STUDIES ON CULTURAL TECHNIQUES OF OYSTER MUSHROOM {*Pleurotus sajor-caju* (Fr.) Singer}"

THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE

REQUIREMENTS FOR AWARD OF

Master of Science (Agriculture)

In

Plant Pathology

Supervisor

Prof.Ram Chandra

Submitted by

Samikshya Adhikari

DEPARTMENT OF MYCOLOGY AND PLANT PATHOLOGY

INSTITUTE OF AGRICULTURAL SCIENCES

BANARAS HINDU UNIVERSITY

VARANASI- 221005

INDIA

ExaminationRoll No. 20412MPP024 **2022** **Enrollment No. 383094**

काशी हिन्दू विश्वविद्यालय

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Prof. Ram Chandra
Professor
E-mail: rcbhumpp@gmail.com
Mobile No: +91-9935658622

Department of Mycology & Plant Pathology
Institute of Agricultural Sciences
Banaras Hindu University
Varanasi - 221005

Ref. No. …… **Date: ……..…...**

CERTIFICATE

To,

The Joint Rergistrar (Academic),
Banaras Hindu University,
Varanasi-221005

Through: **The Head, Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, B.H.U., Varanasi.**

Dear Sir,

I have great pleasure in forwarding the thesis entitled "***Studies on cultural techniques of oyster mushroom {Pleurotus sajor-caju (Fr.) Singer}***" submitted by Miss.Samikshya Adhikari, (Examination Roll No. 20412MPP024; Enrolment No. 383094) in partial fulfillment of the requirements for the award of the degree of Master of Science (Agriculture) in Plant Pathology, Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (U.P.).

I certify that the entire scheme of investigation presented herein, was planned and carried out solely by the candidate under my guidance and supervision. The data presented in thesis, to the best of my knowledge and belief, are genuine and original. No part of the work has been submitted for any degree or distinction.

Yours Faithfully,

Forwarded by

(Head)

(Prof. Ram Chandra)

Supervisor

BHU capital of knowledge

स्वच्छ भारत
एक कदम स्वच्छता की ओर

STUDIES ON CULTURAL TECHNIQUES OF OYSTER MUSHROOM {*Pleurotus sajor-caju* (Fr.) Singer}"

by
Samikshya Adhikari

Thesis submitted in partial fulfillment of the requirements for

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DEPARTMENT OF MYCOLOGY & PLANT PATHOLOGY
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VARANASI - 221005 INDIA

2022

THESIS APPROVED BY ADVISORY COMMITTEE

CHAIRMAN	**Dr. Ram Chandra** **Professor** Department of Mycology and Plant Pathology Institute of Agricultural Sciences, BHU, Varanasi.
MEMBER	**Dr. S. P. Singh** **Assistant Professor** Department of Mycology and Plant Pathology Institute of Agricultural Sciences, BHU, Varanasi.
MEMBER	**Dr. R. S. Meena** **Assosciate Professor** Department of Entomology and Agricultural Zoology Institute of Agricultural Sciences, BHU, Varanasi.

EXTERNAL EXAMINER

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Above all, my humble and whole hearted prostration to Lord Shiva and Goddess saraswati for their blessings.

I hereby declare that my work incorporated in the present thesis entitled ***"Studies on cultural techniques of oyster mushroom {Pleurotus sajor-caju (Fr.) Singer}"*** *is my own work and data presented in the thesis are original and genuine. This study (in part or in full) has not been sumitted to any university for the award of degree.*

Date: .....

Place: Varanasi ***(Samikshya Adhikari)***

List of Abbreviations

%: Per cent

^{o}C: Degree Celsius

@: At the rate

A. D: Anno Domini

ANOVA: Analysis of Variance

B. C.: Before Christ

B.E.: Biological Efficiency

BOD: Biological Oxygen Demand

CD: Critical difference

cm: Centi Metre

CRD: Completely Randomised Design

et al: Co-workers

FAO: Food and Agriculture Organisation

Fig.: Figure

GA: Giberillic Acid

Gm: Gram

hrs.: Hours

i.e.: That is

IAA: Indole-3- Acetic Acid

IBA: Indole-3-Butyric Acid

ICAR: The Indian Council of Agricultural Research

kg: Kilo Gram

L: Litre

M: Maize Straw

Mg: Milli Gram

ml: Milli Litre

mm: Milli Metre

MEA: Malt Extract Agar

MT: Metric Tonne

NHB: National Horticulture Board

PDA: Potato dextrose Agar

pH: Power of Hydrogen ion concentration

psi: Pound per square inch

ppm: Part per million

PSC: *Pleurotus sajor-caju*

rpm: Revolutions per minute

SE(m): Standard Error

spp.: Species

USA: United States of America

USD: US Dollar

viz.: Namely

WP: Wettable Powder

WEA: Wheat Extract Agar

W: Wheat Straw

R: Rice Straw

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Chapter-1

INTRODUCTION

Nutritional security is becoming a challenging task because of increasing world population. This challenge can be fulfilled with the help of alternative source of nutritious food against the malnutrition.In the unique circumstance, mushroom play a vital role to combat against the nutritional scarcity.

Significance of mushroom consumption has been increased during the pandemic SARS-CoV-2 more than ever because specific vaccine or treatment isnot available to fight against it.Research showed that regular intake of mushroom products like *Inonotus obliquus, Grifola frondosa, Lentinula edodes* with effective anti-inflammatory and antiviral properties, can minimize deadly effects of SARS-CoV-2 (Shahzad *et al*. 2020).

Mushroom is fleshy,spore bearing macrofungus which possesses distinct sporocarp which is big enough to be seen by naked eyes and to be plucked by hands. Sporocarp may be either epigeous (aboveground) or hypogeous (underground).

It was reported that out of 1.5 million known fungal species,1,60,000 species produce fruiting body of suited size and structures to be studied as macro fungi or mushrooms (Hawksworth 2012; Murugesan 2017). Of the 16,000 known species of mushroom (Hawksworth 2012), about 7000 species exhibit various ranges of edibility, around 3000 species are considered as primary edible mushrooms, and about 700 mushrooms are found to be of therapeutic or medicinal use (Chang and Wasser 2017; Li *et al*. 2021).

Out of 200 species of mushroom being consumed as superfood globally (Kalac 2013), 35 species are cultivated commercially and about 10 species are produced at industrial level in different countries worldwide (Aida *et al*. 2009; Xu et al. 2011; Chang and Wasser 2017).

For the first time in 1978, a frenchman nearby Paris propagated *Agaricus bisporus* in quarries and achieved its commercial production. In 1984, functional committee was developed in Pennsylvania, mushroom center of the world (Beyer 2003) to exploit the mushroom farming.

Hieroglyphics of the tombs of the Pharaohs shows that the ancient Egyptians considered mushroom to be “the plant of immortality”. Some South American Amazon tribes have considered mushrooms as equivalent to meat in terms of nutrition. Ancient Romans referred mushrooms as “food of the gods”. Chinese culture has perceived mushrooms as a healthy food,

an “elixir of life.” Ancient Greeks considered that mushrooms provided strength for their warriors in war.The Mayans included mushrooms in religious ceremonies.

Mushrooms are biologically unique. Not quite animals, not quite plants.They are dissimilar to plant in that they don’t have green chlorophyll. Mushrooms, being achlorophyllous, grow on dead and decaying material in nature and on substrate of various agrowastes when commercially grown. Furthermore, fungi contain chitin, a important biopolymer, also present in the exoskeleton of crustaceans and insects, not cellulose as in plants, and posses the unique ergosterol, sterol rather than cholesterol as in mammalian cells. Mushrooms are hence placed in a separate kingdom of their own i.e. “Kingdom of Fungi”.

Mushroom cultivation technology has revolutionized the world. By adding this non-conventional crop in subsisting farming systems, social and economic position of needy farmers can be uplifted. Mushrooms cultivation is considered second largest economical microbial technology after yeast (Pathak *et al*. 2009). Because of this technology, the global production of truffles and mushrooms increased from 6.90 to 10.24 million metric tons in past 10 years (Ho *et al.* 2020). The worldwide influence of edible and medicinal mushrooms on being is taken as a move towards the non-green revolution (Chang 1999).

Various types of mushrooms such as parasitic, saprophytic, and mycorrhizal can be found in nature but for artificial cultivation saprophytic mushrooms are preferred generally (Stamets 2000) such as *Agaricus bisporus* (J.E. Lange) Imbach, *Pleurotus sajor-caju* (Fr.) Sing., *Pleurotus ostreatus* (Jacq.) P. Kumm., *Grifola frondosa* (Dicks.) Gray, *Lentinula edodes* (Berk.) Pegler, *Volvariella volvacea* (Bull.) Singer, *Auricularia auricula-judae* (Bull.) Quél, *Flammulina velutipes* (Curtis) Singer, and *Tremella fuciformis* Berk (Miles and Chang 2004).

Oyster mushrooms

Pleurotus sps (oyster mushroom) is one of the most popular type of mushrooms produced globally. Oyster mushroom belongs to Class Basidiomycetes and Family Agaricaceae and commonly known as ‘dhingri’ in India and naturally grows in the tropical and temperate forests on dead and decaying plant parts. These mushrooms consists of typically thin, broad, oyster or fan shaped cap (pileus) in various colour ranges like white, gray or tan and long ridges and furrows are present underside the cap called as gills or lamellae. From the edge of the cap, the gills extends down to the stalk and produce spore. The caps have sometimes frill-edge and can be present in clusters of small mushrooms or separately as larger mushroom.

A lateral or central stipe is present of various length that supports the cap and increase height of mushroom so spores can spread in a wider space. The spores are cylindrical, smooth and germinate very easily on any type of fungal media within 48-96 hrs. The mycelium is pure white in colour.

Oyster mushroom production has several benefits comparing to other edible mushrooms. It grows in less time over a wide range of pH (6-8) and temperature (10-30° C); produces plethora of enzymes that are able to degrade lignocellulosic biomass of agrowastes; requires very less environmental control; can colonize substrates in a short time period; does not require composting of the substrate; has higher yield capacity and poses high nutritional and medicinal worth. Moreover, the substrate used for its production requires only pasteurization (does not need a more costly method- sterilization), their fruiting bodies are not generally affected by pests and diseases, and often, they can be grown in very easy and economical ways. There are many species of Pleurotus viz. *Pleurotus ostreatus, P. florida, P. eryngii, P. cystidiosis, P. flabellatus, P. cornucopie* and *P. sajor-caju* growing on commercial scale (Ram 2007).

Pleurotus sajor-caju

Pleurotus sajor-caju (Fr.) Singer is a type of oyster mushrooms. It was seen growing naturally on succulent tissues of *Euphorbia royleans* Boiss., in the foothills of the Himalayas in 1974) and was found to produce sporophores on chopped paddy straw and banana pseudostems by artificial culture (Jandaik 1974). This mushroom is considered a great delicacy and appreciated for its taste and flavour. Rangaswami *et al*. (1975) noted that also unsterilized plant residues may be colonized by this mushroom. Kurtzman (1979) has given a comprehensive review of the possibilities of nitrogen fixation by Pleurotus.

In modern mycology there are two meanings of name "*Pleurotus sajor-caju".* *Pleurotus sajor-caju* (Fr.) Singer is a strict taxonomic meaning, a synonym nomenclature of *Lentinus sajor-caju* (Fr.) Fries., a recent member of the genus *Lentinus*. But a more widely distributed mention has rather a biotechnological meaning, which initially denoted to a strain reported to be *Pleurotus sajor-caju* but that genuinely belongs to the *Pleurotus* genus. This problematic *Pleurotus* strain has an Indian origin (Zmitrovich and Wasser 2016).

Systematic Position of *Pleurotus sajor-caju* (Lini *et al.* 2021)

Sub-division : Besidiomycotina

Class : Hymenomycetes

Sub class : Holobasidiomycetidae

Order : Agaricales

Family : Trichlomataceae

Genus : *Pleurotus*

Species : *sajor-caju*

Fruiting body structure of *Pleurotus sajor-caju*

Cap

- Initially oblong and becomes convex with time,smooth.
- Hairless,moist and fragrant & 4-20 cm in diameter.
- Slight wavy and smooth margin.
- Colour can range from white to brown to grey.

Flesh

- Flesh can be thick or thin depending upon variety.
- Most common variety has white colour.
- Wild varieties have yellow, blue, pink and grey colour and they can be grown.
- Chewy texture like an oyster.

Gills and stem

- Gills are attached to stem,decurrent and hairless.
- Short stems ,arising from substrates and may produce stub like,short lateral stalk
- Gills and stem colour range from white to pale yellow with time.

Spores

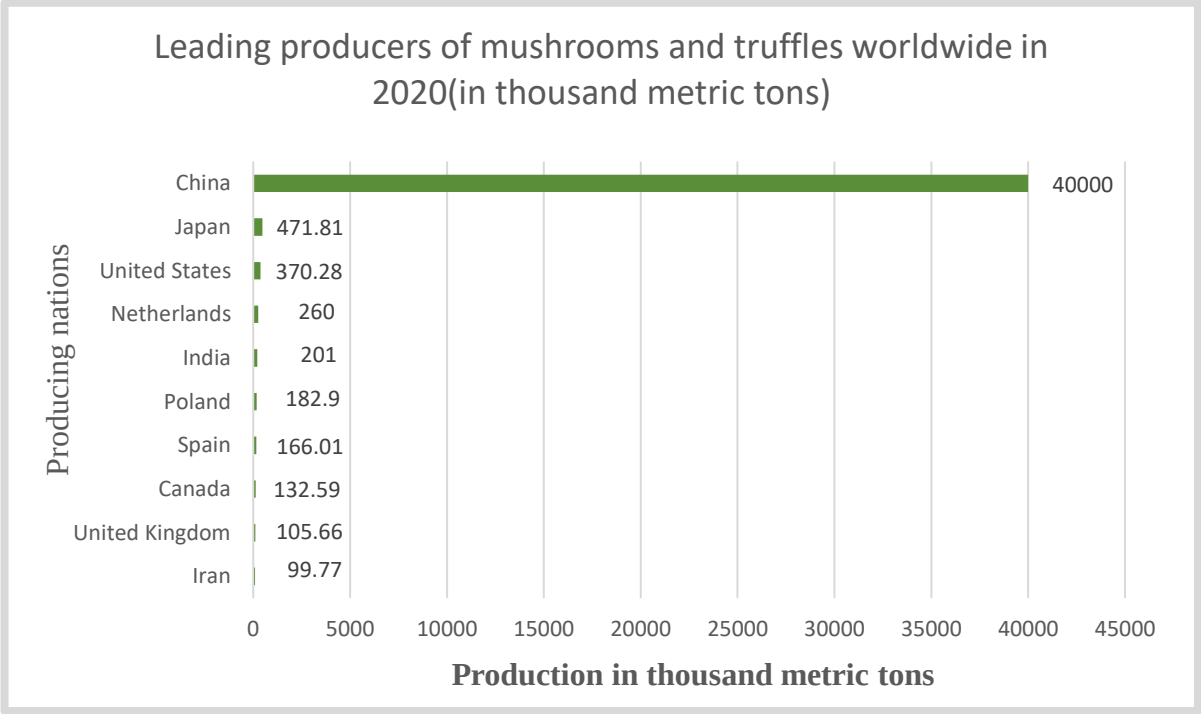
- On dark media spores form white to lilac-grey print

Mycelia

- White in colour.

Status of mushroom production around the globe

According to the FAO statistics 2020, China was ranked first in global mushroom production, producing 40 million metric tons of mushrooms which accounts for roughly 94% of the total production worldwide. This was followed by Japan and the United States. In 2020 nine nations produced more than 100k metric tons of mushrooms.



(Source:Shahbandeh,2022)

Fig.1.1.Leading producers of mushrooms and truffels worldwide in 2020.

Due to massive rise in cultivation of shiitake, oyster mushroom, wood ear mushroom etc particularly in East Asian countries, button mushroom is now no more in first position in terms of share in global mushroom production. Based on data from various sources, world mushroom production in 2018-19 was estimated to be 43 million tonne with *Lentinula edodes* contributing 26%, *Auricularia* spp 21% *Pleurotus ostreatus* 16%, *Agaricus bisporus* 11%, *Flammulina velutipes* 7%, *P. eryngii* 5%, *Volvariella volvacea* 1% and others 13%. Other important share holders were *Agrocybe aegerita, Tremella fuciformis, Pholiota nameko, Hypsizygus marmoreus* etc. Shiitake, Pleurotus species etc which are mostly produced in Asian countries, have started making inroads in Europe, Canada, America and Australia where *Agaricus bisporus* is major contributor. Global mushroom production is likely to cross 50 MT by 2025 (ICAR-DMR, Solan,2021).

Status of mushroom production in India

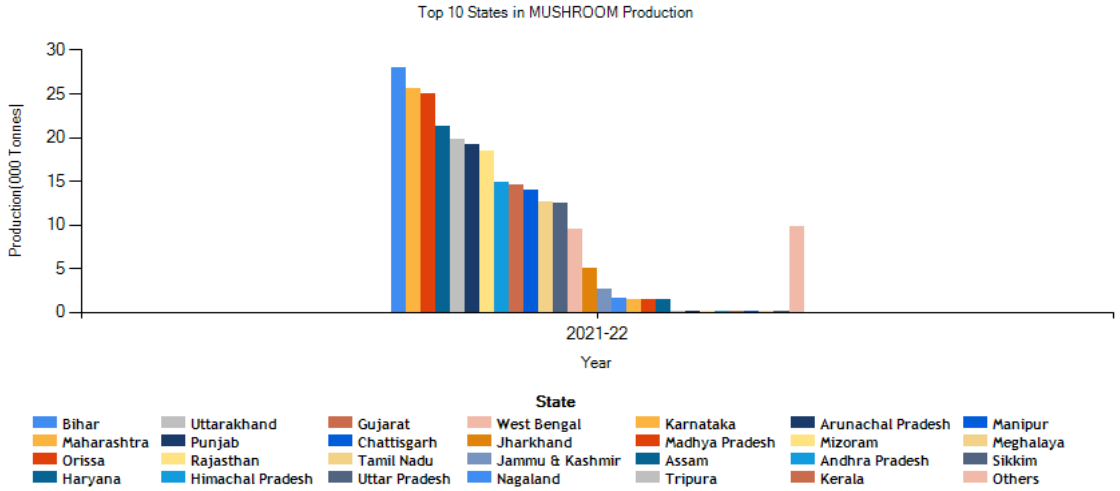


Fig.1.2.Top mushroom producing states of India

According to the data published by National Horticulture Board in year 2021-22,Bihar ranked number one with total mushroom production of 28000 metric tonnes, which is 10.82% of the total mushroom produced in the country. This is followed by Maharashtra and Orissa with total production of 25600 and 25000 metric tonnes respectively.Table below shows top ten states for mushroom production in India for year 2021-22 as per NHB.

Table.1.1.Top 10 mushroom producing states of India

S.No	State	Production(2021-22)	Share(%)
1.	Bihar	28.00	10.82
2.	Maharashtra	25.60	9.89
3.	Orissa	25.00	9.66
4.	Haryana	21.20	8.19
5.	Uttrakhand	19.80	7.65
6.	Punjab	19.15	7.40
7.	Rajasthan	18.40	7.11
8.	Himachal Pradesh	14.80	5.72
9.	Gujarat	14.50	5.60
10.	Chhattisgarh	13.90	5.37

(Source:-National Horticulture Board,2021-22)

Nutritional composition of oyster mushroom

Pleurotus mushrooms are considered a functional food and are valued for their proximate composition and nutritional properties as they are rich source of proteins, fibers, vitamins, and minerals with low-fat levels. Additionally, the food quality may increased due to richness of umami-flavour in oyster mushrooms.

These mushrooms contains four influential nutrients which includes vitamin D, glutathione, ergothioneine, selenium and thay are well known to serve as antioxidants and abate oxidative tension (CNN Health 2018).

It is important to know that the stage, growth characteristics and postharvest situation may influence the nutritional value and chemical composition of edible mushrooms. Also great variations exists both within and among species.

Proteins and amino acids

Pleurotus genus are rich in palatable proteins, especially good for vegetarians. Chitin, amino acids and nucleic acids are the form of non protein nitrogen present. Protein content mainly ranges from 9.29 to 37.4 g/100 g dry weight of fruit bodies depending upon species. All the essential amino acids such as leucine, valine, glutamine, glutamic and aspartic acids etc are present, except the sulfur-containing amino acids methionine and cysteine.

Few amino acid are known to enhance taste in mushroom, making them flavourful. Atri et al. noted rich amino acid profile in wild species of *PSC*, *PP*, *PF and PC*. *Pleurotus* species also posses high amounts of ornithine and γ-aminobutyric acid (GABA) ,a nonessential amino acid needed for mental activity and brain functioning.

Carbohydrates

Oyster mushrooms contain high amounts of carbohydrates ranging from 24.95 to 75.88%. The chitin and polysaccharides shares a major portion of mushroom nutrients. In *PSC* and *PF*, the carbohydrates percentage is 39.82 and 42.83 respectively. Carbohydrates constitute of fibre, polysachharides, beta-glucans, hemicelluloses, chitin and pectic substances. Fructose, trehalose, mannitol, myo-inositol are the sugars present in oyster mushrooms.

Table.1.2. Proximate composition of some *Pleurotus sps*

Species	Content gm/100 gm dried mushroom						
	Moisture	**Protein**	**Carbohy-drate**	**Fat**	**Ash**	**Crude fiber**	**References**
Pleurotus ostreatus(PO)	88.5	32	50.9	3.1	6.1	6.2	Yehia (2012)
Pleurotus flabellatus(PFL)	91	21.6	57.4	1.8	10.7	11.9	Khan and Tania(2012)
Pleurotus florida(PF)	87.5	20.56	42.83	2.31	9.02	11.5	Ahmed *et al.* (2009)
Pleurotus sajor-caju(PSC)	87.0	24.63	39.82	2.29	8.28	10.9	Alam *et al*. (2008)
Pleurotus citrinopileatus(PC)	88.9	30	42.5	3.9	7.65	20.78	Ghosh and Chakravarty (1990).
Pleurotus eryngii(PE)	91	11.95	39.85	7.50	4.89	28.29	Akyuz and Kirbag (2010)
Pleurotus pulmonarius(PP)	78.8	20.3	34	2.62	7.33	9	Silva *et al.* (2002)
Pleurotus eous(PEO)	86.81	24.10	45.59	4.73	9.84	15.91	Kortei and Wiafe-Kwagyan (2015)
Pleurotus djamor var.roseasus(PDR)	79.52	35.5	44.75	1.72	5.90	14.60	Jegadeesh *et al.(*2018)
Pleurotus tuber-regium(PTR)	87.13	22.10	63.03	1.06	2.97	10.86	Ifeoma *et al*. (2009)

Fats and Lipids

Mushrooms provide low amounts of fat, being linoleic, oleic and palmitic the main fatty acids. Lavelli et al. reported fat content in Pleurotus species to be in range of 0.9–7.5%.The possible cause for this variation may be the agro-waste used in the production process. Earlier research found Pleurotus species as a good option for hypocholesterolemia and anti-inflammation. Schneider et al. noted that the PO stopped the accumulation of very-low-density lipoprotein and low-density lipoprotein and significantly decrease the total cholesterol values in human body.

Vitamins

Pleurotus mushrooms are relatively high in folic acid (Vitamin B9) which cannot be produced in the body and must be provided through the diet. In addition to this,they also consist small quantity of Vitamin B1 and C and traces of Vitamin B12 and D2. Aditionally,mushroom have the chemical precursor to Vitamin D and similar to humans their level of Vitamin D rise dramatically, when exposed to sunlight.

Minerals

Prime mineral elements in oyster mushrooms are K, Ca, P, Na, Mg while minor elements present are Cu, Fe, Zn, Cd, Mo. Minerals proportions differs according to age, species, substrates and diameter of fruiting body. Higher concentration of potassium and lower concentration of sodium makes mushroom best for anti-hypertensive diet.

Medicinal Importance/Health Benefits

Animals and mushrooms (fungi) used to share a common evolutionary ancestry before 465 million years . Thus, humans and mushrooms are prone to some similar microbes and posses risks of some same infection. This may described as why humans got so much health advantage from mushrooms; the same compounds protect us from infectious agents that mushrooms produce to protect themselves,when we consume mushrooms or mushroom products.

Mushrooms might exhibit health-boosting qualities with immunostimulation (Vaz *et al*. 2011), hypocholesterolaemic activity (Han *et al.* 2011), antihyperlipidemic activity (Opletal *et al.* 1997, anti-tumorigenic (Kim *et al.* 2015), antifungal activity (Ye *et al.* 1999), anti-nociceptive, antioxidation (Roupas *et al*. 2012), antigenotoxicity (Wang *et al*. 2005), anti-

hypertensive, stress-relieving characteristics and is also beneficial for diabetic patients(Akata *et al*. 2012).

Immune booster

It has been found that beta-glucan compound present in oyster mushrooms that boosts the immune system .It trains the immune system to fight against abnormal cells and lowers the harmful effects of chemo and radiation therapies.Commercially available purified beta-glucan extract from these mushrooms also enhance immunity.

May Lower Cholesterol

As per 2015 study,it has been found that accumulation of triglyceride and bad cholestrol in the body may be reduced by the dietary fiber present on mushrooms.These mushrooms are best substitute of red meat with an advantage of reduced fat,calorie and cholestrol (Caz *et al.* 2015).

May Reduce Risk of Cancer

Some preliminary research shows cancer-fighting properties of oyster mushrooms. A study in 2012 demonstrated that breast cancer and colon cancer growth and spread in human cells might be supressed by regular intake of oyster mushrooms. Still researchs are ongoing and more studies are needed to understand the relationship fully (Xu *et al*. 2012).

Improves Metabolic Health

Health professionals often recommend to intake plenty of fiber-rich diets to maintain a healthy weight. But mushrooms may provide an additional advantage of maintaining better metabolic health. The effects of mushrooms on obesity was examined in one study. Researchers found that " metabolic syndrome, including obesity can be effectively treated by regular intake of mushrooms." However, they suggest that it needs to be along with regular physical exercise and dietary and lifestyle alterations (Ganesan and Xu 2018).

Lowers blood pressure

Mushrooms often provides savory taste that lowers the requirement of extra added salt which helps to keep blood pressure low.

Gut health benefits

A 2021 study reported the decreased growth of pathogenic bacteria and increased formation of valuable short-chain fatty acids in the gut of obese rat with supplementation of oyster mushrooms in their diet (Maheshwan *et al.* 2021).

Anti-inflammatory effects

Oyster mushrooms contain anti-inflammatory compounds. A 2020 study on rat revealed that oral treatment with extract of *P. ostreatus* significantly decreased induced paw inflammation (Jayasuria *et al.* 2020).

Packed with antioxidants

As per the University of pennsylvania, oyster mushrooms are one of the best mushroom sources of ergothioneine,an amino acid and an antioxidant which lowers systemic inflammation.According to 2010 research published on "*Journal of Medicinal food*" ergothioneine might prevent plaque buildup in arteries which causes cardiovascular disease.

Increase brain health

A study in Singapore shows that intake of more than two standard portion of mushroom a week(one standard portion is approx 150gram)may reduce the risk MCI and Alzheimer's disease by 57% due to the presence of ergothioneine.

May promote blood sugar regulation

A study in 2007 in 30 type 2 diabetes patients found that consuming 150 grams of cooked P. ostreatus on daily basis for 7 days decreased fasting blood sugar by 22% and post-meal blood sugar by an approx of 23% (Khatun *et al*. 2007).

Also fasting and post-meal blood sugar elevated by an average of 13% and 20% respectively when participants stopped the mushroom intake for 1 week. The treatment also decreased patients cholesterol, blood pressure and triglyceride levels.

A 2020 review shows that these potential blood sugar-reducing effects may be due to the high concentration of beta-glucans in mushrooms, as this kind of fiber lowers down carbohydrate digestion and absorption (Dicks and Ellinger 2020).

May promote heart health

Oyster mushrooms may benefit heart health by decreasing heart disease risk factors like high blood pressure and high cholesterol.*Pleurotus sps* are especially high in fibers called beta-

glucans that promote heart health. A 2020 review demonstrate that *P. ostreatus* consumption help to reduce blood sugar, blood pressure, triglycerides, and insulin levels, which could lower risk of heart disease (Schneider *et al*. 2011).

Utilization of agro wastes for production of oyster mushroom

Crop residues left in the field and agro-industrial leftovers from processing companies are the two categories of agricultural crop wastes.The various agro-waste products are made from plant components such roots, stems, stalks, straw, leaves, and branches that are collected from the field. The by-products produced during the post-harvest processing of crops that produce residues like shell, husk, bran, cob, straw, and other fibrous materials are known as agro-industrial residues.

More than 500 million tonnes of crop waste are produced on and off farms each year in India, according to the Ministry of New and Renewable Energy (MNRE), including 352 Mt by cereals, 66 Mt by fibres, 29 Mt by oilseeds, 13 Mt by legumes, and 12 Mt by sugarcane crop. Wheat, maize, and millets collectively account for about 70% of all crop residue in India among the several cereal crops grown (MNRE, 2009). Crop residue is available as surplus in amounts of roughly 178 Mt, and each year, about 87 Mt of this surplus is burned on farms (Datta *et al*., 2020)

Waste from agricultural biomass contains certain complex polymer such as lignin, cellulose, hemicellulose. Due to the chemical structure and decomposition properties, handling and disposal of these lignocellulosic compounds often becomes problematic. Their accumulation on land causes environmental pollution. Additionally, crop residues incineration causes health issues as well as leads to global warming. Hence, there is always a high necessity of finding an agricultural waste management process which is economical and contribute less in environmenal hazards. Mushroom growing on agricultural wastes actualized these requirements.

These wastes can be recycled and upgraded to higher value and useful products by chemical or biological processes.The bioconversion of agro-wastes to a value-added outcome is a feasible way of their usage. Edible mushrooms are saprophytic fungi and have the ability to degrade lignocellulosic materials by their extensive enzymes and then utilize them to derive nutrients for growing and fructification. Utilization of these wastes by mushrooms as substrate depend on their potentiality to manufacture relevant oxidative and hydrolytic enzymes.

Mushroom cultivation can be taken as the most efficient bioremediation and economical way of converting these lignocellulosic rich agricultural wastes into consumable, protein-rich biomass,.Obtaining edible product by growing mushrooms on industrial or agricultural wastes is an important step in the process of microbial biotechnology, which may otherwise poses environmental hazards such as leaching, global warming etc.

In this context the mushroom cultivation represents one of the economically viable processes for the bioconversion of agricultural wastes in to protein rich food making it a powerful weapon against malnutrition which is most frequently found in developing countries like India.

Combinations of agricultural substrates used for cultivation

In addition to the use of supplements with agricultural wastes as a substrate, various combinations of agricultural wastes are also used for the cultivation and are reported to be optimal substrate. Vegetable waste when used in combinations with paddy straw resulted in high yield of oyster mushroom . To cultivate *P. ostreatus* sawdust in addition to rice husks is reported as an optimal substrate . The quality of *P. eryngii* was significantly affected by substrate ingredients. On barley straw and sugar beet pulp substrate complemented with rice bran, highest mushroom fresh weight and moisture content were achieved . For *Pleurotus sajor-caju*, combination of soybean straw, wheat straw showed significantly highest yield while soybean straw and saw dust combinations showed significantly lesser yield .

Mushroom market insight and COVID-19 impacts

The global mushroom market is expected to reach 24.05 million tonnes in 2028 from 15.25 million tonnes in 2021 at a CAGR of 6.74% during the period 2021-2028. Although COVID-19 has been astonishing and unprecedented, but mushroom witnessed a positive impact on popular demand amid pandemic. 6.3% rise in global market was observed in 2020 than the average yearly growth during 2017-2019.

The consumption behaviour of people changed after pandemic outbreak and mushroom became one of the best vegetarian diet due to nutritional attributes and medicinal benefits like immuno-stimulating, anti viral properties etc. Due to strict lockdown and restriction of peoples mobility,50-80% decline in revenue for mushroom manufacturers were observed during initial months of COVID-19. But gradually, shifting of consumers towards e-commerce platforms and resuming normal functioning of food service sector lead to growth of mushroom market. 816,367 pounds of product were shipped in 2020 according to the U.S Department of

Agriculture with a increase in sales value by 3% to meet USD 1.15 billion (Fortune Business Insights, 2022).

Scope of mushroom cultivation

Mushrooms can be a significant part of a healthy diet and can help rural and peri-urban residents improve their standard of living by generating revenue and ensuring access to food. A wide variety of mushrooms can be grown in India due to its abundant genetic resources and varying environmental conditions around the country. (Sharma *et al.,* 2017).

From a dietary standpoint, mushrooms are a specific cuisine in India, where vegetarians are prevalent. India itself is a huge market for mushrooms with a population of more than one billion people. The production and consumption of mushrooms in India will rise as more people become aware of their nutritional and therapeutic benefits

The technology can be profitably taken up in urban and rural areas where land is limited and chiefly available of agro-wastes. In addition, mushroom cultivation also provides opportunities for improving the sustainability of small farming systems through the recycling of organic matter and then returned to the land as fertilizer. Recently, unemployment is increasing rapidly both in developed and developing countries. In this situation, self-employment can be one important way to increase employment. Mushroom processing and storage can be another option of an enterprise as this is labour consuming and skill oriented. Mushroom cultivation not only provides a gainful employment to Indian rural youths, but the cost of mushroom production per unit area will be greatly reduced (Karthick and Hamsalakshmi, 2017).

The present study entitled **"Studies on cultural techniques of oyster mushroom {*Pleurotus sajor-caju* (Fr.) Singer}"** was done with following objectives:-

- To determine the effect of different media on mycelial growth of oyster mushroom (*Pleurotus sajor-caju).*
- To determine the effect of different substrate on growth parameters and yield potential of oyster mushroom (*Pleurotus sajor-caju).*
- To determine the effect of substrate moisture content on growth period and yield of oyster mushroom *(Pleurotus sajor-caju).*

Chapter-2

REVIEW OF LITERATURE

This chapter provides a brief overview of the key scientific works done in the field of oyster mushroom (*Pleurotus sajor- caju*) production technology. An attempt has been made to present pertinent literature related to the topic **"Studies on cultural techniques of oyster mushroom {*Pleurotus sajor-caju* (Fr.) Singer}".**

A..Evaluation of mushroom culture media and spawn production:-

Nasim *et al*. (2001), examined three different culture media, including malt extract agar medium (MEA), murashige and skoog's medium (MS), and potato dextrose agar medium (PDA), to grow mycelium of the mushrooms *Pleurotus ostreatus* var.*sajar*, *Pleurotus ostreatus* var.*caju*, *Pleurotus ostreatus* var.*citydeosus*, and *Volvariella volvacea* to see how they affected the growth of oyster and Chinese mushroom mycelia. Plates with MEA medium had the quickest mycelial growth, while those with PDA medium expanded most slowly.

Hussain and Hussain (2004), studied mycelial growth of *Pleurotus ostreatus* on potato dextrose agar (PDA), maize meal agar (CMA), malt extract agar (MEA), and potato dextrose ispghol (PDI) medium and spawn run on sorghum, wheat and corn seeds. The results revealed that potato dextrose agar was the optimal medium for fungal development. The fastest mycelial growth was noted on sorghum, significantly different from wheat and corn seeds.

Asghar *et al.* (2007) examined mycelial growth of pure culture of *P. sajor-caju* at 25°C to determine the optimal medium among PDA and MEA. MEA was found to be the best for quicker mycelial growth, with an average colony diameter of 8.60 cm in just 7 days. The size of the colony diameter was found considerably affected by both medium and time intervals, according to the analysis of variance.

Amin *et al*. (2008) used four growth mediums namely potato dextrose agar (PDA), potato dextrose yeast agar (PDYA), malt extract agar (MEA) and yeast extract agar (YEA) for the generation of *Cordyceps sinensis* mycelium. *Cordyceps sinensis* had the best mycelium growth in PDA. The thickness of mycelial development in PDA and MEA media was substantially larger at P 0.05 than in PDYA and YEA media.

Ukoima *et al*.(2009) investigated mycelial development of *Pleurotus sajor-caju* on various culture media viz. rice bran/soil culture media, cassava/soil culture media, palm fibre culture media, potato dextrose agar culture media and yeast agar culture media. On rice bran/soil culture media, *P. sajor caju* showed the maximum mycelia growth (7.8 cm), according to the findings. On potato dextrose agar culture media and yeast agar culture media, the smallest mycelia development (1.5-4.4 cm) was found.

Munsur *et al.* (2012) evaluated various media such as PDA, YMA, MS for the mycelial colony proliferation of several mushroom species such as oyster, milky and button and various substrates namely wheat, rice and wheat bran for spawn production of oyster mushroom. The best mycelial development was observed in oyster mushroom with PDA media at 16 DAI, while the lowest mycelial growth was observed in button mushroom with MS at 2 DAI in the first trial. It was found that wheat substrate was the ideal substrate for spawn production of oyster mushroom which was followed by rice and wheat bran.

Yadav and Chandra (2014) investigated five culture media viz. potato dextrose agar(PDA), malt extract agar(MEA), wheat extract agar(WEA), compost extract agar(CEA), Yeast extract potato dextrose agar(YPDA) for mycelial development of five strains PL-1, PL-2, PL-3, Psc-1 and Psc-2 of *Pleurotus* species and found that mycelia growth on YPDA was better after eight days of inoculation.

Hoa *et al*. (2015) explored the effects of nutritional circumstances on the mycelium growth of the Pleurotus ostreatus (PO) and Pleurotus cystidiosus (PC). The experiment revealed that potato dextrose agar (PDA) and yam dextrose agar (YDA) were the best media for oyster mushroom (PO), while four media (PDA, YDA, sweet potato dextrose agar, and malt extract agar medium) were not significantly different in supporting oyster mushroom (PC).

Sardar *et al.* (2015) cultivated *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm, Pleurotus *sajor-caju* (Fr.) Singer, Pleurotus *eryngii* (DC.) Quel, Pleurotus *columbinus* (DC.) Quél (exotic), and Pleurotus *sapidus* (Schulzer) Sacc. (FW-133) on different agar medium, namely PDA (Potato dextrose agar), WEA (Wheat extract agar) and MEA (Malt extract agar). PDA was found to be the best medium for the growth of mycelium of all Pleurotus species, outperforming MEA and WEA. PDA may have more carbon sources and nutrients for mushroom mycelia in petri plates.

Tudses *et al*. (2016) compared three different yam-based culture media, i.e. DEDA (*D. esculenta* Dextrose Agar), DADA *(D. alata* L. Dextrose Agar), and DPDA (*D. pentaphylla* Dextrose Agar) with PDA (Potato Dextrose Agar) for mycelial growth of different mushrooms. The highest mycelial growth was 9.00±0.00 cm on DPDA-30 for *V. volvacea*, 5.47±0.42 cm on DADA-20 for *P. sajor-caju* (Fr.) Sing., 3.73±0.06 cm on DADA-25 for *L. edodes*. However thin mycelia was observed on DADA, DEDA and DPDA medium than on PDA.

Shendge and Surywanshi (2016) investigated the effect of different culture media (Ashby's manitol agar, Yeast extract agar, Czapeck's dox agar, Yeast manitol agar, Malt extract agar, Potato malt agar, Corn meal agar, and Potato dextrose agar) and aqueous extracts of cereal and pulse grains (wheat, sorghum, maize, mungbean, pearl millet, pigeonpea, chickpea and soyabean) for mycelial growth of *Pleurotus florida* in vitro. Potato dextrose agar had the highest mycelial growth (90.00 mm) among the culture media examined, followed by Czapeck's dox agar (77.33 mm) and Yeast extract agar (66.67 mm). Yeast manitol agar, on the other hand, produced the least amount of growth (21.90 mm). Sorghum grain extract (79.21 mm) had the highest mycelial growth among the grains aqueous extracts that were examined, followed by wheat grain extract (77.21 mm), maize grain extract (76.55 mm), and mungbean grain extract (74.70 mm).

Ishaq *et al*. (2017) determined the optimal medium and best cereal grains for creating pure cultures and spawn respectively. Potato Dextrose Agar (PDA), Potato Sucrose Agar (PSA), Malt Extract Agar (MEA), Corn Meal Agar (CMA), Yeast Extract Agar (YEA), and Water Agar (WA) were used to make pure culture. Potato dextrose agar had the fastest mycelial growth (11.100 mm/day) of all the media. sorghum, wheat, oat, bajra, bean, maize, soybean, chick pea, and pegion pea grains were utilised in three ways for the preparation of spawn: Control, Addition of Lime 2 %, and Gypsum 4 % + Lime 2 %. The Sorghum grains with Gypsum 4 % + Lime 2 % showed the greatest mycelial growth, 10.658 mm/day.

Mahadevan and Shanmugasundaram (2018) compared *Pleurotus sapidus* growth on a variety of culture media, including Potato Dextrose Agar Medium (PDA), , Glucose Peptone Agar Medium (GPA), Malt Extract Agar Medium (MEA) Yeast Malt Agar Medium (YMA), Saboraud's Dextrose Agar Medium (SDA), and Czapek Dox Agar (CDA). *Pleurotus sapidus* had the maximum mycelial diameter 9.00±0.01cm, density 5+, and growth rate 1.1 cm/day on

YMA media, followed by PDA media with colony diameter 8.98 ±0.04cm, density 5 +, and growth rate 1.1 cm/day on 8th day. On SDA medium, growth was slowest(34.8mm).

Kumar *et al.* (2018) employed six culture media viz wheat extract agar, potato dextrose agar, malt extract agar, Hawkers, Richards, and Czpack dox to investigate mycelial growth parameters. *P. sajor-caju* had the highest average mycelia growth (61.97mm) on wheat extract agar, followed by potato dextrose agar (50.13 mm) and malt extract agar (37.75 mm). After 6 days, Richards had the least amount of mycelial growth.

Naik *et al*. (2020) evaluated the suitability of different culture media for *Pleurotus ostreatus* mycelia growth i.e. wheat extract agar, yeast extract agar, and potato dextrose agar medium , with results showing that wheat extract agar medium supported faster growth, taking 9 days to completely cover a 9 cm diameter petri plate, compared to PDA and Yeast agar medium, which took 10 and 11 days respectively.

Nguyen *et al.* (2020) examined the effectiveness of various culture media, including potato dextrose agar (PDA), yeast malt agar (YMA), and malt extract agar (MEA) and selected grains (wheat, rye, barley and oat) to stimulate the mycelial development and spawning of *P. eryngii* and *P. ostreatus* . PDA media was the best for *P. eryngii* mycelial growth, whereas YMA and MEA media were superior for *P. ostreatus* mycelial growth. While oat grains were the best source for enhancing *P. ostreatus's* mycelial extension and density levels, barley and rye grains were the most favourable for *P. eryngii's* mycelium growth.

Kannaujia *et al.* (2020) studied effect of growth regulators on spawn quality of *Pleurotus sajor-caju.* GA, IAA, and NAA growth regulators were utilised at 10 and 20 ppm in seven treatments with three replications. In GA 20 ppm, the maximum spawn growth (90.00 mm) was recorded. However, the control group had the smallest spawn growth (67.00 mm). In GA 20 ppm, the maximum growth rate (6.00 mm/day) was reported. In the control , however, the minimal growth rate (4.46 mm/day) was recorded.

Godse *et al.* (2021) recorded data on the influence of several growth regulators and micronutrients on *Pleurotus sajor-caju* mycelial growth in vitro. Data collected 9 days after inoculation (DAI) showed that GA 20 ppm had the largest mycelial colony diameter of 90.00 mm, followed by GA 15 ppm with a colony diameter of 89.98 mm. Indole-3-acetic acid (IAA) 20 ppm, indole-3-butyric acid (IBA) 20 ppm, IBA 15 ppm, GA 10 ppm and IBA 10 ppm were

the following treatments in order of efficacy, with colony diameters of 80.70, 80.60, 80.45, 80.32 and 70.95 mm, respectively. In control plates, the colony diameter was significantly smaller, at 50 mm.

B.Evaluation of agricultural waste:-

Bisaria *et al.* (1987) cultivated *Pleurotus sajor-caju* on a variety of agricultural leftovers and combinations. Sarkanda leaves, paddy straw, wheat straw, banana leaves, sugarcane bagasse, guar straw, jowar straw, bajra straw, cotton seeds and ficus fruits were all obtained from local sources. Paddy straw combined with cotton seeds was shown to have the highest biological efficiency.

Ragunathan *et al.* (1996) investigated the growth and yield potential of *Pleurotus sajor-caju, Pleurotus platypus* and *Pleurotus citrinopileatus* on a variety of agricultural wastes, including paddy straw, maize stover, sugarcane baggase, coir pith, and a combination of these wastes. *P.sajor-caju* yielded the most from paddy straw, while *P.citrinopileatus* and *P.platypus* yielded the most from sugarcane baggase and coir pith, respectively.

Madan and Patrabansh (1997) employed three different types of biomass, namely *Populus deltoides, Eupatorium adenophorum* and sericulture waste for the production of *Pleurotus sajor-caju* separately and in combination with paddy straw. Except for sericulture waste, *P. sajor-caju* had excellent colonisation potential on various substrates when applied alone. When *P. deltoides* and *E. adenophorum* were grown on pure substrate, their biological efficiency was 75 and 77 percent respectively, but when *P. sajor-caju* was grown in a 1:2 mixture with paddy straw, it climbed to 102 percent. The percentage of cellulose, hemicellulose, and lignin degradation revealed that *P. sajor-caju* can utilise all three primary components.

Tupatkar and Jadhao (2006) cultivated oyster mushrooms on wheat and paddy straw, bajara, maize, jowar, cotton, soybean stalks and leaves, and groundnut creepers with wheat and soybean straw (1:1), as well as groundnut creepers alone. Cotton stalks and leaves were discovered to have much larger numbers and weights of sporophores (5.12 g) as well as yield of sporophores (914 g/kg of dry straw) than other substrates. Other substrates that produced the highest yields were paddy straw (613 g/kg of dry straw), soybean straw (557 g/kg of dry straw), and a 1:1 w/w combination of soybean straw and wheat straw (508 g/kg of straw). The treatment of groundnut creepers had the lowest yield (258 g/kg dry substrate).

Mane et al. (2007) cultivated *Pleurotus sajor-caju* on cotton stalks, groundnut halves, soybean straw, pigeon pea stalks and leaves and wheat straw, either alone or in combination. Cotton stalks, pigeon pea stalks, and wheat straw were found to be more suited for cultivation than groundnut haulms and soybean straw, either alone or in combination. Organic additions such peanut oilseed cake, gram powder and rice bran improved growth metrics while also increasing yields.

Fanadzo *et al.* (2010) conducted a study to see effect of different substrates such as wheat straw, maize stover, thatch grass and oil/protein rich supplements (maize bran, cottonseed hull} on the biological efficiency of two oyster mushroom species (*Pleurotus sajor-caju* and *P. ostreatus).* When it came to cultivating *P. sajor-caju,* wheat straw outperformed maize stover and thatch grass.

Patil (2012) produced *Pleurotus sajor-caju* on a number of agro wastes including soybean straw, paddy straw, wheat straw, groundnut straw, sunflower stalk, and pigeon pea stalk in order to assess their appropriateness for yield and biological efficiency. *P. sajor-caju* yielded the most when grown on soybean straw (845.66 gm/kg straw) with 84.56 percent B.E., followed by paddy straw (836.66 gm/kg straw) with 83.66 percent B.E., and pigeon pea stalk (716.33 gm/kg straw) with 83.66 percent B.E.

Ashraf *et al.* (2013} cultivated *Pleurotus sajor-caju*, *P. ostreatus* and *P. djmor* on three different substrates: cotton waste, wheat straw, and paddy straw to investigate the effect of different agricultural wastes on mushroom development and yield. Cotton waste had the quickest spawning, primordial initiation, harvesting stage, largest number of fruiting bodies, and maximum yield in the shortest amount of time. Paddy straw had the highest yield in the first flush, with no significant changes from cotton waste, whereas cotton waste had the highest yield in the second and third flushes.

Dehariya and Vyas (2013) cultivated *Pleurotus sajor-caju* on a variety of agro-wastes including soybean straw, wheat straw, paddy straw, sugarcane bagasses, sunflower stalks, maize stalks, domestic waste, used tea leaves, fruit waste, semal flowers, news paper, bamboo leaves, saw dust and their mixtures in 1:1 proportions to see how these agro-wastes affected yield, growth, and biological efficiency. The highest yielding crop was soybean straw. Semal flowers had a shorter spawn run (14.33 days) and pin-head formation (18.33 days) duration.

Soybean straw + wheat straw yielded the most (87.3 percent B.E.) of all the combinations, while soybean straw + saw dust yielded the least (87.3 percent B.E.). Other test substrates, besides saw dust and news paper, were found to be suitable for growth of *Pleurotus sajor-caju*.

Ram *et al*.(2013) compared the growth patterns and prospective yields of five different oyster mushrooms. Spawn run duration varied from 18-21 days and the pinhead initiation was noted between 21-26 days after inoculation. The first flush was collected between 26 to 29 days, the second flush between 35 to 41 days, and the third flush between 48 to 53 days. It was observed that the yield of first and second flush were comparatively more than the yield of third flush.

Pokhrel *et al*.(2013) studied on mycelial growth, colonisation time, primordial initiation, harvesting time, yield, mushroom size and biological efficiency (BE) of *Pleurotus sajor-caju* on three different substrates: maize stalk, pea residue (tendrils), and banana leaves, with and without rice bran and chicken manure supplementation. Maize stalk with rice bran produced the fastest mycelial development and the highest yield (348.13 g per 25 cm x 15 cm bag) with 87.03 percent BE, whereas pea residue with rice bran produced the second best yield (299.53 g) with 74.88 percent BE. Maize stalk revealed to be the most effective substrate, followed by pea waste and banana leaves.

Nurudeen *et al.* (2015) investigated the effect of different substrates on yield of *Pleurotus sajor-caju*. The substrates for mushroom cultivation were sawdust, coconut husk, and maize cob. The mean yield (g) produced from corn-cob substrates was found to be greater, followed by sawdust and coconut husk with yield values of 108.74±7.87, 60.76±4.62 and 56.66±3.48 respectively. The length of the stipe, diameter of the pileus, and mushroom height indicated that the mushrooms grown from all of the substrates were of a marketable size.

Borkar *et al*.(2015) employed a variety of readily available local substrates, including paddy straw, coconut husk, arecanut husk, banana pseudostem, groundnut shells, sugarcane bagasse, and wheat straw, separately and in combination with rice bran and wheat bran. Highest biological efficiency of *P. pulmonarius* was found on paddy straw (76.30%) which was followed by wheat straw (74.53%). The maximum biological efficiency of the mushroom was observed on paddy straw treated with wheat bran (85.40%) in the case of supplemented

substrates. Following this were paddy straw supplemented with rice bran (82.63%) and wheat straw supplemented with wheat bran (82.26%).

Chand *et al*.(2015) examined various substrates, including paddy straw, wheat straw, maize stalk, lentil straw, banana pseudostem and water hyacinth for growth and yield of *Pleurotus sajor-caju.* Paddy straw had the highest yield (296.0 g/500 g) and biological efficiency (60%), followed by wheat straw (209.5 g/500 g) and biological efficiency (42%). Water hyacinth yielded lowest fruit (17 g/500 g) while lowest biological efficiency was on lentil straw (13.1%).

Jatwa *et al*. (2016) cultivated *P. florida, P. eous,* and *P. sajor-caju* on various agro-wastes, including paddy straw, wheat straw, sorghum straw, soybean straw, paddy + wheat straw (1:1), paddy + sorghum straw (1:1), paddy + soybean (1:1), wheat + soybean straw (1:1), wheat + sorghum straw (1:1) and soybean + sorghum straw (1:1). Paddy straw produced the highest yields of 1248.3, 1348.7, and 1275.7 g/1.5 kg of dry substrate for *P. florida, P. eous,* and *P. sajor-caju* respectively.

Masevhe *et al*. (2016) looked at using suitable substrates for growing oyster mushrooms. Wheat straw (control), wood chips, and thatch grass were examined. Wheat straw and thatch grass had a considerably larger cumulative number of flushes, caps and fresh mass of oyster mushrooms than wood chips. The findings showed that thatch grass could be a feasible replacement to wheat straw, which is typically utilised.

Hossain (2017) investigated how different substrates, such as paddy straw, wheat straw, banana leaves, sugarcane bagasse, sugarcane leaves, newspapers, and maize stalks and leaves affected spawn running time, primordial initiation time, fruiting body formation time, yield performance and biological efficiency of *Pleurotus sajor-caju*. Sugarcane bagasse had the shortest time required for spawn running, primordial initiation, and fruiting body formation followed by newspapers, paddy straw, banana leaves, and wheat straw. Paddy straw had the best production and biological efficiency among the substrates followed by banana leaves, wheat straw, sugarcane bagasse, newspapers, and sugarcane leaves. Maize stalks and leaves produced the least amount of mushroom.

Toppo and Chandravanshi (2018) employed locally accessible agrowastes such maize stover, wheat straw and paddy straw singly and in combination as a substrate for *Pleurotus florida*. Numerous factors were evaluated including spawn run time, formation of pinheads, formation of fruit bodies and yield. Results showed maize stover provided better result than wheat straw, sugarcane bagasse and paddy straw for spawn running(15-20 days) and pinhead formation (18-25 days) whereas, total cropping period of *Pleurotus florida* was higher in paddy straw and wheat straw + maize stover (34 days). Highest yield (595.8 grams) with maximum biological efficiency of 66.2% was noted for paddy straw+ corn stover followed by wheat straw + maize stover (60.99%).

Dubey *et al.* (2019) studied how different substrates affected oyster mushroom (*Pleurotus sajor- caju)* performance. Rice straw, wheat straw, banana leaves and sugarcane bagasse were used as substrates for oyster mushroom cultivation. Rice straw produced the highest yield (1515 gram) with the longest stipe length (4.86 cm) and largest cap diameter (5.14 cm), followed by other substrates. Wheat straw and banana leaves had a shorter colonisation period (19 days), while wheat straw had a shorter fruiting period (20.5 days).

Soni *et al*.(2020) investigated the potential use of wheat straw, rice straw, mustard straw, maize straw and mixture of wheat and rice straw were used for the cultivation of oyster mushroom. Wheat straw performed significantly better on growth and yield of oyster mushroom (*P. florida)* compared to other substrate. Other substrates such as wheat straw + rice straw, mustard straw and maize straw was found effective as alternative substrates for the cultivation of *P. florida*.

Khatana *et al*. (2022), conducted a study to evaluate the efficacy of relatively low-cost, readily available crop wastes such as cotton, rice, wheat, mustard, and water chestnut for *Pleurotus eryngii* strains P9 (China) and P10 (PSU-USA) for yield and nutrition enhancement . When compared to other substrates, cotton waste had much better morphological qualities, such as mycelium run, fruit growth, yield, and biological efficiency.

2.3.Evaluation of different substrate moisture content :-

Chang and miles (2004) recommended that in order to allow *Pleurotus* spp. to grow satisfactorily, the proper moisture content in the substrate should be in a range of 50% -75%.

Oei and Nieuwenhuijzen (2005) advocated that water is one of the main factors that influence the success in mushroom growth. Nutrients are transported from the mycelium to the fruiting bodies by a steady moisture flow. Low moisture content will result in the death of the fruiting body.

Souza *et al.* (2006) examined the effect of initial substrate moisture on growth of *P. pulmonarius* and found that the initial moisture content has a significant impact on fungal growth. Visual growth was noticed earlier with moisture level of 75–86% than in cultures with a moisture content of 45–72%. The fungal hyphae penetrated and adhered securely to the substrate in cultures with an initial moisture level of less than 80%. Growth was characterised by the production of a massive mycelial mass above the substrate in cultures with an initial moisture content greater than 86 percent, which could be easily removed from the substrate with a spatula. Maximum laccase production (9,600 U/g substrate) was obtained using cultures of 75% initial moisture content after five days of cultivation.

Shen *et al.*(2008) examined the impact of three different substrate moisture contents (50 percent, 55 percent, and 60 percent) on mushroom production (g/log) and biological efficiency in two crops of shiitake (Crops 1 and 2).The formulation with a substrate moisture content of 55% produced the highest yield and BE. When compared to logs with a moisture level of 60% in Crop 1, yields on logs with a moisture content of 55% or 50 % were 16.1–16.7 percent higher. In Crop 2, yields were 21.1–14.1% higher on logs with a moisture content of 50–55% compared to logs having 60% moisture.

Patel *et al*.(2009) suggested increasing moisture level reduced the porosity of the substrate, thus limiting oxygen transfer. For this reason, the use of high moisture content limited the growth within the whole substrate, resulting in surface growth.

Buah *et al.*(2010) drained off excess moisture from the substrates to obtain 65-75% moisture level to investigate the cultivation of oyster mushroom (*Pleurotus ostreatus*) on different substrates.

Samuel *et al.(*2012) made around 65% moisture content on various substrates composition to evaluate growth performance and yield of oyster mushroom *(P.ostreatus*) in Buea South West Cameroon 1.

Yang *et al.(*2013) adjusted water content of the final mixture to 65% (w/w) to cultivate oyster mushroom (*Pleurotus ostreatus*) on rice straw basal substrate, wheat straw basal substrate, cotton seed hull basal substrate alone or with supplements.

Tesfaw *et al.* (2015) maintained moisture content of the substrates (wheat straw, barely straw, sinar straw and saw dust) from 69.8% to 74.5% for mycelial growth of *P. ostreatus.*

Significantly the highest mycelium run rate was recorded at 70% moisture level.

Girmay *et al.(*2016) drained off excess water until the moisture content was brought to 65–70% in four substrates (cotton seed, paper waste, wheat straw, and sawdust) to test their efficacy in oyster mushroom production.

Pathania *et al*.(2017) removed the excessive moisture from the substrates to get 65-75% moisture level to investigate the cultivation of oyster mushroom on horticultural waste i.e. apple pomace and wheat straw mixed in the different ratio.

Pani *et al.* (2017) carried out an experiment to ascertain the impact of substrate moisture on the growth of the straw mushroom *(Volvariella volvacea)*. Prior to spawning, the substrate was kept at various moisture contents, including 0 (dry straw without soaking), 10, 20, 30, 40, 50, 60, 70, 80, and 90 percent. When compared to other treatments, it was discovered that soaked paddy straw with 60 percent moisture maintained the best production of mushrooms (1214.7 g, 12.1% BE). The yield increases in direct proportion to the rise in moisture content, which ranges from 10% to 60%. As the moisture content rose from 60 to 90 percent, the yield gradually decreased.

Pani *et al.* (2017) allowed the top/covering layer's moisture content to vary, ranging from 0 (dry straw, without soaking), to 10, 20, 30, 40, 50, and 60%, while the remaining three layers contained 60% moisture. The moisture content of 30 percent on top layer was found to be ideal for supporting the biggest amount of fruiting (463.7 g) on top, which resulted in the bed's best overall production (1416.4 g, 14.1 percent BE. This was substantially greater than the standard practise of having layers with a moisture content of 60% (1140.7 g, 11.4 percent BE).

Chapter-3

MATERIALS AND METHODS

3.1. Collection and Maintenance of mushroom culture:-

The pure culture of oyster mushroom, *Pleurotus sajor-caju* (strain DMRP-112) was obtained from the ICAR-Directorate of Mushroom Research (DMR), Chambaghat, Solan, Himachal Pradesh. The pure culture was sub-cultured and maintained on potato dextrose agar (PDA) medium in BOD incubator at 25^{o}C for further investigation.All experiments were carried out at Mushroom Spawn Laboratory, Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University.

3.2.Preparation of culture media:-

Five culture media: Potato Dextrose Agar (PDA), PDA+Indole Butyric Acid (IBA), Chickpea Dextrose Agar (ChDA), Corn Dextrose Agar (CDA) and Rice Dextrose Agar (RDA) were prepared to study the mycelial growth of oyster mushroom (*Pleurotus sajor-caju).*

Table 3.1.Composition of different culture media used for the mycelial growth of *Pleurotus sajor-caju*

Ingredients	PDA+IBA	PDA	CDA	RDA	ChDA
Pieces/grain	200 g	200 g	200 g	200 g	200 g
Dextrose	20 g	20 g	20 g	20 g	20 g
Agar	20 g	20 g	20 g	20 g	20 g
Distilled water	1000 ml	1000 ml	1000 ml	1000 ml	1000 ml
IBA	2 ml	-	-	-	-

3.2.1.Potato dextrose agar medium

Peeled and sliced potatoes were boiled in 500 ml of water for 20 minutes and filtered with muslin cloths. 20 gm dextrose and 20 gm agar were added in the filtrate and distilled water was added to make the final volume of 1000 ml. The mixture was stirred until homogeneous and cooked until boiled. The prepared medium was then filled in clean conical flasks(500ml) at $2/5^{th}$ of their capacity and plugged with non-absorbent cotton. The medium was sterilized in autoclave at 121^0C (15psi) for 30 minutes.

3.2.2.Alternative culture media

Alternative culture media such as chickpea dextrose agar medium, corn dextrose agar medium and rice dextrose agar medium were prepared in the laboratory. Each grain material was washed under running water and drained. Chickpea/corn/rice as much as 200 grams was added to 500 ml of distilled water and boiled for 30 minutes. The decoction was filtered using muslin cloth. 20 gm dextrose and 20 gm agar was added to the filtrate and made it to a final volume of 1000 ml by adding distilled water. Media were sterilized in autoclave at 121^0C (15psi) for 30 minutes.

3.3.Assessment of radial growth

The assessment of radial growth of *Pleurotus sajor-caju* was done using Potato Dextrose Agar, Chickpea Dextrose Agar, Corn Dextrose Agar, Rice Dextrose Agar and PDA+IBA media. For evaluation of radial growth of mycelium, approximately 20 ml of culture medium was poured in each petri plate. A mycelial bit of 5mm was cut from pure culture of mushroom using a cork borer and transferred top down onto the center of the formulated media namely CDA, PDA, ChDA, RDA and PDA+IBA. This process was performed in laminar flow under aseptic conditions. These inoculated petridishes were then incubated at 25 ± 2°C and the radial mycelial growth was noted in every 2 days interval for 8 days. Three replications were maintained for each culture media.

3.4. Mycelial Density

The mycelial density was observed as described as follows

\+ = Very Scanty mycelial density

2+ = Scanty mycelial density

3+ = Moderate mycelial density

4+ = Abundant mycelial density

5+ = Very abundant mycelial density.

3.5.Mycelial growth

Mycelial growth was determined using a ruler across the Petri-dish horizontally

3.6.Preparation of mushroom spawn:-

Requirements:-

Wheat grains

Calcium sulphate

Calcium carbonate

Saline glass bottles(500ml)

Non absorbent cotton

Pure culture of *Pleurotus sajor-caju*

Procedure:-

i. Well-cleaned and healthy wheat grains were washed thoroughly on running tap water and boiled for 30 minutes.

ii. Excess water was drained off after boiling and the grains were evenly spread on a plastic tray for cooling.

iii. After cooling calcium carbonate (chalk powder) and calcium sulphate (gypsum) were mixed at the rate of 1 and 2.0% respectively on dry weight basis of wheat grains to maintain pH and avoid clumping amongst the grains.

iv. Thereafter, about 330 gram grains per bottle were filled in saline glass bottles (500 ml capacity) and plugged with non- absorbent cotton.

v. The plugs were wrapped with aluminum foil and then grain-filled bottles were steam sterilized in autoclave at 121°C (15 psi) for 1 hour and after that allowed to cool at room temperature.

vi. Sterilized and cooled grain filled bottles were aseptically inoculated with mycelial bits of 10 days old mushroom culture of *Pleurotus sajor-caju* maintained on PDA.

vii. These inoculated bottles were incubated at 25 ± 2°C in B.O.D. incubator till the white mycelium covered all grain surfaces.

viii. These bottles were shaken at 4 days interval to allow proper spread of the mycelium between the grains.

ix. The prepared mushroom spawn was used for spawning.

3.7.Assessment of growth parameters and yield potential on different substrate and moisture content:-

Six different substrates namely wheat straw, rice straw, wheat straw + rice straw (1:1), wheat straw + maize straw (1:1), maize straw + rice straw (1:1) and wheat straw + maize straw + rice straw (1:1:1) and five different moisture levels viz. 50%, 55%, 60%, 65% and 70% on wheat straw were evaluated for assessment of growth and yield potential of *Pleurotus sajor-caju*.

Preparation of substrates:-All the substrates used for mushroom cultivation were collected from Agro farm, B.H.U. For preparation of substrate, chopped wheat, rice and maize straw were soaked in water overnight along with 10 gm of carbendazim and 120 ml of formalin. Next day, excess water was drained off and substrates were spread as thin layer on cemented floor to get rid of chemicals. Well prepared substrate were used for spawning.

Moisture content determination:- Wheat straw was used to study the suitable substrate moisture content for better growth and yield of *Pleurotus sajor-caju.* Different moisture content of the substrate i.e. 50%, 55%, 60%, 65%, 70% were maintained for spawning. Dry weight of wheat straw was determined by oven - drying method. Moist weight was maintained by drying over-night water soaked substrate as per the needed moisture content.

Spawning and cropping:- The prepared substrates were well mixed with freshly prepared mushroom spawn. Spawning was done in five layers and spawning rate was 3.5% of wet substrate. The bags were sealed with rubber bands. 8-10 holes were made on the polythene bags for proper aeration.The substrate bags were kept inside the growing room having temperature of 25-30^{0}C and relative humidity of 85-90%. After complete colonization of substrate by mushroom mycelium (spawn run),the polythene bags were cut with a sharp sterilized blade and removed fully from the substrates. Water was sprayed on the bed from

second day of opening using an hand atomizer. The watering was withheld a day before harvesting.Three replications were done for each treatment.

Harvesting:- The pinhead was initiated after 5-6 days of the bag opening. First flush of the mushroom was obtained within 3-4 days of pinhead appearance. Mature sporophores were picked up just before the edges of the pileus begin to fold or curl upwards. Picking was done by slight twisting and pulling of sporophores.

Observations:-

Following observations were recorded during experiment

A.Growth behavior(in days)

Spawn run period

Pinhead formation

First harvesting

Second harvesting

Third harvesting

Total crop period

B.Growth parameters

Average number of fruiting bodies

Average weight of fruiting body(gm)

Average stalk length(cm)

Average cap diameter(cm)

Average stalk diameter(cm)

C.Yield potential(gm)

Yield of first harvest

Yield of second harvest

Yield of third harvest

Total yield

3.7.Statistical analysis

The experiment was set up in a completely randomised design (CRD) with three replications maintained for each treatments.The results obtained from experiments were statistically analysed. The analysis of variance (ANOVA) was done to understand the critical difference (C.D.) between the parameters of different objectives at 5% level of significance.

Chapter-4

RESULTS AND DISCUSSIONS

The present experiment entitled **"Studies on cultural techniques of oyster mushroom {*Pleurotus sajor-caju* (Fr.) Singer}**" was conducted in Mushroom Spawn Laboratory, Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University during the year 2021-22. This chapter presents and discusses the findings of experiments conducted in accordance with all the objectives of this study.

4.1. Suitability of different culture media for mycelial growth of oyster mushroom (*Pleurotus sajor-caju*)

Various indicators for assessing the growth of mushroom mycelia on culture media includes colony diameter, growth rate and mycelial density.

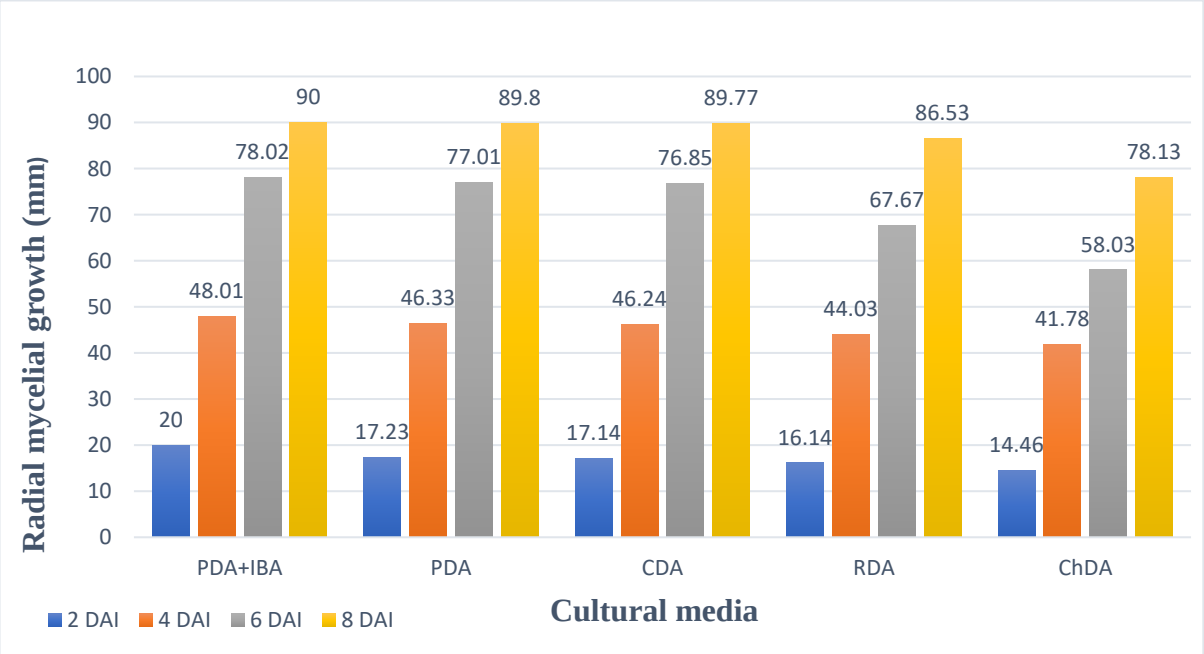
4.1.1.Radial mycelial growth

The tests of different culture media on the mycelial growth of *Pleurotus sajor-caju* showed the results as mentioned in **Table 4.1.** Mycelial growth was assessed at 2 days interval for 8 days on five different types of culture media namely Potato Dextrose Agar (PDA), Potato Dextrose Agar + Indole Butyric Acid (PDA+IBA), Corn Dextrose Agar(CDA), Chickpea Dextrose Agar(ChDA) and Rice Dextrose Agar(RDA). All tested media were able to support the growth of *Pleurotus sajor-caju*. Three media namely PDA+IBA, PDA and CDA were found very close in supporting radial mycelial growth and maximum mycelial growth (90.00 mm) was recorded on PDA+IBA media on 8th day followed by 89.90 mm in PDA medium and 89.77 mm on CDA medium respectively. Moreover, colony diameter was 86.53mm on RDA media. On ChDA media, the tiniest growth was observed, with a colony diameter of 78.13 mm.

Table 4.1.Radial growth measurement of oyster mushroom (*Pleurotus sajor-caju*) on different culture media.

Culture medium	Radial mycelial growth(mm)			
	2nd Day	4th Day	6th Day	8th Day
PDA+IBA	20	48.01	78.02	90
PDA	17.23	46.33	77.01	89.80
CDA	17.14	46.24	76.85	89.77
RDA	16.14	44.03	67.67	86.53
ChDA	14.46	41.78	58.03	78.13
SEM	0.011	0.008	0.009	0.006
CD(5%)	0.035	0.025	0.029	0.018

Where, PDA+IBA-Potato Dextrose Agar + Indole Butyric Acid, PDA-Potato Dextrose Agar, CDA-Corn Dextrose Agar, RDA-Rice Dextrose Agar and ChDA-Chickpea Dextrose Agar.



4.1.2.Mycelial growth rate

Mycelial growth rate was determined by dividing colony diameter on the last day with total number of days observed. Similar mycelial growth rate of 11.25 mm/day was observed on three

culture media viz. PDA+IBA, PDA and CDA. This was followed by RDA media with mycelial growth rate of 10.82 mm/day. ChDA media showed least growth rate of mycelium i.e. 9.77 mm/day. **Table 4.2.**

4.1.3.Mycelial density

On PDA+IBA and PDA media, mycelial density of mushroom mycelia was quite high (5+). It was plentiful (4+) on ChDA, but moderate (3+) on CDA and RDA. **Table 4.2.**

Table 4.2. Mycelial growth rate and density of *Pleurotus sajor-caju* on various culture media

S.No.	Culture medium	Mycelial growth rate(mm/day)	Mycelial density
1.	PDA+IBA	11.25	5+
2.	PDA	11.25	5+
3.	CDA	11.25	3+
4.	RDA	10.82	3+
5.	ChDA	9.77	4+

Where, 5+ = Very abundant, 4+ = Abundant, 3+ = Moderate

Overall, PDA medium supplemented with Indole Butyric Acid(IBA) was found superior among all the treatments in all the cases i.e. colony diameter, growth rate and mycelial density.

The results are in accordance with findings of Syamsia *et al.(*2021), who reported similar fungal growth rate in media like potato dextrose agar, corn dextrose agar and rice dextrose agar but on medium corn and rice, the texture was slightly thin.

Mahadevan and Shanmugasundaram (2018) reported the colony diameter of *Pleurotus sapidus* 8.98 ±0.04cm, density 5 +(very abundant), and growth rate 1.1 cm/day on PDA media which matched our findings.

Results are also similar with findings of Tudses *et al.* (2016) who investigated that growth of *Pleurotus sajor-caju* mycelium was supported by different yam-based media and growth rate was also found comparable to PDA. However, the mycelia were thinner on these media than on PDA.

Our results are also in accordance with findings of Godse *et al.* (2021) who observed the increased mycelial growth of *Pleurotus sajor-caju* when supplemented with growth regulators like IBA in PDA rather than in PDA alone.

4.2. Effect of different substrates on growth parameters and yield potential of *Pleurotus sajor-caju*:-

The growth behaviour of the oyster mushroom, *Pleurotus sajor-caju* with regard to spawn run period, pin head initiation, harvesting of three flushes and total crop duration was studied using six different substrates, namely wheat straw, rice straw, wheat straw + rice straw (1:1), maize straw + wheat straw (1:1), maize straw + rice straw (1:1), and wheat straw + maize straw + rice straw (1:1:1). The average number of fruiting bodies, average weight of fruiting bodies, average cap diameter, average stalk length and average stalk diameter were various yield parameters being observed. The yield of first, second, third and total yield were also recorded. (**Table 4.3., 4.4. and 4.5.**).

4.2.1. Spawn run period:-

The time period for the spawn run was estimated from the date of spawning to the day when the substrate had been fully invaded by mushroom mycelia. According to the findings, the shortest spawn run period for the wheat straw+ maize straw+ rice substrate (1:1:1) was 18 days followed by 19 days for wheat straw and wheat + rice straw (1:1), 19.67 days for wheat straw+maize straw (1:1), 22 days for rice straw respectively. The maximum spawn run period recorded was 24 days for maize straw+rice straw (1:1) (**Table 4.3.).**

4.2.2. Pinhead initiation:-

Pinhead started initiating quicker in the combination substrate of wheat straw, maize straw, and rice straw (1:1:1) at day 23. It was followed by a substrate made of wheat straw, for which

pinhead initiation took 24 days. Wheat straw+rice straw (1:1) and wheat straw+maize straw (1:1) completed the pinhead initiation in 25 and 25.67 days respectively. The maize straw+rice straw (1:1) substrate took 29 days, which was the maximum duration taken for emegence of pin heads, among the experimented substrates (**Table 4.3.**).

4.2.3. Harvesting and Total Crop Period:-

Three flushes of mushroom fruiting bodies in all were harvested. **Table 4.3.** lists the period of harvesting of these three flushes and the entire crop period. Depending on the substrate, the initial harvesting took place between 26 and 32 days. In a substrate of wheat straw + maize straw + rice straw (1:1:1), the first harvest took 26 days to complete. It was followed by different substrates namely wheat straw, wheat straw+rice straw (1:1), wheat straw+maize straw (1:1), rice straw and maize straw+ rice straw (1:1) in 27, 28, 28.67, 31 and 32 days respectively.

Similar to the first harvesting, the second harvesting was finished first for wheat straw+maize straw+rice straw (1:1:1) in 35 days and took 39, 39.67, 41, 42, and 43 days for wheat straw, wheat straw+maize straw (1:1), wheat straw+rice straw (1:1), maize straw+rice straw (1:1), rice straw respectively.

Similarly, third harvesting was completed in the shortest duration of time (48 days) when wheat straw + maize straw + rice straw (1:1:1) was used as a substrate. This was followed by substrate made of wheat straw, wheat straw+maize straw (1:1), maize straw+rice straw (1:1), wheat straw+rice straw (1:1) and rice straw in 52, 53.67, 54, 55, and 57.67 days respectively.

The bags with wheat straw + maize straw + rice straw (1:1:1) as substrate showed shortest total cropping period of 48 days followed by bags with wheat straw (52 days), wheat straw + maize straw (1:1) (53.74 days), and maize straw + rice straw (1:1) (54 days), wheat straw + rice straw (1:1) (55 days) and rice straw (57.67 days) respectively. Although, spawn run was observed slowest in maize straw + rice straw (1:1) but it had overall less crop period as compared to wheat straw + rice straw (1:1), and rice straw at the end (**Table 4.3.**).

Table 4.3.Effect of different substrates on growth period of *Pleurotus sajor-caju*

Substrate	Growth period (in days)					
	Spawn run period	Pin head initiation	Harvesting			Total crop period
			1st	2nd	3rd	
W+M (1:1)	19.67	25.67	28.67	39.67	53.67	53.67
W+M+R (1:1:1)	18.00	23	26	35	48	48
RICE	22.00	28	31	43	57.67	57.67
WHEAT	19.00	24	27	39	52	52
W+R (1:1)	19.00	25	28	41	55	55
M+R (1:1)	24	29	32	42	54	54
SEM	0.15	0.15	0.15	0.15	0.21	0.21
CD 5%	0.47	0.47	0.47	0.47	0.66	0.66

Where,

W+M-Wheat Straw+Maize Straw

W+M+R-Wheat Straw+Maize Straw+Rice Straw

W+R-Wheat Straw+Rice Straw

M+R-Maize Straw+Rice Straw

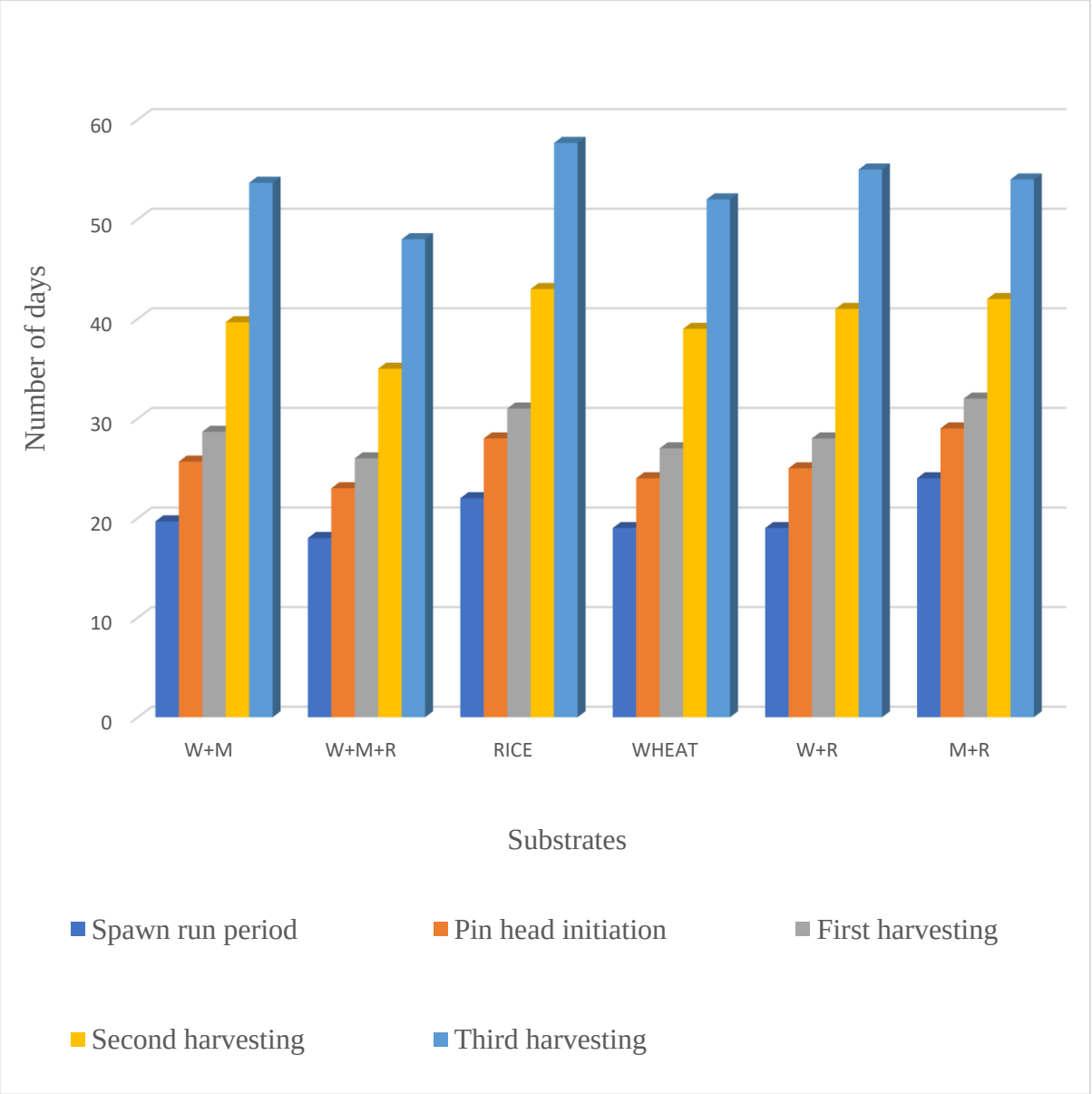


Fig.4.2.Effect of different substrates on spawn run period, pinhead initiation and harvesting period of *Pleurotus sajor-caju*

4.2.4. Average number of fruiting bodies:-

Maximum average number(16.73) of fruiting bodies were obtained while using wheat straw as a substrate. Wheat straw + maize straw (1:1) was found on 2nd place with 15.75 average number of fruiting bodies.It was followed by rice straw, wheat straw + rice straw (1:1), wheat straw + maize straw + rice straw (1:1:1) and maize straw + rice straw (1:1) with total average number of fruiting bodies 14.91, 14.68, 14.27 and 11.80 respectively.

4.2.5. Average weight of fruiting bodies:-

The average weight of fruiting bodies was measured across a range of tested substrates and the highest average weight of 20.33 g was found in bags with a substrate of wheat straw + maize straw + rice straw (1:1:1). Wheat straw + maize straw substrate (1:1) produced fruiting bodies with an average weight of 19 g, which was the second highest. The substrate made of maize straw + rice straw (1:1) had an average weight of 18 gram and was third highest. This is followed by average weights of 17.3 gram for rice straw, 16.37 gm for wheat straw + rice straw (1:1), and 15.27 gm for straw respectively (**Table.4.4**.).

4.2.6. Average stalk length of fruiting bodies:-

Fruiting bodies showed different stalk length in accordance with the varying substrates. Average stalk length was noticed highest (3.03 cm) in mushroom when grown in wheat straw + maize straw + rice straw substrate (1:1:1). This was followed by maize straw + rice straw substrate (1:1), wheat straw + maize straw substrate (1:1), wheat straw + rice straw (1:1), rice straw and wheat straw substrate and their average stalk length observed was 2.80 cm, 2.57 cm, 2.35 cm, 2.30 cm, and 2.00 cm respectively (**Table.4.3**.).

4.2.7. Average cap diameter:-

Substrate combination of wheat straw + maize straw + rice straw (1:1:1) showed highest average cap diameter of the fruiting bodies which was 8.37cm. This was followed by 6.27cm in maize straw + rice straw substrate (1:1). The performance of wheat straw + maize straw (1:1) substrate was also satisfactory, giving the average cap diameter of 6.03 cm.Wheat straw+ rice straw (1:1) and rice straw alone as substrate showed average cap diameter of 5.57 cm and 5.53 cm respectively. The minimum average cap diameter of 5.33 cm was recorded for wheat straw substrate **(Table.4.4.).**

4.2.8. Average stalk diameter:-

The maximum average stalk diameter of 4.44 cm was found in the combination substrate of wheat straw + maize straw + and rice straw (1:1:1), followed by 4.01 cm, 3.27 cm, 2.80 cm, 2.54 cm and 2.50 cm for the substrates made of wheat straw + maize straw (1:1), maize straw + rice straw substrate (1:1), wheat straw + rice straw (1:1), rice straw and wheat straw respectively.(**Table.4.4.).**

Table 4.4. Effect of different substrates on growth parameters of *Pleurotus sajor-caju*

Substrate	Growth parameters				
	Av. no. of fruit bodies	Av. wt. of fruit bodies(gram)	Av. stalk length(cm)	Av. cap diameter(cm)	Av. stalk diameter(cm)
W+M (1:1)	15.75	19	2.57	6.03	4.01
W+M+R (1:1:1)	14.27	20.33	3.03	8.37	4.44
RICE	14.91	17.3	2.30	5.53	2.54
WHEAT	16.73	15.27	2.00	5.33	2.50
W+R (1:1)	14.68	16.37	2.35	5.57	2.80
M+R (1:1)	11.80	18	2.80	6.27	3.27
SEM	0.51	0.2	0.06	0.09	0.03
CD 5%	1.62	0.63	0.18	0.28	0.10

Where,

W+M-Wheat Straw+Maize Straw

W+M+R-Wheat Straw+Maize Straw+Rice Straw

W+R-Wheat Straw+Rice Straw

M+R-Maize Straw+Rice Straw

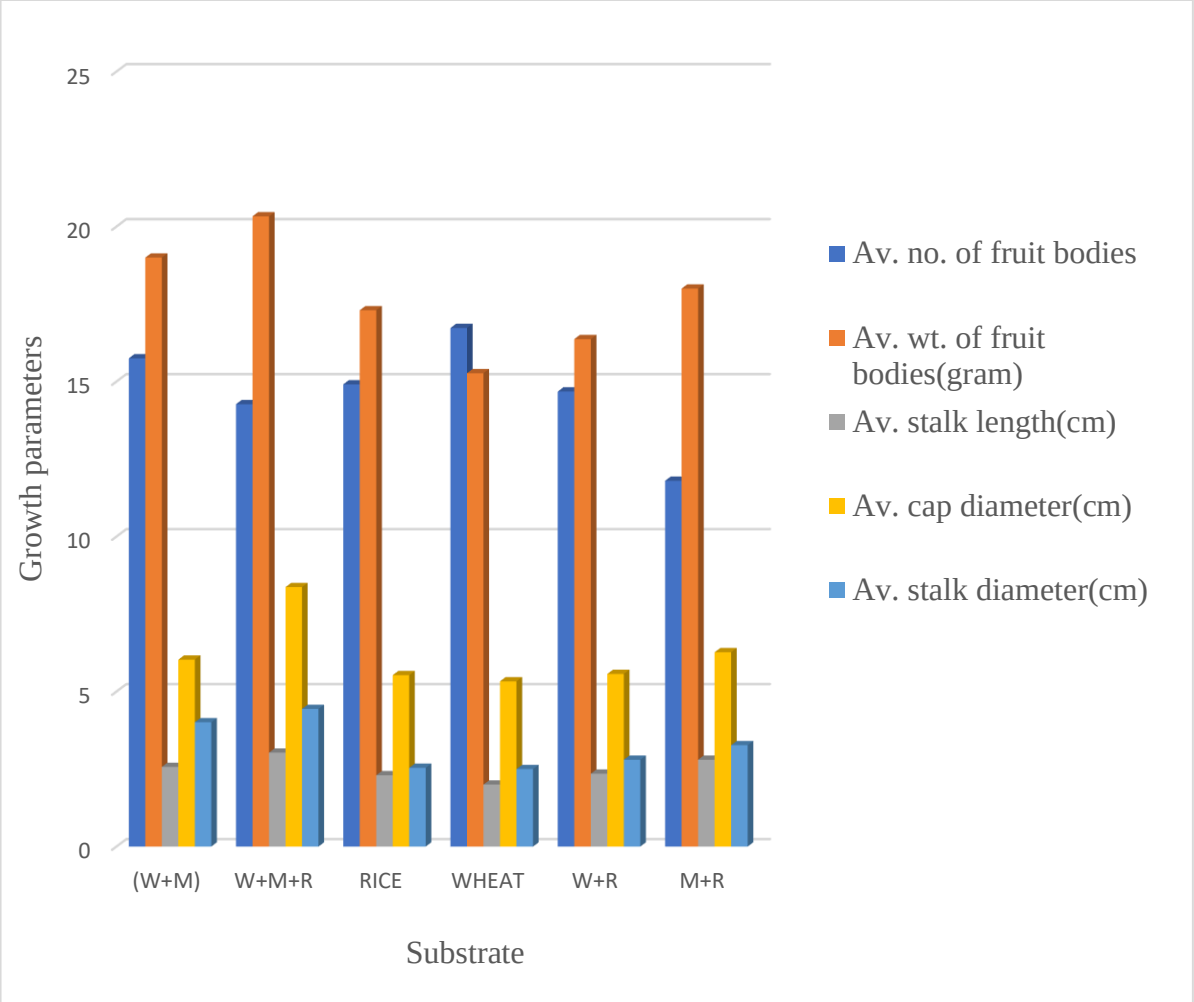


Fig.4.3.Effect of different substrates on growth parameters of *Pleurotus sajor-caju*

4.2.9. Total Yield Potential:-

The maximum yield of 303.33gm in the first harvesting was recorded on the substrate of wheat straw+maize straw (1:1). This was followed by wheat straw+ maize straw+ rice straw(1:1:1), rice straw, wheat straw, wheat straw+rice straw(1:1), and maize straw+rice straw substrate(1:1), with yield of 290 gm, 258 gm, 256.67 gm, 240.33 gm, and 212.33 gm respectively.

Similarly, with a total yield of 250.33 gm, wheat straw+maize straw substrate (1:1) showed best result in second harvesting which was followed by 232.33 gm in the wheat straw+ maize straw+ rice straw substrate (1:1:1), 213.67 gm in rice straw, 210.33 gm in wheat straw, 196.67 gm in wheat straw+rice straw substrate (1:1), and 173.67 gm in maize straw

However, highest yield of third harvesting (181.67 gm) was obtained from combined substrate of wheat straw+ maize straw+ rice straw (1:1:1). This was followed by wheat straw+maize straw (1:1) substrate with yield of 174 gm, rice straw with yield of 167 gm, wheat straw with yield of 121.33 gm, maize straw+rice straw substrate(1:1) with yield of 114.33 gm and wheat straw+rice straw(1:1) with yield of 111.33 gm respectively.

Wheat straw+maize straw (1:1) had a maximum total yield potential of 727.67 gm/bag. Combined substrate of wheat straw+ maize straw+ rice straw (1:1:1) also performed excellently as a substrate and produced a total yield of 704 gm. Rice straw and wheat straw both functioned admirably as a substrate, producing total yields of 638.67 gm and 588.33 gm respectively. In terms of overall yield, wheat straw+rice straw (1:1) yielded 555 gm, placing it in second-to-last position. The minimum weight of the mushroom, 500.67 gm, was taken from the substrate made of maize straw+rice straw (1:1). All information pertaining to yield potential is presented in **Table 4.5.**

Table 4.5.Effect of different substrates on yield potential of Oyster mushroom (*Pleurotus sajor-caju*)

Yield potential(gram/bag)				
Substrate	1st harvesting	2nd harvesting	3rd harvesting	Total yield
Wheat+Maize (1:1)	303.33	250.33	174.00	727.67
Wheat+Maize+Rice (1:1:1)	290	232.33	181.67	704.00
Rice	258	213.67	167.00	638.67
Wheat	256.67	210.33	121.33	588.33
Wheat+Rice (1:1)	240.33	196.67	111.33	555.00
Maize+Rice (1:1)	212.33	173.67	114.67	500.67
SEM	9.44	5.43	5.13	11.48
CD 5%	29.75	17.10	16.18	36.16

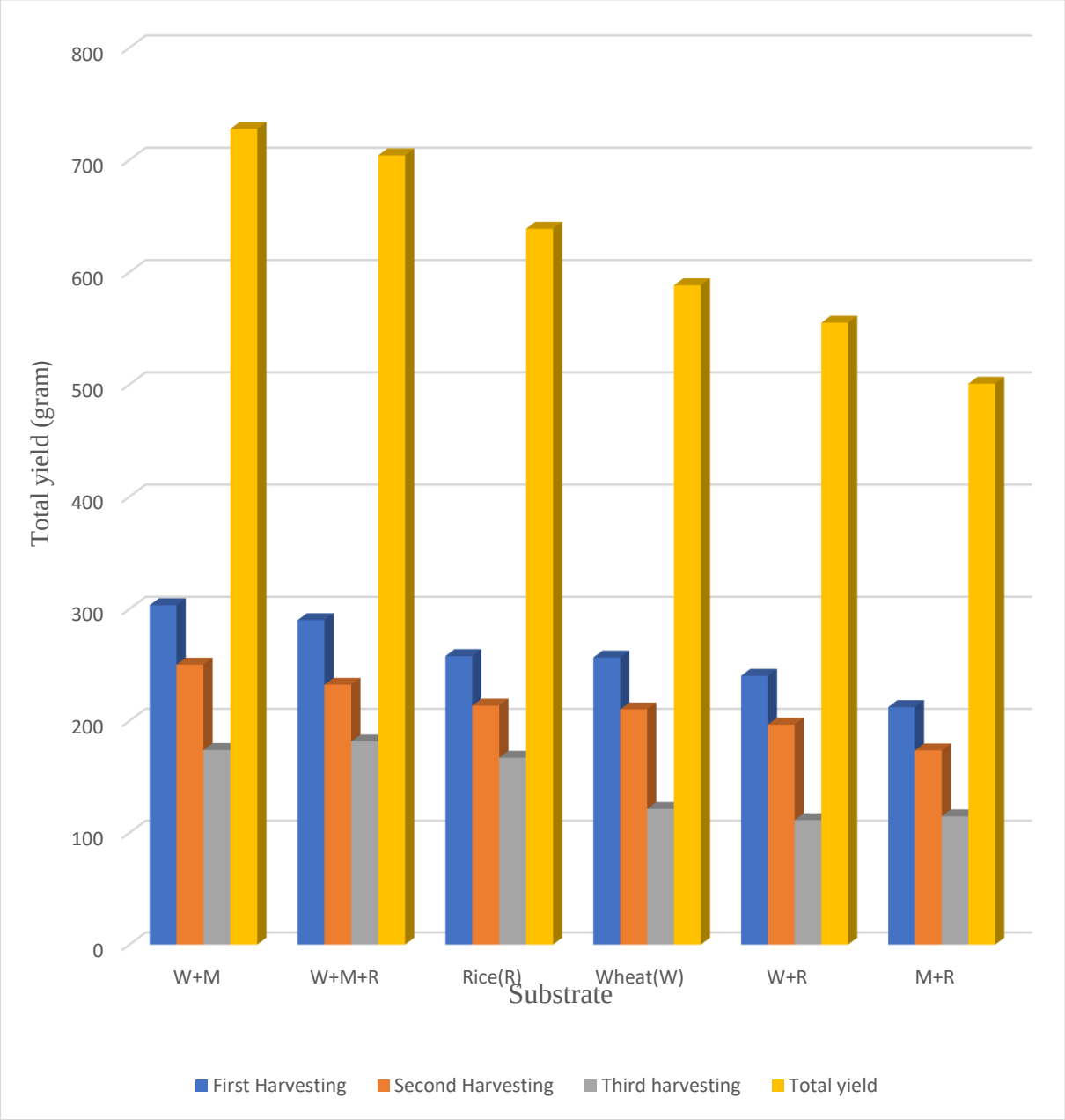


Fig.4.4.Effect of different substrates on yield potential of *Pleurotus sajor-caju*

Our results are similar to the findings of Jatwa *et al. (*2016) who observed paddy straw yielded better than both wheat straw and wheat+paddy straw in combination.

These results are also in accordance with Patil (2012) who found higher yield of *Pleurotus sajor-caju* on paddy straw than on wheat straw substrate.

Other researchers Hossain (2017), Dubey *et al*. (2019) also reported that effect of different substrates on growth and yield of oyster mushroom.

4.3. Effect of substrate moisture content on growth period and yield potential of *Pleurotus sajor-caju*:-

In order to study the growth period and yield potential of *Pleurotus sajor-caju*, wheat straw was chosen as the substrate and five different moisture levels including 50%, 55%, 60%, 65%, and 70% were maintained on it.The growth behaviour of the oyster mushroom, *Pleurotus sajor-caju* was investigated with respect to the spawn run period, pin head commencement, duration of harvesting three flushes and total crop period, and yield of first, second and third harvesting were recorded on various moisture content of substrate.

4.3.1. Spawn run period:-

The findings on the impact of substrate moisture on spawn run period of oyster mushrooms (*Pleurotus sajor-caju)* is reported in **(Table 4.6.).** In terms of spawn run period, substrate with a moisture content of 65% took least time i.e. 17.16 days for complete spawn run followed by moisture content of 60%, 70%, 55% and 50% which took 18.42 days, 19.1 days, 21.26 days and 22.24 days respectively.

4.3.2.Pinhead initiation:-

In comparison to 50–55 percent, 60–70 percent moisture levels had outstanding mycelial development and required less time for pinhead initiation. Least time period required for pinhead initiation was 20.15 days in a substrate with 65% moisture level. Substrate moisture content of 60%, 70%, 55% and 50% required 21.37 days, 23.12 days, 24.26 days and 25.24 days for initiation of pinhead respectively (**Table 4.6.).**

4.3.3.Harvesting and total crop period

Fruiting bodies of mushroom were harvested in total of three flushes. First harvesting was completed in minimum time (23.1 days) with 65% substrate moisture content. Substrate with 60% moisture content gave first flush in 24.37 days and 70% moisture content took 27.11 days. Substrate with moisture content of 55% took 29.25 days. Maximum time (30.22 days) was recorded from substrate prepared with 50% moisture level.

Similar to the first harvesting, the second harvesting was first completed from substrate with 65% moisture level, which took 33.16 days. This was followed by substrate moisture content of 60% in 34.38 days, 70% in 38.1 days, 55% in 40.26 days and 50% in 41.22 days, in that order.

Third harvesting also followed the same pattern i.e. bag with 65% of substrate moisture level harvested in 47.15 days. Harvesting period of 48.40 days, 52.13 days, 54.26 days and 55.22 days was recorded for a substrate with moisture content of 60%, 70%, 55% and 50% respectively.

Table 4.6.Effect of different substrate moisture on growth period of *Pleurotus sajor-caju*

Growth period(in days) after spawning						
Moisture content	Spawn run period	Pin head initiation	Harvesting			Total crop period
			1st	2nd	3rd	
50%	22.24	25.24	30.22	41.22	55.22	55.22
55%	21.26	24.26	29.25	40.26	54.26	54.26
60%	18.42	21.37	24.37	34.38	48.40	48.40
65%	17.16	20.15	23.1	33.16	47.15	47.15
70%	19.10	23.12	27.11	38.1	52.13	52.13
SEM	0.17	0.16	0.16	0.17	0.16	0.16
CD 5%	0.55	0.53	0.53	0.54	0.54	0.54

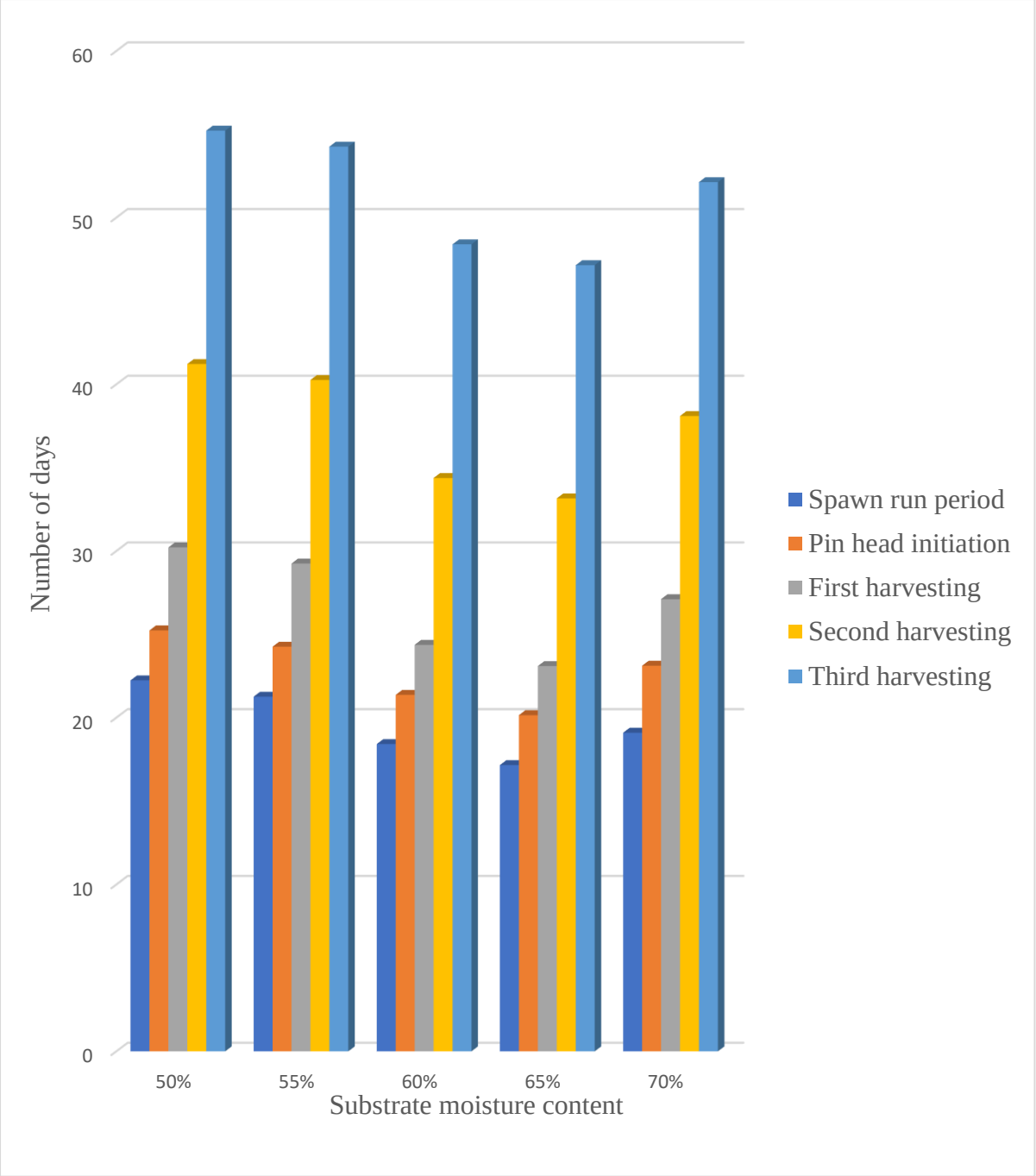


Fig.4.5.Effect of different substrate moisture on spawn run period, pinhead initiation and harvesting period of *Pleurotus sajor-caju*

4.3.4. Total yield potential

Data regarding yield of 1st, 2nd, 3rd harvesting and overall yield are presented in **Table.4.7.** and **Fig.4.6.**

In the first harvesting, maximum yield of 305.33 gm was recorded from the substrate having 65% moisture content. Moisture content of 60% and 70% yielded 289.67 gm and 275.33 gm of mushroom, which was a admirable quantity. Yield 259 gm was obtained from prepared substrate with 55% moisture content. Least quantity of mushroom (243.33 gm) was harvested from a substrate maintained at 50% moisture level.

Similarly, in second harvesting, highest yield, 202.67 gm of mushroom was harvested from substrate having 65% moisture level. This was followed by 189 gm in 60% moisture level, 180 gm in 70% moisture level, 165.33 gm in 55% moisture level and 133 gm in 50% moisture level respectively.

Highest yield of third harvesting (101.83 gm) mushroom was obtained from the substrate maintained at 65% moisture level. In addition, 60% of substrate moisture level produced 91.67 gm of mushroom. 88 gm of mushroom was harvested from substrate having 70% moisture content. 77 gm of mushroom yield was noted on the substrate with moisture level maintained at 55%. Lowest yield (61 gm) was observed in substrate having 50% moisture content.

Prepared substrate with 65% moisture content was found significantly higher in supporting the mushroom production. It was discovered that, when compared to other treatments, a substrate moisture level of 65 percent was ideal for maintaining the largest output of mushrooms (609.83 gram/bag) which was followed by substrate moisture content of 60% with a total yield of 570.33 gram/bag, 70% with total yield of 543.33 gram/bag ,55% with total yield of 498 gram/bag and 50% with total yield of 437.33 gram/bag respectively. The yield increased in direct proportion to the rise in moisture content from 50 to 65 percent. However, as the moisture content increased from 65 to 70 percent, the yield gradually decreased (**Table 4.7.).**

Table 4.7.Effect of substrate moisture content on yield potential of oyster mushroom (*Pleurotus sajor-caju*)

Yield potential(gram/bag)				
Moisture content	1st harvesting	2nd harvesting	3rd harvesting	Total yield
50%	243.33	133	61	437.33
55%	259	165.33	73.67	498
60%	289.67	189	91.67	570.33
65%	305.33	202.67	101.83	609.83
70%	275.33	180	88	543.33
SEM	3.76	2.36	2.15	6.46
CD 5%	12.25	7.69	7.0	21.07

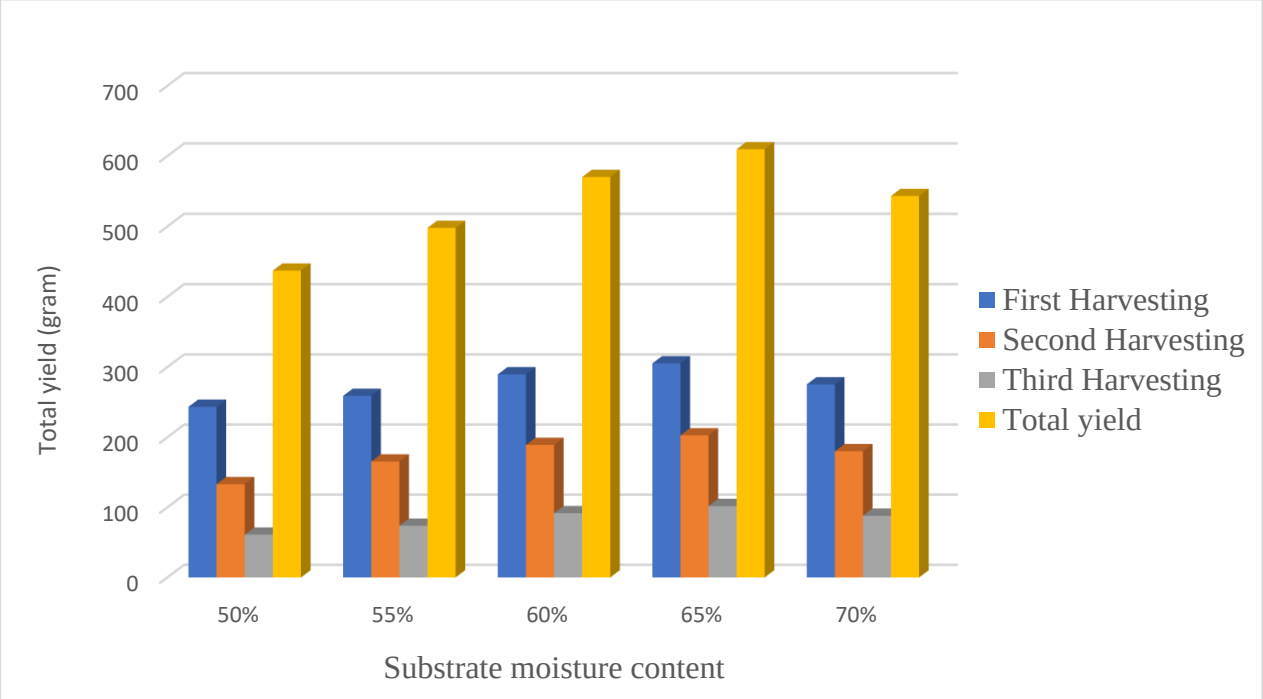


Fig.4.6.Effect of different substrate moisture on yield potential of *Pleurotus sajor-caju*

Above results have confirmity with findings of Samuel *et al*. (2012) and Yang *et al*. (2013) who observed optimum growth performance and yield of *Pleurotus ostreatus* when substrates were maintained at 65% moisture level.

Also suggested for preparation of substrate with different moisture content for better growth and yield of oyster mushroom.

Chapter-5

SUMMARY AND CONCLUSION

In India, agriculture is the main economic sector, and from decades it has always made a massive contribution to the national economy. However, rapid population increase and food demand have encouraged intensive agriculture, which has resulted in enormous agricultural waste that is mainly being discarded. Approximately, 87 Mt of extra crop residue is burned on-farm each year which leads to a variety of risks, including oxygen-deficient environment, respiratory illnesses such as allergic bronchitis, asthma and tuberculosis, as well as impaired night time visibility.

Management of crop waste must be our prime concern for the long-term stability of Indian agriculture. The exponential growth in the current level of agro-wastes production highlights the urgent need of a solution to this problem. Therefore, it is necessary to avoid burning wastes and instead adopt alternative measures to enhance farmer's revenue and lessen their influence on the environment.

With minimal money and scientific expertise, it is possible to develop a sustainable agro-residues management strategy. Mushroom farming is one such promising agro-residues management technology that appeals to farmers. Mushroom cultivation technology can be better termed as health and wealth

The abundant availability of cereals waste (straw) gained more importance in mushroom cultivation. These waste contains certain complex polymer such as cellulose, hemicellulose and lignin and their accumulation on land causes environmental pollution. Cultivation of mushroom on various residual wastes is one of the eco-friendly practices to combat the malnutrition and environmental pollution caused by these agricultural wastes.

The mushrooms degrades polymers into simple form by producing extracellular enzymes and produces functional food rich in protein, vitamins, minerals, antioxidants with low fat contents.

In addition, cereals straw could be used alone or in combination of with other residues in order to enrich the nutrients required for mushroom growth. Studies have reported that the supplementation of substrates would increase the yield compared to single substrate.

As a result of using this technique, farmers can increase their income, create jobs, engage in short-term agricultural ventures, utilize their own farm residues and add value to them, improve community nutrition, avoid burning stubble and harming the environment, dispose residues in an eco-friendly manner, access organic manure from spent mushroom substrate and raise their standard of living.

Oyster mushroom ranks second among commercially grown mushroom, mainly in Southeast Asia, India, Europe, and Africa. It possesses several benefits comparing to other edible mushrooms. It grows in less time over a wide range of pH (6-8) and temperature (10-30° C) ; produces plethora of enzymes that are able to degrade lignocellulosic biomass of agrowastes; requires very less environmental control; can colonize substrates in a short time period; does not require composting of the substrate; has higher yield capacity and poses high nutritional and medicinal worth.

To fulfill the above objectives, present study entitiled "Studies on cultural techniques of oyster mushrom {*Pleurotus sajor-caju* (Fr.) Singer**"** was conducted to find out the in-vitro growth requirements,conditions for optimum cultivation (*Pleurotus sajor-caju*).

The experimental findings of this study are summarized as below:-

- Mushroom culture was observed on five different media(potato dextrose agar + IBA, potato dextrose agar, corn dextrose agar, rice dextrose agar and chickpea dextrose agar).Maximum mycelial growth was observed in potato dextrose agar + IBA media with radial growth of 90 mm in 8th day of inoculation.
- *S*ix different agricultural substrates (wheat straw+maize straw+rice straw (1:1:1), wheat straw, wheat straw+rice straw(1:1), wheat straw+maize straw (1:1), maize straw+ rice straw (1:1) and rice straw) were used for *Pleurotus sajor-caju* mixture substrate (wheat straw+maize straw+rice straw (1:1:1) best for growth parameters and highest yield potential.
- Total crop period and total yield potential was also evaluated for *Pleurotus sajor-caju* using wheat substrate at different moisture level of 50%, 55%, 60%, 65% and 70%.It

was found that substrate moisture of 65% was ideal for cultivation of *Pleurotus sajor-caju* with shortest spawn run period and highest yield potential.

The above findings gave an insight into common culture media, locally available agricultural substrates and optimum level of water content that should be maintained in substrates for oyster mushroom production in order to achieve the highest yield potential in shorter period of time and to avoid the risk of contamination by diseases and pests.Knowing of factors that affect mushroom production in various stages can provide one with maximum return in minimum investment.

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