PHYSIOLOGICAL AND CULTURAL STUDIES ON BLUE OYSTER MUSHROOM (*Hypsizygus ulmarius* (Bull.:Fr.) Redhead)

SUMI I. (2014-11-142)

DEPARTMENT OF PLANT PATHOLOGY COLLEGE OF AGRICULTURE VELLAYANI, THIRUVANANTHAPURAM-695522 KERALA, INDIA

2016

PHYSIOLOGICAL AND CULTURAL STUDIES ON BLUE OYSTER MUSHROOM (*Hypsizygus ulmarius* (Bull.:Fr.) Redhead)

by SUMI I. (2014-11-142)

THESIS

Submitted in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture Kerala Agricultural University



DEPARTMENT OF PLANT PATHOLOGY COLLEGE OF AGRICULTURE VELLAYANI, THIRUVANANTHAPURAM-695522 KERALA, INDIA

> **2016** ii

DECLARATION

I, hereby declare that this thesis entitled "PHYSIOLOGICAL AND CULTURAL STUDIES ON BLUE OYSTER MUSHROOM (*Hypsizygus ulmarius* (Bull.:Fr.) Redhead)" 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 to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellayani, Date: 29.08.2016 Sumi I. (2014 - 11-142)

CERTIFICATE

Certified that this thesis entitled "PHYSIOLOGICAL AND CULTURAL STUDIES ON BLUE OYSTER MUSHROOM (*Hypsizygus ulmarius* (Bull.:Fr.) Redhead)" is a record of research work done independently by Mrs. Sumi. I. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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ACKNOWLEDGEMENT

This is perhaps the easiest and hardest chapter that I have to write. First and foremost I bow my head to the God Almighty for giving me the strength, health and good spirit to complete this work successfully. It is a great pleasure for me to express gratitude to several personalities who guided and extended all help required directly or indirectly during my study.

I express my deep sense of gratitude to my major advisor, **Dr. D. Geetha**, Professor (Pl. Path.), Instructional Farm, College of Agriculture, Vellayani for her meticulous guidance, transcendent suggestions, constructive criticism, remarkable patience, support, care and constant encouragement right from the conceptualization of the research work to preparation of the thesis. I proudly acknowledge that this manuscript was gained its completeness under the kind supervision and inspiration of my guide and she has been a valuable support for both academic and personal level, for which I am extremely grateful.

I owe my sincere thanks to Dr. V. K, Girija, Professor and Head, Department of Plant Pathology, College of Agriculture, Vellayani and member of advisory committee for all the support and suggestions, expert advice and constructive criticism during the course of my study.

I am deeply indebted to Dr. Susha S. Thara, Assistant Professor, Department of Plant Pathology, College of Agriculture, Vellayani and member of advisory committee for her valuable suggestions and advice, constant encouragement, support and care to complete the thesis in time.

I am grateful to Dr. C. Nirmala, Associate Professor, Department of Home Science, College of Agriculture, Vellayani and member of advisory committee for their valuable and expert suggestions, critical evaluation, everlasting patience and constant encouragement for the present investigation.

I gladly acknowledge Dr. C. Gokulapalan, retired Professor and Head, Department Plant Pathology, College of Agriculture, Vellayani for his friendly approach, constant encouragement and valuable suggestions and support not only for the present study but for my entire college life. I express my heartfelt acknowledgement and sincere thanks to Dr. K, K, Sulochana, (former Head), Dr. A. Naseema, retired Professor, Department Plant Pathology, for rendering suggestions and support during the course of my study. I am extremely grateful to Dr. K, Umamaheshwaran, Dr. Lulu Das, Professors, Department Plant Pathology, Dr. G. Heera, Assistant Professor, Department Plant Pathology, Dr. M. Joy, Associate Professor, Department of Plant Pathology for their constant encouragement, ever willing help and valuable suggestions during the entire study period.

I express my sincere gratitude to Dr. P. Babu Mathew, Professor and Head, Instructional Farm, College of Agriculture, Vellayani for granting the permission and facilities for the smooth conduct of research work. I am also thankful to Sheeja chechi and Selvi chechi for their immense help and mental support throughout my study.

I am grateful to Dr. Kamala Nayar, Professor (Pl. Path.), Instructional Farm, College of Agriculture, Vellayani for her valuable suggestions and advice during my M. Sc. programme. I also express my sincere gratitude to all the teaching and non teaching staff of Instructional Farm, College of Agriculture, Vellayani for their support without which I wouldn't complete my study successfully.

I deeply express my special wholehearted thanks to Radhika mam, Assistant Professor who helped me a lot with valuable advices and suggestions throughout the course of my study. I am also thankful to Deepa chechi for her kind support and suggestions during the research period. My sincere thanks to Manal and Nisha, project fellows of my department, Rejitha chechi and Saritha chechi, office staff, Department of Plant Pathology, College of Agriculture, Vellayani for their unlimited help throughout the programme.

I would likely to take this opportunity for express my gratitude towards Dr. Vijayaraghavakumar, Professor and Head, Department of Agricultural Statistics for the proper guidance and help for getting the research data analysed. I am also thankful to all the teachers from the Department of Agricultural Entomology for their constant support and help during my study.

I am extremely grateful to Dr. Mini. C., Professor and Head and Dr. Geetha Lekshmi Assistant Professor, Department of Processing Technology and Dr. Arya, K., Professor and Head, Department of Plant Breeding and Genetics for their motherly care, friendly approach, loving behaviour and support not only for the present study but for my entire college period. My loving and wholehearted thanks to Dr. K, M. Abdul Khader, retired professor, Department of Plant Breeding and Genetics for his profound help, immense support, constant encouragement and fatherly care which gave me more strength in my difficult times.

I am also indebted to the Dean, College of Agriculture, Vellayani for providing me all the necessary facilities from the university during the whole course of study.

I shall be missing something if I don't acknowledge the support and caring of my beloved seniors Asha chechi, Lishma, Shilpa and Priya for their continuous encouragement and guidance. I am also thankful to all the juniors of my department especially Brinda for the mental support and constant encouragement during the course of my study.

I sincerely thank my colleagues in plant pathology who helped me a lot to complete the course works as well as research works in time. I cherish with appreciation the joyful and cheerful company of my colleagues Shymi, Pravi, Arya, Aaruni, Theresa, Parvathy, Niki and Priya who always cheered me in the moments of despair and supported me in difficult times not only in academic works but also in personal life and made the things much smoother.

I will forever be grateful to my sincere friends Vichu, Adersh chettan, Jithu, Eldhose, Mridul, Prasu, Kutoos, Elizabeth, Sameeha, Amrutha and Anjali for being there when I needed them most. I am extremely thankful to them for their unwavering friendship and support. Also I will forever be thankful to all my batchmates for their selfless help and moral support throughout the period of my study. I owe immense gratitude to Kerala Agricultural University and its staff for the financial, academic and technical support, especially in the award of a KAU Post Graduate Fellowship, contingency fund that provided the necessary financial support for this work.

Most importantly none of this would have been possible without the love and care of my family to whom this dissertation is dedicated to, has been a constant source of love, concern, support and strength for all these years. I am deeply indebted to my husband, Aneesh for his profound help, immense support, loving nature, constant encouragement and care without which this work would not be completed in successful manner. I allocate my highest respect and heartful regards from my inner core of hearts to my Chechi, Sarath, Sachu, Achan, Amma, Ratheesh annan and Kishan for their love, inspiration and prayers for me to achieve higher goals in my life.

I express my immense and whole hearted thanks to all my near and dear for their cooperation, help and loving care and concern during the course of study and research.

Sumi.. I.

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%	Per cent
lbs	Pound per square inch
PDA	Potato dextrose agar
mm	Milli meter
⁰ C	Degree Celcius
CD	Critical difference
cm	Centimetre
et al.	And other co workers
g	Gram
h	Hours
<i>i. e.</i>	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
рН	Negative logarithm of hydrogen ions
μm	Micron meter

LIST OF ABBREVIATIONS AND SYMBOLS USED

ppm	parts per million
wt.	weight
B . E	Biological efficiency
temp./T.	temperature
RH	Relative humidity
CRD	Completely Randomized Design

Introduction

1. INTRODUCTION

Mushrooms are macroscopic, spore- bearing fruiting bodies of fungi. They have been valued throughout the world for thousands of years both as food and as medicine. They contain reasonable amount of protein, minerals, vitamins and various novel compounds of medicinal value. Many studies revealed that mushrooms possess biopharmaceutical compounds that can be used for therapeutic applications. They have antifungal, anti-inflammatory, antitumor, antiviral, antibacterial, hepato-protective, anti-diabetic, and hypotensive properties (Nandakumar, 2013). According to Shivashankar and Premkumari (2014) out of the 10,000 known species of mushrooms, 2000 are safe for humans and about 300 of them possess medicinal properties. The mushrooms comprise a large heterogeneous group having various shapes, sizes and colours, all quite different in character, appearance and edibility. Of these large groups with more than 2000 edible species, about 300 species belonging to 70 genera are reported from India (Karthika and Murugesan, 2015).

Mushroom cultivation recycles agro-residues, much of which is otherwise burnt in the field. Since our country has abundant agricultural residues, we can emerge as a major player in mushroom production. In the present agricultural scenario, secondary agriculture is going to play a pivotal role and mushroom fits very well in this category. Mushroom being an indoor crop, utilizes vertical space and requires only 25-30 litre water for production of one kg mushroom, thus offering a solution to shrinking agricultural land and water. The annual world production of button mushroom has reached 6.5 million tonnes and that of all types of mushrooms is estimated to be over 25 million tonnes. Our country has registered twenty-fold increase in production of mushrooms in the last four decades and still our production is only 1.2 lakh tonnes of which, button mushroom continues to occupy a prominent place and contributes about 80% of the total mushroom production of our country (DMR, 2013). India produces about 250 thousand tons of edible mushrooms annually (Dhar, 2014). Oyster mushroom, one of the edible mushroom cultivated in the tropics, has gained much importance in the last decade in many countries including India. During 1990 its contribution was 24.1% of the total world production. According to Royse (2014), oyster mushroom is the second largest cultivated mushroom around the world recording 27 per cent of world production. Oyster mushrooms are cultivated widely as their temperature requirement of $20-30^{\circ}$ C prevails in most of the areas. Among all the cultivated mushrooms *Pleurotus* has the maximum number of commercially cultivated species suitable for year round cultivation.

Hypsizygus ulmarius (Bull.:Fr.) Redhead, commonly called as 'elm oyster' or 'blue oyster' is similar to oyster mushroom, but differ in morphology and biological "Hypsi" means "high" or "on high" and "zygus" means "yoke", efficiency. Hypsizygus, then referring to position of this mushroom often high in the tree. Ulmrefers to "elm" indicating one of the common substrates for this fungus. They often grow in clusters on living elm trees or elm logs in forests. It is a novel species with very large fruiting body, blue coloured pinheads becoming light white on maturity, high yielder, palatable with meaty flavour and attractive keeping quality. This mushroom variety has attractive shape and is fleshy with excellent taste. Despite these attractive qualities, its production in tropical climate has not yet been fully explored. In Kerala, mushroom growers are mainly concentrating on the cultivation of white oyster, *Pleurotus florida* and recently growers started producing H. ulmarius, but its erratic production hinders large scale adoption. In this context, the present study was undertaken with the objectives; to standardize the cultivation technology of *H. ulmarius* and to study its cultural and physiological aspects. Till date, systematic study on the cultural and physiological aspects of this mushroom has not been conducted in Kerala. The present study will generate information on this highly nutritious mushroom species for the growers and consumers of this State.

Review of Literature

2. REVIEW OF LITERATURE

Hypsizygus ulmarius (Bull.:Fr.) Redhead, also known as 'blue oyster' or 'elm oyster' is a high yielding mushroom gaining popularity nowadays. It is one of the important edible mushrooms in the world popularly cultivated in Japan, China and other Asian countries (Chang, 1999). This mushroom has wide uses due to its unique flavour, nutritive value and medicinal properties. Medically it is known for its cardiovascular, anti-tumour and cholesterol controlling properties. This mushroom is wide spread throughout the temperate forests of eastern North America, Europe and Japan. It is a saprophyte on elms, cotton woods, beech, maple willow, oak and occasionally on other hard woods. It is growing solitary or in small clusters on living hardwoods, particularly elm (*Ulmus*) and boxelder (*Acer negundo*). This mushroom closely parallels the morphology of oyster mushroom but it is far better in flavour and texture (Sutha and Eswaran, 2016).

Taxonomy

Gray (1821) cited by Redhead (1984), first named the blue oyster mushroom as *Pleuropus ulmarius* (Bull.). This was later renamed as *Pleurotus ulmarius* (Bull.) in 1871, as *Dendrosarcus ulmarius* (Bull.) in 1898, as *Micromphale ulmarium* in 1916, as *Lyophyllum ulmarium* (Bull.) in 1938 and finally as *Hypsizygus ulmarius* (Bull.) Redhead by Redhead (1984). Singer (1947) first described this genus and placed it under the sub division Basidiomycotina, order Agaricales and family Lyophyllaceae.

Kirk *et al.*, (2001) placed it taxonomically in phylum Basidiomycota, class Basidiomycetes, subclass Agaricomycetidae, order Agaricales and family Tricholomataceae. It is saprobic, growing alone or in clusters, cap 5-15 cm, convex with a slightly curved margin with a sunken centre, gills attached to the stem and not running down it (Kirk *et al.*, 2008).

Meera *et al.* (2011) reported that the mushroom is rich in antioxidants and proved for its antidiabetic activity. Looking like the oyster mushroom, this species was once called *Pleurotus ulmarius* (Bull. ex Fr.) Kummer, later *Lyophyllum ulmarium* (Bull.:Fr.) Kuhner and most recently *Hypsizygus ulmarius* (Bull.:Fr.) Redhead. The fruiting bodies of elm oyster are larger, heavier and white to bluish grey in colour and have a stipe that is off centre to nearly central (Ruchita and Shukla, 2012).

2.1. ISOLATION AND PURE CULTURING

Karthika and Murugesan (2015) isolated the culture of *H. ulmarius* on potato dextrose agar medium in petri plates. They also inoculated the fungus in to 100 ml potato dextrose broth contained in 250 ml conical flask. Cultures maintained on petri plates were later inoculated to test tubes containing potato dextrose agar medium for further studies.

Sutha and Eswaran (2016) conducted a study to find out the effect of various surface sterilants on tissue germination of *H. ulmarius* and contamination. The result of experiment clearly indicated that swabbing of basidiocarp with ethyl alcohol and sodium hypochlorite as surface sterilant (@ 2 per cent concentration recorded the maximum percentage of tissue germination (98.85 %) without contamination. Dipping mushroom bits in sodium hypochlorite @ 3 per cent reduced the germination per cent significantly (68.52 %) without any contamination.

2.2. MORPHOLOGICAL, CULTURAL AND PHYSIOLOGICAL STUDIES

2.2.1. Morphological Studies

Moser (1983) explained the morphological characters of *Hypsizygus* circinatus and *Hypsizygus tessulatus*. Cap of *H. circinatus* appeared as dirty white, thin, convex, margin lobed, 3-5 cm with weak flour smell. Lamellae were very crowded and almost decurrent. The stipe was 2-5/5-10, whitish and central to eccentric and the spores were egg-shaped, almost rounded and 5-6 µm. Cap of *H. tessulatus* was somewhat spotted-marbled, ochraceous- reddish-brown and paling. The stipe was eccentric, short, firm and base tapered and the lamellae were emarginated- adnate, white and yellowish.

Meyers (2004) observed *H. ulmarius* in natural condition and reported that it possess a pileus of 5-15 cm length, which was convex with a slightly in-rolled margin at first, becoming almost flat with a slightly sunken centre; white, turning to creamy buff or tan; cracking and forming small scales or patches. The gills were attached to the stem and not running down, close or nearly distant, whitish becoming cream. The stipe was around 5-10 cm long, 1-2.5 mm thick, dry, smooth to hairy, whitish, solid, stout, off-centre to nearly central, sometimes enlarged at base. The spores were 5-6 μ m in size, smooth, round or nearly round.

According to Kushwaha *et al.* (2011) hyphae of blue oyster mushroom was septate, branched, subhyaline to creamy buff in colour, measuring 1-4 μ m in width. The sporophores (fruit bodies) were medium to large, bluish grey in early (pinhead) stage of development and they fade to creamy buff at maturity. The pileus was 6-15 cm in diameter, convex with slightly inrolled margin at first, becoming almost flat with a slightly sunken centre. The gills were attached to stipe and not running down it, close or nearly distant, whitish, later becoming cream. The stipe was 2-6 cm long and often with eccentric, thick, fleshy, hard when dried and not fragile, solid, stout, off centre to nearly central, sometimes enlarged at base, whitish and smooth to hairy. The spore print was white to buff or pale cream or light ochraceous or pure white

when quite fresh. The basidiospores were small 2.5-6.5 μ m, broad-ellipsoid to ovoid, hyaline and smooth.

Shubhra and Jaitly (2011) observed that the stipe length of *H. ulmarius* fruiting bodies produced on wheat straw was 3.6 cm with a pileus of 5.36 cm length and 7.85 cm width.

The stipe length and pileus width are important morphological characters which determine the quality as well as the quantity of produce. The smallest stipe and larger pileus were considered as good (Ruchita and Shukla, 2012).

2.2.2. Cultural and Physiological Studies

The cultural characters of the fungus was studied by Kushwaha *et al.* (2011) and found that the colony on PDA medium was white to creamy buff, fluffy and circular in nature.

2.2.2.1. Growth of H. ulmarius in Different Media

Growth of *H. ulmarius* on solid media *viz.*, potato dextrose agar, malt extract, soybean malt yeast extract, oat meal agar, Coon's agar and Czapek's (dox) agar and in liquid media viz., potato dextrose solution, malt extract solution, soybean malt yeast extract solution, oat meal solution, Coon's solution and Czapek's (dox) solution was studied by Wange and Patil (2007). He observed that malt extract agar and solution were the most preferred media by the fungus which was on par with soybean malt yeast extract medium. Significantly moderate growth of fungus was recorded on PDA and poor growth was recorded on oat meal agar.

Chandravanshi (2007) reported that both solid and liquid media with potato dextrose supported maximum radial growth and biomass of *H. ulmarius*. Kushwaha *et al.* (2011) evaluated nine media including malt extract agar, wheat extract agar and

potato dextrose agar for their ability to support the growth of *H. ulmarius* and the results showed that malt extract agar and wheat extract agar media supported maximum mycelial growth followed by potato dextrose agar medium. Significantly poor growth was recorded on water agar, yeast extract agar and potato carrot agar medium.

Jatav *et al.* (2012a) evaluated six different media for the mycelial growth of *H. ulmarius* and malt extract agar medium was found to be the best one. Mishra *et al.* (2015) studied the cultural characters of *H. ulmarius* along with ten species of oyster mushroom in potato agar medium and observed cottony growth for *H. ulmarius*.

Sutha and Eswaran (2016) tested various media to find out the best media for the growth of *H. ulmarius*. The different media evaluated included corn meal agar (CMA), Czapek's dox agar (CDA), malt extract agar (MEA), oat meal agar (OMA), potato dextrose agar (PDA), potato malt extract agar (PMEA), potato carrot agar (PCA) and tapioca dextrose agar (TDA). Among these, the maximum growth was observed in potato dextrose agar and potato malt extract agar.

2.2.2.2. Growth of H. ulmarius in Different Temperature

An optimum temperature of 25 0 C was reported for the mycelial growth of *Pleurotus* spp. by Jandaik and Kapoor (1975) and that for *H. marmoreus* by Lomberh *et al.* (2000). Wang and Patil (2007) conducted studies to find out the effect of temperature on the growth of *H. ulmarius* and observed that the fungus failed to grow at 5 0 C and 10 0 C on lower side and at 35 0 C and 40 0 C on higher side. Significantly maximum growth was recorded at 25 0 C and there was decrease in growth on either side of 25 0 C which was the optimum temperature.

The growth of *H. ulmarius* in different temperatures was observed by Kushwaha *et al.* (2011) and they concluded that it could grow at all temperature tested but 25 0 C was found optimum at which the maximum mycelial growth was

recorded and the growth decreased as the temperature increased or decreased. Very low growth was recorded at 35 0 C.

Jatav *et al.* (2012a) evaluated four different temperatures to find out the best temperature giving maximum mycelial growth for *H. ulmarius* and the maximum mycelial growth was observed at 25 ± 1 ⁰C while the least growth was observed at 20 ⁰C and 35 ± 1 ⁰C temperatures.

According to Sutha and Eswaran (2016), a temperature of 25 -30 0 C was found optimum for *H. ulmarius* as it favoured the maximum growth of the fungus and biomass production on 10th day after inoculation. The growth decreased as the temperature increased or decreased beyond 20-30 0 C.

2.2.2.3. Growth of H. ulmarius in Different pH

The pH of the medium has a better impact upon the growth of any organism (Zervakis *et al.*, 2004; Sharma *et al.*, 2005).

Lomberh *et al.* (2000) observed an optimum pH of 6.1 for the mycelial growth of *Hypsizygus marmoreus*. Chandravanshi (2007) reported that a pH of 8 gave superior radial growth and biomass of *H. ulmarius*. Results from the studies of Wange and Patil (2007) indicated that the fungus failed to grow at pH 3 but could grow from pH 4 to 8. Significantly highest mycelium mass was obtained at pH 6 which was the optimum pH for the growth of *H. ulmarius*.

Kushwaha *et al.* (2011) studied the effect of different pH for the growth of blue oyster mushroom and reported a pH level of 7 to be the optimum at which maximum growth occurred which was on par with the growth recorded at pH 8. The poor mycelial growth was recorded at pH 4 which was on par with the growth recorded at pH 5 and 6.

Sutha and Eswaran (2016) reported that the maximum biomass production of *H. ulmarius* was obtained at a pH 7.0 followed by pH 6.5 and 6.0. An increase in pH above 7.5 and below 5.5 showed significant reduction in the growth and biomass production.

According to Jatav *et al.* (2012a), hydrogen ion concentration in the range of 5.0 to 6.0 was found suitable for the mycelial growth of *H. ulmarius*.

2.2.2.4. Growth of H. ulmarius in Light and Dark Conditions

According to Chandravanshi (2007) 24 h darkness in potato dextrose media of pH 8 found optimum for the mycelial growth of *H. ulmarius*. Jatav *et al.* (2012a) clearly observed the effect of light intensities on mycelial growth of *H. ulmarius* and reported that it prefers total darkness followed by natural diffused day light.

2.3. SPAWN PRODUCTION

According to Sinden (1972), spawn is the material used for seeding the beds (compost) and the vegetative growth of which is called as mycelium.

The standard procedure for grain spawn preparation was first described by Sinden (1934). Antonio and Hwang (1971) observed that cereal grains were the common substrates for mushroom mycelium production, whereas Biserka (1972) used rye, wheat and smaller grains for commercial spawn production.

Shah *et al.* (1999) and Singh *et al.* (1999) reported that about 10 to 17 days are required for complete colonization of cereal grains by fungal mycelium.

Ashulata (2007) evaluated cereal grains for spawn production of H. ulmarius and reported that paddy, sorghum and wheat grains took least time (9-10 days) for spawn development, showed dense white, bright mycelial growth overcrossing the substrate as compared to others. From the study it was clear that shelf life of mother spawn was highest (46 days) in lemon grass oiled, lemon grass deoiled and lemon grass + paddy straw (1:1) but it was less (24.75 to 29.75 days) in cereal grains.

Chandravanshi (2007) observed that maize grains, black colour and complete darkness took significantly less period for spawn development of H. ulmarius. Wange and Patil (2007) observed the time taken for mycelial growth of *H. ulmarius* on wheat grain in mother spawn and commercial spawn. The results stated that wheat grain was suitable substrate for spawn preparation and it took 14 days and 9 days for complete mycelial run for mother spawn and commercial spawn respectively.

Spawn prepared using wheat grain was used for the studies conducted by Ruchita and Shukla (2012). Karthika and Murugesan (2015) prepared spawn of H. ulmarius on sorghum grains. The grains were boiled for 10-15 min. and mixed with CaCO₃ and CaSO₄, which completed growth in 10-15 days.

2.4. CULTIVATION

Among the different methods of cultivation, polythene bag method or compact polybag method was found to be the best for *Pleurotus* spp. (Baskaran et al., 1978).

Vijay and Sohi (1987) have reported the most effective chemical pasteurisation method using formalin 500ppm and bavistin (75ppm) in substrate soaking water (18 h soaking). This method is now widely adopted to check weed mould problems and also higher biological efficiency in *Pleurotus* cultivation.

Biological efficiency is the yield of mushroom per kg of substrate on dry weight basis and is calculated as % B.E. = fresh weight of mushroom/ dry weight of substrate \times 100 (Chang *et al.*, 1981). The cultivation of oyster mushroom was standardized in Kerala and suitability of different substrates for spawn production as well as mushroom production was tried (Suharban, 1987).

The various substrates used for cultivation of *H. ulmarius* can be coconut husk, tea dust, sawdust and sugarcane bagasse (Beig and Jandaik, 1989). According to Hanscha *et al.* (2000), *H. ulmarius* could be easily cultivated on various common natural substrates like paddy straw.

Cultivation trials on *H. ulmarius* were conducted by Ashulata (2007) in which first harvesting was recorded in 35.8 days for beds prepared from paddy straw. Based on the studies, she observed that effect of environmental conditions greatly influenced the period of spawn run, pinning and biological efficiency of *H. ulmarius* and no spawn run was observed during May.

Chandravanshi (2007) cultivated *H. ulmarius* on paddy straw from October to May and reported that significantly earlier spawn run and higher yield was observed during the month of January. Significantly less yield and more period for spawn run was recorded for *H. ulmarius* in April and March months whereas no spawn run was observed during May. The study also concluded that nine per cent spawning rate and thorough mixing method was found excellent for higher yield of *H. ulmarius*. Mondal *et al.* (2010) reported highest biological yield (164.4 g) and economic yield (151.1 g) for *P. florida* from rice straw.

Cultivation studies of *H. ulmarius* were done by Shikha *et al.* (2012a) with wheat straw, paddy straw and wheat straw : paddy straw (1:1) pretreated with hot water (80 0 C) and chemicals- carbendazim and formaldehyde in concentrations of 50 ppm + 250 ppm, 75 ppm +500 ppm and 150 ppm + 750 ppm. According to them,

maximum biological efficiency, least spawn run period and pinhead appearance were observed on wheat straw substrate pretreated with hot water.

H. ulmarius was introduced for commercial production for the first time in India by IIHR (IIHR, 2012) and according to the reports the average biological efficiency of blue oyster mushroom was 60-80 per cent.

Biswas and Sanjeeb (2013) reported a biological efficiency of 156 per cent and spawn run period of 15 days for *H. ulmarius* in paddy straw and they found it as the most appropriate mushroom in West Bengal followed by *P. florida* (121.5 %) and *P. sajor-caju* (115.5 %). In this study minimum period (15 days) was taken by *H. ulmarius* for completing spawn run followed by *P. ostreatus* (18 days), *P. sajor-caju* (19 days) and *P. florida* (20 days).

Ranjini and Padmavathi (2013) reported the successful cultivation of H. ulmarius on paddy straw. They reported a suitable temperature of 23-25 0 C and relative humidity of 95-98 per cent for spawn run and harvested first flush in three to four weeks after spawning. According to them, it can be cultivated on low-nutrient agricultural wastes like paddy straw, cotton waste, saw dust and tea dust.

Ranjini and Padmavathi (2015) cultivated blue oyster mushroom on paddy straw and coconut husk and they observed highest yield and biological efficiency for *H. ulmarius* cultivated on paddy straw compared to that in coconut husk.

Senthilmurugan and Krishnamoorthy (2015) conducted studies to identify innovative container system for oyster mushroom cultivation using polypropylene bottles, carton box and polypropylene bags of different sizes, among which polypropylene bottles were found to be significantly superior and recorded an yield of 1484.3 g and 1096.3 g per kg dry weight of paddy straw for *H. ulmarius* and *P. florida* respectively with a biological efficiency of 148.4 per cent and 109.6 per cent. Biological efficiency of 114.7 per cent was recorded for *H. ulmarius* cultivated in 60×30 cm sized polypropylene bags containing paddy straw. When the size of polypropylene bags reduced to 45×30 cm, the biological efficiency recorded was 116.6 per cent.

2.5. DEVELOPMENTAL MORPHOLOGY

The buds of *H. ulmarius* were bluish grey in the early stages of development and they faded to white at maturity indicating the time of harvest. An average yield of 635 g per bed (containing 500 g of dry paddy straw) was recorded in various trials conducted with this mushroom. The mushroom fungus took 18 days for spawn running, 22 days for the pinhead formation and 25 days for the first harvest in paddy straw substrate. The total crop cycle was 45 to 50 days (Anon., 2004).

According to the reports of IIHR (2012), it completed spawn run in 25-30 days in a temperature range of 25-30 ^oC. Pinhead initiation began after 4-7 days of opening of the bags and matured for harvest within 2-3 days. The total cropping cycle of this variety from spawning to harvesting was 37- 42 days.

2.6. COMPARATIVE PERFORMANCE

The elm oyster mushroom is an excellent edible mushroom which can be easily grown either for commercial purpose or for home consumption. It is an excellent source of high quality protein and vitamins (especially Vitamin B). The average biological efficiency of this mushroom was reported to be 60-90 %. The yield, sporophore size, texture and flavour of this mushroom was found to be far superior to the commercial oyster mushrooms *Pleurotus florida* or *P. sajor-caju* presently being grown in India. An additional advantage reported by mushroom growers was its very low spore count (IIHR, 2012). Biswas and Sanjeeb (2013) reported the comparative performance of *H. ulmarius* and *P. florida* in paddy straw substrate. In this, the sporophores produced from *H. ulmarius* weighed an average of 7.98 g where as that of *P. florida* was 6.97 g. Also, spawn run of *H. ulmarius* completed in 15 days and *P. florida* took 20 days for complete spawn run.

Mohapatra and Behera (2013) evaluated the yield performance as well as quality attributes of ten oyster mushroom species and reported the highest biological efficiency of 115.33 per cent for *P. florida* followed by 102.83 per cent for *H. ulmarius*. Maximum weight of sporocarp was recorded by *H. ulmarius* followed by *P. florida*.

Mishra *et al.* (2015) observed bluish grey coloured fruiting bodies which were 13 cm long with a diameter of 8.1 cm in wheat straw substrate. Fruiting bodies of *P. florida* in the same substrate were milky white in colour with a length of 12.5 cm and diameter 6.7 cm. In the same study he compared the yield performance of *H. ulmarius* with nine *Pleurotus* spp. including *P. florida* and he got an yield of 975 g kg⁻¹ substrate from 48 numbers of fruiting bodies with a biological efficiency of 97.5 % for *H. ulmarius* and that of *P. florida* were 531.7 g kg⁻¹ substrate, 96.2 numbers and 53.17 % respectively.

According to Shubhra and Jaitly (2011) *P. florida* completed spawn run in 13 days on wheat straw, produced an average number of 12.67 fruiting bodies having an average weight of 51.34 g and recorded an yield of 650.46 g kg⁻¹ dry substrate whereas *H. ulmarius* yielded 855.52 g kg⁻¹ dry substrate from an average of 18.71 fruiting bodies of 45.73 g.

Senthilmurugan and Krishnamoorthy (2015) reported 12 days for complete spawn run in case of *H. ulmarius* whereas that for *P. florida* was 12.5 days in paddy straw. In the study pinheads of *H. ulmarius* appeared in 22.6 days and the first

harvest was done in 24 days. In case of *P. florida* 22 days were recorded for pinheads formation and 23.5 days were noted for first harvest. Total crop cycle recorded for *H. ulmarius* was 48 days where as that for *P. florida* was 42 days.

2.7. PEST AND DISEASE INCIDENCE

Krishnamoorthy *et al.* (1991) recorded the damage by *Megaselia* sp. to oyster mushroom in Tamil Nadu. *Staphylinus* sp. was observed damaging oyster mushroom in Thiruvananthapuram district in Kerala (Asari *et al.*, 1991). Balakrishnan (1994) also reported the occurrence of sciarid flies and staphylinid beetle as pests of oyster mushroom in Kerala.

The damage caused by springtails was first reported by Gill and Sandhu (1994) in button, oyster and tropical mushrooms. Kumar and Sharma (2001) studied the seasonal abundance of mushroom pests, indicating presence of phorids and sciarids throughout the year in cropping rooms. Another devastating pest of oyster mushroom, *Scaphiosoma nigrofasciatum* was reported for the first time by Deepthi *et al.* (2003).

Chakravarty *et al.* (1982) reported the inhibitory action of carbendazim at 25 ppm against the contaminants of oyster mushrooms. Contamination of *Trichoderma viride* in steam pasteurized straw reduced the mushroom yield which was controlled by using formalin and carbendazim (500 ppm and 75 ppm) for sterilization of straw (Vijay and Sohi, 1987). According to Sharma and Vijay (1996), high incidence of *T. viride* on steam sterilized paddy straw for *Pleurotus* cultivation resulting in 45 per cent yield loss.

Several competitor moulds have been reported occurring in the substrate used for oyster mushroom cultivation and the loss in yield of different *Pleurotus* spp. by these competitor moulds has been reported up to 70 % (Sharma *et al.*, 2007).

2.8. ANALYSIS OF PROXIMATE CONSTITUENTS

The nutritive composition of the mushroom varies with species, strain, type of substrate on which it is grown, the maturity of the fruiting body, the methods of analysis, and the environmental conditions in which it is grown (Beelman *et al.*, 2003).

H. ulmarius is an excellent source of high quality protein and vitamin, especially vitamin B. The yield, flavour, texture and biological efficiency are far superior to *P. florida* and *P. sajor-caju* (IIHR, 2012).

2.8.1. Estimation of Moisture Content

Moisture content of mushrooms ranged from 66.7 to 90.7 per cent (Hung and Nhi, 2012). According to Ragunathan and Swaminathan (2003), the fresh mushrooms contained about 90 % moisture and dry mushrooms contained about 90 per cent dry matter and 10 per cent moisture. Total moisture content of 89.68 per cent was reported by Jatav *et al.* (2012b) for *H. ulmarius* on fresh weight basis.

Rathore and Thakore (2004) reported 89 per cent of moisture content in *P. florida*. Total moisture content of 91.5 per cent was reported in blue oyster mushroom by Shikha *et al.* (2012b). Shivashankar and Premkumari (2014) reported the moisture content of *H. ulmarius* as 10.4 per cent in dried sample.

Ranjini and Padmavathi (2015) reported moisture content of 91.3 % and 90.2 % when elm oyster was cultivated on paddy straw and coconut husk respectively. In a study conducted by Usha and Suguna (2015), moisture content of two strains of *H. ulmarius* was found to vary from 88.3 to 90.6 %. The study conducted by Parisa *et al.* (2015) revealed that *P. florida* contains 91.5 per cent moisture.

2.8.2. Estimation of Carbohydrate

Khanna *et al.* (1992) reported that edible mushrooms are highly valued as a good source of carbohydrates and their contents usually range from 40.6 to 53.3 per cent of dry weight. This variety of oyster mushroom (*H. ulmarius*) contains 52.4 per cent carbohydrates on dry weight basis (Anon., 2004).

Study conducted by Rathore and Thakore (2004) revealed that *P. florida* contains 44.23 per cent carbohydrates. Khan *et al.* (2009) indicated that the carbohydrate content of *H. ulmarius* was 49.9 % which was higher than *Volvariella volvacea* (42 %).

According to Jatav *et al.* (2012b), *H. ulmarius* contained an amount of 52.50 per cent carbohydrate on dry weight basis. Rajeshbabu *et al.* (2012) analysed the proximate constituents in *H. ulmarius* and according to them, it was low in carbohydrate (44.02 % of dry weight) compared to *P. sajor-caju* and *P. ostreatus*.

Total carbohydrates in *H. ulmarius* was 56.1 per cent containing 8.8 per cent total sugars with reducing (4.5 %) and non-reducing (4.3 %) sugars were reported by Shikha *et al.* (2012b). The amount of carbohydrate present in two strains of *H. ulmarius* (*H. ulmarius* CO2 and *H. ulmarius* IIHR Hu 1) was reported by Usha and Suguna (2015) as 28 per cent and 34 per cent respectively. Parisa *et al.* (2015) reported total carbohydrate content of 58 per cent in *P. florida*.

2.8.3. Estimation of Protein

Protein content of mushroom was reported to vary according to genetic structure of species and physical and chemical differences in growing medium (Ragunathan and Swaminathan, 2003). According to Chang and Miles (2004), 60-70 per cent of nitrogen in fungal cell was present as cell protein.

Blue oyster is a new variety of oyster mushroom which contains 23.6 per cent protein on dry weight basis (Anon., 2004). According to Rathore and Thakore (2004) amount of protein present in *P. florida* was 35 per cent. Pushpa and Purushothama (2010) reported that the protein content of *P. florida* was 27.83 per cent.

Shubhra and Jaitly (2011) recorded 17.58 per cent protein content for H. ulmarius and that for P. florida was 20.83 per cent. Jatav *et al.* (2012b) conducted studies on nutritional composition of H. ulmarius fruiting bodies in which the amount of protein was found to be 23.01 per cent on dry weight basis.

According to Rajeshbabu *et al.* (2012) protein content of *H. ulmarius* (24.27 %) was higher than *Agaricus bisporus* (23.9 %) and *Lentinus edodes* (13.4 %). Shikha *et al.* (2012b) estimated crude protein content of 23.2 per cent for blue oyster mushroom using Kjeldahl method. The protein digestibility coefficient of *H. ulmarius* was calculated as 76.7 per cent on 100 g dry weight basis.

H. ulmarius contain large amount of protein (60 mg $100g^{-1}$ dry weight) compared to *P. florida* (Nithiya and Saraswathy, 2014). Mishra *et al.* (2015) analysed ten different species of *Pleurotus* along with *H. ulmarius* and the results showed that the later one has highest amount of protein (33.6 %) compared to all others included in the study.

Usha and Suguna (2015) evaluated two strains of blue oyster mushroom (CO2 and IIHR Hu1) for nutritional composition and results showed that the amount of protein was high in both the strains (47.59 and 54.7 per cent respectively). The amount of crude protein in *P. florida* was estimated as 27 per cent by Parisa *et al.* (2015).

2.8.4. Estimation of Fat

This is a novel variety of oyster mushroom contains 2.2 per cent fat on dry weight basis (Anon., 2004). Rathore and Thakore (2004) reported 2.20 per cent fat content in *P. florida*. The fat content in *H. ulmarius* (0.55 %) was less compared to *P. sajor-caju* (2.0 %) and *P. ostreatus* (2.2 %) (Rajeshbabu *et al.*, 2012).

Nutritional and mineral profile of blue oyster mushroom were studied by Shikha *et al.* (2012b) and reported 4.5 per cent crude fat per 100 g powder with 4 per cent total lipids.

Parisa *et al.* (2015) estimated the fat content of *P. florida* as 1.6 per cent. The fat content reported for two strains of blue oyster mushroom (CO2 and IIHR Hu1) was 3.55 per cent and 4.80 per cent (Usha and Suguna, 2016).

2.8.5. Estimation of Crude Fibre

H. ulmarius, commonly called as blue oyster mushroom contains 12.9 per cent crude fibre on dry weight basis (Anon., 2004). Jatav *et al.* (2012) reported the nutritional composition of blue oyster mushroom and based on the studies, it contained 12.20 per cent crude fibre on dry weight basis.

According to Rajeshbabu *et al.* (2012), in the case of fibre content *H. ulmarius* is superior to *P. sajor-caju* and *P. ostreatus*. Crude fibre content of *P. florida* was estimated as 11.5 per cent (Parisa, *et al.*, 2015).

Rathore and Thakore (2004) reported 9.85 per cent ash content in *P. florida*. The fibre content of dried fruit bodies *H. ulmarius* was reported as 9.16 per cent by Shikha *et al.* (2012b).

Usha and Suguna (2015) analysed crude fibre content of two strains of *H. ulmarius* namely CO2 and IIHR Hu1 and the results obtained were 19.45 per cent and 17.45 per cent respectively.

2.8.6. Estimation of Ash

The ash content in *P. florida* was estimated as 8.72 per cent (Rathore and Thakore, 2004). The studies conducted by Jatav *et al.* (2012b) reported that the ash content present in *H. ulmarius* was 22.05 per cent. Shikha *et al.* (2012b) reported an ash content of 7.1 per cent in blue oyster mushroom which was less compared to *P. florida*, *P. ostreatus* and *P. sajor-caju*.

According to Alam *et al.* (2008), the ash content of fresh mushrooms ranged from 1.1 to 1.28 per cent. Shivashankar and Premkumari (2014) analysed *H. ulmarius* for total ash and the result obtained was 4.31 per cent per 5g of the sample powder. The ash content reported in *P. florida* was 9.3 per cent (Parisa *et al.*, 2015).

Nutrient analysis for two strains of *H. ulmarius* was done by Usha and Suguna (2015) and reported ash content of the two strains was 5.86 per cent and 4.3 per cent.

2.8.7. Estimation of P and K

Limited studies have been conducted in the phosphorous and potassium content of *H. ulmarius*. According to Bano and Rajarathnam (1982), potassium and phosphorous were the main constituents of ash of *Pleurotus* spp.

2.8.7.1. Estimation of P

Bisaria *et al.* (1987) recorded phosphorous content of 1858 mg $100g^{-1}$ dry weight for *P. florida*. The phosphorous content of *H. ulmarius* was found to be 0.68 per cent (Shikha *et al.*, 2012b).

2.8.7.2. Estimation of K

Bisaria *et al.* (1987) reported the potassium content of *P. florida* as 4660 mg $100g^{-1}$ dry weight. According to Shikha *et al.* (2012b), the amount of potassium present in *H. ulmarius* was found to be 1.76 per cent. The fruit bodies were subjected to analysis for different elements in which, the amount of potassium was high compared to other elements such as phosphorous, sulphur, sodium, calcium and iron.

2.9. ORGANOLEPTIC STUDIES

The flavour and taste of blue oyster mushroom have been rated as good to excellent (Anon., 2004). Das (2011) prepared wine from oyster mushroom, milky mushroom and Jew's ear mushroom and reported that the fleshy blue oyster variety (*H. ulmarius*) had been found to be excellent. The wine was of high quality and appealing and delicious to taste.

Kaur *et al.* (2013) carried out works to add value to the bakery products utilizing mushroom along with tomato and curry leaves. According to them, bread prepared after addition of 2 per cent mushroom powder was found most acceptable. Different indigenous products available in the market can be fortified with nutritionally enriched mushrooms to overcome deficiency diseases (Vaidya *et al.*, 2013).

Desai *et al.* (1991) reported poor consumer acceptability of *P. sajor-caju* due to the tough texture of stipe and unattractive colour of pileus but its flavour was good. A comparative study was conducted by Balakrishnan (1994) and reported that *Pleurotus sapidus*, *P. membranaceous* and *P. petaloides* obtained maximum consumer acceptability with respect to colour and flavour. Overall acceptability of these species was significant when compared to *P. sajor-caju* and *P. flabellatus* which were found inferior in all the qualities.

2.10. KEEPING QUALITY

Mehta and Jandaik (1989) reported storage of freshly harvested fruit bodies of *Pleurotus* spp. in non perforated polythene bags up to 72 h at room temperature and at low temperature of 0-5 ⁰C up to 15 days. Under normal conditions, blue oyster mushrooms can be stored up to three days. It experiences minimum damage during packing and transporting when compared to all other oyster mushrooms (Anon., 2004).

According to Kim *et al.* (2006), one effective method to enhance shelf life of mushrooms during postharvest storage and commercialization is modified atmosphere packaging. Mota *et al.* (2006) packed mushrooms in plastic trays over-wrapped with perforated PVC films and stored under refrigerated condition.

The short shelf life of mushrooms is mainly because of their increased respiration, loss of water and fast metabolic activity (Ares *et al.*, 2007). Rai and Arumuganathan (2008) reported that shelf life of mushrooms is less due to its very high respiration rate of about 28.2- 43.6 mg CO₂ per kg fresh weight per hour at 0 $^{\circ}$ C and 280 mg CO₂ per kg fresh weight per hour at 19 $^{\circ}$ C.

Fernandes *et al.* (2012) suggested that gamma, electron beam and UV irradiation are the potential tools in extending the postharvest shelf life of fresh mushrooms like *A. bisporus*, *L. edodes* and *P. ostreatus*. According to Yanjie *et al.* (2013), high oxygen packaging especially with initial 100 per cent oxygen, maintained sensory quality of fresh mushrooms.

Materials and Methods

3. MATERIALS AND METHODS

3.1. ISOLATION AND PURE CULTURING

The pure culture of *H. ulmarius* was isolated from the mushroom beds maintained in the mushroom unit of instructional farm, College of Agriculture, Vellayani using standard tissue culture method (Suharban, 1987) and the original source was Directorate of Mushroom Research, Solan. The pest and disease free, medium aged, healthy mushrooms were thoroughly cleaned and surface sterilized with ethyl alcohol. After splitting longitudinally, a small portion of the tissue from the junction of pileus and stipe was detached using a sterile inoculation needle. The detached tissue was placed in the petri dishes containing sterile solidified potato dextrose agar (PDA) medium aseptically and incubated for a week at room temperature to obtain pure growth of mycelium. These cultures were further purified using hyphal tip method by transferring the hyphal tips of fungal growth aseptically (Rangaswamy and Mahadevan, 2008). Circular discs were cut from the tip portion of mycelia with the help of a sterile cork borer of 5mm size and sub cultured into sterile PDA slants for further studies and preservation.

3.2. MORPHOLOGICAL, CULTURAL AND PHYSIOLOGICAL STUDIES

3.2.1. Morphological Studies

The mushroom was observed for macroscopic characters such as colour and texture of pileus and stipe, nature of gills and microscopic details of hyphae and spore based on the data sheet described in Appendix I.

Spore print was taken by transferring the pileus from medium aged sporocarp on black paper sheets as per the method described by Deepa (2016). The detached pileus was placed upside down black paper with gills facing paper surface. A bell jar was placed over this to maintain humidity. After six hours, the bell jar and pileus were removed and the spore print thus obtained on paper was observed under microscope.

3.2.2. Cultural and Physiological Studies

3.2.2.1. Growth of H. ulmarius in Different Media

To find out the best media for growth of *H. ulmarius*, four different solid media namely Potato Dextrose Agar (PDA) (Ainsworth *et al.*, 1973), Malt Extract Agar (MEA), Oat Meal Agar (OMA) (Booth, 1971) and Carrot Extract Agar (CEA) were used. The compositions of media used are given in Appendix II. The media were prepared and autoclaved at 1.02 kg/ cm² pressure for 20 minutes, allowed to cool and poured into sterile petri dishes under aseptic conditions. The media were then allowed to solidify. A 5mm diameter culture disc from one week old culture was cut out using a cork borer and transferred to the centre of solidified media in the dishes and incubated them at room temperature (28 ± 2 ⁰C) for ten days. For each treatment, four replications were maintained and statistical analysis was carried out using Completely Randomized Design (CRD). The nature of mycelial growth and colony diameter were observed at two days interval.

3.2.2.2. Growth of H. ulmarius in Different Temperature

Effect of different temperature on mycelial growth of *H. ulmarius* was evaluated on PDA medium. Sterile petri dishes were plated with PDA media under aseptic conditions and inoculated with culture discs of 5mm diameter. The inoculated plates were incubated at 20 °C, 25 °C and 30 °C. Four replications were maintained for each treatment and the diameter of mycelial growth, nature of mycelial growth and time taken for complete colonization were recorded. The treatments were statistically analysed using CRD.

3.2.2.3. Growth of H. ulmarius in Different pH

PDA media of pH 5, 6, 7 and 8 were prepared using 0.1 N hydrochloric acid (HCl) or 0.1 N sodium hydroxide (NaOH). Sterilization of media was done in autoclave at 121 ⁰C for 20 minutes and poured in to sterile petri plates of 9 cm diameter after sufficient cooling. Allowed the media to solidify and inoculated with culture discs of 5 mm diameter from seven day old culture. Four replications were maintained for each treatment and the time taken for complete growth as well as

nature of growth were recorded. The treatments were analysed statistically using CRD.

3.2.2.4. Growth of H. ulmarius in Light and Dark Conditions

Sterile molten PDA media was poured into sterile petri plates of 9 cm diameter under aseptic conditions and allowed to solidify. Culture discs of 5mm size were cut out from actively growing culture of *H. ulmarius* and inoculated at the centre of plate containing solidified medium. The plates were incubated in room light and dark conditions. Four replications were maintained for both the treatments and they were statistically analysed using simple t-test. The plates were observed and the time taken for complete mycelial growth and nature of growth were recorded.

3.3. SPAWN PRODUCTION

In order to assess the efficacy of different substrates for spawn production, different grains *viz.*, paddy, wheat, sorghum, ragi and rubber saw dust were tried as per the standard procedure of Sinden (1934) and the treatments were statistically analysed using CRD. The grains were cooked in boiling water until the seed coat just begins to split open. Then the excess water was drained off and the grains were spread on a clear area for drying. Each of this substrate after sufficient drying was mixed with calcium carbonate at the rate of 40 g per kg of grains. These were separately packed in polypropylene bags (12×6 inches) at the rate of 300 g per bag and sterilized by autoclaving at 1.02 kg/cm^2 pressure and $121 \, {}^{0}$ C for 2 h. After cooling, the bags were inoculated aseptically with mycelial bits of equal sizes from 10 days old culture of *H. ulmarius* and incubated at $26 \pm 2 \, {}^{0}$ C for 15 days. The time taken for spawn run, nature of mycelial growth presence of contaminants and keeping quality were observed and recorded. The spawn thus obtained as mother spawn was maintained for further spawn production.

The sawdust spawn was prepared as follows (Deepa, 2016).

Composition

i) Rubber sawdust - 1 kg

ii) Calcium carbonate	- 40 g
iii) Rice bran	- 200 g
iv) Water	- 65 %.

Procedure

After removing the clods and other impurities, fresh rubber sawdust was mixed thoroughly with rice bran and calcium carbonate, by sprinkling water until moisture reaches 65 %. The mixture was then filled in poly propylene bags and sterilized at 121 0 C for 2 hours in an autoclave. Mycelial bits from 10 days old pure culture of *H. ulmarius* was inoculated under aseptic conditions and incubated at 26±2 0 C until complete spawn run occurs. The best substrate for spawn production was selected based on the minimum time required for complete spawn run, nature of mycelial growth and presence of contaminants.

3.4. CULTIVATION

In order to find out the effect of different substrates on mushroom production, studies were conducted using different locally available substrates such as paddy straw, rubber sawdust, banana pseudostem, sugarcane bagasse and neopeat. The statistical analysis was carried out using CRD. Mushroom beds were prepared as per the procedure described by Baskaran *et al.* (1978).

Paddy straw and rubber sawdust were soaked in water containing carbendazim (bavistin) 75 ppm and formalin 500 ppm for 18 hours for sterilization. Then the excess water was drained off and spread it over a silpaulin sheet under sun to reduce the moisture content to 60 %. The beds were prepared by polybag method by using polythene bags of 60×30 cm size. Paddy straw was placed in bag as twists and spawn laid in sides, over which again paddy straw twists were laid and spawning was done. Likewise, four layers were prepared and the upper layer was fully covered with spawn. The bags were made compact, tied at the top and provided with 15 pin holes for air circulation. The same method was used for rubber sawdust also. The bags were then transferred to an incubation chamber having adequate aeration,

temperature and darkness. After complete spawn run, 8-10 one inch slits were put in polybags for the emergence of pinheads.

Banana pseudostem and sugarcane bagasse were prepared by chopping the materials into small pieces and dried properly. Then substrates were sterilized by chemical treatment and beds were prepared following the procedure as stated above. Neopeat blocks were soaked in water first at the rate of 4 litre water per block (650 g), sterilized and beds were prepared as above. One kg substrate and one fifty gram spawn were used for preparing beds. For each treatment, four replications were maintained and the best substrate was selected based on the time taken for complete spawn run, pinhead formation, first flush, total yield, total crop period, average weight of sporocarp, number of sporocarps and biological efficiency.

3.5. DEVELOPMENTAL MORPHOLOGY

Developmental morphology of *H. ulmarius* was studied by observing the developmental changes of sporocarps from pinhead formation upto harvest. The observations recorded include colour, size and shape of the sporocarps and days taken for full bloom stage from pinhead stage.

3.6. COMPARATIVE PERFORMANCE

Comparative performance of H. *ulmarius* and *Pleurotus* florida on the best substrate for spawn and mushroom production was done after selecting the best spawn medium as well as cultivation substrate for H. *ulmarius*. Spawn production was done by inoculating the culture bits of P. florida on the best substrate as per the standard procedure. Likewise, mushroom beds for P. florida were prepared using polybag method similar to that done for H. *ulmarius*. Observations on the time taken for spawn run and nature of mycelial growth were recorded in case of spawn. The time taken for complete spawn run, pinhead formation, first flush, total yield, total crop period, average weight of sporocarp, number of sporocarps and biological efficiency were recorded in case of mushroom production. The results obtained for P. florida and H. *ulmarius* were compared statistically using simple t-test and interpreted.

Beds were laid out in three seasons *i. e.*, February to May, June to September and October to January of 2015-16 in order to find out the effect of different seasons on mushroom production. The weather data in three seasons of 2015-16 is given in Appendix VI. Trials were also carried out at Adimali, Idukki and Puthur vayal, Wayanad in order to study the comparative performance of *H. ulmarius* and *P. florida* in different locations.

3.7. PESTS AND DISEASES INCIDENCE

Pests and diseases incidence were observed during the time of spawn run as well as mushroom production. The beds were monitored daily for their presence and the percent incidence was recorded.

3.8. ANALYSIS OF PROXIMATE CONSTITUENTS

The proximate constituents namely moisture content, carbohydrate, protein, fat, fibre, ash, phosphorous and potassium were estimated for both *H. ulmarius* and *P. florida*.

3.8.1. Estimation of Moisture Content

Two fifty grams (w_1) fresh sample of *H. ulmarius* was dried in an oven until a constant weight was obtained (w_2). Weight of dried sample was noted and the difference between fresh sample weight and dried sample weight gives the result which was converted into percent. The same was repeated for *P. florida* also (Geetha, 1993).

% of moisture content = $\frac{w_1 - w_2}{250} \times 100$

3.8.2. Estimation of Carbohydrate

Estimation of total carbohydrate content was done using anthrone method (Aminoff et al., 1970).

One hundred mg of mushroom powder was weighed in to boiling tube. It was hydrolysed by keeping in a boiling water bath for three hours with 5ml of 2.5N hydrochloric acid. It was cooled to room temperature and neutralised with solid sodium carbonate until the effervescence ceased. The volume was made to hundred ml and centrifuged at 5000 rpm for 10 min. The supernatant was collected and the aliquot was used for analysis. 0.5 ml aliquot was taken from the supernatant and made up to one ml by adding distilled water. Four ml anthrone reagent was added to the solution and heated for eight minutes in a boiling water bath. The solution was cooled rapidly and the absorbance was read at 630 nm in a spectrophotometer.

3.8.3. Estimation of Protein

The standard method described by Bradford (1976) was used for the estimation of protein content in *H. ulmarius* and *P. florida*.

One gram sample was ground in 10 ml of 0.1 M acetate buffer and centrifuged the materials at 5000 rpm for 15 min at 4 ^oC. Further analysis was carried out using the supernatant. Reaction mixture was prepared with 0.5 ml enzyme extract, 0.5 ml distilled water and 5 ml of Coomassie brilliant blue G-250 and used for analysis. The absorbance was read at 595 nm against reagent blank. Standard graphs were prepared using the Bovine serum albumin. Using this graph, the concentration of protein content was estimated.

3.8.4. Estimation of Fat

Estimation of fat was carried out using Soxhlet extraction apparatus (Lees, 1975).

Five g of mushroom powder was taken in a thimble and placed inside the extractor. For the proper distribution of solvent on the sample during extraction, a piece of cotton wool was placed at the top of thimble. Extraction of sample was then carried out with petroleum ether for 16 hours. The extract was transferred into a pre-weighed beaker (w1), cooled in a desiccator and weighed (w2). The percent of fat was determined using the following equation.

% of fat content = $\frac{w^2 - w^1}{5} \times 100$

3.8.5. Estimation of Crude Fibre

Estimation of crude fibre content in *H. ulmarius* and *P. florida* was done by following the steps described by (De, 1965).

Two grams of powdered sample was extracted using petroleum ether to remove the fat content. Then the dried sample was boiled with 200 ml concentrated sulphuric acid (1.25 %) for 30 minutes with bumping chips. The digested sample was filtered through a muslin cloth and washed with boiling water until washings are no longer acidic. The sample was again boiled with 200 ml of sodium hydroxide solution for 30 min. and filtered through muslin cloth and washed with 1.25 % sulphuric acid, three 50 ml portions of water and 25 ml alcohol. The residue was removed and transferred to pre-weighed ashing dish (w1). Dried the residue for two hours at 130 ± 2 °C. It was then cooled in a desiccator and recorded the weight (w2). The residue was further ignited for 30 min. at 600 ± 15 °C, cooled in a desiccator and reweighed.

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

3.8.6. Estimation of Ash

Five gram mushroom powder was transferred to a pre-weighed silica crucible (w1). It was then heated at low flame over a Bunsen burner and when the substrate was charred, transferred the crucible to a muffle furnace. It was then heated to 500 0 C for two h until a white ash was obtained. The weight (w2) was recorded after cooling in a desiccator. Percent ash content was estimated using the following formula (Raghuramulu *et al.*, 1983).

% of ash content = $\frac{w^2 - w^1}{5} \times 100$

3.8.7. Estimation of P and K

Digestion of sample

Digestion of the sample was carried out using kjeldhal's digestion assembly. Weighed 0.5 g of powdered sample in to kjeldhal's distillation flask and added 10 ml of concentrated sulphuric acid containing salicylic acid (1 g salicylic acid in 30 ml concentrated sulphuric acid). The mixture was allowed to stand for overnight. Digestion was started initially on low flame for 10 to 15 minutes until the frothing stops. The digestion was continued the digestion at high flame for 1 to 3 hrs until the liquid became clear which was the indication of complete digestion. The contents were allowed to cool, transferred to a 50 ml volumetric flask through a Whatmann No.1 filter paper and made up the volume to 50 ml.

3.8.7.1. Estimation of P

Working phosphorous standards of 2,4,6,8 and 10 ppm were prepared by pipetting out 2,4,6,8 and 10 ml of 50 ppm stock solution into 50 ml volumetric flasks and made up the volume. Ten ml of digested sample was transferred to a 50 ml volumetric flask and 10 ml of Barton's reagent was added. The volume was made upto 50 ml with distilled water and thoroughly shaken. It was allowed to stand for 30 minutes for full colour development and the intensity of yellow colour was read in a spectrophotometer at 470 nm. The phosphorous concentration was found out with the help of standard curve. A blank was also prepared and read at 470 nm (Sadasivam and Manikam, 1992).

Per cent of phosphorous = X × $\frac{50}{10}$ × $\frac{100}{0.5}$ × $\frac{1}{10,000}$

X - Concentration of phosphorous from the graph

3.8.7.2. Estimation of K

Working standards of 1,2,3,4,5,6,7,8,9 and 10 ppm potassium was prepared from 100 ppm stock solution. Five ml of aliquot was pipetted out into a 50 ml volumetric flask from the digested sample and made up the volume with distilled water. The flame photometer was set up with aspirated working standards and aliquot. The readings were noted down and standard graph was prepared (Sadasivam and Manikam, 1992).

Per cent of potassium = X × $\frac{50}{5}$ × $\frac{100}{0.5}$ × $\frac{1}{10,000}$

X - Concentration of K from the graph

3.9. ORGANOLEPTIC STUDIES

Fresh mushroom was cooked by sauting method and organoleptic characters including colour, appearance, texture, flavour and taste were recorded. Hedonic rating scale was used for the preference study of the cooked sample as per the reference given by Jellinick (1985). The overall acceptability was calculated by taking average of all the five parameters. Appreciation per cent for both the varieties (*H. ulmarius* and *P. florida*) was also calculated using the following formula (Geetha *et al.*, 1995).

Appreciation per cent = $\frac{\text{Sum of individual scores}}{\text{Total score}} \times 100$

Rating scale of 5 point score card for each character and 9 point Hedonic rating scale are given in Appendix III and IV respectively. The recipe and method of preparation is given in Appendix V.

3.10. KEEPING QUALITY

In order to assess the keeping quality of *H. ulmarius* and *P. florida*, both mushrooms of medium maturity were harvested, cleaned and used. Packing was done in perforated polypropylene covers with ten holes of 5mm diameter and one set was stored at room temperature $(26 \pm 2 \ ^{0}C)$ and another set in refrigerated condition (4 ^{0}C). Visual observations on the changes in colour, smell and texture were recorded at 24 h interval.

Results

4. RESULTS

H. ulmarius is a novel species with very large fruiting body having attractive size and shape, high biological efficiency, excellent taste and keeping quality. The name blue oyster is due to the blue coloured pinheads which become light white on maturity. But its variable morphology and biological efficiency needs indepth study and hence the present work was undertaken in College of Agriculture, Vellayani on the physiological, cultural and cultivation aspects of *H. ulmarius*.

4.1. ISOLATION AND PURE CULTURING

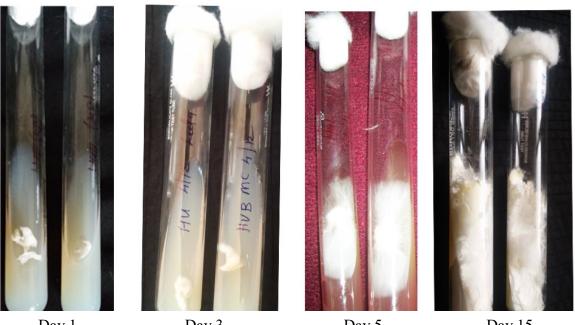
The medium aged, pests and disease free, good quality mushrooms were collected and isolation was done by tissue culturing as per the standard method described under 3.1. Hair like white mycelial growth started after 48 h (Plate 1) from the inoculated bits on PDA medium. The growth was ashy white and branched which completed in 14 to 15 days.

4.2. MORPHOLOGICAL, CULTURAL AND PHYSIOLOGICAL STUDIES

4.2.1. Morphological Studies

Macroscopic studies like colour and texture of pileus and stipe and nature of gills and microscopic studies of hyphae and spore were done.

- **Sporocarps** : Medium to large in size having a dark blue colour (Plate 2.1a) in the pinhead stage, became creamy white on maturity.
- Pileus: 5-15 cm diameter, irregular shape, creamy white on maturity,
convex, depressed towards the base (Plate 2.1b).
- Stipe: 1.5-10 cm long, thick, solid, cylindrical, smooth, eccentric or
lateral, creamy white coloured (Plate 2.1c).



\

Day 1

Day 3

Day 5

Day 15

Plate 1. Isolation by tissue culturing and purification of *H. ulmarius*



Pinheads



Mature Sporocarps Plate 2.1a. Sporocarps of *H. ulmarius*





Plate 2.1c. Stipe of *H. ulmarius*

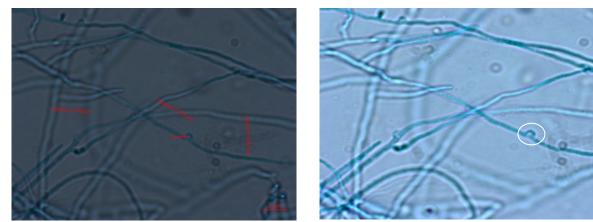




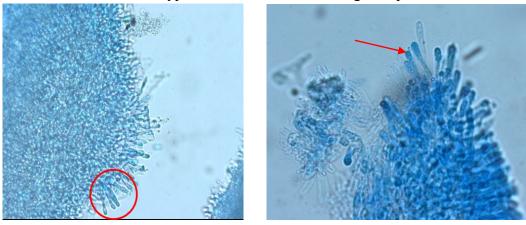
Plate 2.1b. Pileus of H. ulmarius



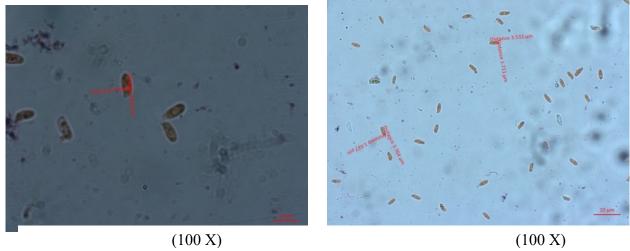
Plate 2.1d. Gills of H. ulmarius



(100 X) (100 X) Plate 2.1e. Hyphae of *H. ulmarius* showing clamp connection



(40 X) (100 X) Plate 2.1f. Basidia of *H. ulmarius*



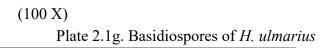




Plate 2.1h. Spore print of H. ulmarius

Gills	: Attached to stem but not running down, not decurrent, creamy		
	white in colour, 14 numbers per cm (Plate 2.1d).		
Hyphae	: Nodose- septate, branched, hyaline, aerial as well as submerged,		
	only generative hypha present, 1.5 to 3 μ m width, clamp		
	connections present (Plate 2.1e)		
Basidium	: Club shaped (Plate 2.1f)		
Cystidia	: Absent		
Basidiospore	: 2.30-3.94 \times 0.77-1.54 μm in size, oval shaped, smooth, hyaline		
	(Plate 2.1g)		
Spore print	: White (Plate 2.1h)		

4.2.2. Cultural and Physiological Studies

4.2.2.1. Growth of H. ulmarius in Different Media

Among the different media tested for the mycelial growth of *H. ulmarius*, potato dextrose agar was found to be the best medium followed by malt extract agar.

The least time for complete mycelial growth was recorded by potato dextrose agar medium (8.75 days) followed by malt extract agar (9.25 days) medium (Table 1). These were followed by oat meal agar (10.5 days) and carrot extract agar (11.5 days) media which were significantly different from each other. The maximum time for complete mycelial growth was taken by carrot extract agar followed by oat meal agar (Plate 2.2a (A)). After 20 days of inoculation, a number of primordia were observed on the potato dextrose agar medium (Plate 2.2a (B)).

On 9th day of inoculation the maximum radial growth was noted for potato dextrose agar (9cm) followed by malt extract agar (8.75 cm) and oat meal agar (8.5 cm) and these three were statistically on par. Carrot extract agar showed the least mycelial growth (7.88 cm) which was significantly different from others. Thick, dense white mycelial growth was observed in PDA medium compared to the sparse growth in malt extract agar. In oat meal agar the growth was faster,

Sl. No.	Media	Time taken for complete	Radial growth on	Nature of mycelial	Colour of mycelia
		mycelial	9 th day	growth	5
		growth (days)*	(cm)*	C	
1.	Potato Dextrose				
	Agar	8.75°	9.00^{a}	+ + + +	White
2.	Malt Extract				White
	Agar	9.25°	8.75 ^a	+ + +	
3.	Oat meal Agar				Milky
		10.5 ^b	8.50^{a}	++	white
4.	Carrot Extract				Greyish
	Agar	11.5 ^a	7.88 ^b	+	white
	CD (0.05)	0.832	0.532		

Table 1. Influence of different media on mycelial growth of H. ulmarius

* Average of four replications Means followed by similar superscripts are not significantly different at 5% level

++++ - Thick and fluffy +++ - Thick and sparse ++ - Thick + - Thin and slow



Plate 2.2a (A). Influence of different media on mycelial growth of H. ulmarius

MEA – Malt Extract Agar

OIVIA – Oat IVICal Agai CEA – Carrot Extract Agar



PDA- Potato Dextrose Agar MEA – Malt Extract Agar OMA – Oat Meal Agar CEA – Carrot Extract Agar

Plate 2.2a (B). Primordial formation of H. ulmarius on PDA medium after 20 days of inoculation

thick and milky white coloured while on carrot extract agar medium the growth was feeble and greyish white mycelia was observed in circular fashion. In carrot extract agar medium, mycelium was not fluffy but the circles were very clear and uniform and up to five circles could be observed on 9^{th} day of inoculation.

4.2.2.2. Growth of H. ulmarius in Different Temperature

A temperature of 25 0 C was found to be the best for mycelial growth of *H. ulmarius* at which complete colonisation was observed within 8 days, followed by 30 0 C (8.8 days)which was on par with the former one (Table 2). The maximum time taken for complete colonisation was at 20 0 C (10.2 days). Thick and fluffy mycelial growth was observed both in 25 0 C and 30 0 C which was denser compared to 20 0 C (Plate 2.2b).

4.2.2.3. Growth of H. ulmarius in Different pH

Among the different pH tested, thick fluffy growth of mycelia was observed in media having pH 8 on 9th day of inoculation which was statistically on par with pH

Figure 1. Influence of different media on mycelial growth of H. ulmarius

the growth was fluffy but less denser compared to pH 8 (Plate 2.2c). The least growth (6.82 cm) was observed on media having pH 5 which was on par with pH 6 (6.87 cm). The mycelial growth was uniform in pH 6 compared to pH 5.

- -

4.2.2.4. Growth of H. ulmarius in Light and Dark Conditions

Among the two conditions tested, dark condition favoured the maximum mycelia growth compared to culture grown under light conditions. Thick and sparse mycelial growth in maximum time (8.9 days) was observed in culture grown under light conditions (Table 4) where as thick and cottony growth in

Sl. No.	Temperature (⁰ C)	Time taken for complete mycelial growth (days) *	Nature of mycelial growth
1.	20	10.20 ^a	++
2.	25	8.00 ^b	+++
3.	30	8.80 ^b	+++
	CD (0.05)	1.095	

Table 2. Influence of different temperature on mycelial growth of H. ulmarius

* Average of five replications Means followed by similar superscripts are not significantly different at 5% level ++++ - Thick and fluffy

++- Thick

Table 3. Influence of different pH on mycelial growth of *H. ulmarius*

-	Table 5. Influence of different pri on influence growth of 11. aumarias			
S1.		Time taken for	Radial growth	Nature of mycelial
No.	pН	complete mycelial	(cm)*	growth
	1	growth (days)*		5
1.	5	8.93ª	6.82 ^b	Thick, not uniform
				and slow
			_	
2.	6	8.88^{a}	6.87 ^b	Thick, uniform and
				slow
				Medium thick,
3.	7	8.38 ^b	8.77 ^a	circular and fast
4.	8	7.78°	8.82^{a}	Thick, fluffy and
				fast
	CD			
	(0.05)	0.485	0.891	

*Average of four replications

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



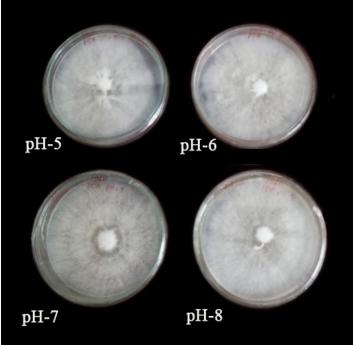


Plate 2.2b. Influence of different temperature on mycelial growth of *H. ulmarius*

Plate 2.2c. Influence of different pH on mycelial growth of *H. ulmarius*

Table 4. Influence of light and dark conditions on mycelial growth of H. ulmarius

Sl. No.	Condition	Time taken for complete mycelial growth (days)*	Nature of growth
1.	Light	8.9	+ + +
2.	Dark	8.4	+ + ++
	t (0.05)	1.56	

*Average of ten replications ++++ - Thick and fluffy +++ - Thick and sparse







Light

Plate 2.2d. Influence of light and dark conditions on mycelial growth of *H. ulmarius*

minimum time (8.4 days) was observed on that grown under dark conditions (Plate 2.2d).

4.3. SPAWN PRODUCTION

Five substrates (Plate 3.1) were evaluated for production of spawn on the basis of time taken for spawn run, nature of mycelial growth and presence of contaminants. The substrates tested were paddy grains, wheat, sorghum, ragi and rubber saw dust. Among these, least time taken to complete the spawn run was recorded by wheat (15.25 days) and paddy grains (15.50 days) and there was no significant difference between these two treatments (Table 5). It was followed by sorghum (16.25 days) and ragi (17.50 days), which were statistically on par. Rubber saw dust differed significantly from all other treatments which recorded maximum time (31.75 days) for completing the spawn run. Thick and fluffy growth of mycelia was observed in paddy grains, wheat and sorghum where as thin and feeble growth was noted for ragi (Plate 3.2). In case of rubber saw dust, very thick mycelium was observed but the growth was very slow.

Presence of contaminants like Trichoderma sp., Aspergillus sp., Penicillium sp., *Rhizopus* sp. and *Bacillus* sp. was recorded in all the substrates (Plate 3.3). However Trichoderma was predominant in paddy grains, Aspergillus sp.in wheat and saw dust and *Rhizopus* sp.and *Penicillium* sp. in ragi. The contamination problem was more in spawn prepared from wheat (40 %) followed by ragi (25 %). The least contamination was recorded in sorghum spawn (10 %) followed by paddy grains (20 %). Spawn prepared from rubber saw dust recorded 15 per cent infection by Aspergillus sp. Maximum keeping quality was observed for spawn prepared from rubber saw dust (104.30 days) followed by paddy grains spawn (87.10 days). The least keeping quality was recorded for wheat spawn (25.65 days) which was on par with sorghum spawn (31.40 days). Spawn prepared from ragi recorded a keeping quality of 58.55 days. Except wheat and sorghum, all



Plate 3.1. Substrates used for spawn production

Sl.	Substrates	Time taken for	Nature of	Keeping quality of	Spawns	Contaminants
No.		spawn run (days)*	mycelial growth	spawn(days)*	contaminated (%)	observed
1.	Paddy grains					<i>Trichoderma</i> spp.,
		15.50 ^c	Thick and fluffy	87.10 ^b	20	<i>Penicillium</i> sp.
2.	Wheat	15.25°	Thick and fluffy	25.65 ^d	40	Aspergillus sp.
3.	Sorghum	16.25 ^{bc}	Thick and fluffy	31.40 ^d	10	Bacillus sp.
4.	Ragi	17.50 ^b	Thin and sparse	58.55°	25	<i>Rhizopus</i> sp., <i>Penicillium</i> sp.
5.	Rubber sawdust	31.75ª	Thick and dense	104.30 ^a	15	Aspergillus sp.
	CD (0.05)	1.835		8.820		

Table 5. Influence of different substrates on spawn production of *H. ulmarius*

*Average of four replications Means followed by similar superscripts are not significantly different at 5% level



Plate 3.2. Influence of different substrates on spawn production of *H. ulmarius*



Aspergillus sp.

Penicillium sp.





Bacterial infection

Plate 3.3. Contaminants observed in different spawn substrates

Trichoderma sp.

other treatments differed significantly each other with respect to the keeping quality of spawn.

Considering the factors like time taken to complete spawn run, nature of mycelial growth, contamination problem and keeping quality, paddy grains was found to be the best substrate for spawn preparation of *H. ulmarius*.

4.4. CULTIVATION

Among the different substrates used (Plate 4.1), paddy straw was found to be the best substrate with respect to time taken for complete colonisation (22.6 days), pinhead formation and total yield. Rubber saw dust took maximum (26.15) days for complete colonisation followed by neopeat which took 25.15 days and these two substrates were statistically on par (Table 6). Paddy straw and banana pseudostem took 22.6 days for complete colonisation and the growth was thick and fluffy. The least time was recorded by sugarcane bagasse to complete spawn run (2 0.7 days). No significant difference was observed for beds prepared from paddy straw and banana pseudostem in case of spawn run. After complete spawn run, the beds were fully covered with white tuft of mycelia (Plate 4.5a & b), later it changed to yellow colour intermingled with white colour. Compared to all other substrates, very thick mycelial mat was observed in rubber saw dust.

As the name indicates, light blue coloured pinheads were formed in beds prepared from different substrates. The maximum time for pinhead formation (60.8 days) was recorded by rubber saw dust even though it yielded well and was significantly different from all other treatments. It was followed by banana pseudostem (47.7 days) and paddy straw (45.9 days) which were statistically on par. The least time for pinhead formation was recorded by sugarcane bagasse (37.35 days) followed by neopeat (40.7 days) and were on par. Harvesting of first flush was done after five days of pinhead formation. Rubber saw dust took maximum time foR harvest (67.25 days) which differed significantly from all other treatments. Banana pseudostem took 52.2 days for first harvest from the date of spawning which was on par with paddy straw (48 days). Sugarcane bagasse recorded the least time (42.4 days) for first harvest and was on par with neopeat (45.75 days) and paddy straw. The total crop period was maximum for rubber saw dust (93 days) followed by neopeat (80 days), which differed significantly each other. The total crop period was less for banana pseudostem (74.25 days) and paddy straw (71.45 days), which were on par. The minimum crop period was recorded for sugarcane bagasse (60.48 days), but beds were heavily infected with *Trichoderma* spp. Eventhough the crop period and spawn run period were minimum for sugarcane bagasse, the yield recorded was very poor mainly due to the contamination by *Trichoderma* spp.

Sporocarps obtained from different substrates differed in their size, weight and number. An average of 5-12 sporocarps was observed in each bunch. Number of sporocarps per bunch was more in paddy straw (27.15) (Plate 4.4) followed by saw dust (18.55) (Plate 4.5). There was no significant difference in the number of sporocarps harvested from banana pseudostem (Plate 4.6) and neopeat (Plate 4.7). Both the size and number of sporocarps (Plate 4.8) were very less in sugarcane bagasse which differed significantly from all other treatments in case of average number of sporocarps (Table7). The maximum yield was recorded by paddy straw (985 g) which was on par with rubber saw dust (905 g). There was no significant difference between sugarcane bagasse (255 g) and neopeat (315 g) in terms of yield. Banana pseudostem differed significantly from all other treatments which recorded an yield of 500 g per kg dry weight of substrate. The maximum biological efficiency was recorded by paddy straw (98.5 %) followed by rubber saw dust (90.5 %). The average weight of sporocarp was more in neopeat compared to banana pseudostem but, the number of sporocarps was less and recorded a biological efficiency of 31.5 per cent. Sugarcane bagasse recorded the least biological efficiency (25.5 %) with less number of sporocarps followed by neopeat (31.5 %). Among the different

substrates used, paddy straw was found to be the best substrate for cultivation of blue oyster mushroom followed by rubber saw dust and banana pseudostem.

Sl. No.	Substrates	Time taken for complete spawn run (days)*	Time taken for pinhead formation (days)*	Time taken for first flush (days)*	Total crop period (days)*
1.	Paddy straw	22.60 ^{bc}	45.90 ^{bc}	48.00 ^{bc}	71.45 ^c
2.	Rubber saw dust	26.15 ^a	60.80 ^a	67.25 ^a	93.00 ^a
3.	Banana pseudostem	22.60 ^{bc}	47.70 ^b	52.20 ^b	74.25 ^{bc}
4.	Sugarcane bagasse	20.70°	37.35 ^d	42.40°	60.48 ^d
5	Neopeat	25.15 ^{ab}	40.70 ^{cd}	45.75°	80.00 ^b
	CD (0.05)	2.719	5.226	6.398	7.663

Table 6. Influence of different substrates on the duration of growth stages of *H. ulmarius*

*Average of four replications Means followed by similar superscripts are not significantly different at 5% level

Sl. No.	Substrates	Average weight of sporocarp (g)*	Number of sporocarps*	Total yield from three harvest (kg kg ⁻¹ dry weight of substrate)*	Biological efficiency (%) (Fresh wt. of sporocarps/ dry wt. of substrate used ×100)
1.	Paddy straw	44.95ª	27.15 ^ª	0.985 ^a	98.5
2.	Rubber sawdust	63.70 ^a	18.55 ^b	0.905 ^a	90.5
3.	Banana pseudostem	37.30 ^b	10.90°	0.500 ^b	50.0
4.	Sugarcane bagasse	36.55 ^b	6.20 ^d	0.255°	25.5
5	Neopeat	39.55 ^b	9.25°	0.315°	31.5
	CD (0.05)	9.344	2.864	0.176	

Table 7. Influence of different substrates on the yield parameters of *H. ulmarius*

*Average of four replications Means followed by similar superscripts are not significantly different at 5% level



Paddy straw

Rubber sawdust

Banana pseudostem



Sugarcane bagasse



Neopeat

Plate 4.1. Substrates used for mushroom production



Plate 4.2. General view of mushroom house







Plate 4.3a. Influence of different substrates on cultivation of *H. ulmarius*





Plate 4.3b. Influence of different substrates on cultivation of *H. ulmarius*



Plate 4.4. Production of sporocarps of *H. ulmarius* on paddy straw



Plate 4.5. Production of sporocarps of *H. ulmarius* on rubber saw dust



Plate 4.6. Production of sporocarps of *H. ulmarius* on banana pseudostem



Plate 4.7. Production of sporocarps of *H. ulmarius* on neopeat



Plate 4.8. Production of sporocarps of *H. ulmarius* on sugarcane bagasse

4.5. DEVELOPMENTAL MORPHOLOGY

The sporocarps took an average of five days from the day of pinhead formation to complete maturity (Table 8). Pileus of the pinheads formed on beds was dark blue coloured and a creamy white stipe which was too short (1.32 cm). Gradually the colour faded and became creamy white in colour on full bloom stage (Plate 5). The stipe length and thickness increased gradually up to third day of pinhead formation. On the third day, stipe became bulged at the centre. Later as the pileus opened fully, the length of stipe reduced. At the time of pinhead formation, the pileus was circular with clear margin which became large and irregular on maturity. Sporocarps were produced either singly or as a bunch. Even within a bunch, the sporocarps differed in their size and morphology. The maturity of sporocarps was greatly influenced by factors such as temperature, humidity and aeration. High temperature and low humidity inside the cultivation room lead to the drying of pinheads on the very next day of formation.

4.6. COMPARATIVE PERFORMANCE

Since *P. florida* is the most acceptable oyster mushroom species and widely cultivating one, comparative studies were conducted with *H. ulmarius* on paddy grains for spawn production and on paddy straw for mushroom production. Paddy grain was found to be the best substrate for spawn production of *H. ulmarius* which completed the spawn run in 18.5 days with thick, white cottony growth with buff coloured mycelial patches (Plate 6.1) whereas, *P. florida* took only 13.5 days for completion of spawn run and uniformly thick, silky white growth was recorded (Table 9). Cultivation trials on paddy straw (Plate 6.2) indicated that *H. ulmarius* completed spawn run in 22.4 days, produced pinheads in 38.1 days and the first harvesting was done in 42.4 days. The total yield recorded for *H. ulmarius* was 1.096 kg kg⁻¹ dry weight of paddy straw whereas that for *P. florida* was 0.976 g. The average number of sporocarps recorded was 26.3 with an average mushroom weight of 41.7 g (Table 10). Compared to *H. ulmarius*, *P. florida* took less time for spawn run (17 days), pinhead formation (23.9 days) and first harvest (26.7 days). *P. florida* yielded more number of

Stages of development	Dimension of pileus (cm)*	Length of stipe (cm)*	Weight of sporophore $(g)^*$	Days after spawning	Morphological characters
Ι	0.42×0.42	1.32	0.37	36	Dark blue coloured pileus with creamy white stipe, pileus circular with clear margin
II	1.3× 1.2	3.86	2.93	37	Light blue coloured, circular pileus, stipe not straight
III	5.4× 4.6	4.11	11.28	38	Stipe bulged at the centre, convex, creamy white coloured pileus with a depression in centre
IV	7.6 imes 6.8	3.66	30.66	39	Irregular margin, reduced stipe, creamy white coloured pileus
V	12.1× 9.5	3.12	44.95	40	Short stipe, irregular margin, fleshy, creamy white coloured pileus

Table 8. Developmental morphology of *H. ulmarius*





Plate 5. Developmental morphology of *H. ulmarius*

spawn pro	spawn production in paddy grains							
Treatments	Time taken for spawn	Nature of mycelial growth						
	run (days)*							
		Thick, white fluffy growth						
H. ulmarius	18.50	with buff coloured patches						
		of mycelia						
		Uniformly thick, silky white						
P. florida	13.5	growth						
t (0.05)	9.48**							

Table 9. Comparative performance of *H. ulmarius* and *P. florida* forspawn production in paddy grains

*Average of ten replications ** Treatments are significantly different at 5% level

Table 10. Comparative performance of *H. ulmarius* and *P. florida* for cultivation in paddy straw

Treatments	TSR*	TPF*	TFF*	TCP*	W*	N*	TY*	BE
H.ulmarius	22.40	38.10	42.40	68.60	41.70	26.20	1.096	109.6
P. florida	17.00	23.90	26.70	46.80	19.94	49.30	0.976	97.6
t (0.05)	4.59**	9.78**	11.54**	10.78^{**}	9.32**	11.99**	6.01**	6.01**

*Average of ten replications

** Treatments are significantly different at 5% level

TSR-Time taken for complete spawn run (days)

TPF- Time taken for pinhead formation (days)

TFF- Time taken for first flush (days)

TCP- Total crop period (days)

W- Weight of sporocarp (g)

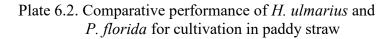
N- Number of sporocarps

TY- Total yield from three harvests (kg)

BE- Biological Efficiency (Fresh wt. of mushroom/ dry wt. of substrate used×100)



Spawn of H. ulmarius on
paddy grainsSpawn of P. florida on paddyPlate 6.1. Comparative performance of H. ul@TâtMas and
P. florida for spawn production in paddy grains



• 7	Seasons (2015-16)							
Variety	February- Ma	ıy	June – September		October – January			
	First harvest (days)*	Total Yield (g kg ⁻¹)*	First harvest (days)*	Total Yield (g kg ⁻¹)*	First harvest (days)*	Total Yield (g kg ⁻¹)*		
H. ulmarius	51.60	152.20	45.00	337.40	37.20	922.20		
P. florida	26.40	700.50	24.40	732.70	23.40	808.40		
t (0.05)	9.31**	13.62**	10.48**	11.50**	5.94**	3.44**		

Table 11. Seasonal variation in production of *H. ulmarius* and *P. florida*

*Average of five replications ** Treatments are significantly different at 5% level

Weather parameters						
Seasons		Out	tdoor		Indoor	
	Temperature (⁰ C)		RH (%)	Rain	Temp.	RH
	Max.	Min.		(mm)	(8 am)	(%)
February– May	32.2	23.94	90.04	644.70	27.16	78.52
June - September	31.45	24.42	90.20	768.10	26.84	81.80
October - January	31.67	23.45	93.53	912.90	22.58	93.83

Table 12. Weather data in three seasons of 2015-16



Plate 6.3. Abnormal sporocarps of *H. ulmarius* produced in unfavourable conditions

Variety	Idukki		Wayanad		Vellayani	
5	(October	- January)	•	(October- January)		- January)
	First	Total	First	Total	First	Total
	harvest	Yield	harvest	Yield	harvest	Yield
	(days)*	$(g kg^{-1})^*$	(days)*	$(g kg^{-1})^*$	$(days)^*$	$(g kg^{-1})^*$
H. ulmarius	27.60	803.80	28.00	816.30	37.80	914.10
P. florida	18.40	736.40	18.80	730.90	24.40	800.60
t (0.05)	6.96**	2.08	6.15**	2.71**	9.25**	4.63**

Table 13. Comparative performance of *H. ulmarius* and *P. florida* in Idukki,Wayanad and Vellayani

*Average of five replication ** Treatments are significantly different at 5% level

sporocarps (49.3) than *H. ulmarius* with an average weight of 19.94g. *H. ulmarius* recorded more yield and higher biological efficiency (109.7 %) than that of *P. florida* (97.6 %) in favourable climatic conditions. Both the mushrooms differed significantly in all the yield parameters observed.

Climatic variations greatly influenced the cultivation of blue oyster mushroom (Table 11). The maximum time taken for first harvest (51.6 days) and very low yield (142g kg ⁻¹ dry weight of paddy straw) were recorded in February-May. When cultivated in June to September, first harvest was done in 45 days after spawning and yield recorded was 337g kg⁻¹ paddy straw. The maximum yield (922g) and minimum time for first harvest (37.2 days) were recorded in October-January. Compared to *H. ulmarius*, the time taken for first harvest and yield were almost stable for *P. florida* throughout the year. The data on weather parameters indicated that that less temperature (22.58 $^{\circ}$ C) and high relative humidity (93.83 %) inside the cropping room in October-January season favoured the maximum production of blue oyster





Plate 6.4a. Comparative performance of *H. ulmarius* and *P. florida* in Idukki



Plate 6.4b. Comparative performance of H. ulmarius and P. florida in Wayanad





Plate 6.4c. Comparative performance of *H. ulmarius* and *P. florida* in Vellayani (October - January)

(Table 12). The sporocarps produced in February-May were smaller in size and funnel shaped with elongated stipe. Multilocational trials conducted in Idukki, Wayanad and Vellayani (Plate 6.4a, 6.4b, 6.4c) revealed that blue oyster can be cultivated throughout the year except April-May in cool climate of Idukki and Wayanad whereas in Vellayani it can be profitably cultivated only in the months of October to January (Table 13). Since there was high temperature and low RH in other two seasons, the beds failed to produce sporocarps or abnormal sporocarps were produced (Plate 6.3) which revealed that blue oyster can be profitably cultivated throughout the year if a temperature of 24 to 26 ^oC and relative humidity more than 90 per cent can be maintained in the mushroom house.

4.7. PESTS AND DISEASES INCIDENCE

Phorid flies (*Megaselia* sp.) were the major problem in the cultivation of blue oyster mushrooms. All the beds prepared from different substrates observed were attacked by phorids (Table 14; Plate 7.1). Neopeat recorded maximum (35 %) attack by different pests like phorid flies, grubs of staphylinid beetles and spring tails followed by rubber saw dust (20 %). Grubs of staphylinid beetles inhabited in gills and made small irregular holes in the hymenium and stipe in initial stages and later on damaged the developing mushrooms. In addition to the common pests, black ants were also observed in rubber saw dust. Paddy straw and banana pseudostem recorded similar infestation (15 %) by pests like phorids, spring tails and staphylinid beetles. No infestation of staphylinid beetles was observed in paddy straw beds.

The competitors observed on different substrates were *Trichoderma* sp., *Coprinus* sp. and *Aspergillus* sp. (Plate 7.2). The maximum per cent infection (80 %) by competitor moulds was recorded in sugarcane bagasse (Table 15), that too only by *Trichoderma* sp. which spread fast uniformly and hindered mushroom production. Initially the infested areas were covered with white mycelia which latter turned to dark green. Incidence was found at the time of spawn run and fruit body formation.

Contamination with *Trichoderma* spp. was noticed in paddy straw and neopeat also, but the per cent infestation was less compared to sugarcane bagasse. About 50 per cent neopeat beds were contaminated with *Trichoderma* sp. and algal mat. The least per cent contamination (20 %) by fungi like *Aspergillus* sp. and *Coprinus* sp. was recorded in rubber saw dust. Paddy straw beds were contaminated by *Trichoderma* sp., *Aspergillus* sp. and *Coprinus* sp. and the contamination recorded as 25per cent. The infection by *Aspergillus* sp. started as black dots which later extended to form scattered patches.

4.8. ANALYSIS OF PROXIMATE CONSTITUENTS

Proximate constituents namely moisture, carbohydrates, protein, fat, fibre and ash were analysed for both *H. ulmarius* and *P. florida* (Table 16; Plate 8). The results indicated high amount of moisture, carbohydrates, protein, fat and fibre in *H. ulmarius* compared to *P. florida*.

Sl. No.	Substrates	Beds infested by pests (%)	Major pests observed	
1.	Paddy straw	15.00	Phorid flies (<i>Megaselia</i> spp.), Springtails (<i>Seira</i> spp.)	
2.	Rubber saw dust	20.00	Phorid flies, Springtails, Staphylinid beetles, black ants	
3.	Banana pseudostem	15.00	Phorid flies, Springtails, Staphylinid beetles	
4.	Sugarcane bagasse	10.00	Phorid flies, Springtails	
5.	Neopeat	35.00	Phorid flies, Springtails, Staphylinid beetles	

Table 14. Incidence of pest infestation in blue oyster mushroom production

Table 15. Incidence of infection by competitor moulds in blue oyster mushroom production

Sl. No.	Substrates	Beds infected by competitor moulds (%)	Competitors observed
1.	Paddy straw	25.00	<i>Trichoderma</i> sp., <i>Aspergillus</i> sp., <i>Coprinus</i> sp., Bacteria
2.	Rubber sawdust	20.00	Aspergillus sp., Coprinus sp.
3.	Banana pseudostem	Nil	Nil
4.	Sugarcane bagasse	80. 00	Trichoderma sp.
5.	Neopeat	50.00	<i>Trichoderma</i> sp., Bacteria, Algal mat



Phorid flies (Megaselia sp.)

Grubs of staphylinid beetles

Plate 7.1. Pests observed on mushrooms



Bacterial contamination on mushroom and bed



Trichoderma sp. on beds

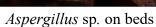


Coprinus sp. on beds



Algal mat on beds

Plate 7.2. Incidence of infection by competitor moulds in blue oyster mushroom production





Sl. No.	Proximate constituents	Percentage (%)		
		H. ulmarius	P. florida	
1.	Moisture ^{\$}	90.37	93.95	
2.	Carbohydrate [#]	29.00	10.50	
3.	Protein [#]	32.00	20.05	
4.	Fat [#]	2.96	1.50	
5.	Fibre [#]	17.69	10.49	
6.	Ash [#]	8.00	10.60	
7.	Phosphorous [#]	0.69	0.68	
8.	Potassium [#]	1.98	2.45	

Table 16: Analysis of proximate constituents

\$ - % in fresh weight basis# - % in dry weight basis



Fresh mushrooms

Dried mushrooms

Mushroom powder

Plate 8. Preparation of sample for analysis of the proximate constituents in H. ulmarius

4.8.1. Estimation of Moisture Content

The moisture content of *H. ulmarius* was found to be 90.37 per cent on fresh weight basis which was high compared to that of *P. florida* (93.95 %).

4.8.2. Estimation of Carbohydrates

Estimation of carbohydrates was done using anthrone method. Less amount of carbohydrates was found in *P. florida* (10.5 %) compared to *H. ulmarius* which recorded 29 per cent carbohydrate on dry weight basis.

4.8.3. Estimation of Protein

Bradford method was used to find out the protein content of dried mushroom. *H. ulmarius* recorded high amount of protein (32 %) on dry weight basis which was almost double compared to *P. florida* (20.05 %).

4.8.4. Estimation of Fat

Soxhlet extraction apparatus was used for the estimation of fat content in mushroom powder. The fat content estimated for *H. ulmarius* was 2.96 per cent on dry weight basis and that of *P. florida* recorded as 1.5 per cent.

4.8.5. Estimation of Crude Fibre

Studies conducted on analysis of crude fibre content revealed that *H. ulmarius* is more fibrous (17.69 %) than *P. florida* (10.49 %) on dry weight basis.

4.8.6. Estimation of Ash

H. ulmarius recorded an ash content of 8 per cent on dry weight basis and that of *P. florida* was 10.6 per cent.

4.8.7. Estimation of P and K

Phosphorous and potassium were estimated for both *H. ulmarius* and *P. florida*.

4.8.7.2. Estimation of P

Amount of phosphorous estimated for both *H. ulmarius* and *P. florida* was almost similar on dry weight basis (0.69 and 0.68 % respectively)

H. ulmarius and P. florida			
Sl. No.	Parameters	H. ulmarius*	P. florida*
1	Appearance	3.8	3.5
2	Colour	3.5	3.4
3	Flavour	3.6	2.8
4	Texture	3.4	3.0
5	Taste	3.8	2.7
	Overall acceptability	3.6	3.0
	Appreciation per cent (sum of individual scores/ maximum score×100)	90.5	77.0

Table 17. Sensory scores (mean values) of recipes developed from

*Average of ten replications (0-5 scores)

 Table 18. Preference (per cent) of recipes developed from *H. ulmarius* and

 P. florida

Particulars	Samples (%)	
	H. ulmarius	P. florida
Like Extremely	30	10
Like Very Much	50	40
Like Moderately	20	30
Like Slightly		20
Neither Like nor Dislike	-	-
Dislike Slightly		
Dislike moderately		
Dislike Very Much		
Dislike Extremely		



H. ulmarius

P. florida

Steam cooked mushroom

Plate 9.1. Sensory evaluation of recipes prepared from *H. ulmarius* and *P. florida*



Sauted mushroom

Plate 9.2. Preference of recipes developed from H. ulmarius and P. florida

4.8.7.3. Estimation of K

P. florida recorded more potassium content (2.45 %) than *H. ulmarius* (1.98 %) based on dry weight analysis.

4.9. ORGANOLEPTIC STUDIES

Among the two mushrooms, *H. ulmarius* recorded higher scores for appearance (3.8), colour (3.5), flavour (3.6), texture (3.4) and taste (3.8) compared to *P. florida* (3.5, 3.4, 2.8, 3.0 and 2.7 respectively). The overall acceptability was more (3.6) for blue oyster than white oyster (3.0). *H. ulmarius* recorded an appreciation per cent of 90.5 whereas that for *P. florida* was 77 per cent (Table 17; Plate 9.1). In the preference study conducted for both the mushrooms, 30 per cent of evaluators extremely liked *H. ulmarius*, 50 per cent liked it very much and 20 per cent liked moderately. Among the evaluators, 10 per cent extremely liked *P. florida*, 40 per cent liked it very much, 30 per cent liked moderately and 20 per cent liked slightly (Table 18; Plate 9.2).

2.10. KEEPING QUALITY

In order to assess the keeping quality, both *H. ulmarius* and *P. florida* were kept in ordinary condition as well as in refrigerated condition. It was noted that mushrooms kept in refrigerated condition had better shelf life compared to that kept in room temperature. *H. ulmarius* packed in perforated polythene bags could be kept fresh for eight hours in normal atmospheric condition whereas *P. florida* could be kept only for six hours. There was no discolouration or bad smell for *H. ulmarius* kept inside perforated polypropylene bags kept in refrigerator up to five days (Table 19; Plate 9.3a) and was good for cooking. After five days of harvesting, 18.16 per cent reduction in fresh weight was recorded for *H. ulmarius* which increased as 35.91 %, 49.48 % and 60.98 % on 10th day, 15th day and 20th day respectively. On 15th day, small fungal outgrowths were seen on the edges of blue oyster which were absent in *P. florida*. In case of *P. florida*, the mushroom kept in refrigerated condition recorded a keeping quality of three days (Table 20).

S1.	Time after	danty of <i>II. umurus</i> in terrigerated cond	Per cent reduction
No.	harvest	Observation	in fresh weight
1	One day	Fresh, white, no smell, no watery appearance	2.05
2	Two days	Fresh, white, no smell, no watery appearance	3.80
3	Three days	Fresh, white, no smell, no watery appearance	6.17
4	Five days	Fresh, white, no smell, no watery appearance	18.16
5	10 days	Off white colour, no smell, no watery appearance	35.91
6	15 days	Creamy white colour, no smell, no watery texture, small pinhead like growth started from edges	49.48
7	20 days	Light yellow colour, slight watery texture, no smell, small outgrowths were more prominent	60.98

Table 19. Keeping quality of *H. ulmarius* in refrigerated condition (4 ⁰C)

Table 20. Keeping	auality of P	. <i>florida</i> in	n refrigerated	condition $(4 {}^{0}C)$

14010 2011			
Sl. No.	Time after harvest	Observation	Per cent reduction in fresh weight
1	One day	Fresh, no yellow colour, no smell, no watery appearance	Nil
2	Two days	Fresh, no yellow colour, no smell, no watery appearance	1.04
3	Three days	Fresh, no yellow colour, no smell, no watery appearance	2.84
4	Five days	Light yellow colour, no smell, slight watery appearance	8.46
5	10 days	Yellow colour, no smell, watery appearance	16.75
6	15 days	Yellow colour , foul smell, watery appearance	26.86



Fresh mushroom (0 day after harvest) 3 days after harvest

5 days after harvest

10 days after harvest



15 days after harvest



20 days after harvest

Plate 9.3a. Keeping quality of H. ulmarius in refrigerated condition



2 days after harvest



Fresh mushroom (0 day after harvest)



3 days after harvest Plate 9.3b. Keeping quality of P. florida in refrigerated condition





5 days after harvest

Discussion

5. DISCUSSION

Hypsizygus ulmarius popularly called blue oyster mushroom is widely cultivated throughout the world especially in Asia and Europe as a temperate mushroom (Mane *et al.*, 2007). Due to the simple and low cost production technology and higher biological efficiency, its cultivation is gaining popularity. Unlike the common oyster mushroom (*Pleurotus* spp.), the production of fruiting bodies of *H. ulmarius* is highly influenced by environmental factors. A little effort was made by Rai (2004) to standardize the cultivation technology in India, but it could not reach commercial level. The present study was mainly focussed on the standardization of its cultivation technology with special emphasis on morphological and physiological aspects.

5.1. ISOLATION AND PURE CULTURING

Isolation of *H. ulmarius* was done by tissue culture method after sterilizing with 99.9 per cent ethyl alcohol. The tissue was selected from the junction of pileus and stipe and hair like mycelial growth started after 48 h of inoculation, in potato dextrose agar medium. Purification was done by hyphal tip method. Singer (1961) developed the pure cultures through tissue culture method from freshly harvested fruit bodies of cultivated mushrooms. Karthika and Murugesan (2015) isolated the culture of *H. ulmarius* on potato dextrose agar medium in petri plates. Similar method was used by Sutha and Eswaran (2016) and they used 80 per cent ethyl alcohol as surface sterilant. The study concluded that swabbing of basidiocarp with ethyl alcohol or sodium hypochlorite as surface sterilant (@ 2 per cent concentration recorded the maximum percentage of tissue germination (98.85 %) without contamination.

5.2. MORPHOLOGICAL, CULTURAL AND PHYSIOLOGICAL STUDIES

5.2.1. Morphological Studies

The studies on macroscopic characters of pileus, stipe and gills revealed that sporocarps were medium to large in size, fleshy and dark blue coloured in pinhead stage and changed to creamy white on maturity. The pileus was convex with irregular margin, depressed towards the base and the stipe was eccentric, smooth, solid and cylindrical in nature. The gills were attached to stem, not decurrent and creamy white coloured. The microscopic studies of hypahe, basidia and spores, assured the presence of septate, branched, hyaline, generative hyphae with clamp connections. Club shaped basidia were present and the spores were oval shaped, hyaline and smooth. The cystidia were absent. These results were almost similar with the morphology of *H. ulmarius* reported by Meyers (2004). Kushwaha *et al.* (2011) observed the morphological characters of *H. ulmarius* and reported that hyphae of blue oyster mushroom is septate, branched, subhyaline to creamy buff in colour, measuring 1-4 μ m in width.

5.2.2. Cultural and Physiological Studies

5.2.2.1. Growth of H. ulmarius in Different Media

To find out the best media for the growth of *H. ulmarius*, different media like potato dextrose agar, malt extract agar, oat meal agar and carrot extract agar were tested. Among these, the maximum mycelial growth in minimum days with thick, cottony mycelial growth was recorded for potato dextrose agar medium followed by malt extract agar (Figure 1). Primordial formation was observed on culture grown in potato dextrose agar medium after 20 days of inoculation. The results obtained were in accordance with Mishra *et al.* (2015) who reported potato dextrose agar as the best medium for cottony growth for *H. ulmarius*. Similar results were observed for different *Pleurotus* spp. by Rafique *et al.* (1999).

Singh *et al.* (2000) observed potato dextrose agar as the best medium for the radial growth of *P. sajor-caju* and *P. sapidus*. Chandravanshi (2007) reported that

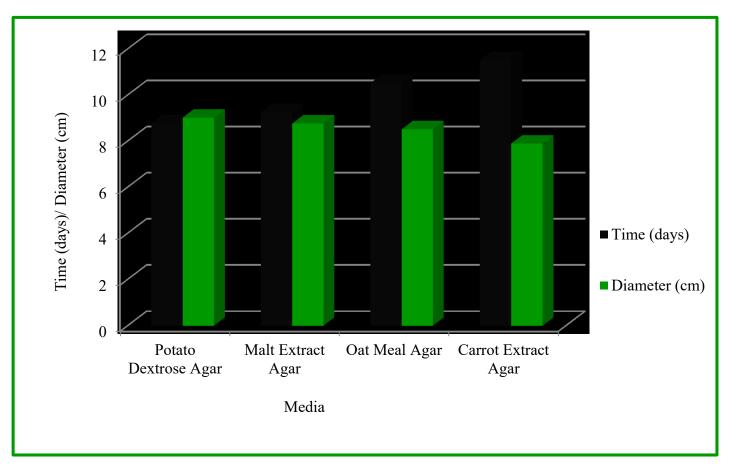


Figure 1. Influence of different media on mycelial growth of *H. ulmarius*

both solid and liquid media with potato dextrose supported maximum radial growth and biomass of *H. ulmarius*. In similar studies conducted by Kushwaha *et al.* (2011), the results showed that malt extract agar and wheat extract agar media supported maximum mycelial growth followed by potato dextrose agar medium. Sharma and Jandaik (1984) reported better mycelial growth of *Pleurotus eryngii* on potato dextrose agar medium.

5.2.2.2. Growth of H. ulmarius in Different Temperature

Out of the three temperatures tested in potato dextrose agar medium, 25 0 C was found to be the best temperature for the growth of *H. ulmarius*, followed by 30 0 C (Figure 2). Thick cottony mycelial growth was observed in 25 0 C. According to Chang and Miles (2004), high temperatures inactivate the enzymes with a resulting effect on metabolism and consequently on growth. Similar results were obtained in the studies of Wange and Patil (2007) and Kushwaha *et al.* (2011).

Jandaik and Kapoor (1975) reported the maximum mycelial growth at 25 0 C for *Pleurotus* spp. and Lomberh *et al.* (2000) reported the same for *H. marmoreus*. The optimum temperature for the mycelial growth of *Lentinula edodes* was 20-25 0 C as observed by Song *et al.* (1987); Lata and Sharma (2012) and Sharma *et al.* (2013b). Lishma (2015) reported the maximum mycelial growth for *Agaricus* spp. at 25 0 C.

Jatav *et al.* (2012a) observed the maximum mycelial growth of *H. ulmarius* at 25 ± 1 ⁰C while the least growth was observed at 20 ⁰C and 35 ± 1 ⁰C temperatures. Sutha and Eswaran (2016) reported that a temperature of 25-30 ⁰C was found optimum for *H. ulmarius* as it favoured the maximum growth of the fungus and biomass production on 10th day after inoculation. The growth decreased as the temperature increased or decreased beyond 20-30 ⁰C. Optimum temperature reported for maximum mycelial growth of *Auricularia polytricha* was 20-34 ⁰C (Kurtzman, 1979).

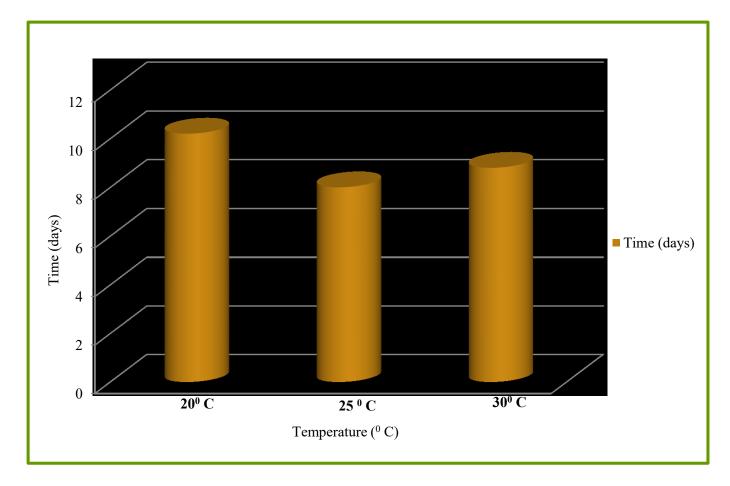


Figure 2. Influence of different temperature on mycelial growth of *H. ulmarius*

5.2.2.3. Growth of H. ulmarius in Different pH

Four different hydrogen ion concentrations like 5, 6, 7 and 8 were evaluated to find out the best pH for the growth of mycelia in which, slightly alkaline pH 8 gave the maximum mycelial growth for *H. ulmarius* followed by pH 7 (Figure 3). Acidic pH decreased the mycelial growth. Usually fungal growth occurs in pH range from 4 to 8, which influences the metabolic activities and thereby the ability to utilize certain substances (Chang and Miles, 2004). These results were in accordance with Chandravanshi (2007) who reported a pH 8 for superior growth and biomass production for *H. ulmarius*. Prathibha (2013) reported a pH 8 for the maximum mycelial growth of *Tricholoma giganteum*.

The maximum mycelial growth of *H. ulmarius* was obtained at pH 7 (Kushawaha *et al.*, 2011). Similar results were also given by Sutha and Eswaran (2016). According to Wange and Patil (2007), *H. ulmarius* could grow in a pH ranging from 4 to 8 and optimum growth was recorded in pH 6.

5.2.2.4. Growth of H. ulmarius in Light and Dark Conditions

Dark and light conditions were provided for the growth of *H. ulmarius* in potato dextrose agar medium and the result concluded that dark condition favoured the growth of *H. ulmarius* compared to light conditions. This was confirming the reports given by Chandravanshi (2007) and Jatav *et al.* (2012a) who observed that darkness favoured the growth of *H. ulmarius*. Deepa (2016) reported the maximum radial growth and biomass production for *L. edodes* in darkness. Chang and Miles (2004) reported that strong light inhibited or even killed the fungus. Present study indicated that the fungus can grow in both light and dark conditions, but faster, thick mycelial growth was observed in dark conditions than in presence of light.

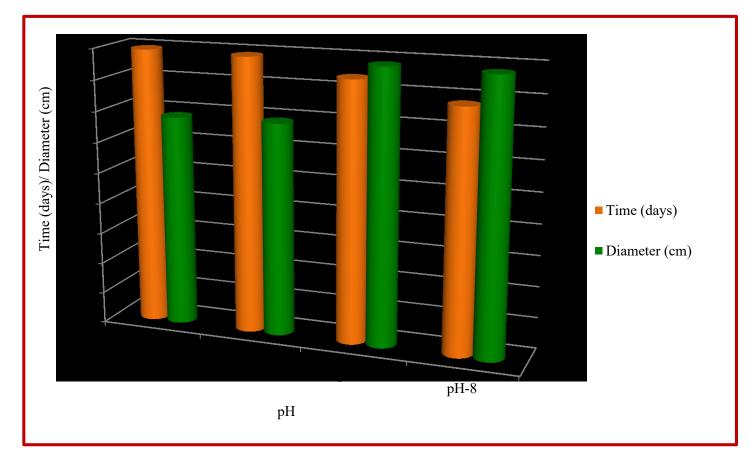


Figure 3. Influence of different pH on mycelial growth of *H. ulmarius*

5.3. SPAWN PRODUCTION

The success or failure of mushroom cultivation depends on the availability of good quality spawn. The yield and quality of spawn of the cultivated mushroom is governed mainly by the genetic makeup of the strain and technology used for spawn production which include the nature of substrates also (Kumar, 1995).

In order to find out the best substrate for spawn production, five different substrates like paddy grains, wheat, sorghum, ragi and rubber saw dust were evaluated after adding calcium carbonate (40 g kg⁻¹ grains). The quality was assessed in terms of time taken for spawn run, nature of mycelial growth, presence of contaminants and keeping quality (Figure 4). Paddy grains was found to be the best media for preparation of spawn as it recorded the minimum time for complete spawn run (15.50 days), less contaminants (20 per cent) and good keeping quality (87.10 days). Ashulata (2007) reported that paddy, sorghum and wheat grains took least time for spawn development in *H. ulmarius*. She also reported a shelf life of 24.75 to 29.75 days for *H. ulmarius* spawn prepared in cereal grains.

Contradictory results were also obtained by Chandravanshi (2007) who observed that maize grains were the best substrate for spawn production of *H. ulmarius*. Lishma (2015) reported paddy grains as the best substrate for spawn production of *Agaricus* spp. which completely covered the substrate within 12 days of inoculation. Sharma and Kumar (2011) reported that wheat is the most widely used grain for spawn production all over the world.

Stoller (1962) added calcium carbonate to prevent the sticking of grains and to adjust the pH. Gayatri *et al.* (2004) reported that addition of calcium carbonate in spawn grains not only helped in vegetative growth but also absorbed excess moisture, helped in pH adjustment and prevented clump formation. Pandey *et al.* (2000) observed that addition of calcium did not effect the spawn but it helped in breaking of lumps.

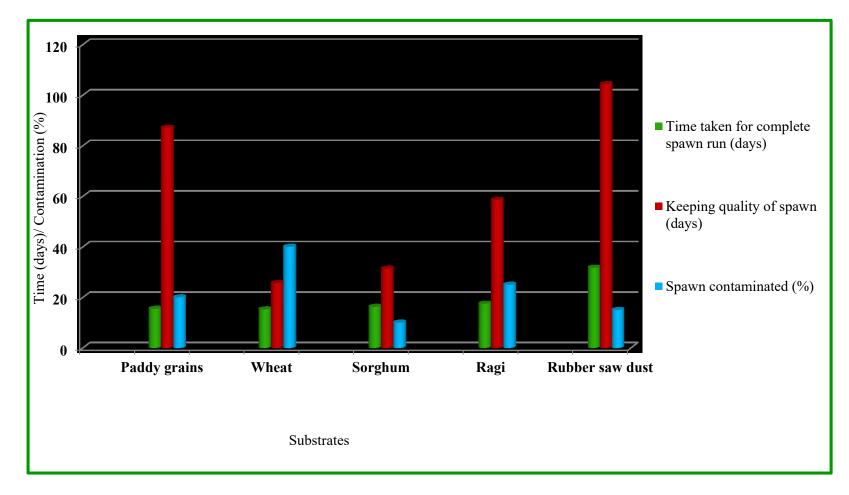


Figure 4. Influence of different substrates on spawn production of H. ulmarius

Based on the studies conducted by Sharma *et al.* (2013a), an increase in amount of calcium carbonate in wheat grains resulted in increased pH, enhanced mycelial growth and improved quality of *Agaricus bisporus* spawn and the quality improvement of spawn may be due to the increased availability of Ca^{2+} ions, favourable pH and micro climate in the substrate.

The mycelial growth in rubber saw dust was very thick than in any other substrates but it took maximum time for complete spawn run (31.75 days). This is contradictory to the findings of Smith *et al.* (1987) and Bhandal and Mehta (1989) who reported saw dust as the best substrate for *Auricularia polytricha*. Sorghum, wheat and paddy grains were reported as good quality substrates for *Pleurotus* spawn production (Mathew *et al.*, 1996).

Sharma and Kumar (2011) reported contamination of *Agaricus bisporus* spawn predominantly by *Bacillus* sp., *Alternaria* sp., *Penicillium* sp., *Trichoderma* sp. and *Aspergillus* sp. According to them, the introduction of contamination into the spawn was mainly from raw grains. This is in accordance with the present study.

5.4. CULTIVATION

Nilanjana *et al.* (2001) reported that the yield of mushroom depends on the quality of substrate, cultural practices, genetic makeup of the strain used and the environmental conditions during cultivation. Availability of nutrients from the substrate is very important for getting maximum yield.

Five substrates namely paddy straw, rubber saw dust, banana pseudostem, sugarcane bagasse and neopeat were tested for the cultivation of *H. ulmarius* after chemical sterilization with formalin (500 ppm) and carbendazim (75 ppm). Similar study was conducted by Vijay and Sohi (1987) and reported chemical pasteurisation method using formalin 500 ppm and bavistin (75 ppm) in substrate soaking water (18 h soaking) as the most effective method of sterilization of substrates.

Balakrishnan and Nair (1995) reported polybag method as the best method for the production of *Pleurotus* spp. Among the different substrates evaluated for mushroom cultivation, paddy straw was found to be the best for cultivation of H. *ulmarius* (Figure 5). This may be due to the better availability of nitrogen, carbon and minerals from this substrate (Shah *et al.*, 2004). Similar findings were given by Hanscha *et al.* (2000) who reported that *H. ulmarius* can be easily cultivated on various common natural substrates like paddy straw. Deepika and Sharma (2005) reported rice straw as the best substrate for the cultivation of *Pleurotus* spp. in terms of yield and biological efficiency.

Paddy straw being a rich source of cellulose favours mycelial growth and fruitbody production. It contains 37 % cellulose, 24 % hemicellulose and 14 % lignin abundant biomass and the yield of mushroom is proportional to the amount of cellulose (Nguyen, 2004). The superiority of paddy straw over the other substrates was also reported earlier by Pal and Thapa (1979) and Bano *et al.* (1978). This is in accordance with the findings of Sivaprakasam and Kandaswamy (1981) who reported a positive correlation of sporophore yield to the cellulose content and cellulose – lignin ratio. Superiority of paddy straw over other substrates for the cultivation of *Pleurotus sajor-caju* was reported by Desai (1982) and Bisaria *et al.* (1987).

In the present study, spawn run in paddy straw and banana pseudostem was completed in 22.6 days and first harvesting was done in 48 days. This is in accordance with the findings of Daniel et al. (1991) who reported 28 days for spawn run in banana pseudostem for P. sajor-caju. Biswas and Sanjeeb (2013) reported a spawn run period of 15 days for *H. ulmarius* in paddy straw. Cultivation trials on *H.* ulmarius were conducted by Ashulata (2007) in which first harvesting was recorded by paddy straw beds in 35.8 days. Rao (1991) reported a biological efficiency of 46 per cent in chopped banana pseudostem for P. florida which is in accordance with the present study (Figure 6). Suharban et al. (1996) tried pseudostems of different banana varieties for cultivation of P. florida and reported highest biological efficiency in red pseudostem followed rasakadali. banana by

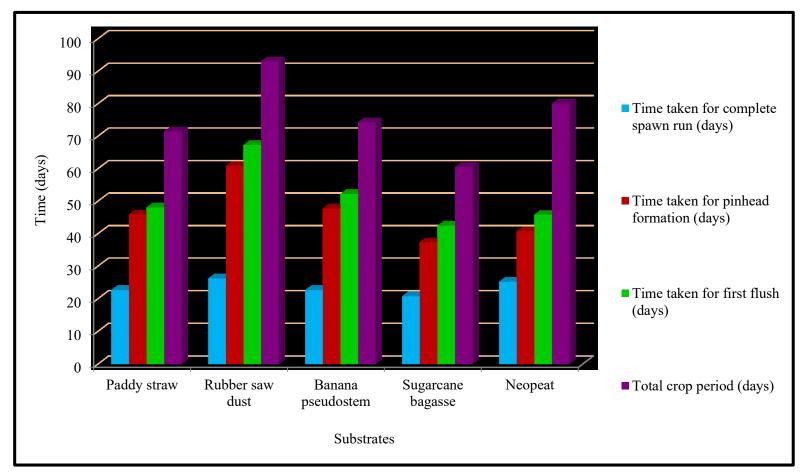


Figure 5. Influence of different substrates on the duration of growth stages of H. ulmarius

Studies on the cultivation of blue oyster mushroom were conducted in different seasons throughout the year and the maximum yield and biological efficiency were observed during the months of October to January. Chandravanshi (2007) cultivated *H. ulmarius* on paddy straw from October to May and reported that significantly earlier spawn run and higher yield was observed during the month of January. This might be due to the climatic conditions such as low temperature and high relative humidity which favoured the production of fruiting bodies of *H. ulmarius*.

Successful cultivation of *H. ulmarius* on paddy straw was reported by Ranjini and Padmavathi (2013). They reported a suitable temperature of 23-25 0 C and relative humidity of 95-98 per cent for spawn run and harvested first flush in three to four weeks after spawning. Studies were conducted on the influence of environmental factors in the production of *Pleurotus* spp. Rangad and Jandaik (1977) recorded the maximum mycelial growth of *P. florida* at 30 0 C. Bano and Rajaratnam (1982) recorded maximum yield for *Pleurotus* spp. from Mysore during rainy seasons, when the temperature was 20-26 0 C and relative humidity 70-90 per cent.

In the present study, rubber saw dust also favoured good mushroom production with 90.5 per cent biological efficiency, but more time was taken for production of fruiting bodies. The superiority of saw dust was reported earlier by many workers. The nutrient status of rubber sawdust is higher than other sawdust. It contains 1.68 % N, 0.48 % P, 1.18 % K, 0.12 % Ca and 0.04 % Mg. This sawdust has uniform size and structure which facilitates enrichment of substrate. Rubber sawdust is recognized to be very suitable for cultivation of *Pleurotus* spp., *Auricularia* spp., *Lentinula* spp. and *Ganoderma* spp. (Nguyen, 2004). Mathew *et al.* (1991) also reported the suitability of rubber sawdust for oyster mushroom cultivation.

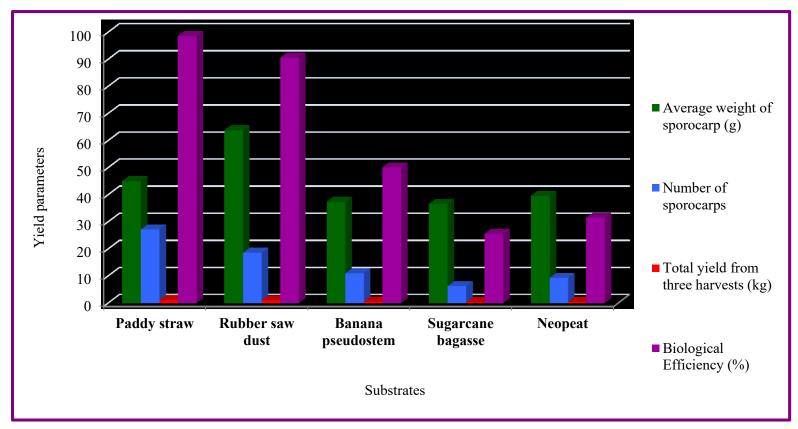


Figure 6. Influence of different substrates on the yield parameters of H. ulmarius

The present study recorded a biological efficiency of 98.5 per cent for *H. ulmarius* in paddy straw. Contradictory results such as 60 - 80 per cent biological efficiency was reported from IIHR (2012). Biswas and Sanjeeb (2013) reported a biological efficiency of 156 per cent for *H. ulmarius* in paddy straw. The lowest yield was recorded from beds prepared on sugarcane bagasse. This is in accordance with the findings of Karnawadi (2006) who reported the lowest yield of *Pleurotus* spp. in beds made from sugarcane bagasse. Sugarcane bagasse contains 45-55 % cellulose, 20-25 % hemicelluloses and 18-24 % lignin and is a rich source of xylan which favours the growth of competitive saprophytic fungi (Nguyen, 2004). The lowest productivity of oyster mushroom in sugarcane trash was also reported by Patil and Jadhav (1991).

Neopeat is the registered trade name of cocopeat. Cocopeat is the byproduct obtained after processing coconut husk and removing the long fibres and has a pH of 5.7 to 6.5. The cocopeat can hold large quantities of water, just like a sponge and is rich in cellulose and lignin (Mason, 2003). Sherin (2003) reported that coirpith did not support sporophore production of both *Pleurotus florida* and *Calocybe indica*. This is in accordance with the present study. The low yield in coirpith is due to the poor ability of the fungus to degrade and utilize hard lignocellulosic fibre.

5.5. DEVELOPMENTAL MORPHOLOGY

The mushroom development can be divided two stages; the vegetative and the reproductive stage. The transition from vegetative to reproductive stage is principally controlled by environmental conditions *viz.*, light, CO₂ concentration, temperature and humidity.

The present study indicated that the pinheads of *H. ulmarius* took five days for attaining full maturity. Similar findings were given for *Pleurotus* spp. by Bahukhandi and Mundal (1989). They reported that the pinheads of oyster mushroom started appearing after five days of complete spawn run and harvesting could be done in another five days. The ashy blue colour in pileus faded gradually and became creamy white on maturity. This was in accordance with previous reports (Anon., 2004).

The pinheads were very small with circular pileus and cylindrical stipe at first which later changed both the size as well as morphology. Large sporocarps with irregular pileus and reduced stem were produced either singly or in cluster. Prathibha (2013) reported that 5.75 to 6.75 days were needed for *Tricholoma giganteum* from pinhead formation to maturity. From the reports given by IIHR (2012), it was clear that blue oyster took 3 days for maturity from pinhead formation. This is contradictory to the present result.

5.6. COMPARATIVE PERFORMANCE

Since paddy grains and paddy straw were found to be the best substrates respectively for spawn production and cultivation of blue oyster, comparative studies were made with *P. florida* on these substrates. The results indicated that *H. ulmarius*

took more time for spawn run in paddy grains. In cultivation trials on paddy straw, *H. ulmarius* completed spawn run in 22.4 days and the average weight of sporocarp recorded was 41.7 g. The average weight of sporocarp recorded for *P. florida* was 19.94 g and it completed spawn run in 17 days (Figure 7a &b).

According to the reports given by Chang *et al.* (1981), paddy straw was the best substrate for *Pleurotus* spp. Biswas and Sanjeeb (2013) reported fifteen days and twenty days for complete spawn run in case of *H. ulmarius* and *P. florida* respectively. The average weight of sporocarp recorded was 6.97 g for *P. florida* and 7.98 g for *H. ulmarius*. In the present study, the weight of *H. ulmarius* sprocarps was very high and much more than that of *P. florida*.

H. ulmarius took 38.1 days for pinhead formation in paddy straw which was more compared to *P. florida* (23.9 days). Senthilmurugan and Krishnamoorthy (2015) reported 22.6 days for the pinhead formation of *H. ulmarius* and 22 days for *P. florida*.

Present study recorded an yield of 1.096 kg kg⁻¹ dry weight of paddy straw with a biological efficiency of 109.6 % for *H. ulmarius* and 0.976 g with 97.5 % biological efficiency for *P. florida*. This is almost in accordance with the report of Mishra *et al.* (2015), with an yield of 975 g kg⁻¹ of wheat straw for *H. ulmarius*. They also reported biological efficiency of 97.5 % for *H. ulmarius* and 53.17 % for *P. florida*. Mohapatra and Behera (2013) reported the highest biological efficiency of 115.33 per cent for *P. florida* followed by 102.83 per cent for *H. ulmarius*.

Multilocational trials conducted in Idukki, Wayanad and Vellayani revealed that blue oyster can be cultivated throughout the year except April-May in cool climate of Idukki and Wayanad . Environmental factors highly influenced the production of sporocarps. Maximum production of *H. ulmarius* was recorded in October-January season (Figure 8, 9 (A&B) and 10). This is in conformity with the reports of Chandravanshi (2007) who reported earlier spawn run and higher yield during January.

5.7. PEST AND DISEASE INCIDENCE

There are opportunities for the organisms to enter and grow in the substrate, or on the mycelium, or on developing sporocarp at various stages of mushroom production such as spawning, spawn running and mushroom development and these occurrences ultimately cause diseases (Chang and Miles, 2004).

Phorid flies (*Megaselia* spp.), staphylinid beetles and springtails were the major pests observed during the cultivation of blue oyster. Similar findings were made by Deepthi *et al.* (2003) and they reported these as common pests of oyster mushroom in Kerala. Pest infestation was noticed during all the stages of mushroom production from spawn run period to sporocarp formation. This is in accordance with the report of Krishnamoorthy *et al.* (1991) who stated severe damage of oyster mushroom beds in Tamil Nadu due to the attack of *Megaselia* sp. The maggots of *Megaselia* sp. were reported to feed on the mycelium in beds during spawn run period.

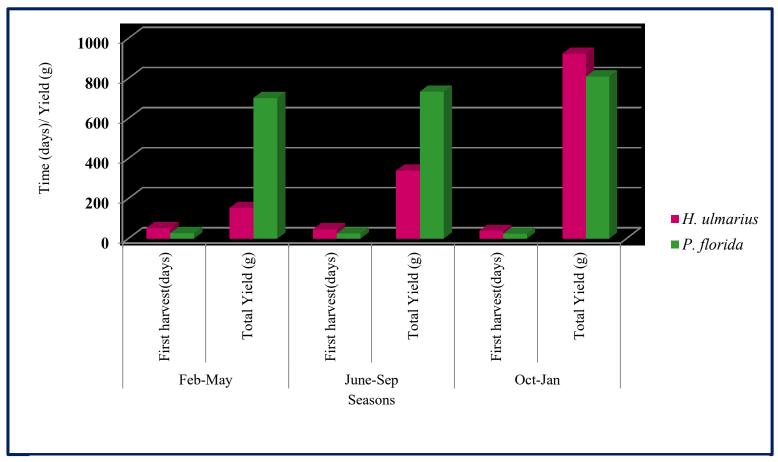


Figure 8. Seasonal variation in production of H. ulmarius and P. florida

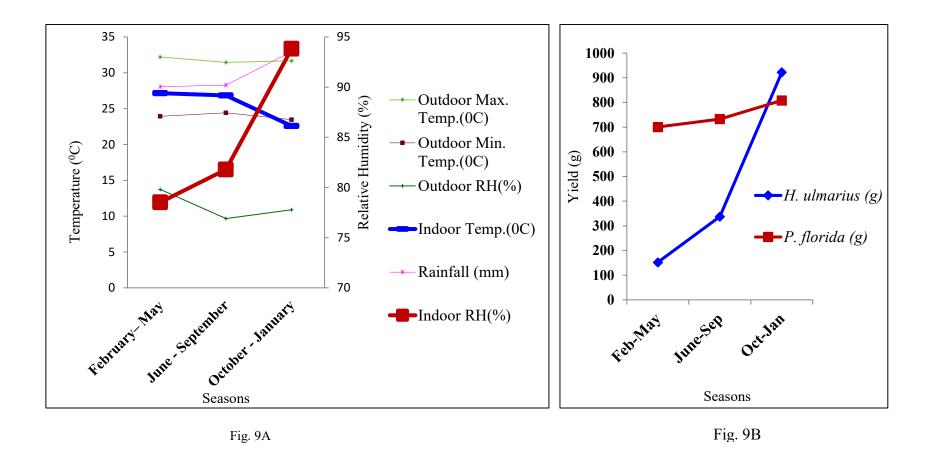


Figure 9 (A &B). Seasonal variation in production of *H. ulmarius* and *P. florida*

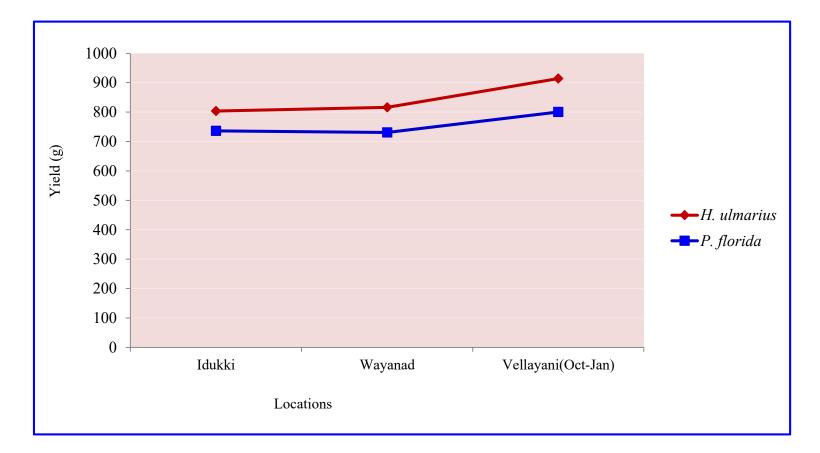


Figure 10. Comparative yield performance of *H. ulmarius* and *P. florida* in Idukki, Wayanad and Vellayani

Present study recorded an yield of 1.096 kg kg⁻¹ dry weight of paddy straw with a biological efficiency of 109.6 % for *H. ulmarius* and 0.976 g with 97.5 % biological efficiency for *P. florida*. This is almost in accordance with the report of Mishra *et al.* (2015), with an yield of 975 g kg⁻¹ of wheat straw for *H. ulmarius*. They also reported biological efficiency of 97.5 % for *H. ulmarius* and 53.17 % for *P. florida*. Mohapatra and Behera (2013) reported the highest biological efficiency of 115.33 per cent for *P. florida* followed by 102.83 per cent for *H. ulmarius*.

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The present study recorded early and severe infestation of phorid flies in the beds prepared from substrates *viz.*, paddy straw, rubber saw dust and neopeat. This is in accordance with the findings of Deepthi (2003) who reported the decaying of oyster mushroom beds due to severe attack of sciarids and phorids. The intensity of attack was severe in the months of October to January when the temperature inside the cropping room was about 22 ⁰C and relative humidity 93 per cent. This may be the reason for heavy infestation of phorids in cropping period based on the findings given by Kumar and Sharma (1999). They reported that phorid flies were observed in the temperature ranging from 18.5 ^oC to 25 ^oC. Heavy infestation of phorids in beds was controlled by spraying 2 per cent neem oil.

The grubs of staphylinid beetles inhabiting between the gills made irregular holes through continuous feeding. The infestation was noticed in younger sporocarps. Occurence of staphylinid beetles on mushroom beds was also reported by Asari *et al.* (1991) and Balakrishnan (1994).

As people became more interested in mushroom cultivation and started growing mushrooms, the problems of associated microflora infecting mushroom beds were also increased. Water and oxygen availability is not a problem in mushroom production room which help the fungi to produce their asexual spores in countless qualities. Some fungi used the mycelium and sporocarps of the mushroom as food, often leading to the detriment of the mushroom (Chang and Miles, 2004). Several competitor moulds have been reported in oyster mushroom cultivation which vary in number and types due to the use of variety of substrates, different methods of substrate preparation and conditions and containers used for cultivation (Sharma, 1995).

Trichoderma sp., Aspergillus sp. and Coprinus sp. were predominant competitors in the cultivation of blue oyster. Das and Suharban (1991) reported Aspergillus niger, A. flavus, A. fumigatus, Penicillium sp., Rhizopus stolonifer, Trichoderma viride etc. in oyster mushroom beds. Presence of Trichoderma spp., Aspergillus sp. and Coprinus sp. in oyster mushroom beds was also reported by Deepthi (2003).

Trichoderma was the most serious competitor during the cultivation of *H. ulmarius* and it was noted in the later stages of infection as the infected sites turned dark green colour due to sporulation. Similar findings were given by Das *et al.* (1993); Thapa and Sharma (1994) and Balakrishnan (1994). They reported that the most severe problem in oyster mushroom cultivation was infection of *Trichoderma* spp. High relative humidity and dead mushroom tissues encouraged infection (Sohi, 2004). In the present study, infection with *Trichoderma* spp. was severe in beds prepared from sugarcane bagasse and that may be the main reason for the reduced yield in sugarcane bagasse.

Presence of *Coprinus* sp. was also noticed in spawn run period of *H. ulmarius*. Kaul *et al.* (1978) reported the appearance of ink caps during spawn run in mushroom beds. Similar findings were reported by Garcha (1984) and Sohi (1986). Gupta and Raina (2008) reported infection of oyster mushroom beds by *Trichoderma* spp. (15-18 %) followed by *Penicillium* (12-14 %) and *Coprinus* spp. (7-9 %) during spawn run period.

Bacteria can also behave as pathogens in mushroom cultivation. Bacterial rot has been reported by Biswas *et al.* (1983) on *Pleurotus sajor-caju* as water soaked areas and yellow brown discolouration of sporocarps. Similar symptoms were observed in the present study also. Gill and Tsuneda (1997) reported bacterial soft rot in *Agaricus bitorquis, Lentinula, Pleurotus, Flammulina* and *Hypsizygus*.

5.8. ANALYSIS OF PROXIMATE CONSTITUENTS

The fast growing mushrooms have gained a remarkable amount of interest recently with the realization that they are good source of delicious food with appreciable amount of nutritional attributes. Determination of nutritional value needs analysis of proximate constituents. Chang and Miles (2004) reported that composition of a mushroom was affected by diversity in genetic makeup, environmental conditions and nature of the substratum. It was also influenced by the stage of development and the manner of postharvest storage.

5.8.1. Estimation of Moisture Content

Moisture content considerably affects the nutritional value of any food item. It is the most variable component in mushrooms and is influenced by genotype, stage of growth, environmental conditions and postharvest storage before analysis (Rai, 1995). They also observed that fruit bodies collected from an atmosphere with high humidity showed high moisture content and vice-versa.

The present study revealed that *H. ulmarius* contains 90.37 per cent on fresh weight basis which was lower compared to that of *P. florida* (93.95 %). The results were comparable with that of Rathore and Thakore (2004) who reported 89 per cent moisture content in *P. florida*. Shikha *et al.* (2012b) reported 91.5 % moisture in *H. ulmarius*. Rai and Sohi (1988) reported 90.2 per cent moisture on fresh weight basis in *P. sajor-caju* and 90.1 % in *Agaricus bisporus* and *Volvariella volvacea*.

5.8.2. Estimation of Carbohydrate

Carbohydrates of the mushrooms are not nutritionally important so far as calories are concerned. But recently much interest has been noticed in the characterization of components in water-soluble polysaccharides obtained from mushrooms due to their ability to inhibit tumour growth (Chang and Miles, 2004).

Nutritive content of *H. ulmarius* revealed 29 % carbohydrate which was comparable with the findings of Usha and Suguna (2015). They reported carbohydrate content of 28 per cent and 34 per cent for two strains of *H. ulmarius* (*H. ulmarius* CO2 and *H. ulmarius* IIHR Hu 1 respectively). Rai *et al.* (1988) reported 3.09 % carbohydrate in *P. florida* on fresh weight basis and in the present study it was recorded as 10.5 %.

5.8.3. Estimation of Protein

In general mushroom possesses high protein content which has been recognised by Food and Agriculture Organisation (FAO) (Rai, 1995). The chitin nitrogen is responsible for high protein value (Crisan and Sands, 1978; Bano and Rajarathnam, 1982; Rai *et al.*, 1988). Chang (1980) reported that the protein content of four popular edible mushrooms, *Agaricus bisporus*, *Lentinula edodes*, *Pleurotus* spp. and *Volvariella volvacea* ranged from 1.75 % to 3.63 % on fresh weight basis.

Chang and Miles (1989) reported that the protein content of edible mushrooms ranged from 19 to 35 % on dry weight basis. This is in accordance with the present study in which *H. ulmarius* and *P. florida* recorded protein content of 32 % and 20.05 % respectively. Mishra *et al.* (2015) reported 33.6 % protein content in blue oyster mushroom. Protein content of 20.83 per cent was recorded for *P. florida* by Shubhra and Jaitly (2011).

5.8.4. Estimation of Fat

Crude fat in mushrooms represents all classes of lipid components. The present study revealed that *H. ulmarius* contains 2.96 % fat which was almost double to that of *P. florida* (1.5 %). Similar report was given by Crisan and Sands (1978). Huang *et al.* (1985) reported a fat content of 1.6 % in *P. sajor-caju* on dry weight basis.

The fat content of *P. florida* was estimated by Parisa *et al.* (2015) as 1.6 per cent. Usha and Suguna (2015) reported the fat content of *H. ulmarius* as 3.55 % on dry weight basis. Darwin *et al.* (2014) reported fat content of 2.10 \pm 0.02 % in *V. volvacea* on dry weight basis.

5.8.5. Estimation of Crude Fibre

Fibre is considered to be an important ingredient in a balanced diet. Chang and Miles (2004) reported that fibre content of *Pleurotus* spp. ranges from 7.4 to 27.6 %. This is in accordance with the present study which revealed a fibre content of 17.69 % for *H. ulmarius* and 10.49 % for *P. florida*.

5.8.6. Estimation of Ash

In the present study, ash content of *H. ulmarius* recorded was 8.0 % and that for *P. florida* was 10.6 % on dry weight basis. This was comparable with the

findings of Shikha *et al.* (2012) who reported an ash content of 7.1 % in *H. ulmarius*. Deepa (2016) reported that ash content of different strains of *Lentinus edodes* ranged from 2.7 to 4.4 %. The ash content content of *Agaricus bisporus* was reported as 9.37 % by Lishma (2015) which is comparable with the present result. Devina (2012) reported an ash content of 6.98 % in freeze dried button mushrooms on dry weight basis.

5.8.7. Estimation of P and K

5.8.7.1. Estimation of P

The results obtained in this study for amount of phosphorous in both *H*. *ulmarius* (0.69 %) and *P. florida* (0.68 %) were similar to the findings given by Shikha *et al.* (2012). George *et al.* (2014) reported that fruiting bodies of shiitake contained a phosphorous content of 7.8 g to 54.5 g kg⁻¹. Usually fungal cell contain low phosphorous content in mycelium (Chang and Miles, 2004).

5.8.7.2. Estimation of K

The potassium content of *H. ulmarius* was recorded as 1.98 % in the present study which was in accordance with the result obtained by Shikha *et al.* (2012). They reported a potassium content of 1.76 % in *H. ulmarius*. *P. florida* recorded 2.45 % of potassium on dry weight basis. Potassium and phosphorous are the most abundant minerals present in fungal cell (Chang and Miles, 2004).

5.9. ORGANOLEPTIC STUDIES

Considering the pleasing flavour, adequate protein and health value, mushrooms represent one of the world's greatest relatively untapped sources of nutritious and palatable food for the future. The palatability can be judged by colour, texture, flavour and taste. Mushrooms can be used as an ingredient in the diet which enhance the taste, texture and nutrients (Kumar and Barmanray, 2007). Jaziya (2011) reported that incorporation of oyster mushroom was very acceptable to the respondents and it did not cause any difficulty in acceptability of dishes prepared during the study. *H. ulmarius* was screened for the characters such as colour, appearance, flavour, texture and taste in steam cooked mushroom using five point score card and comparative studies were done with *P. florida*. The present study recorded an overall acceptability score of 3.6 for *H. ulmarius* compared to 3.0 for *P. florida*. Geetha *et al.* (1995) reported a higher overall acceptability score for *P. djamor* than *P. citrinopileatus*. Prabhu (1991) reported an appreciation per cent of 80 for pink *Pleurotus* and 56.66 % for *P. sajor-caju*.

A preference study was also conducted in *H. ulmarius* and *P. florida* using Hedonic rating scale. Inorder to retain the characteristic taste of mushroom, it was sauted with minimum ingredients and cooked. *H. ulmarius* recorded four minutes as cooking time compared to *P. florida* (two minutes) and most of the evaluators preferred *H. ulmarius* than *P. florida*.

Arumuganathan *et al.* (2005) evaluated the value added products of button mushroom for different attributes *viz.*, colour, appearance, flavour, taste, texture and overall acceptability on the ten point Hedonic scale by a panel of ten judges.

5.10. KEEPING QUALITY

The keeping quality of horticultural products can be increased by harvesting them before ripening which is not applicable in case of mushrooms. Since mushroom is a highly perishable commodity, preservation is having much importance, when there is a glut in the market.

In the present study, *H. ulmarius* could be stored fresh for eight hours and *P. florida* for six hours in room temperature. Saxena and Rai (1990) observed that *A. bisporus* had a shorter shelf life of about 24 h at the ambient temperature due to high moisture content, delicate texture and unique physiology like blackening and autolysis.

The higher moisture content of the species may be attributed to the fast deterioration. Even after harvesting, mushrooms continue to respire, grow, mature which results in weight loss and microbial spoilage (Lal and Sharma, 1995; Rai, 1986; 1995). The very high respiration rate of 28.2- 43.6 mg CO₂ per kg of fresh

weight per hour is mainly responsible for the shorter shelf life of mushrooms (Hammond and Nichols, 1975).

Storage under low temperature is an excellent method for restricting deterioration of harvested mushrooms for a limited period of time (Rai and Arumuganathan, 2008). The major changes occurring in harvested mushrooms include wilting, ripening, browning, liquefaction, lose of moisture and loss of texture, aroma and flavour. These changes are preceded by an increase in the rate of respiration which combined with other reactions and cessation of the nutrient supply will trigger a series of irreversible reactions damaging the mushrooms (Cho *et al.*, 1982). The shelf life of mushroom can be extended by pre-treatments or storage at chilling temperature. Depending on the species, the shelf life may vary from one day to two weeks. In the present study harvested mushrooms were kept inside polythene bags after proper ventilation under refrigeration (4° C).

Hammond and Nichols (1975) reported that the storage of mushrooms in plastic bags with perforations is essential to avoid suffocation of mushrooms. The button stage could have then shelf life extended by storing at 8 0 C in plastic bags. The effect of perforation is to establish equilibrium between CO₂ and O₂ which reduces rate of respiration and thereby the loss of moisture. It was found that mushrooms were best kept at 8 to 10 0 C in 100 gauge polyethylene bags of 30 µm thickness. Similar recommendations were given by Chopra *et al.* (1985) who stored button mushrooms in polythene bags of 100 gauge thickness with 0.5 % ventilation in refrigerated condition. Contradictory observations were given by Saxena and Rai (1988) who reported that mushrooms could be stored in non-perforated polypropylene bags of 100 gauge thickness.

The shelf life of mushrooms is more in refrigerated condition than in ordinary condition. This is due to the fact that low temperature retards the growth of micro organisms, decreases the rate of postharvest metabolic activities of the mushroom tissues and minimizes the moisture loss (Rai and Arumuganathan, 2008). The selection of cooling system mainly depends upon the quantity of mushroom to be

stored. *H. ulmarius* could be kept under refrigeration for five days in perforated polythene bags with no visual symptoms of deterioration where as *P. florida* could be kept only for three days. Shiitake mushrooms were stored upto 10 days in perforated polybags under natural condition and upto 30 days in refrigerated condition by Ramkumar *et al.* (2010). Dhar (1992) reported better shelf life of mushrooms in non perforated polythene bags at low temperature compared to perforated bags at high temperature.

Summary

6. SUMMARY

The fruiting bodies of *H. ulmarius* were collected from the beds maintained in the mushroom unit of Instructional Farm, College of Agriculture, Vellayani. Isolation of the fungus was done in potato dextrose agar medium by tissue culture method and it was purified by hyphal tip method. Hair like mycelial growth was observed after 48 h of inoculation and the growth was completed in 14 to 15 days.

Morphological studies of the fruiting bodies showed that sporocarps were medium to large in size, produced either singly or as a bunch. The pileus was fleshy, dark blue coloured in pinhead stage became creamy white on maturity having non decuurent gills with creamy white, solid, eccentric, cylindrical stipe. Presence of nodose- septate, branched hyaline hyphae with clamp connections was also observed. The spore print was white in colour with feeble spore load. Microscopic studies of spores revealed that they were oval shaped with an average size of 2.30-3.94×0.77-1.54 μ m, smooth and hyaline.

Growth of *H. ulmarius* was evaluated in four different media *viz.*, potato dextrose agar, malt extract agar, oat meal agar and carrot extract agar revealed that maximum mycelial growth (9 cm) was occurred in potato dextrose agar in 8.75 days with thick, cottony growth followed by malt extract agar (8.75 cm). Feeble and slow growth was noticed in carrot extract agar medium.

Studies on influence of different temperature *viz.*, 20 0 C, 25 0 C and 30 0 C on mycelial growth of *H. ulmarius* indicated that 25 0 C was the ideal followed by 30 0 C. More time was taken to complete the growth in 20 0 C.

Maximum mycelial growth (8.82 cm) of *H. ulmarius* was recorded in a pH of 8 in 7.78 days of inoculation followed by pH 7 (8.77 cm). Least growth was recorded in a pH of 5 followed by pH 6. Thick and cottony mycelial growth was observed in dark conditions.

Different substrates *viz.*, paddy grains, wheat, sorghum, ragi and rubber saw dust were evaluated for the attributes like time taken for complete spawn run, nature

of mycelial growth, keeping quality and presence of contaminants. Among the substrates tested, paddy grains was found to be the best medium which completed spawn run in 15 days followed by sorghum (16 days). The maximum time for spawn run (31.75 days) was recorded for rubber saw dust. Wheat recorded high contamination (40 %) followed by ragi (25 %). *Trichoderma* sp., *Aspergillus* sp., *Penicillium* sp. and *Bacillus* sp. were predominantly contaminated the grain spawns.

Cultivation studies were conducted with substrates like paddy straw, rubber saw dust, banana pseudostem, sugarcane bagasse and neopeat to evaluate the best medium for mushroom production of *H. ulmarius*. The minimum time for spawn run (20.7 days) and first harvest (42.4 days) was recorded for sugarcane bagasse and maximum for rubber saw dust. Even though the average weight of sporocarp was higher (63.7 g) for beds prepared from rubber saw dust, more number of sporocarps (27.15) was recorded from paddy straw beds. Biological efficiency of 98.5 % was noted for paddy straw followed by rubber saw dust (90.5 %). Very low yield was recorded for sugarcane bagasse (255 g) followed by neopeat (315 g).

H. ulmarius took an average of five days for harvesting from pinhead formation. Dimension of pileus and weight of the sporocarp increased gradually and reached maximum at the day of harvest. The stipe length increased upto third day and thereafter decreased.

When compared with *P. florida*, *H. ulmarius* needed more time (18.5 days) for spawn run in paddy grains with thick, white cottony growth having buff coloured patches of mycelia. Except the number of sporocarps, all other attributes like time taken for complete spawn run and pinhead formation, first harvest, crop period, weight of sporocarps and total yield were higher for *H. ulmarius* compared to *P. florida*. Under favourable climatic conditions of low temperature and high relative humidity inside the cropping room during the months of October to January, *H. ulmarius* recorded a biological efficiency of 109.7 % which was higher that of *P. florida*. Comparatively lesser yield and more spawn run period was recorded during February to September in case of *H. ulmarius*. However, *P. florida* recorded almost similar yield and spawn run period throughout the year. Trials conducted in Idukki, Wayanad and Vellayani revealed that blue oyster can be cultivated throughout the year as *P. florida* in Idukki and Wayanad whereas in Vellayani it can be profitably cultivated in the months of October to January.

Phorid flies (*Megaselia* sp.), staphylinid beetles and springtails were the major pests observed in beds during the time of spawn as well as fruit body formation. Maximum pest attack (35 %) was noticed in neopeat followed by rubber saw dust (20 %). The predominant competitors observed were *Trichoderma* sp., *Aspergillus* sp. and *Coprinus* sp. Maximum (80 %) infection by *Trichoderma* sp. was recorded in beds prepared from sugarcane bagasse. About 25 % infection was observed in paddy straw by *Trichoderma* sp., *Aspergillus* sp. and *Coprinus* sp.

Analysis of proximate constituents revealed that *H. ulmarius* consisted 90.37 % moisture on fresh weight basis. The other nutritive components estimated were 29 % carbohydrate, 32 % protein, 2.96 % fat, 17.69 % fibre, 8 % ash, 0.69 % phosphorous and 1.98 % potassium on dry weight basis. *P. florida* recorded 93.95 % moisture, 10.50 % carbohydrate, 20.05 % protein, 1.5 % fat, 10.49 % fibre, 10.6 % ash, 0.68 % phosphorous and 2.45 % potassium.

Studies on sensory evaluation and cooking quality on both *H. ulmarius* and *P. florida* revealed *H. ulmarius* was superior to *P. florida* for the organoleptic characters such as appearance, colour, flavour, taste and texture. The preference study showed that 30 per cent of evaluators extremely liked *H. ulmarius*, 50 per cent of them liked it very much and 20 per cent liked moderately. Instead, 10 per cent extremely liked *P. florida*, 40 per cent of them liked it very much, 30 per cent liked slightly.

The studies regarding keeping quality of mushrooms in perforated polythene bags indicated a shelf life of eight hours for *H. ulmarius* in normal atmospheric condition which was higher compared to *P. florida* (six hours). Under refrigerated condition (4 0 C), *H. ulmarius* recorded a keeping quality of 5 days without any visible symptoms of damage and was good for cooking. After five days, considerable

weight reduction was recorded. In the same condition, *P. florida* recorded a keeping quality of three days. After three days of storage under refrigeration, cooking quality of *P. florida* was reduced.

The blue oyster mushroom (*H. ulmarius*) can be profitably cultivated in Kerala on locally available materials like paddy straw and rubber sawdust from by maintaining low temperature, good aeration and high RH inside the mushroom house. The attractive size and shape of sporophores, high biological efficiency, keeping quality and nutritional value are its added advantages over the common oyster mushroom (*P. florida*).



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PHYSIOLOGICAL AND CULTURAL STUDIES ON BLUE OYSTER MUSHROOM (*Hypsizygus ulmarius* (Bull.:Fr.) Redhead)

by SUMI I. (2014-11-142)

Abstract of the thesis Submitted in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture Kerala Agricultural University



DEPARTMENT OF PLANT PATHOLOGY COLLEGE OF AGRICULTURE VELLAYANI, THIRUVANANTHAPURAM-695522 KERALA, INDIA

2016

ABSTRACT

The present study entitled "Physiological and cultural studies on blue oyster mushroom (*Hypsizygus ulmarius* (Bull.:Fr.) Redhead)" was carried out in the mushroom unit, Instructional Farm, College of Agriculture, Vellayani during 2014-2016, with the objective to standardize the technology for cultivation of *Hypsizygus ulmarius* and to study its morphological and physiological aspects. The initial culture of *H. ulmarius* was isolated from the mushroom beds maintained in the mushroom unit of instructional farm through tissue culture method and purified by hyphal tip method.

Morphological studies of *H. ulmarius* showed that the sporocarps were medium to large in size having a dark blue colour in the pinhead stage which became creamy white on maturity with an irregularly shaped, convex pileus with gills attached to the stem, but not decurrent and cylindrical, smooth and eccentric stipe. Microscopic studies revealed septate hypahe with clamp connection, oval shaped, hyaline basidiospores and the spore print was white. Studies on developmental morphology showed that *H. ulmarius* took an average of five days from the day of pinhead formation to complete maturity.

The maximum mycelial growth was recorded on potato dextrose agar. A temperature of 25 ⁰C, pH of 8 and dark conditions are found favourable for maximum mycelial growth. Evaluation of different substrates for spawn production revealed that paddy grains was the best medium in which spawn run was completed in fifteen days with thick fluffy growth and recorded less contaminants followed by wheat and sorghum.

Evaluation of different substrates for mushroom production revealed that paddy straw was the best material for the cultivation of blue oyster with a total yield of 985 g kg⁻¹ from three harvests followed by rubber sawdust (905 g kg⁻¹). The minimum time for mushroom production was recorded for sugarcane bagasse and the maximum time for rubber sawdust. The average weight of sporocarp was maximum in mushrooms harvested from rubber sawdust and the maximum number of sporocarps was recorded in paddystraw. Beds prepared from sugarcane bagasse were heavily contaminated with *Trichoderma* sp.

When compared with *Pleurotus florida*, *H. ulmarius* took more time (18 days) for complete spawn run in paddy grains and the yield was higher on paddy straw (1.096 kg kg⁻¹) than *P. florida* (976 g kg⁻¹).

Infestation of pests *viz.*, phorid flies (*Megaselia* sp.) and staphylinid beetles were prevalent during spawn run as well as sporocarp formation. The competitor moulds recorded were *Trichoderma* sp., *Aspergillus* sp. and *Coprinus* sp.

Analysis for the proximate constituents in *H. ulmarius* revealed that it contains appreciable amount of carbohydrate (29 %), protein (32 %) and fibre (17.69 %).

Sensory evaluation was done on steam cooked mushrooms for attributes like appearance, colour, texture, flavor and taste using five point score card and an overall acceptability score of 3.6 was obtained for *H. ulmarius* compared to *P. florida* (3.0). In the preference study conducted for both the mushrooms using Hedonic rating scale, 30 per cent of evaluators extremely liked *H. ulmarius* than *P. florida* (10%).

The study on the keeping quality of mushrooms in normal atmospheric condition indicated a shelf life of eight hours for *H. ulmarius* compared to six hours for *P. florida*. The study also showed that blue oyster mushrooms stored under refrigeration (4 0 C) in perforated polythene covers had better shelf life (5 days) compared to *P. florida* (3 days).

The present study indicated that blue oyster mushroom can be cultivated successfully in tropical areas on locally available materials like paddy straw and rubber saw dust under favourable climatic conditions *viz.*, 26-28 ⁰C temperature, more than 90 per cent relative humidity and good aeration. The variety is superior to the presently growing oyster mushroom (*P. florida*) in terms of yield, presence of appreciable amount of proximate constituents and keeping quality.

<u>kw{Klw</u>

\oe-Nn-,n-¡q-Wnsâ (ln-]vkn-ssk-Kkv AÄta-cn-b-kv) Irjn-co-Xnbpw ^nkn-tbm-f-Pnbpw kw_-Ôn¨ KthjW]T\w 2014-þ16 Ime-b-f-hn shÅm-bWn ImÀjnI tImtf-Pnse C³kv{S-£-W ^man \S-⁻p-I-bp--mbn. \oe-þ-Nn-,n-¡q-Wnsâ Irjn-co-Xn-IÄ imkv{Xo-b-ambn \nP-s,-Sp-⁻pI, Ah-bpsS _mly-im-kv{X-]-c-amb]T-\-§Ä \S-⁻pI F**J** n-hbm-bn-cp**J**p]T-t\m-t±-iyw.

C³kv{S-£-W ^man \ne-\nÀ⁻n-bn-cpJ 1q-X-S-§-fn \nJpw tiJ-cn⁻ 1qWp-IÄ Snjyp-IÄ⁻Ä {]{In-bbvjv hnt[-b-am-jn-bmWv {]m[-an-1-ambn 1q- X'p-jsf hfÀ⁻n-sb-Sp-⁻-Xv. 1q- 1Ä⁻-dnsâ bYmÀ^o Dd-hnSw Ub-d-lvStdäv Hm^v ajvdqw dnkÀ⁻v, tkmf³ Bbn-cp-Jp. Cu 1qWp-IÄ hep-,-ta-dn-bXpw Ch-bpsS kvt]mdp-IÄ shÅ-\n-d-⁻n-ep-Å-h-bp-am-Wv. \oe-\n-d-⁻n-epÅ 1q-ap-1p-f-§Ä A©p Znhkw s1m-v]qÀ[®] hfÀ⁻-sb-⁻n, Cfw shÅ \nd-am-bn-⁻o-cp-Jp.

\oe-Nn-,n-jq-Wp-1-fpsS lq¬ X′p-jÄ \¶mbn hf-cp-¶Xv s]m«-täm-sU-lvkvt{Smkv AKmÀ am²y-a-īn-em-Wv. lqSmsX 25 Un{Kn skÂjykv Xm]-hpw, AÂ]w £mc-Xzhpw (]n.-F`v) 8) Ccp«pw hfÀ¨sb A\p-lq-en-jp¶ LS-l-§fm-lp-¶p. lq¬ hnīpÂ]m-Z-\-īn\p Gähpw tbmPn¨ am[y-a-ambn ls--īnb s\--Wn-l-fn lq¬X-´p-jÄ Gl-tZiw 15 Znhkw slm-v]qÀ®-h-fĨ {]m]n-jp-¶-Xmbn Cu]T-\-īn ls-īn. s\-Wn lgn-ªm slmÅm-hp¶ am[y-a-§Ä tKmX¼pw aWn-t¨m-f-hp-am-Wv.

lq¬lr-jn-jmbn hnhn-[-Xcw am[y-a-§Ä]co-£n-"-Xn hbvtjm-emWv Gähpw A\p-tbmPyw Fgp Is--"p-1bp--m-bn. aqgp hnf-sh-Sp-,n-eqsS Hcp Intem{Kmw hbvtjm-en \ngpw 985 {Kmw lq-hsc DXv]m-Zn-,n-jphm³ km[n-"p. dºÀ ac-s,m-Sn-bn CXv 905 {Kmw Bbn-cp-gp. km[m-cW lrjn-sN-;pg ¹qtdm-«kv ^vtfmdnU Fg C\s⁻ At]-£n[°]v \oe-Nn-,n-jq-Wp-IÄ AXypÂ]m-Z\ tijn-bp-Å-Xm-sW**9**p Ch-bpsS Xmc-X-ay-]-T-\-\$Ä sXfn-bn-jp-**9**p.

lq¬lr-jn-bn {][m\ loS-§-fmbn ls--⁻n-bXv t^mdn-Up-lfpw lss^-en-\n-Up-l-fp-am-Wv. lqSmsX ss{Stjm-sUAa, Bkvs]APn-Ã-kv, slmss{]-\kv apX-emb lpan-fp-lfpw lq-X-S-§-fn lmW-s,-«p. \oe-Nn-,n-jq-WnÂ KWy-amb Af-hn AJPw (29%), amwkyw (32%), \mcp-lÄ (17.7%) FJnh AS-§n-bn-cn-jp-J-Xmbn cmk-þ-A-]-{K-Y-\-⁻n ls--⁻p-l-bp--m-bn. kpjn-c-§-fn« t]mfn-⁻o³ lh-dp-l-fn-emjn {^nUvPn (4 Un{Kn skÂjy-kv) kq£n-⁻mÂ A©p Znhkw hsc Cu lqWp-lÄ tlSp-lq-Sm-sX-bn-cn-jpw.

tlcfⁿ Xt±i e`y-amb hbvt;mÂ, dºÀ ac-s,mSn apX-emb ImÀjnImhin-jvS-§-fp-]-tbm-Kn⁻v A\p-Iq-e-amb Imem-h-Ø-bn hfsc hnP-b-I-c-ambn \oe-Nn-,n-;q- Irjn-sN-;m-hp-J-Xm-Wv. Cu IqWp-IÄ \ne-hn-epÅ Nn,niq-Wp-Isf At]-£n⁻v hnf-hn-epw, AJ-Pw, amwkyw, \mcp-IÄ XpS-§nb LS-I-§-fnepw anIhp]peÀ⁻p-J-Xmbn]T-\-§Ä sXfn-bn-;p-Jp. aäp Nn,n-;q-Wp-I-tf-;mÄ kq£n-,p-Imew IqSp-X-ep-s--Jp-ÅXpw \oe-Nn-,n-;q-Wp-I-fpsS {]tXy-I-X-bm-Wv.



APPENDIX – I DATASHEET

		Date of collection
Collected by		Locality
	GEN	IERAL
Substrate	:	
Habitat	: Terrestrial / Lignicolous /	Epixylose / Coprophilous
Habit	: Solitary / Scattered / Greg	garious
Pileus		
Shape	: Convex/Infundibuliform/	Umbonate/Petaloid/Flabelliform/Depressed
Colour	:	
Size	: Diameter	
	Thickness	
Texture	: Soft/ Brittle/ Fleshy/ Frag	ile/ Coriaceous/ Membraneous
Stipe		
Shape	: Clavate	
Size	: Length :	

 Size
 : Length :

 Diameter :
 Diameter :

 Attachment to pileus
 : Lateral/ Eccentric/ Central/ Resupinate

 Surface
 : Glabrous/Scaly/Smooth/Pubescent/Fibrillose

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

Туре	: Present/ Absent
Annulus	
Туре	: Present/ Absent
Volva	
Туре	: Present/ Absent
Spore print	
Colour	:
Spores	
Colour	:
Shape	: Ovate/Elliptical/Globose/Epiculate/Cylindrical/Fusiform/
	Angular/Echinulate/Recticulate/Ovoid/Pyriform
Reaction with	
Cotton blue	: Cyanophilic/ Acyanophilic
Melzer's reagent	: Amyloid/ Dextrinoid/ Nonamyloid

APPENDIX - II

Composition of different media

a) Potato Dextrose Agar (PDA)

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

b) Malt Extract Agar (MEA)

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

c) Oat Meal Agar (OMA)

Oat flakes	:	30 g
Agar-agar	:	20 g
Distilled water	:	1 litre

d) Carrot Extract Agar (CEA)

Grated carrot	:	20 g
Agar-agar	:	20 g
Distilled water	:	1 litre

Score card for the evaluation of cooked mushro Parameters Score		
Appearance		
Excellent	5	
Very good	4	
Good	3	
Fair	2	
Poor	1	
Colour		
Excellent	5	
Very good	4	
Good	3	
Fair	2	
Poor	1	
Flavour		
Highly acceptable	5	
More acceptable	4	
Acceptable to certain extend	3	
Less acceptable	2	
Not acceptable	1	
Texture		
Very soft and fleshy	5	
Soft and fleshy	4	
Slightly fibrous	3	
Fibrous	2	
Very fibrous	1	

APPENDIX – III Score card for the evaluation of cooked mushroom

Taste		
Excellent	5	
Very good	4	
Good	3	
Fair	2	
Poor	1	
APPENDIX – IV		

APPENDIX – IV Hedonic rating scale for the evaluation of mushroom recipe

Particulars	Score
Like Extremely	9
Like Very Much	8
Like Moderately	7
Like Slightly	6
Neither Like nor Dislike	5
Dislike Slightly	4
Dislike moderately	3
Dislike Very Much	2
Dislike Extremely	1

APPENDIX – V

Preparation of sauted mushroom

Ingredients		Amount
Mushroom	-	100 g
Big onion	-	10 g
Green chilli	-	5 g
Tomato	-	20 g
Coconut oil	-	1 tsp
Pepper powder	-	1/8 tsp
Turmeric powder	-	a pinch
Red chilli powder	-	a pinch
Salt	-	to taste
Curry leaves	-	one sprig

Method

Heat the oil, sauted big onion, green chilli and tomato. Added mushroom pieces, salt and other ingredients. Then sauted and cooked by covering the vessel. Served hot.

	Wea	ather data - 2	2015-16	
	Weather parameters			
Month	T. (⁰ C)		RH (%)	Rain (mm)
	Max.	Min.		
January	31.30	21.92	91.90	8.00
February	31.56	22.34	91.79	0
March	32.40	23.65	89.94	56.10
April	32.74	24.47	87.73	182.60
May	32.13	25.31	90.71	406.00
June	31.43	24.48	90.83	346.90
July	31.31	24.63	89.13	53.50
August	31.68	24.57	89.45	77.90
September	31.41	24.40	91.40	289.80
October	32.33	24.80	95.58	399.1

APPENDIX - VI Weather data - 2015-16

November	31.55	23.79	92.70	254.10
December	31.48	23.28	93.94	259.30
January (2016)	32.34	21.64	91.90	0.40