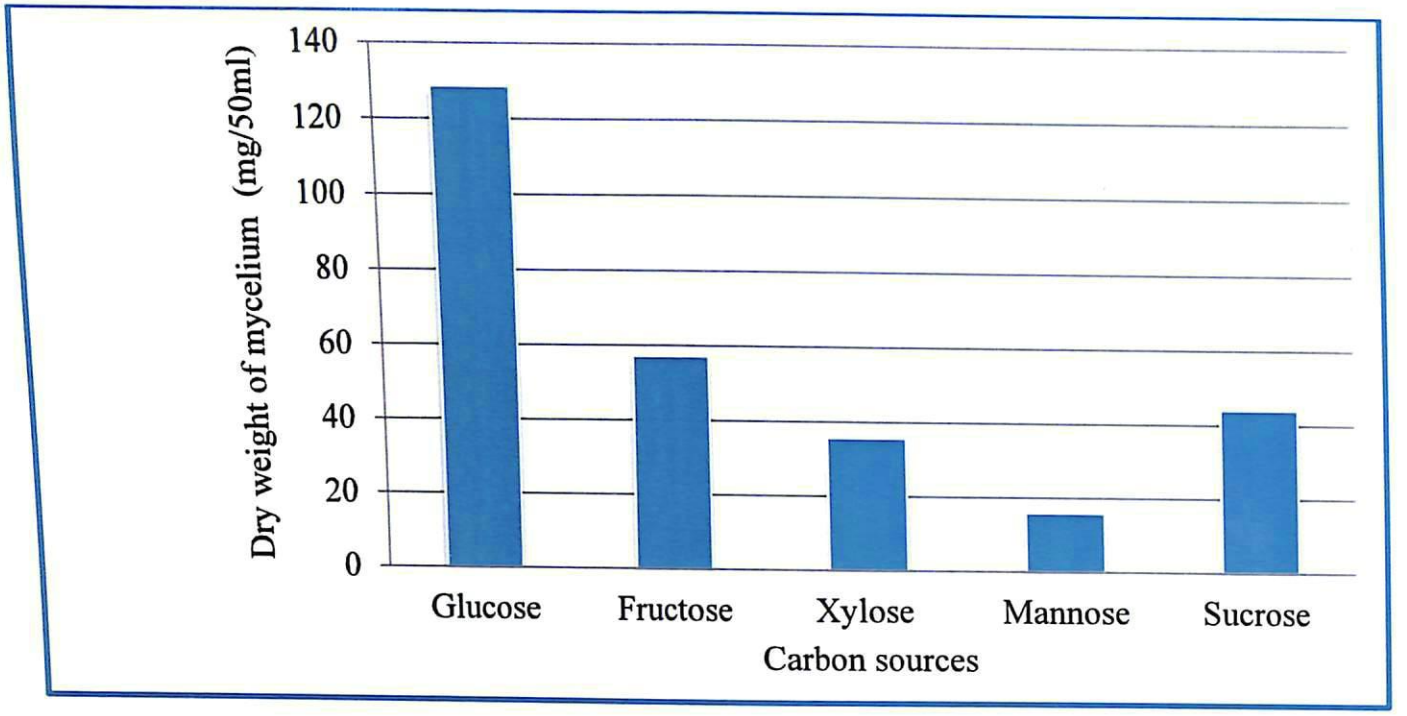


Figure 6. Growth of *A. bisporus* in liquid media using different carbon sources

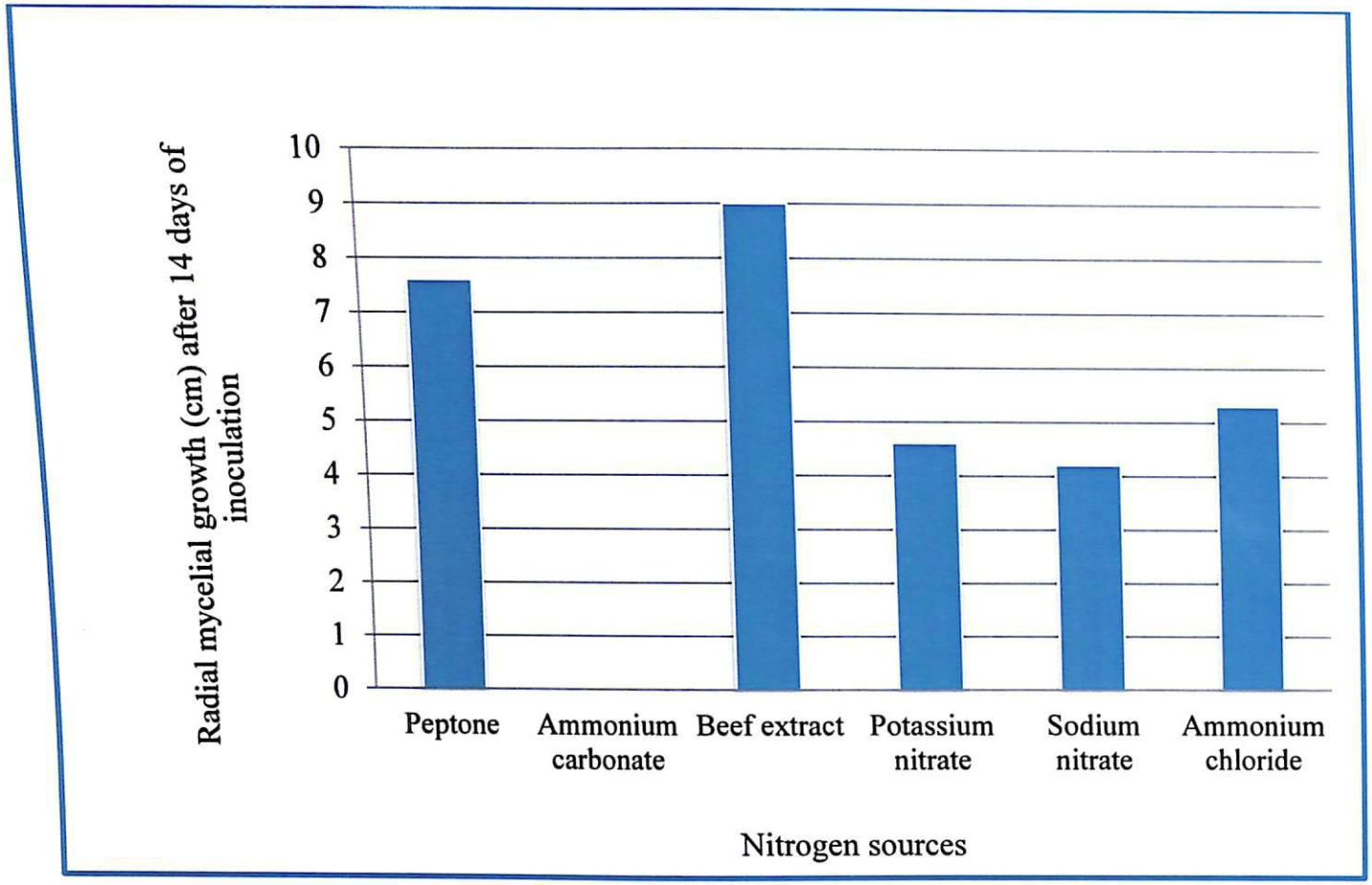


Figure 7. Growth of *A. bisporus* in solid media using different nitrogen sources

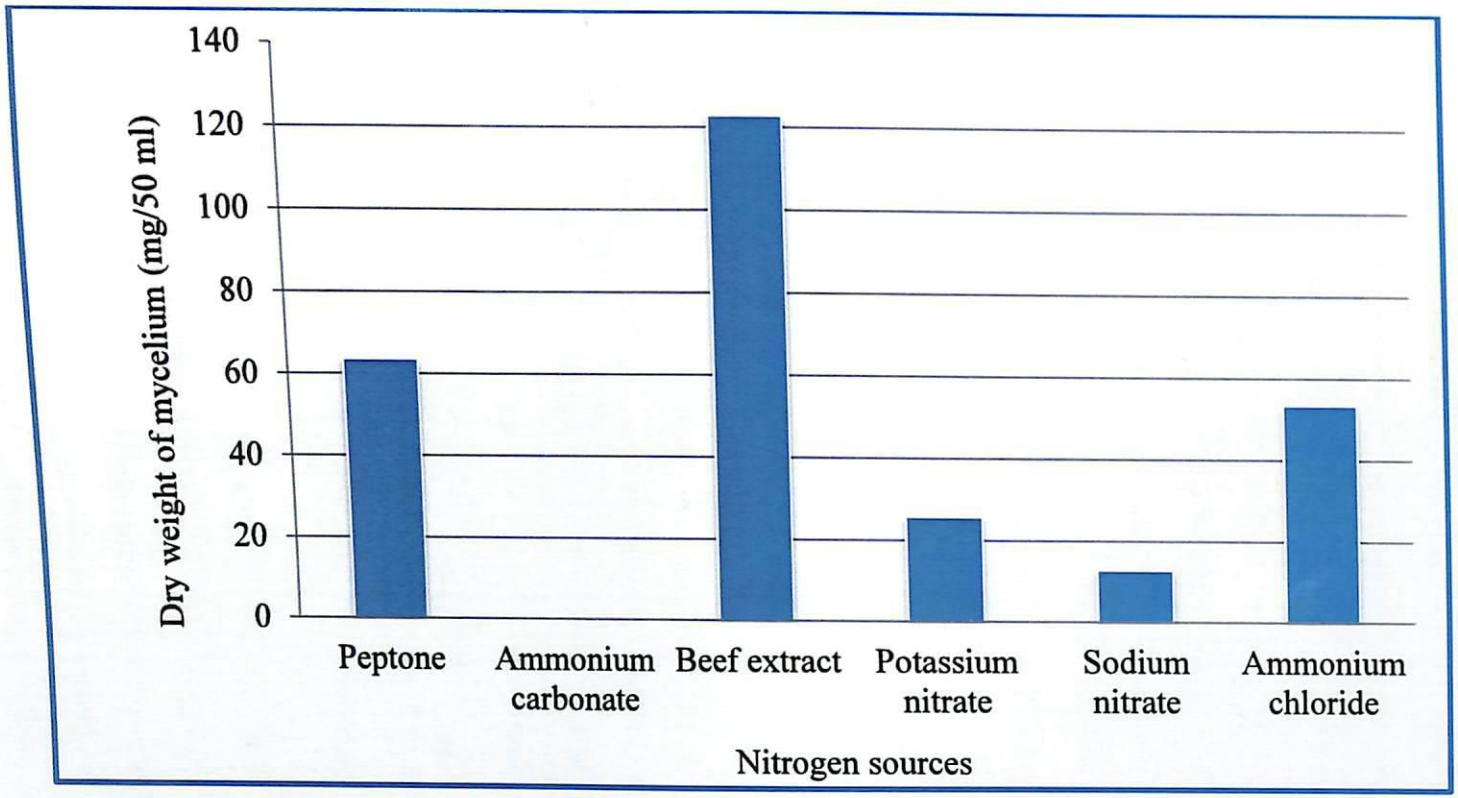


Figure 8. Growth of *A. bisporus* in liquid media using different nitrogen sources

(1986) evaluated various nitrogen sources for the growth of *A. bisporus* and stated that it can utilize protein as the best source of nitrogen where catabolite repression was observed when media were added with ammonium chloride and protein. Khan *et al.* (1991) tried various nitrogen sources for the growth of *A. bisporus* and observed that peptone was the best source. However Baurs *et al*., (1994) observed that *A. bisporus* cannot utilize organic nitrogen containing substances as only source of nitrogen. Liu and Wang (2009) recorded maximum growth of *A. blazei* when wheat bran was used as the nitrogen source. Mantovani *et al*. (2007) observed that nitrogen from urea had better effect on the *in vitro* mycelia growth of *A. brasiliensis* in different C : N ratios in the dark at 28°C. They measured the radial mycelial growth after 8 days and reported that nitrogen from urea boosted growth of this mushroom better than ammonium sulfate or any other mixture of nitrogen. Accordng to the present investigation the organic nitrogen sources like beef extract and peptone were found to be superior in supporting the growth of *A. bisporus* isolate DMRO 707.

In the study conducted to determine the influence of different H^+ ion concentrations in both solid (Figure 9) and liquid media (Figure 10) on the growth of *A. bisporus*, pH six was observed to be best for the growth of isolate DMRO 707. Mehta and Kumar (1985) also recorded maximum mycelial growth of *A. brunnescens* at a pH ranging from 6-7. Singh *et al*. (2000) stated that optimum pH for the growth of *A. bisporus* was found to be six. Thara (2001) observed that *Agaricus* showed increased growth up to pH six and thereafter its growth declined as pH increased.

In order to determine the suitable temperature for optimum growth of *A. bisporus*, the isolate DMRO 707 was grown in different temperature conditions both in solid (Figure 11) and liquid (Figure 12) media. Among the different temperature conditions (5 ºC, 10 ºC, 15 ºC, 20 ºC, 25 ºC and 30ºC) tested, maximum radial growth was observed at 25ºC. Growth of the *Agaricus* was highest at 25 ºC (Losel, 1964; Thara, 2001). It was also in accordance with the results obtained by Fritsche

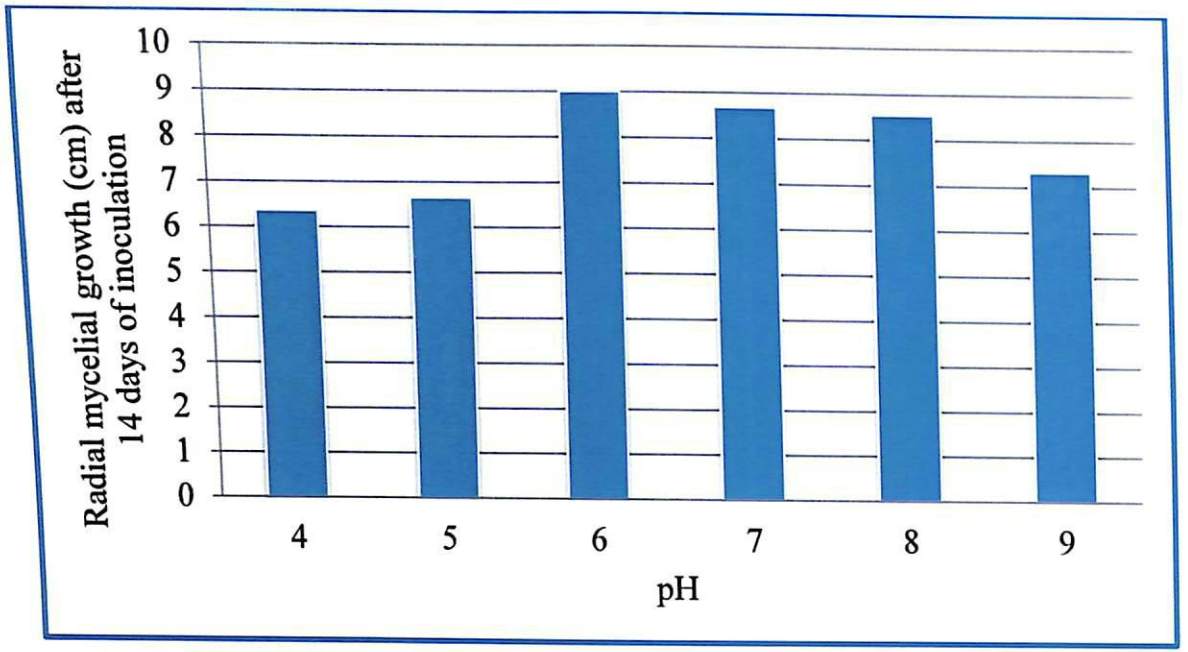


Figure 9. Growth of *A. bisporus* in solid media using different pH levels

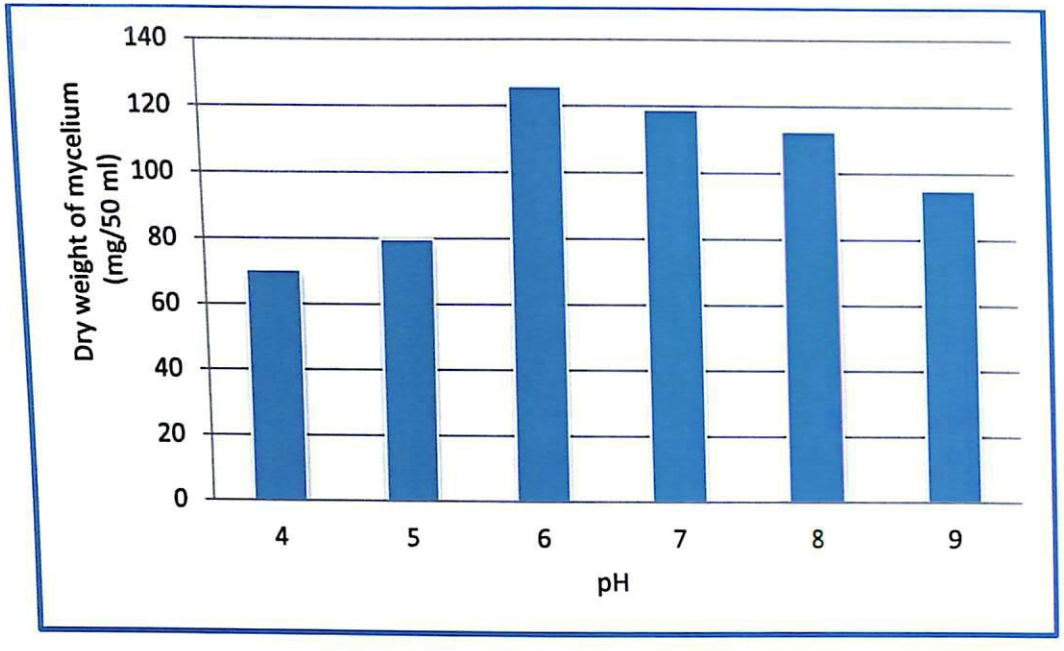


Figure 10. Growth of *A. bisporus* in liquid media using different pH levels

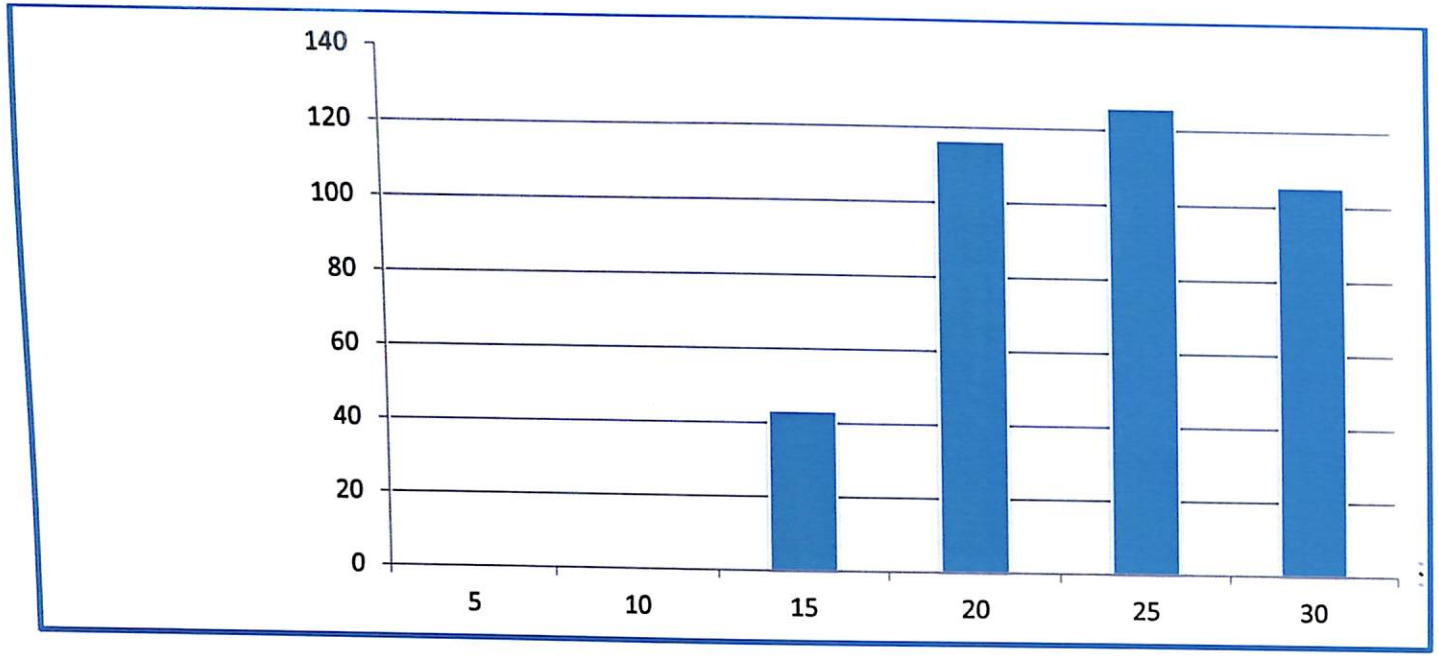


Figure 11. Growth of *A. bisporus* in solid media at different temperature

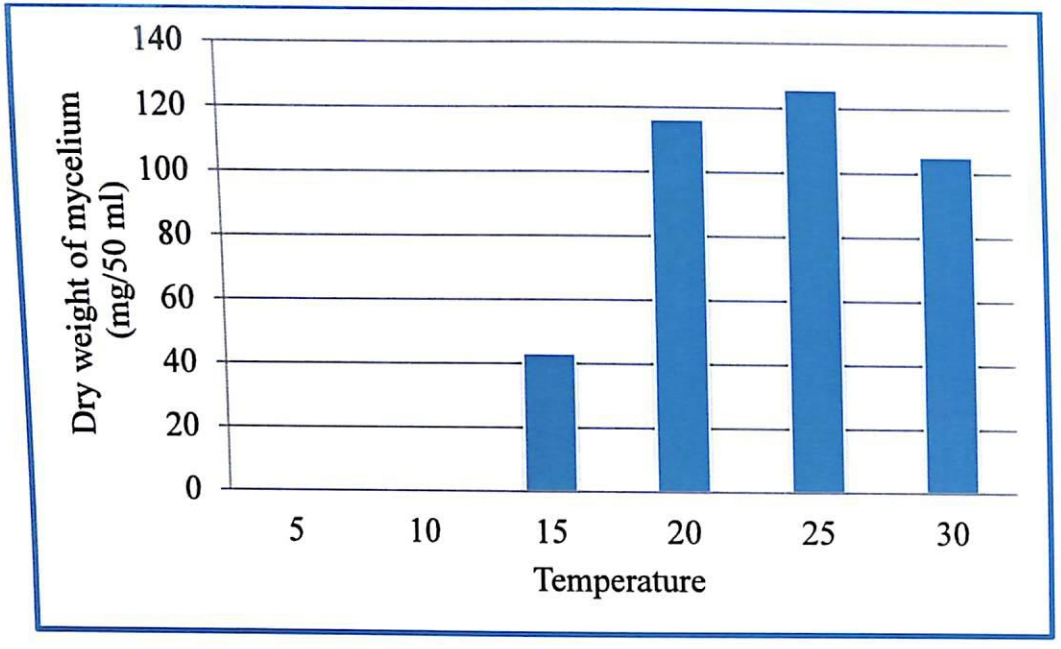


Figure 12. Growth of *A. bisporus* in liquid media at different temperature

(1968) who evaluated the temperature requirement of *A. bisporus* and *A. bitorquis* and found that they grew best at 24^{0}C and 20-30^{0}C respectively. Hayes (1972) conducted a detailed study on temperature requirement of *A. bisporus* and revealed that minimum growth was obtained at 3^{0}C but the maximum limit for most of the strains was found to be 30^{0}C with an optimum at 24 - 25^{0}C. *A. bitorquis* showed maximum growth at 25^{0}C in all media (Iqbal, 1988). Khan *et al.* (1991) stated that the optimum temperature for growth of *A. bisporus* was found to be 25^{0}C on malt extract. Contradictory results were obtained by Furlan *et al*. (1997) who investigated the temperature requirement of *A. bitorquis* and concluded that it showed maximum growth rates at 30^{0}C than at 20 or 25^{0}C. Largeteau *et al*., (2011) stated that the commercially cultivated button mushroom *A. bisporus* requires 16-19°C during the fruiting period. Maximum biomass production of some strains of *A. bisporus* occurred from 15-18^{0}C whereas the strain AVT 06 of *A. bisporus* produced consistently maximum growth at 24 °C to 27 °C (Kaur *et al.*, 2014).

Three different ranges of light like ambient condition, darkness and fluorescent light were tested for the growth of *A. bisporus* in both solid (Figure 13) and liquid media (Figure 14). Maximum biomass production and radial growth were observed in darkness. *A. bitorquis* showed rapid mycelial growth in the darkness than in the presence of light (Furlan *et al*., 1997).

Five different substrates comprising of grains of paddy, wheat, sorghum, ragi, and maize were evaluated separately for their effects on the growth of *A. bisporus*, on the basis of number of days taken for maximum spawn run and the nature of mycelia growth on the spawn (Figure 15). Paddy grain was the best substrate for spawn run as the mycelial growth of the isolate DMRO 707 completely covered the substrate within a minimum period of 12 days. In trials on the growth of different strains of *A. bitorquis* on various spawn substrates, Guleria *et al.* (1989) indicated that spawn made of jowar grains supported maximum yield followed by bajra grains

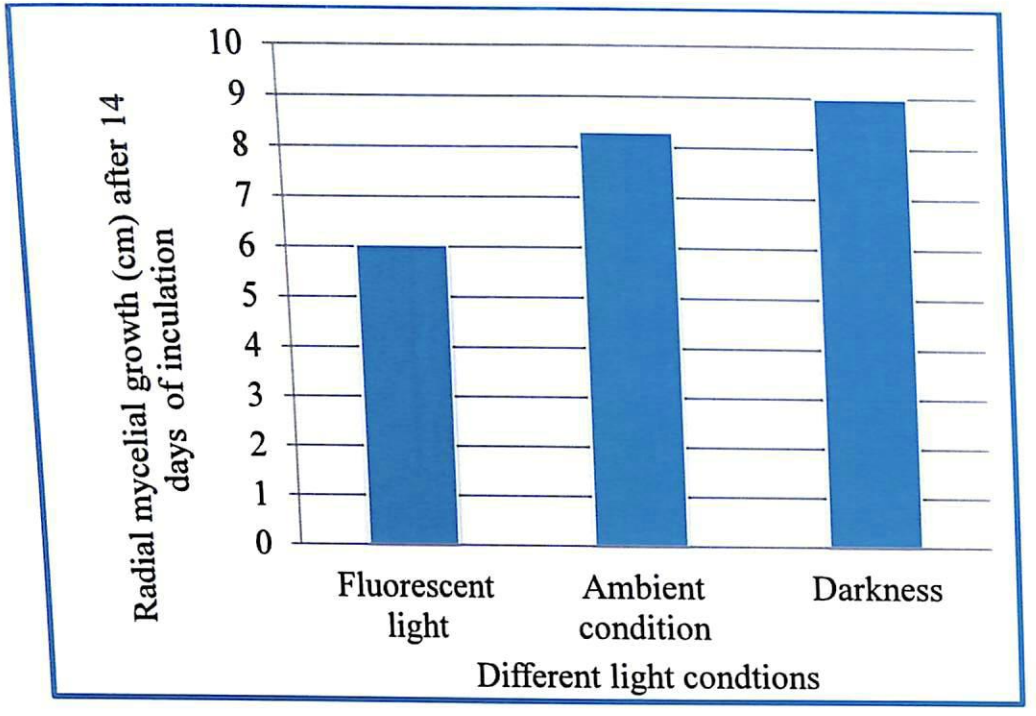


Figure 13. Growth of *A. bisporus* in solid media under different light sources

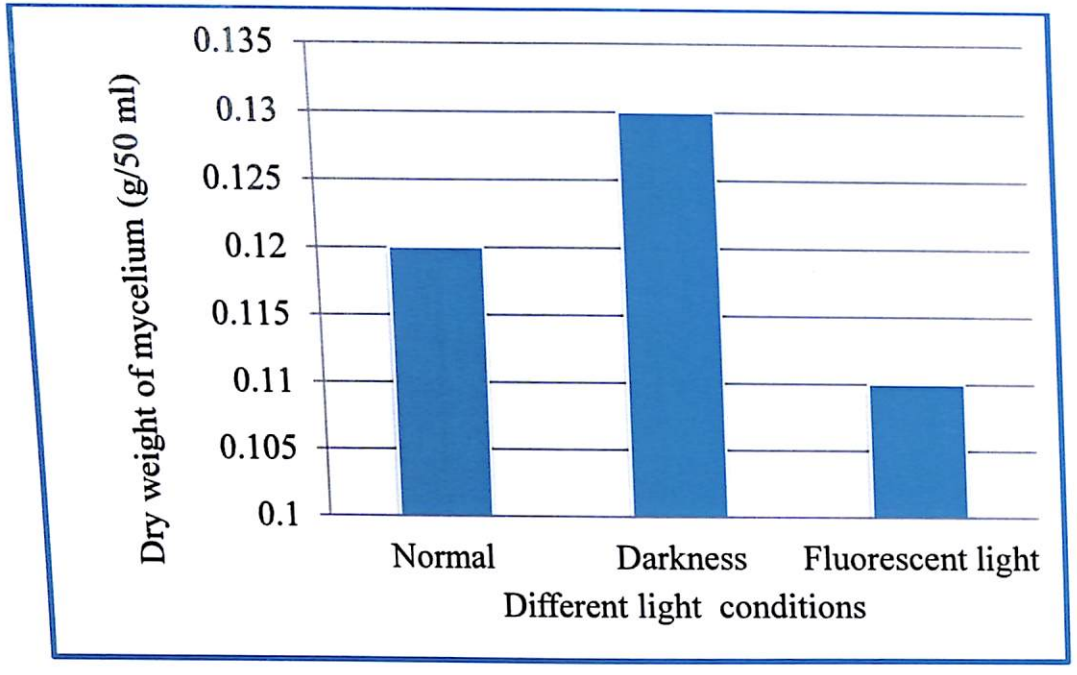


Figure 14. Growth of *A. bisporus* in liquid media under different light sources

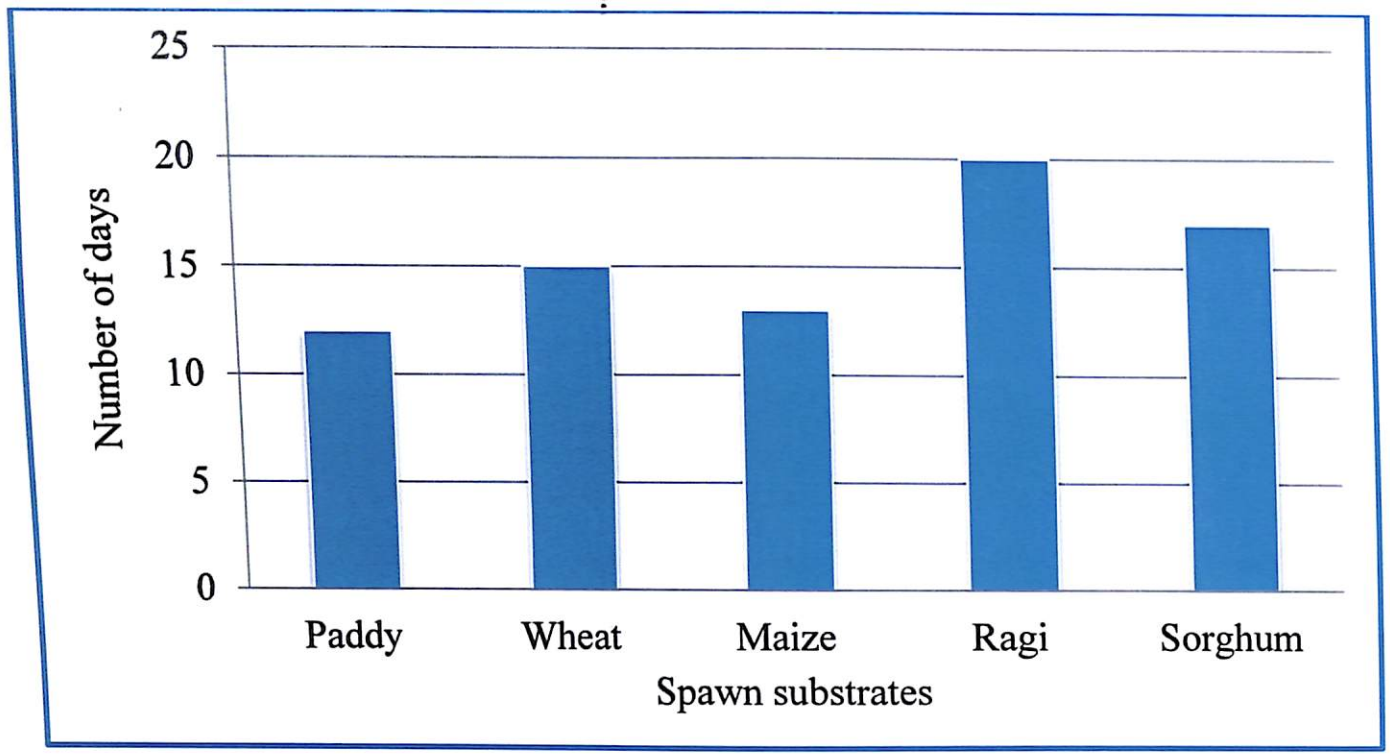


Figure 15. Time taken for complete mycelial run of *A. bisporus* on different spawn substrates

supplemented with shelled maize cob in the ratio 1:1. Mushroom spawn can be prepared on any of the grain like wheat, rice, bajra, sorghum, ragi, etc. (Rai *et al*. 2005).

Yield performance study of *A. bisporus* isolate DMRO 707 was conducted by estimating the growth and yield parameters such as days taken for complete mycelial run, number of sporocarps produced, average weight of sporocarp, total yield per bed after three harvests and average yield on six different compost substrates in combination with four casing materials. Each of the six different substrate used for composting namely paddy straw, sugarcane bagasse, spent mushroom substrate, saw dust, wheat bran and peal millet straw was added with poultry manure, urea and gypsum in the ratio 10: 4: 0.15: 0.3. The casing materials evaluated in the study were vermi compost, tea waste, coir pith compost and red soil + sand + cow dung (1:1:1).

In the comparative evaluation of above mentioned substrates in inducing a quick spawn run it was observed that pearl millet straw was the best substrate as the spawn run was completed on this composted substrate within a minimum period of ten days. This was closely followed by the substrate, sugarcane bagasse compost, on which the spawn run was completed within 12 days. Spawn run on compost using paddy straw was completed within13 days. Vijay and Gupta (1992) stated that a mixture of wheat and paddy straw can be used for compost preparation. Have *et al*. (2003) indicated that sugarcane bagasse and wheat straw are the potential composting substrates for the cultivation of *Agaricus*. Baysal *et al.* (2007) cultivated *A. bisporus* on wheat straw based compost and obtained highest mushroom yield when the wheat straw mixed with pigeon manure and peat. Uddin *et al*. (2013) investigated the effect of various composts namely wheat: paddy (1:1) straw compost, paddy straw compost and decomposed cow dung on growth and yield performance of white button mushroom (*A. biporus*) and reported maximum yield in wheat: paddy straw compost

and the lowest in decomposed cow dung. Use of paddy straw as composting material in the cultivation of button mushroom was found feasible, economically viable and environmentally desirable (Dahiya *et al.*, 2013). The production of quality compost for *A. bisporus* using alternative and agricultural waste beyond the search for productive strains are the main factors related to improve yield (Jesus *et al.*, 2013).

In the present study a longer period was required for complete mycelial run when spent mushroom was used as a substrate for composting in bed preparation. However Favara *et al*. (2014) observed that spent mushroom substrate compost was a good ingredient for the production of compost for *A. blazei* cultivation.

There was no mycelial run on wheat bran or saw dust and therefore these substrates were not suitable for the preparation of spawned compost cultivation of *A. bisporus* isolate DMRO 707. Yigitbasi *et al.* (2006) cultivated *A. bisporus* in compost made from waste tea leaves along with wheat bran and chicken manure. They reported that wheat bran based compost was not appreciable for cultivation of *A. bisporus.* Dehariya *et al*. (2013) reported that compost prepared from saw dust was a poor substrate for the cultivation of *A. bisporus.*

The mushroom (*A. bisporus*) has a requirement for a "casing layer" that has specific physical, chemical and microbiological properties, which stimulate and promote the initiation of primordial. These primordia then may develop further into sporophores, involving differentiation of tissue (Noble *et al*., 2003).

A comparative evaluation of different casing materials in combination with the various spawned compost substrates was conducted by recording the yield parameters like number of sporocarps produced, average weight of sporocarp, total and average yield per bed after three harvests. All the yield parameters were recorded maximum in the spawned compost prepared from pearl millet straw which was overlaid with coir pith compost as casing material. This was followed by the combination of same spawned compost and tea waste as casing material. Lowest

yield parameters were recorded in the combination of spawned compost prepared from spent mushroom substrate with vermi compost as casing material. Gupta (1997) suggested that coir pith compost either alone or in a mixture of farm yard manure and spent mushroom substrate can be used as casing material for cultivation of mushrooms. In several studies conducted, coir pith compost was observed to be a highly suitable casing material in the cultivation of *A. bisporus* (Gulser and Peksen, 2003; Suman and Paliyal, 2004). Raina *et al*. (2002) concluded that coir pith and tea waste are potential casing materials in the cultivation of A. bisporus. Dhar *et al*. (2003) also suggested coir pith compost as a suitable casing material in the cultivation of *A. bisporus*.

In the present study beds cased with vermi compost gave lowest yield when compared to other casing materials used. However Garcia *et al.,* (2005) recorded maximum yield when vermi compost was used as the casing materials for *A. bisporus*. Similarly study Ratnoo and Doshi (2012) successfully used Farm Yard Manure (FYM), Spent Mushroom Compost (SMC), vermi compost as casing materials for the cultivation of *Agaricus*.

Yield performance of isolate DMRO 707 in each of the other combinations of spawned compost and casing materials were not appreciable.

Incidences of a few pests and diseases were observed during cultivation of *A. bisporus*. Pests that observed during the cultivation trial included sciarids and staphylinids. Four per cent of the mushrooms beds were infested with sciarids whereas thirteen per cent of beds were infested with staphylinid beetles. Incidence of pests was negatively correlated with temperature but was not influenced by relative humidity. Shandilya *et al*. (1975) observed sciarid flies feeding on compost, mycelium and fruiting bodies of *A. bisporus*. White *et al*. (2000) reported that mushroom sciarid flies are pests of *A. bisporus* and that their uncontrolled population can lead to unsuccessful cultivation of *A. bisporus*. Fletcher and Gaze (2008) stated

that the sciarid fly (*Lycoriella auripila*) is one of the most destructive pests of cultivated mushroom, *A. bisporus* (Lange) Imbach.

The fungal incidences observed during the cultivation of *A. bisporus* in the present study, included *Trichoderma* sp. and *Coprinus* sp. Fourty one per cent of *Trichoderma* sp. and seventy nine per cent of *Trichoderma* sp. were observed in the total number of beds *Agaricus*. Incidence of *Coprinus* sp. was negatively correlated with temperature whereas the growth of *Trichoderma* sp. was positively correlated. *Trichoderma viride* and *Coprinus* were reported to cause the yield loss of *A. bitorquis* up to 45 and 2 per cent respectively (Sharma, 1991).

Shelf life of *A. bisporus* was determined by keeping mushrooms at room temperature and also in refrigerated condition (Figure 16). Mushroom packed in polypropylene covers with and without perforation was kept in room temperature and refrigerated conditions. The study showed that the mushrooms stored in refrigerated condition had better shelf life when compared to room temperature. Maximum shelf life (7.50 days) was recorded when mushrooms were kept in polypropylene covers without perforation in refrigerator.

The result of this study was confirmatory with the findings of Saxena and Rai (1988); who conducted a study on short term storage of white button mushrooms (*A. bisporus)* in polythene bags with and without perforations at different temperature. They concluded that the mushrooms can be kept in non-perforated bags for 4 days at 5^{o}C, 2 days at 10^{o}C and 1 day at 15^{o}C without spoilage. Ratnoo and Doshi (2013) reported that post-harvest dip treatment of *A. bisporus* in 0.25 per cent citric acid showed better keeping quality as it retains excellent whiteness and no veil opening up to 96 hours of storage at 5°C, good whiteness and no veil opening up to 96 hours of storage at 12°C.

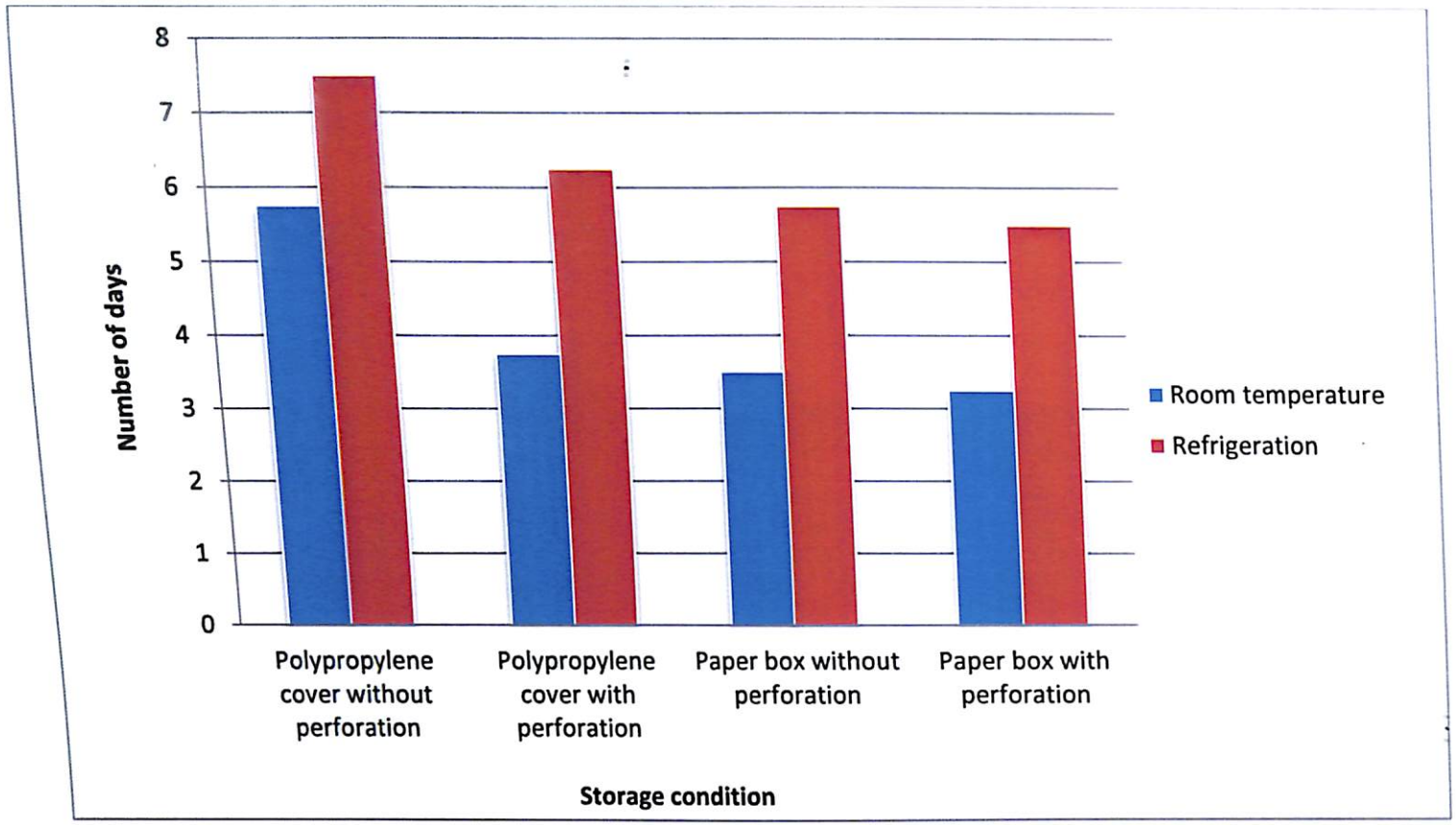


Figure 16. Shelf life of *A. bisporus*

The proximate constituents present in *A. bisporus* were analyzed (Figure 17). The moisture content of *Agaricus* was found to be 90.03 per cent (fresh mushroom), 29.1 per cent protein, 9.37 per cent ash, 22.63 per cent fibre, and 2.10 per cent fat, 4.58 per cent carbohydrate, 3.76 per cent nitrogen, 0.58 per cent phosphorus and 1.54 per cent potassium. This study was supported by the results of experiments done by Singh *et al*. (1999) on nutritive content of *Agaricus* and they stated that it consisted of 90.10 per cent moisture, 3.75 per cent protein, 0.53 per cent crude fibre and 4.59 per cent carbohydrate.

This study was also supported by Masamba and Mwale (2010); estimated the proximate constituents of *A. bisporus* and they concluded that this mushroom consisted of 3 per cent protein, 0.8 per cent fat, 2.2 per cent calcium, 8.4 per cent iron, and 0.2 per cent magnesium on wet basis.

Organoleptic studies were conducted by preparing recipes of both fresh and dried *Agaricus* mushrooms and subjected to sensory evaluation. Three different recipes *viz.,* cutlet, mushroom masala and soup were prepared. The mushroom recipes were evaluated for their characters like colour and appearance, texture, flavour, taste and overall acceptability using a five point score card. Among the various products, soup ranked first for colour and appearance, texture, flavour and overall acceptability. As far as taste is concerned mushroom masala scored significantly higher rank when compared to other products. Das (2011) reported that recipes prepared from mushroom *Agaricus* scored good consumer acceptability in case of colour, flavour, taste and texture. Singh *et al.* (2013) assessed the effect of value addition of *A. bisporus* powder to various baked products like buns and cakes. It was observed that the use of powdered mushroom up to a concentration level of 15 per cent in both the products was found to be in acceptable limits.

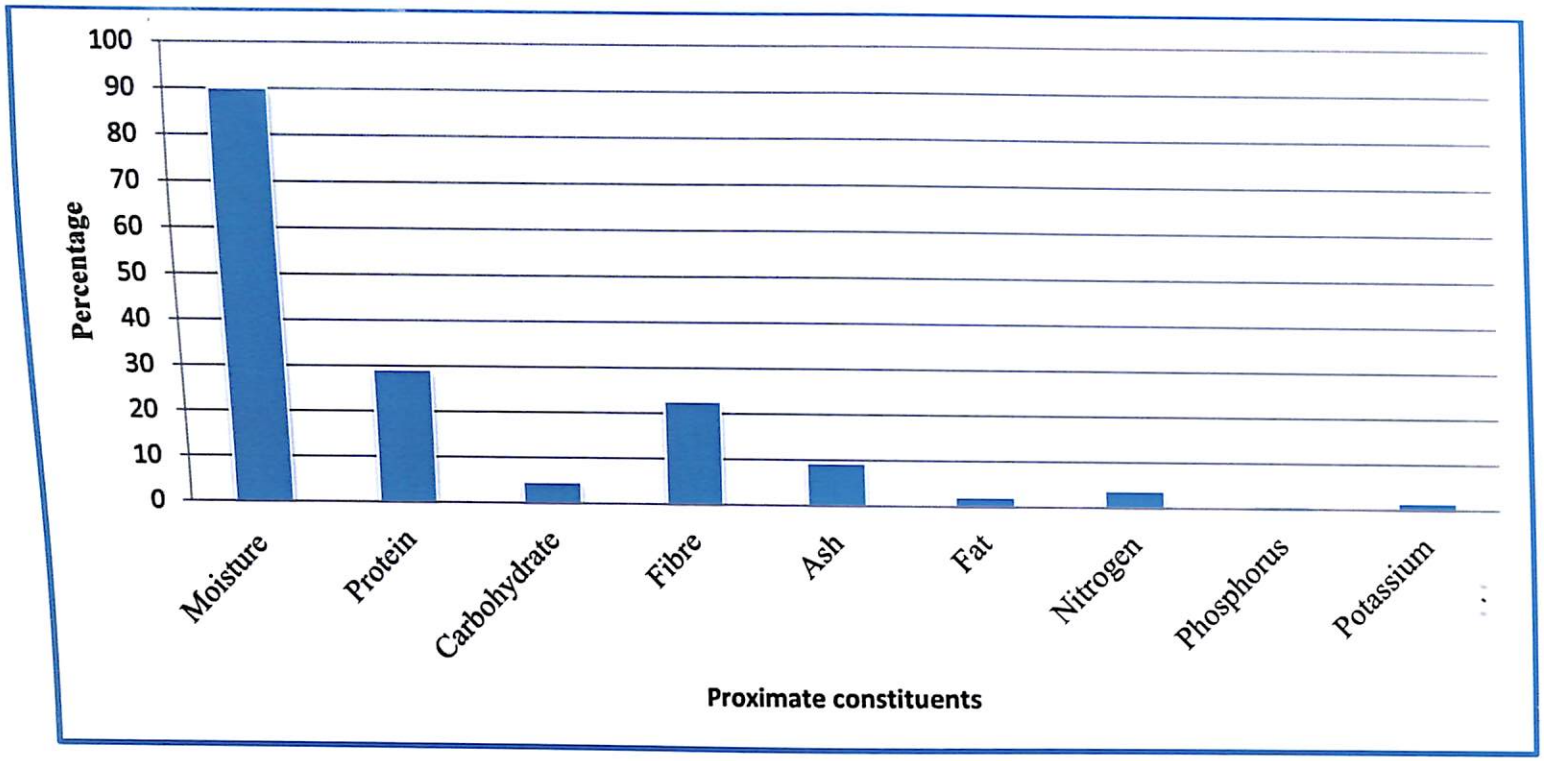


Figure 17. Proximate constituents present in *A. bisporus*

Summary

6. SUMMARY

Agaricus spp. was collected from South Kerala after the Southwest and Northeast monsoons. The habit of these strains varied from solitary to gregarious type and they were terrestrial. Organic matter rich soil was found to be the usual spots for the occurrence of *Agaricus*. The period of occurrence of these mushrooms were from June – October. Collected isolates along with two strains already present in the AICRP centre were sent to Directorate of Mushrooms for getting accession numbers. The selected isolate selected after preliminary trials was given the accession number DMRO-707 and identified as *Agaricus bisporus* (Lange) Imbach from Directorate of Mushroom Research, Solan.

Growth of *A. bisporus* was tried on different solid media namely potato dextrose agar (PDA), oat meal agar, malt extract agar, carrot agar and Czapek's Dox agar for testing their efficacy in supporting the radial mycelia growth of *A. bisporus*. The radial growth of *Agaricus* after 14 days indicated that potato dextrose yeast agar was superior with 9.0 cm of radial growth. Potato dextrose agar (8.43 cm) was found to be next best media which was followed by malt extract agar (6.43 cm), oat meal agar (5.30 cm) and Czapek's Dox agar (4.88 cm). No growth was found in carrot agar media.

The biomass production of *A. bisporus* in liquid media after 14 days of incubation indicated that potato dextrose broth was found to be the most promising treatment with 124.33 mg / 50 ml of biomass production.

Studies on the effect of carbon sources on radial mycelial growth and biomass production of *A. bisporus* in solid and liquid media revealed that glucose and fructose were the best in both solid (with a radial growth of 4.53 and 3.30 cm respectively) and liquid media (with a biomass production of 128.60 mg / 50 and 57.17 mg / 50 ml respectively). The isolate could not effectively utilize xylose and mannose.

Beef extract (9.00 cm) was found to be the significantly superior nitrogen source when compared to remaining treatments which was followed by peptone (7.63 cm). Same trend was seen in liquid medium also. The organic nitrogen source beef extract was found to be the best producing highest biomass of 122.76 mg which was followed by peptone with a biomass of 63.49 mg.

Growth of *A. bisporus* in petri dish was recorded 14 days after incubation for growth in different hydrogen ion concentration. The study indicated that pH 6 was best for the growth by producing a radial growth of 9.00 cm which was significantly superior to the rest of the treatments. In liquid medium, it was observed that maximum biomass was obtained at pH 6 (125.88 mg/50 ml) which was followed by pH 7 (118.94 mg).

Six different temperature conditions of 5 ºC, 10 ºC, 15 ºC, 20 ºC, 25 ºC and 30ºC were tested for the efficacy in the production of maximum radial growth and biomass. At a temperature of 25ºC *A. bisporus* completed full growth (9.00 cm) on petri dish after 14 days and it was significantly superior over other treatments. In liquid media, it was observed that temperature of 25 ºC supported maximum mycelia growth and biomass production of 125.87 mg which was followed by 20 ºC, 30 ºC and 15 ºC.

The influence of different sources of light and darkness on the radial mycelial growth and biomass production of *A. bisporus* was evaluated and it was found that darkness was more efficient in enhancing the radial growth (9.00 cm) and biomass production (0.13g/50 ml). Fluorescent light was very poor in supporting the mycelia growth.

Five different grain substrates were evaluated for their time taken for maximum spawn run and nature of mycelia growth. The spawn substrates tried were paddy, wheat, sorghum, ragi and maize. Paddy was found to be the best substrate for spawn run with a minimum of 12 days required for maximum spawn run with thick, white mycelia growth on the grains. It was followed by maize and wheat which took 13 and 15days for maximum spawn run with a thick

and fluffy growth on grains. Ragi grain was found to be a poor substrate and took maximum days for spawn run.

Six different composts prepared from paddy straw, sugarcane bagasse, spent mushroom substrate, saw dust, wheat bran and peal millet straw as base materials; each with the addition of poultry manure, urea and gypsum in a proportionate manner (10:4:0.15:0.3). Different casing materials namely coir pith compost, vermi compost, tea waste and red soil + sand + cow dung (1:1:1) were used for cultivation trials of *A. bisporus.* The compost prepared from pearl millet as main component took only ten days for complete spawn and gave maximum number of sporocarps when cased with coir pith compost. No mycelia growth was observed in composts prepared from sawdust and wheat bran. Average weight of sporophores ranged from 8.88 g to 22.66 g. The maximum average weight (22.66 g) being in pearl millet compost cased with coir pith compost and lowest was found in spent mushroom substrate compost with vermi compost as casing material. Total yield per bed for different bed substrates varied from 56.92 to 325.97 g, highest being in pearl millet compost with coir pith as casing material which was significantly superior from rest of the treatments. Average yield on different substrate and casing material combination was found between 18.98 g to 108. 66 g. Maximum average yield was observed in pearl millet compost with coir pith as casing material which was on par with same compost with tea waste as casing material.

Pest and disease incidence was very low during the study. Pests like sciarids and staphylinid beetles were mostly found in mushrooms. Their incidence was commonly found during low temperature. The major diseases observed during the study were the incidence of *Trichoderma* sp. and *Coprinus* sp. on mushroom beds during spawn run, case run and fruiting.

The nutrient content of *A. bisporus* was tested to find out the proximate constituents. The findings revealed the nutrient composition of *A. bisporus* as having 90.03 % moisture (fresh weight), 29.1% protein, 9.37% ash, 22.63% fibre,

2.10% fat, 4.58% carbohydrate, 3.76%nitrogen, 0.58% phosphorus and 1.54% potassium. The shelf life study of *A. bisporus* showed that this can be stored in polypropylene covers without perforations for 5 days in room temperature and seven days in refrigerated conditions.

A. bisporus was screened for their organoleptic properties like colour and appearance, texture, taste and flavour. Three different recipes were prepared and they were subjected to evaluation by 10 judges based on a five point score card. Though all the recipes were delicious and tasty the overall acceptability was high for button mushroom soup.

The technology of cultivating *A. bisporus* on compost substrate prepared from pearl millet as base material was found to be best with coir pith compost as casing material and it can be recommended as a suitable domestication package which will be transferred to the farmers, for cultivation in hilly areas where the temperature is considerably low.

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Appendices

APPENDIX – I

DATA-SHEET

Date of collection ……

Collected by……. Locality ……...

GENERAL

Substrate :

Habitat : Terrestrial / Lignicolous / Epixylose / Coprophilous

Habit : Solitary / Scattered /Gregarious

Pileus

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

Colour :

Size : Diameter

Thickness

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

Stipe

Shape : Clavate/Cylindrical/Solid/Hollow/Slender

Size : Length :

Diameter :

Attachment to pileus : Lateral/Eccentric/Central/Resupinate

Surface : Glabrous/Scaly/Smooth/Pubescent/Fibrillose

Basal part : Globular/Bulbous/Fusoid/Cylindrical

APPENDIX – I (Continued)

Gills

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

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

Margin : Smooth/Wavy/Serrate/Fimbriate/Dentate

Size : Number per cm

Veil

Type : Present/Absent

Annulus

Type : Present/Absent

Volva

Type : Present/Absent

Spore print

Colour :

Spores

Colour :

Shape : Ovate/Elliptical/Globose/Epiculate/Cylindrical/Fusiform/ Angular/Echinulate/Recticulate/Ovoid/Pyriform

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APPENDIX – II
Composition of different media

a) Potato dextrose agar (PDA)

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

b) Malt extract agar

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

c) Oat meal agar

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

d) Potato dextrose yeast agar

Potato	:	200 g
Dextrose	:	20 g
Yeast	:	1.5 g
Agar-agar	:	20 g
Distilled water	:	1 l

e) Carrot agar

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

f) Czapek – Dox Agar

Na NO_3	:	2 g
K_2 H PO_4	:	1 g
Mg SO_4. $7H_2O$	:	0.5 g
K Cl	:	0.5 g

APPENDIX – II (Continued)

Fe SO_4	:	0.01 g
Sucrose	:	30 g
Agar	:	20 g
Distilled water	:	1 l

APPENDIX – III

Score card

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

APPENDIX - IV

Recipes of different products

Soup

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

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

Cutlets

Agaricus	-	200 g
Onion finely chopped	-	1 medium
Garlic crushed	-	3 cloves

APPENDIX – IV (Continued)

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

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

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

APPENDIX – IV (Continued)

Mushroom masala

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

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

APPENDIX – IV (Continued)

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

STANDARDIZATION OF TECHNIQUES FOR CULTIVATION OF BUTTON MUSHROOM (*Agaricus* spp.) IN KERALA.

Lishma N. P.

(2013-11-151)

Abstract of the thesis

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ABSTRACT

The work entitled "Standardization of techniques for cultivation of Button mushroom (*Agaricus* spp.) in Kerala" was conducted during the period 2013 - 2015 at College of Agriculture, Vellayani. The aim of study was to explore the possibility of cultivation of *Agaricus* species and develop a modified technique for the production of quality compost with alternative agricultural waste. A preliminary survey was conducted for the collection of various isolates of *Agaricus* spp. during and after south - west monsoon in different locations. The five isolates obtained along with the two isolates available at AICRP centre, Vellayani were sent to DMR and accession numbers were obtained for all the isolates.The isolate 7 identified as *Agaricus bisporus* (Lange) Imbach which showed maximum growth on potato dextrose agar as well as intensive spawn run on paddy grains was selected for further studies.

Studies revealed that maximum vegetative growth was observed on potato dextrose agar and it preferred a temperature of 24 °C with a pH of 6. It was also observed that dark phase favoured the growth of *A. bisporus.* Among the different carbon and nitrogen sources tested, glucose and beef extract supported maximum growth of *A. bisporus*.

The study on evaluation of different substrates for spawn production revealed that in paddy grains spawn run was completed within twelve days which was the best.

Evaluation of various compost and casing material combinations revealed that pearl millet straw, poultry manure, urea and gypsum + coir pith was the best with a total yield after three harvests of 325.97 g per kg bed followed by pearl millet straw, poultry manure, urea and gypsum + tea waste (301.76 g per kg bed). No mycelial growth was observed in all combinations of sawdust, poultry manure, urea and gypsum as well as wheat bran, poultry manure, urea and gypsum with different casing materials.

The pest and disease incidence during the cultivation period of *A. bisporus* was studied and it was observed that sciarid flies and staphylinid beetles were prevalent during sporocarp formation. The competitive fungi recorded were *Trichoderma* sp. and *Coprinus* sp. during spawn run, casing and sporocarp formation.

The study also showed that mushrooms stored under refrigeration (4 °C) had maximum shelf life of 7 days compared to those stored at room temperature.

Sensory evaluation of mushroom recipes were carried out by a panel of judges for attributes like colour, texture, flavor and taste using a five point score card. Among the various products mushroom soup obtained an overall acceptability of 4.7.

From this study it can be concluded that growth of *A. bisporus* was maximum in potato dextrose agar at a temperature of 24 °C with a pH of 6 in dark phase. Glucose and beef extract were found to be the best carbon and nitrogen sources respectively. Compost prepared from pearl millet straw, poultry manure, urea and gypsum overlaid with coir pith compost as casing material supported maximum growth and yield of *A. bisporus.*