EVALUATION OF DIFFERENT STRAINS AND SUBSTRATES FOR CULTIVATION OF LION'S MANE MUSHROOM

BY

RENU DEVI
(J-18-M 576)

Thesis submitted to Faculty of Agriculture

in partial fulfillment of requirements

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PLANT PATHOLOGY

DIVISION OF PLANT PATHOLOGY

Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu
Main Campus, Chatha, Jammu-180009

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CERTIFICATE-I

This is to certify that the thesis entitled **"Evaluation of Different Strains and Substrates for Cultivation of Lion's Mane Mushroom"** submitted in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture (Plant Pathology)** to the Faculty of Post Graduate Studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, is original work and has similarities with published work not more than minor similarities as per UGC norms of 2018 adopted by the University. Further the level of minor similarities has been declared after checking the manuscript with urkund software provided by the University.

The work has been carried out by **Ms. Renu Devi,** Registration No. **J-18-M-576,** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma. It is further certified that help and assistance received during the course of thesis investigation have been duly acknowledged.

30/6/21

Dr. Sachin Gupta
Major Advisor

Place: Jammu
Date: 30-06-2021

Dean

Head of division

CERTIFICATE-II

We, the members of Advisory Committee of **Ms. Renu Devi,** Registration No. **J-18-M-576,** a candidate for the degree of **Master of Science in Agriculture (Plant Pathology),** have gone through the manuscript of the thesis entitled **"Evaluation of Different Strains and Substrates for Cultivation of Lion's Mane Mushroom"** and recommend that it may be submitted by the student in partial fulfillment of the requirements for the degree.

30/6/21

Dr. Sachin Gupta
Major Advisor and
Chairman Advisory
Committee

Place: Jammu
Date: 30-6-2021

Advisory Committee Members:

Member from Major Subject
Dr. Ranbir Singh
Associate Professor
Division of Plant Pathology

Member from Minor Subject
Dr. Moni Gupta
Associate Professor
Division of Biochemistry

Dean's Nominee
Dr. Devinder Sharma
Assistant Professor
Division of Entomology

CERTIFICATE-III

This is to certify that the thesis entitled **"Evaluation of Different Strains and Substrates for Cultivation of Lion's Mane Mushroom"** submitted by **Ms. Renu Devi**, Registration No. **J-18-M-576**, to the Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, in partial fulfillment of the requirements for the degree of **M.Sc.** in **Agriculture (Plant Pathology)**, was examined and approved by the advisory committee and external examiner(s) on **04-08-2021**.

Dr. Shaheen Kausar Jan
Professor & Head
Division of Plant Pathology
Faculty of Agriculture
SKUAST-K, Wadura
External Examiner

Dr. Sachin Gupta
Major Advisor

.....4/8/21.....

Head
Division of Plant Pathology

.....

Dean, Faculty of Agriculture,
SKUAST-J, Jammu

.....

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Place: Jammu

Date: 04-08-2021

Renu

Renu Devi

ABSTRACT

Title of Thesis	:	**EVALUATION OF DIFFERENT STRAINS AND SUBSTRATES FOR CULTIVATION OF LION'S MANE MUSHROOM**
Name of student	:	Renu Devi
Registration No.	:	J-18 -M-576
Major Subject	:	Plant Pathology
Name and Designation of major Advisor	:	Dr. Sachin Gupta Associate Professor, Plant Pathology
Degree to be Awarded	:	M.Sc. Plant Pathology
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ABSTRACT

The present study was conducted in regard to "Evaluation of Different Strains and Substrates for Cultivation of Lion's Mane Mushroom". Under *in vitro* conditions radial growth of five different strains of *Hericium erinaceus* was evaluated on PDA and it was observed that He-03 strain showed maximum mycelial growth of 86.86 mm after 18 days of incubation with average mycelial growth rate of 4.23 mm per day. Three substrates *viz*., wheat straw, saw dust and rice straw were tested alone and in combination with supplements to compare their efficacy in the growth pattern, yield and yield related pattern of fruiting bodies of *H. erinaceus* strains. The results indicated that He-03 strain observed minimum days (38.66) for complete spawn run and minimum days (51.33) for fruit initiation observed in He-05 strain on a substrate combination of rice straw + wheat straw and wheat straw + saw dust, respectively. He-03 strain recorded maximum diameter of clumps of spines (8.20 cm) and maximum number of fruit bodies (15.50) on saw dust whereas maximum average weight of fruit body (34.76) were observed in wheat straw + saw dust in He-05 while minimum diameter of clumps of spines (4.70 cm) was recorded in He-03 strain. Minimum number of fruit bodies (10.10) was recorded on rice straw + wheat straw in He-05 strain whereas minimum average weight of fruit body (24.60 g) was observed in rice straw. Maximum yield for first flush (178.00 g), second flush (167.33 g) was recorded on saw dust in He-05 strain while maximum yield for third flush (160.00 g) was recorded on saw dust in He-03 strain. In He-05 strain, saw dust substrate gave highest total yield of 502.99 g /1000 g of dry substrate with corresponding 50.29 per cent biological efficiency while the least effective substrate was rice straw substrate which produced a total yield of 316.65 g/1000 g of dry substrate in He-03 strain. Maximum cost: benefit ratio (1:4.05) was recorded in wheat straw + saw dust in He-05 strain while minimum cost: benefit ratio (1:2.92) was observed in rice straw substrate in He-03 strain. Biochemical analysis of fruit bodies of *H. erinaceus* strain He-03 showed maximum moisture content (91.66 %) on rice straw + wheat straw while maximum crude fat content (4.83 %) and maximum ash content (12.86 %) was observed in fruit bodies grown on rice straw while maximum crude protein content (27.50 %) and crude fibre (8.10 %) were recorded in fruit bodies grown on wheat straw and saw dust in He-05 strain while minimum moisture content (87.66 %), crude protein content (24.83 %), crude fat content (3.16 %), crude fibre (7.00 %) and ash content (10.23 %) were observed in fruit bodies grown on saw dust, rice straw, saw dust, rice straw + wheat straw and rice straw + wheat straw respectively in He-03 strain. Maximum carbohydrates (61.92 %) were recorded in fruit bodies of He-05 strain cultivated on saw dust while minimum carbohydrates (58.50 %) were observed in fruit bodies of He-05 strain grown on wheat straw.

Key words: *Hericium erinaceus,* Mycelial growth, Nutritional parameters, Substrate

Signature of Major Advisor

Renu

Signature of Student

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CHAPTER-I

INTRODUCTION

Mushrooms provide a highly nutritious source of food and many of these mushrooms produce metabolites of intense interest to the nutraceutical, pharmaceutical and food industries. In addition to being edible, mushrooms have been shown to have medicinal properties making them a prime candidate for the specialty mushroom market. *Hericium* species are common saprophytic basidiomycete fungi that belong to the Hericiaceae family. It is also known as lion's mane mushroom or tooth fungus and grows on hardwood of old or dead broadleaf trees. All members of the genus produce more or less globoid white fruiting bodies covered in downward cascading spines. The colour of this mushroom is white and turns brownish or yellowish with age. This mushroom has been known as Chinese medicine or food in China and Japan without harmful effects on humans for treatment of gastritis in traditional Chinese medicine for more than 1000 years (Mizuno, 1999).

The taste of lion's mane mushroom is highly desired by chefs and is said to resemble lobster and seafood. There is currently a limited commercial market for *Hericium erinaceus* which is produced indoors on sawdust substrate, but practically no forest (log) production. Fungi of the genus *Hericium* contain various ingredients possessing antibacterial activity, cytotoxic effect on cancer cells and compounds that stimulate the synthesis of the Nerve Growth Factor (NGF). It also has various pharmaceutically active substances such as phytosterols (β-sitosterol and ergosterol) which lower the content of Low-Density Lipoproteins (LDL) and triglycerides (Gerbec *et al*., 2015).

Hericium erinaceus has various biological activities such as anti-microbial effect, anti-tumor activities, immunomodulatory effect, antioxidant properties, cytotoxic effect, hypolipidemic effect and promoting the synthesis of the neurogrowth factor. In addition, this fungus has been used for treating and curing gastric ulcers, gastro duodenal ulcers and chronic gastritis among other ailments. It has been reported that fruit bodies of lion's mane

mushroom contain various constituents including terpenoids (erinacine, hericenone), lectins, phenols, proteins, lipids, polysaccharides, fibres, ash etc. Since the polysaccharides from mushrooms are known to have no toxic side effects, unlike the existing anti-cancer chemical medications, treatment using polysaccharide-based therapies has shown very promising results in prolonging the life span of a patient. They strengthen the immune system, relieve gastritis and gastrointestinal infections, reflux and upset stomach due to stress.

Currently, there are very few strains of lion's mane that are commercially available. Keeping in view the importance of this mushroom and to further strengthen its potential as cultivated mushroom, the present study was taken up for cultivation of strains of *Hericium erinaceus* and evaluation of yield related parameters. Different locally available substrates were evaluated for cultivation of lion's mane mushroom and the fruit bodies of *H. erinaceus* of different strains cultivated on different substrates were analysed for nutritional parameters *viz*., moisture content, fibre content, protein, fat, ash contents and total carbohydrates. The present study had following objectives:

- Evaluation of different strains and potential substrates for cultivation of Lion's mane mushroom
- Comparison of different strains of Lion's mane mushroom for yield parameters.
- Assessment of nutritional parameters of different strains of Lion's mane mushroom.

Chapter-II

Review of Literature

CHAPTER-II

REVIEW OF LITERATURE

The present chapter deals with the brief review of literature related to the various aspects of the present study "Evaluation of Different Strains and Substrates for Cultivation of Lion's Mane Mushroom". Literature pertaining to the mycelial growth assay, media and substrate evaluation, strain evaluation, cultivation studies, biochemical and enzymatic studies is being reviewed critically.

2.1 Mycelial growth assay

Figlas *et al*. (2007) studied the mycelial growth rate in linear growth assay, yield and mushroom productivity of *Hericium erinaceus* in substrates containing sunflower seed hulls as the main energy and nutritional component, with the addition of different levels of Mn (II) or NH_4^+. The mycelial growth rate in substrates possessing different sunflower seed hull sizes with or without the addition of wheat bran showed that irrespective of the presence of wheat bran, higher mycelial growth rate was observed with the larger sunflower seed hull size. Adding growth-limiting mineral nutrients such as Mn (II) (20 or 100 ppm) or NH_4^+ (200 or 500 ppm) increased the mycelial growth rate by 8-16 per cent. No statistical differences were detected between accumulated biological efficiencies coming from different substrate formulations with the addition of wheat bran, barley straw or poplar sawdust compared to the sunflower seed hull control, but a tendency for higher yield was observed for the substrate supplemented with 20 ppm Mn and 200 ppm NH_4^+. Sunflower seed hulls without supplementation constitute a very good basal substrate, so this substrate by itself constitutes a very good source of energy and nutrition for *H. erinaceus* growth and development.

Imtiaj *et al*. (2008) observed the vegetative growth of four different strains of *Hericium erinaceus* and reported the temperature suitable for optimal mycelial growth to be 25^0C, with growth observed in the extended temperature range of 20-30^0C. The different strains of *H. erinaceus* showed distinct pH requirements for their optimum vegetative growth with the most favorable growth observed at pH 6. For vegetative

mycelial growth, PDA, YM, Hennerberg, Hamada, and Glucose peptone were the most favorable media for these mushroom strains. With the exception of lactose, most of the carbon sources assayed demonstrated favourable vegetative growth of *H. erinaceus*. For mycelial growth, the most suitable nitrogen source was alanine. Oak sawdust medium supplemented with 10-20 per cent rice bran was the best for mycelial growth of the mushroom. Kwon *et al.* (2008) conducted an experiment by utilizing the byproducts after manufacturing fresh vegetable juice. *H. erinaceus* was cultured using *Angelica keiskei* (AK) and the byproducts of *Angelica keiskei* (BAK) as a nutrient and the chemical composition of the culture extract was analysed. When *H. erinaceus* was cultured using culture media of AK and BAK, it showed 107-112 mm of growth during 40 days. The moisture content of AK and BAK extracts were 94.36 and 97.36 per cent, respectively; however, those of extracts of *H. erinaceus* cultured in AK and BAK decreased to 90.95 and 94.20 per cent, respectively.

Siwulski *et al.* (2009) determined the effect of sawdust substrates and their enrichment with glucose on the mycelium growth and yield of three *Hericium erinaceus* strains. The strains of *H. erinaceus* were designated as 'H1', 'D5' and 'D9' and pine and beech sawdust supplemented with glucose in the amount of 1 per cent, 2 per cent and 3 per cent were used as cultivated substrates. The experiment revealed that the mycelium grew more rapidly on substrate with glucose addition in comparison to substrate without glucose addition. The 'H1' and 'D5' strains gave higher yield than 'D9'. Highest yield was recorded on beech sawdust substrate with 3 per cent addition of glucose. Liang *et al*. (2011) studied the cultivation of *H. erinaceus* on camellia seed coats and chestnut shells as components of substrates. It was found that highest mycelium growth rates were recorded when the growth substrate included 20 per cent and 40 per cent powdered *Camellia oleifera* seed coats. Kang *et al*. (2016) investigated the effects of high concentrations of various plant oils with their major components of fatty acids on mycelial growth and pinhead formation of *H. erinaceus* and reported that palmitic acid was most stimulatory for the mycelial growth (18.3 %) after 3 weeks of incubation. Olive oil was also stimulatory and was 11.2 per cent longer than the control. The addition of high levels of coconut oil and lauric acid drastically suppressed mycelial growth. All lipid additives tested for pinhead formation, except linoleic acid had a positive effect that

shortened the period of pinhead formation by 10 per cent to 25 per cent compared to the control. Cottonseed and palm oils were equally the most effective for pinhead formation of *H. erinaceus.*

2.2 Media and Substrate evaluation

Han *et al*. (2005) carried out an experiment by using several agricultural by-products as supplements of sawdust substrate for the production of *Hericium erinaceus.* All the tested supplements (rice bran, wheat bran, barley bran, chinese cabbage, egg shell, and soybean powder) were found to be suitable for the mycelial growth of all the tested species. For mycelial growth, soybean powder was the best supplement for *H. ericanus* and *H. coralloides* while barley bran was the best for *Hericium alpestre*, *Hericium laciniatum,* and *H. erinaceus*. For *H. abietis,* rice bran and Chinese cabbage was the best. The possibility of mushroom production on oak sawdust substrate with 20 per cent rice bran supplement was demonstrated with *H. coralloides, H. americanum, H. erinaceus, and H. erinaceus* which showed 26–70 per cent biological efficiency. The results showed that strain selection are important to improve biological efficiency and mushroom yield in *Hericium* cultivation.

2.3 Strain evaluation

Gryganski *et al*. (2000) conducted a cultivation experiment with 14 heterokaryotic strains of *Hericium erinaceus* and revealed wide variation in yield, quality and colour of fruiting bodies. The fruit body colours, oxidoreductases were investigated in vegetative mycelia on agar and liquid media. Results showed that the colour of fruiting bodies correlated with the presence and activity of the enzyme laccase. There was no correlation between fruiting body colour and presence of tyrosinase, the enzyme responsible for browning of white button mushroom fruiting bodies. The data obtained are important for selection and breeding of new *H. erinaceus* strains with good fruiting body qualities. Wang *et al.* (2018) carried out an experiment and screened the ten strains of *H. erinaceus* for cultivation with eucalyptus sawdust as the main material. Ten strains of *H. erinaceus* were selected, their mycelial growth, agronomic characteristics of the fruiting bodies, resistance to contamination and yield were comparatively studied. There were

differences in the various traits of the tested strains. The better comprehensive characters were displayed by *H. erinaceus* Changshan strain and *H. erinaceus* BJ strain. The average yield of *H. erinaceus* Changshan strain and *H. erinaceus* BJ strain was higher than other strains, which was 276.19 and 245.10 g/bag, respectively. The weight of single mushroom body was better, which was 88.41 and 77.60 g respectively. In addition, the other traits of both the strains showed good performance and the performance of *H. erinaceus* Changshan strain was better than the *H. erinaceus* BJ strain. Thus, *H. erinaceus* Changshan strain and *H. erinaceus* BJ strain are suitable for cultivation using eucalyptus sawdust as main raw material.

Grace *et al*. (2015) worked on the production of *Hericium erinaceus* on a forest farming system. They compared yield of mushrooms from four different strains and one commercial *H. erinaceus* and three strains of *H. americanum* isolated locally. Each strain was inoculated and mushroom production was monitored for five years. The result revealed that significantly fewer logs fruited on the commercial strains compared to the local isolate strains and there were no significant differences among the four strains with respect to the yield of mushrooms per log but yield varied significantly depending on the number of years after inoculating with peak production during the third and fourth years. Ajchara *et al.* (2017) conducted the experiment in which six strains (AF, DA1, DA2, AMC, DPRS and KKU) of monkey's head mushrooms were selected having properties suitable for growing and gave high yield in East of Thailand. The strains gave fruit bodies if temperature during mycelium growth (incubation time) and induced fruiting time was 26-30ºC. Higher temperature affected mycelium growth slowly and did not yield fruit bodies and growing media was highly contaminated. It was observed that strains AF and DA2 had adaptability in East of Thailand especially at Chanthaburi higher than the other strains, the yield per bag was 211.42 and 180.6 g respectively, while DA1, DPRS, KKU and AMC had the yield per bag of 146.98, 111.94, 95.31 and 84.77 g, respectively. Rice straw substrate gave yield higher than sawdust. Increasing rice bran ratio in growing media also increased protein content in fruit bodies.

2.4 Cultivation studies

Chang *et al*. (1999)[a] evaluated yield and biological efficiency of *Hericium erinaceus* and reported that in the case of bottle cultivation the yield was 356 g/850 ml and the biological efficiency was 147.8 per cent while in pot culture, the yield was 810 g/2500 ml with biological efficiency of 114.3 per cent respectively. Chang *et al.* (1999)[b] carried an experiment to investigate the physiological characters of *H. erinaceus* in sawdust media wherein the optimum temperature was 25^0C and optimum pH was 5. They observed that the mycelial growth and density of *H. erinaceus* was quite good when oak tree sawdust was used as cultural substrate. However, the best mycelial growth in *H. erinaceus* was observed when wheat pollard was added as supplement on sawdust substrate. The optimum supplement ratio of wheat pollard and magnesium sulphate was 20 per cent and 0.1 per cent respectively. Siwulski *et al*. (2005) studied the yield of two *H. erinaceus* strains (CS 91 and DSM 11325) on beech sawdust substrate enriched with wheat bran (20 %), rye grain (25 %), soyabean meal (7 %), rape meal (10 %) or meat-osseous flour (6 %). Highest yields were obtained on the substrate with wheat bran and soyabean meal. The yields of carpophores on these substrates were more than 52 g/100 g dry matter of substrate.

Hassan (2007) studied *Hericium erinaceus* grown under local conditions in Egypt using the available lignocellulosic wastes as growing media. Incubation time, yield, biological efficiency (B.E %) were determined during three consecutive growing seasons. The incubation time for the tested growing media ranged from 37 to 46 days. The highest yield of *H. erinaceus* (184 g / kg media) and B.E of 50.3 per cent were obtained when grown on sawdust. It was observed that using a mixture of sawdust with wheat straw as growing medium gave a good yield (165 g/ kg medium) and an overall Biological Efficiency of 46.5 per cent. Song *et al.* (2009) discussed the effects of different media formulations on cultivation of *H. erinaceus* on leaf and stem of banana replacing cotton seed hull. It was observed that replacing cotton seed hull by leaf and stem of banana to cultivate *H. erinaceus* was feasible.

Qiong *et al*. (2014) conducted a cultivation test trial to compare mycelium growth conditions, yield and quality of four strains of *Hericium erinaceus viz.,* Ha, Hb, H1 and

H2 in efficient cultivation. The result revealed that mycelium growth rate of Ha, Hb and H1 was 0.32-0.34 cm/d, while that of H3 was 0.21 cm/d, however, the yield of Ha was ranked highest at 232.1 g/bag with the biological efficiency at 66.3 per cent followed by H1 at 225.8 g/bag and 64.5 per cent respectively which was significantly higher than those of other strains. Atila and Tuzel (2016) conducted an experiment to determine the effects of different substrates on mycelial growth, fructification, yield, sizes and colours of fruit bodies of *Hericium* isolates (HE- Ankara, HE- Denizli, HE, HE-Trabzon, HE-İzmit, HC, HE- Amerika). Experiments were conducted with seven different substrates prepared with oak sawdust (MT), wheat bran (BK), cotton seed hulls (PK) and olive press cake (ZP) (80MT:20BK, 90MT:10PK, 80MT:20PK, 70MT:30PK, 90MT:10ZP, 80MT:20ZP, 70MT:30ZP). 1 kg (wet weight) of substrates was packed in polypropylene autoclavable bags of 25x45 cm and sterilized in autoclave at 121^0C for 90 minutes. Maximum yield and B.E were reported from oak sawdust medium supplemented with 20 and 30 per cent cotton seed hulls on HE-Ankara, HE-Denizli, HE- İzmit and HE-Amerika isolates while the best yield and BE were detected from 70MT:30ZP on Trabzon isolate. Significant differences were observed among substrates regarding yield, BE, average mushroom weight, fruit body size and colour of *Hericium* isolates.

2.5 Biochemical and enzymatic studies

Kwon *et al.* (2008) conducted an experiment by utilizing the by products after manufacturing fresh vegetable juice. *Hericium erinaceus* was cultured using *Angelica keiskei* (AK) and the byproducts of *Angelica keiskei* (BAK) as a nutrient. Vitamin A content of AK extract was 20.78 IU/100 mL and was higher than those of other extracts. However, vitamin A was not detected in extracts of *H. erinaceus* cultured with AK and BAK. In contrast, vitamin B2 and C in the extracts of *H. erinaceus* cultured were higher than those of AK and BAK. Total ash content including Fe, P, Mg, and Ca increased in the extracts of *H. erinaceus* cultures when compared with AK and BAK extracts. Total amino acid content was also higher in the extracts of *H. erinaceus* cultures (231.08 and 372.25 mg %) than those in the extracts of AK (177.17 mg %) and BAK (149.99 mg %). Wong *et al.* (2009) investigated the cultivation and processing conditions that may affect the medicinal, antimicrobial and antioxidant properties of locally grown *H. erinaceus*.

The fruit bodies that were fresh, oven-dried or freeze-dried were extracted with methanol and their properties compared to those exhibited by mycelium extract of the same mushroom. Various extracts of *H. erinaceus* inhibited the growth of pathogenic bacteria but not of the test fungi.

Lee *et al*. (2010) observed that *H. erinaceus* has considerable antitumor, immuno-modulatory, and cytotoxic effect. It has been postulated that the fruiting body of *H. erinaceus* contains a polysaccharide that is similar to β -D- glucan, which is known to have antitumor activity against Sarcoma 180. Khan *et al*. (2013) stated mushrooms to be nutritionally functional foods and source of physiologically beneficial medicines. *H. erinaceus*, also known as Lion's Mane Mushroom or Hedgehog Mushroom is an edible fungus which has a long history of usage in traditional Chinese medicine. This mushroom is rich in some physiologically important components, especially β-glucan polysaccharides which are responsible for anti-cancer, immuno-modulating, hypolipidemic, antioxidant and neuro-protective activities of this mushroom. *H. erinaceus* has also been reported to have anti-microbial, anti-hypertensive, antidiabetic, wound healing properties among other therapeutic potentials. They discussed the potential health beneficial activities of this mushroom with the recognition of bioactive compounds responsible for these medicinal properties.

Guang *et al*. (2014) evaluated the anticancer efficacy of two extracts (HTJ5 and HTJ5A) from the culture broth of *H. erinaceus* against three gastrointestinal cancers such as liver, colorectal and gastric cancers in both *in vitro* cancer cell lines and *in vivo* tumor xenografts and discovered the active compounds. Sokół *et al*. (2016) reported that fruit bodies of Lion's mane mushroom contain 57 per cent carbohydrates, 3.52 per cent fats, 7.81 per cent fiber, 22.3 per cent protein and 9.35 per cent ash per dry matter. Moreover, other soluble sugars were also found Arabitol at 127.17 mg/g, glucose at 11.35 mg/g, mannitol at 12.98 mg/g, inositol at 1.43 mg/g and trehalose at 9.71 mg/g d.m. They also found that fourteen amino acids are also present in the fruit bodies of this species. Among the detected amino acids the highest share was recorded for L-alanine at 2.43 mg/g d.m. and L-leucine at 2.38 mg/g d.m. The lowest contents were found for L-tryptophan at 0.10 mg/g d.m. and L-phenylalanine at 0.20 mg/g d.m. L-isoleucine and L-tyrosine were not

detected. Lion's mane mushroom contain considerable amounts of potassium and phosphorus, *viz.*, 254 and 109 mg/100 g dry matter, respectively. Manganese, copper and zinc were found in *Hericium* very low, trace amounts. Contents of heavy metals, *viz*., arsenic, lead, copper, and cadmium, were higher in mycelium than in fruit bodies. Analyses were also conducted on contents of aroma compounds in fruit bodies of lion's mane mushroom. The total content of volatile aroma compounds was determined in fruit bodies of lion's mane mushroom. The dominant compound was 1-octen-3-ol which accounted for 56–60 per cent total content of aroma substances. In another study, it was showed that the dominant compounds are 2-methyl-3-furanthiol, 2-ethylpyrazine and 2,6-diethylpyrazine. Sixteen aroma substances were identified containing nitrogen or sulphur, aldehydes, ketones, alcohols, and esters**.**

Chapter-III

Materials And Methods

CHAPTER-III

MATERIAL AND METHODS

The material and methods adopted during the course of present investigation are described here as under:

3.1 Experimental site

The cultivation studies were carried out at Mushroom Research and Training Centre, Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Main campus, Chatha during the year 2020-2021. *In vitro* studies related to the present research were conducted in the Mushroom Biology Laboratory, Division of Plant Pathology and the studies related to the analysis of nutritional parameters were carried out at the Division of Biochemistry, Faculty of Basic Sciences, SKUAST-J.

3.2 Cleaning and sterilization of glassware and media

Glass wares were cleaned with a cleaning mixture (100 g potassium dichromate + 400 ml hot water + 600 ml of concentrated sulphuric acid) and subsequently washed under tap water. The washed glass wares were sterilized by dry heat in hot air oven at 180^0C for 90 minutes while the media was sterilized by wet heat in an autoclave at 15 lb per square inch (psi) for 20 minutes during the course of experimentation.

3.3 Cleaning and disinfection of growing room

The concrete flooring of cropping room was scrubbed with a wire brush, swept with bamboo broom and thoroughly washed with detergent and water. The disinfection of flooring, concrete walls and air space was done with 5 per cent formaldehyde. The air space of growing room was disinfected by spraying 2.5 liter of above solution for each $100m^2$ of air space. The room was aerated two days before placing the bags in the cropping room.

3.4 Sources of master culture and its maintenance

Five strains of *Hericium erinaceus* used in the present study (He-01, He-02, He-03, He-04, He-05) were procured from the Directorate of Mushroom Research, Solan (Himachal Pradesh). The pure cultures were maintained by repeated sub culturing on Potato Dextrose Agar (PDA) media at 20 days interval and incubated at 25±1^{0}C. After full growth, these slants were kept in refrigerator till further use.

3.5 Spawn preparation

Wheat grain based spawn was used during the course of present investigation. To prepare the spawn, clean and healthy wheat grains were boiled for approximately 15 minutes or till they became soft but did not split apart. Excess water was drained through steel mesh and the grains were allowed to dry slightly under shade. These grains were mixed with 0.5 per cent calcium sulphate and 2 per cent calcium carbonate on dry weight basis of grains. About 250 gm of these grains were filled in half litre glass bottles, plugged with non absorbent cotton and sterilized at 15lb psi for 2 hours. Each bottle after cooling was shaken to remove clumps and inoculated under aseptic conditions with 30 days old pure culture bits of *Hericium* species prepared as per 3.4. Bottles were incubated in B.O.D incubator at 25±2^{0}C till full spawn run which was achieved in about 15 days. These bottles served as mother spawn and from each bottle of master spawn were used for inoculating 10 commercial spawn bottles which was further used for inoculating the substrate bags of *H. erinaceus*.

3.6 Cultivation studies

3.6.1 Substrate preparation and spawning

Production trials of *Hericium erinaceus* were carried out on three different lignocellulosic wastes *viz*. wheat straw, paddy straw, saw dust and their combinations. Hard wood sawdust of Sheesham (*Dalbergia sissoo*), rice straw and wheat straw were used singly as well as mixed (1:1) for preparation of substrate media.

The substrates were soaked in a solution of carbendazim (75 ppm) and formalin 40 per cent (500 ppm) for 16-18 hours. The excess water was drained off by spreading

PLATE-1

Mycelial cultures of *Hericium erinaceus* strains (He-01,He-02, He-03, He-04, He-05)

Mycelial growth of *Hericium erinaeus* strains (He-01,He-02, He-03, He-04, He-05)

Spawn of He-03 and He-05 strains

Culture and Spawn of *Hericium erinaceus*

the wet substrates on sloppy cemented floor. The partially dried substrates (63-64 % moisture) used singly as well as in combination (1:1) were mixed with wheat bran, $CaCO_3$ and sugar in following proportions:

Sawdust + 20 % wheat bran + 1 % $CaCO_3$ + 1 % sugar.

Rice straw + 20 % wheat bran + 1 % $CaCO_3$ + 1 % sugar.

Wheat straw + 20 % wheat bran + 1 % $CaCO_3$ + 1 % sugar.

Sawdust + rice straw + 20 % wheat bran + 1 % $CaCO_3$ + 1 % sugar.

Sawdust + wheat straw + 20 %wheat bran + 1 % $CaCO_3$ + 1 % sugar.

Rice straw + wheat straw + 20 %wheat bran + 1 % $CaCO_3$ + 1 % sugar.

The mixture was then filled in autoclavable polypropylene bags (12″ × 20″) and autoclaved at 121^0C for 1 hour. Once the sterilized substrate was cooled down, the bags were inoculated by the previously prepared grain spawn 2 per cent (w/w) and then incubated at 22-27^0C for spawn run (mycelium growth). The spawned bags were kept in a dark room for the completion of spawn run. Once the spawn run was complete in inoculated bags, the bags were opened and subjected to the fruiting conditions *viz*. exposure to scattered light, watering by daily water spraying, good ventilation, adjusting relative humidity to 85–90 per cent and temperature of 20-25^0C.

3.6.2 Harvesting

Fruit bodies were harvested when they had matured fully by twisting them gently clockwise and anti clockwise so that young developing sporocarps were not damaged. The crop was picked after 14-20 days from the end of incubation time in consecutive flushes at intervals of 15-20 days. After harvesting, following yield and yield related parameters of *Hericium erinaceus* were recorded:

- Days of spawn run
- Days of fruit initiation
- Number of fruit bodies
- Average weight of fruit body

- Diameter of clump of spines
- Number of flushes
- Total yield
- Biological Efficiency
- Analysis of Benefit: Cost ratio

3.7 Biochemical analysis

Harvested fruit bodies of *Hericium erinaceus* were brought to the laboratory for analysing flowing biochemical parameters:

3.7.1 Estimation of moisture per cent of fruit bodies

The moisture content of fruit bodies was determined by drying the fruit bodies in hot air oven at 55^0C till constant weight was achieved. The per cent moisture content was calculated by the formula:

$$\text{Moisture \%} = \frac{\text{Weight of fresh sample} - \text{Weight of dry sample}}{\text{Weight of fresh sample}} \times 100$$

The dried samples after grinding and moisture estimation were stored in airtight containers for further estimation of various proximate compositions.

3.7.2 Estimation of crude fat

Crude fat was estimated by standard method of analysis (AOAC, 1990) using the Soxhlet Extraction apparatus (SOCS PLUS; SSC-6).

Procedure:

- Weighed amount (3 g) of dry sample was taken in a beaker and was transferred to an extraction thimble.
- The thimble was placed in a soxhlet extractor fitted with a condenser and flask containing sufficient hexane (BP 60-70^{o}C)

- After 3-4 hours of extraction, beaker was removed from the extraction apparatus and dried in a hot air oven to a constant weight, cooled in a desiccator at room temperature and then weighed.
- Loss of weight of beaker indicated the amount of fat in the sample.

The fat per cent was calculated by the following relationship as under:

$$\text{Crude fat (\%)} = \frac{\text{Final weight of the beaker – empty weight}}{\text{Weight of sample}} \times 100$$

3.7.3 Estimation of crude fibre

Crude fibre was estimated by standard method of analysis (AOAC, 1990).

- A known quantity of fat free sample was transferred to a beaker to which 175 ml of distilled water followed by 25 ml of 2.04 N H_2SO_4 were added.
- This was refluxed for 30 minutes, filtered through a muslin cloth and washed repeatedly with hot distilled water.
- The washed residue was transferred to a spotless beaker with the help of a jet of distilled water.
- To this 175 ml of distilled water and 25 ml of 2.5 N NaOH solution were added.
- This was refluxed for 30 minutes and filtered through a pre weighed Gooch crucible under vacuum.
- The crucibles were dried over night at 100 ± 2^0C and weighed.
- The residue in Gooch crucibles was ashed at 550-600^0C in a muffle furnace for 3 hours.
- The difference in oven dried and ashed weights gave us the weight of crude fibre and was calculated as follows:

$$\text{Crude fibre (\%)} = \frac{a - b}{w} \times 100$$

where,

a = Weight (g) of crucible plus oven dried residue left after acid and alkali digestion

b = Weight (g) of crucible plus ash

w = Weight (g) of oven dried sample

3.7.4 Estimation of Total ash

Total ash was estimated by standard method of analysis (AOAC, 1990).

- A known quantity of ground sample was taken in a pre weighed silica basin and charred over the heater to make it smoke free.
- The crucible along with the sample was ignited at 600^0C for 3 hours in muffle furnace.
- When muffle furnace was slightly cooled, the crucible with ash was taken out and kept in a dessicator to cool down and weighed to constant weight.
- The difference between the weight of silica basin as empty and with ash was the amount of total ash.
- The per cent ash was calculated from the following formula:

$$\text{Ash \%} = \frac{\text{Weight of ash}}{\text{Weight of sample on dry matter basis}} \times 100$$

3.7.5 Estimation of Crude protein

Protein in the mushroom samples was estimated by Kjeldahl method suggested by Jackson (1973).

3.7.5.1 Reagents used

a) Commerical sulphuric acid

b) Digestion mixture (1 part copper sulphate: 10 parts potassium sulphate)

c) 40 per cent sodium hydroxide

d) Indicator (10 ml of methyl red and bromocresol green was added to 1000 ml of 2 % boric acid solution)

e) H_2SO_4 (N/100 or N/200)

3.7.5.2 Procedure

The method for estimation of crude protein includes digestion, distillation and titration.

3.7.5.3 Digestion

a) A known quantity of each processed dried sample was taken in a 50 ml Kjdeldahl flask.

b) About 20- 50 ml of commercial sulphuric acid was added.

c) 5-10 g of digestion mixture was added.

d) The contents were boiled for 2-3 hours on digestion bench placed in a digestion chamber till the solution became clear without leaving any undigested black particles. Adhering materials inside the walls of flask were given one or two washings in between after cooling.

e) The digested material present was transferred after cooling by dissolving with nitrogen free tap water into volumetric flask (250 ml) followed by 5-6 repeated washings. The final volume was made up after cooling to 250 ml.

f) Similarly a blank was also run without sample.

3.7.5.4 Distillation

a) A conical flask containing 10 ml of Tashiro's indicator was placed at the end of condenser of micro Kjeldahl distillation apparatus. The tip of condenser was kept completely dipped inside the indicator to avoid escape of released ammonia during distillation.

b) 5-10 ml of aliquot of digested sample was pipette out from the volumetric flask into the distillation unit.

c) 10-20 ml of 40 per cent NaOH was added which was sufficient to make the contents alkaline (*viz*. till contents turn blue or black). This was washed with a small quantity of distilled water and receiving end was closed with a pinch cock. The funnel was sealed with a little amount of distilled water to avoid escape of ammonia.

d) The contents of distillation unit were steam distilled by boiling the water in a round bottom flask.

e) Around 30-50 ml of distillate was collected to ensure that all nitrogen in the form of ammonia was distilled. Red colour turned green.

f) The conical flask with distillate was removed after washing the tip of condenser with a few ml of distilled water.

g) The distillation unit was washed 2-3 times with distilled water with the help of black suction developed by vacuum due to displacement of boiling flask from the heater to make the apparatus ready for distillation of next sample.

3.7.5.5 Titration

a) The distillate in the conical flask was titrated against N/10 H_2SO_4 solution taken in a burette till the red colour just reappears.

b) The volume of N/10 H_2SO_4 consumed was noted.

3.7.5.6 Calculation

1ml of 1 N H_2SO_4 = 14 mg of nitrogen

1ml of N/10 H_2SO_4 = 1.4 mg of nitrogen or 0.004 g of nitrogen

$$\text{Protein \%} = \frac{A \times (B\text{-}B1) \times 0.0014 \times 6.25}{C \times W} \times 100$$

where,

A = Volume (ml) made out of digested sample

B = Volume (ml) of standard (say N/10 H_2SO_4) consumed for titration of blank distillate

C = Volume (ml) of aliquot taken for distillation

W = Weight of sample (g)

3.7.6 Total carbohydrate

Total carbohydrate was calculated by subtracting the sum of percentage of total moisture (M), total crude protein (CP), crude fiber (CF), total fat (TF) and total ash (TA) from dry matter (Gopalan *et al.*, 1971).

Total carbohydrates = 100- (M % + CP % + CF % + TF % + TA %)

Chapter-IV

Results

CHAPTER-IV

RESULTS

The result of the present investigation on **"Evaluation of Different Strains and Substrates for Cultivation of Lion's Mane Mushroom''** are described as under:

4.1 *In vitro* evaluation for mycelial growth of different strains of *Hericium erinaceus*

4.1.1 Evaluation for mycelia growth of *Hericium erinaceus* strains on PDA

Different strains of *Hericium erinaceus* (He-01, He-02, He-03, He-04 and He-05) were studied for mycelial growth on Potato Dextrose Agar (PDA).The results of mycelial growth of different strains are presented in Table 1 which revealed that the maximum colony diameter of 86.86 mm was observed in He-03 strain followed by 78.06 mm in He-05. However, minimum colony diameter 22.43 mm was observed in He-01. Data presented in table 4.1 shows that all the strains were significantly different from each other at all the days of evaluation.

Table 4.1. Mycelial growth of *Hericium erinaceus* on PDA

Strain	Colony diameter (mm) in days								
	2	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**
He-01	2.03	5.60	8.86	11.33	14.30	16.20	17.80	19.33	22.43
He-02	3.30	6.21	9.10	12.20	15.23	18.56	21.86	25.03	28.33
He-03	8.20	16.40	23.90	32.13	40.16	48.16	62.10	74.40	86.86
He-04	5.20	10.83	16.30	24.26	32.33	40.46	48.50	58.03	70.63
He-05	7.23	16.40	23.33	30.66	37.20	44.10	56.13	67.86	78.06
C.D ($p < 0.05$)	0.22	1.10	1.11	1.03	12.54	0.99	1.46	0.89	0.81

4.1.2 Mycelial growth rate of *Hericium erinaceus* strains

Mycelial growth rate based on the mycelial growth of different strains of *Hericium erinaceus* on PDA was calculated and has been depicted in Table 4.2. It was observed that He-03 showed the maximum average mycelial growth rate of 4.23 mm per day followed by 3.91 mm per day in He-05. However, the minimum average growth (1.30 mm) was observed in He-01. Table 4.2 shows that their was a significant difference between all the strains at day 2, day 4, day 6, day 8, day 10, day 12, day 14, day 16 and day 18.

Table 4.2. Mycelial growth rate of *Hericium erinaceus* on PDA

Mycelial growth rate (mm/day)										
Strain	**Days**									**Average Mycelial growth rate**
	2	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	
He-01	1.01	1.40	1.47	1.41	1.43	1.35	1.27	1.20	1.24	1.30
He-02	1.65	1.55	1.51	1.52	1.52	1.54	1.56	1.56	1.57	1.55
He-03	4.10	4.10	3.98	4.01	4.01	4.01	4.43	4.65	4.82	4.23
He-04	2.60	2.70	2.70	3.03	3.20	3.37	3.46	3.62	3.90	3.17
He-05	3.61	4.10	3.88	3.80	3.72	3.60	4.00	4.24	4.30	3.91
C.D ($p < 0.05$)	0.31	0.46	0.45	0.45	0.41	0.71	0.98	0.75	0.29	

4.2 Evaluation of different substrates for the cultivation of Lion's mane mushroom

Different substrates *viz*., wheat straw, sawdust, rice straw alone and in combination sawdust + wheat straw, sawdust + rice straw and wheat straw + rice straw with wheat bran as supplement were evaluated for the cultivation of *Hericium erinaceus*.

4.2.1 Days taken for spawn run of *Hericium erinaceus*

Among the five different strains (He-01, He-02, He-03, He-04 and He-05), two strains (He-03 and He-05) based on their fast and vigorous mycelial growth rate were selected and consequently evaluated for the cultivation parameters of *Hericium erinaceus*. Data presented in Table 4.3 shows that in He-03, there was a significant difference in all the substrates while wheat straw and rice straw, rice straw + saw dust and rice straw + wheat straw were non significant to each other. Similarly, in case of He-05 all the substrates were significant to each other. The minimum (earliest) days for spawn run (38.66 days) were observed with He-03 on wheat straw + rice straw substrate followed by wheat straw + saw dust (41.00 days), rice straw + saw dust (42.00 days), wheat straw alone (45.00 days), rice straw (46.33 days) and sawdust (48.66 days). However, maximum spawn run period of 48.66 days was observed in sawdust substrate alone. In case of strain He-05, the earliest mean spawn run was observed with rice straw + wheat straw in 39.00 days followed by 41.66 days in wheat straw + sawdust; 42.33 days in rice straw + sawdust; 45.00 days in case of rice straw alone and 46.33 days with wheat straw alone. Maximum spawn run period of 47.33 days was observed with sawdust alone.

4.2.2 Days taken for fruit initiation of *Hericium erinaceus*

Data presented in Table 4.3 indicates that the strains He-03 and He-05 were non significant in regard to days taken for fruit initiation in case of wheat straw and rice straw, rice straw and rice straw + saw dust, rice straw and rice straw + saw dust, wheat straw + saw dust and saw dust, wheat straw + saw dust and rice straw + wheat straw. Minimum number of days recorded for fruit initiation by He-03 were (52.33 days) with wheat straw + sawdust followed by 52.66 days in case of wheat straw + rice straw; 55.33 days in rice straw + saw dust; 57.66 days in wheat straw alone and 58.00 days in rice straw alone. However, maximum days for fruit initiation (65.00 days) were observed with sawdust substrate with strain He-03. In case of He-05 strain, minimum days for fruit initiation (51.33 days) was recorded in wheat straw + rice straw followed by 54.33 days for wheat straw + saw dust; 56.66 days in rice straw alone; 58.33 days with rice straw + saw dust and 58.66 days in wheat straw alone. Maximum days for fruit initiation (63.33 days) were observed in sawdust.

Table 4.3. Effect of various substrates on cropping period of *Hericium erinaceus*

Substrate	Days of spawn run		Days of fruit initiation	
	Strain		Strain	
	He-03	He-05	He-03	He-05
Saw Dust	48.66	47.33	65.00	63.33
Wheat Straw	45.00	46.33	57.66	58.66
Rice Straw	46.33	45.00	58.00	56.66
Rice Straw + Saw Dust	42.00	42.33	55.33	58.33
Wheat Straw + Saw Dust	41.00	41.66	52.33	54.33
Rice Straw + Wheat Straw	38.66	39.00	52.66	51.33
C.D ($p < 0.05$)	2.64	2.87	4.83	4.58

4.3 Evaluation of various substrates on yield related parameters of *Hericium erinaceus*

Different yield related parameters of *Hericium erinaceus* like diameter of clump of spines, number of fruit bodies and average weight of fruit bodies obtained on being cultivated on different substrates were evaluated.

4.3.1 Diameter of clump of spines of *Hericium erinaceus*

The data presented in Table 4.4 showed maximum diameter of clumps of spines (8.20 cm) in fruit bodies of He-03 cultivated in sawdust followed by 7.20 cm in wheat straw; 6.60 cm in rice straw + sawdust; 6.50 cm rice straw and 5.40 cm in wheat straw + sawdust. However, minimum diameter of clump of spines (4.70 cm) was observed in rice straw + wheat straw. In case of strain He-05, maximum diameter of clump of spines (7.60 cm) was observed in sawdust followed by 7.00 cm in wheat straw; 6.80 cm in rice straw + sawdust; 6.40 cm diameter in rice straw and 5.20 cm diameter in wheat straw + sawdust. However, minimum diameter of 5.00 cm was observed in wheat straw + rice straw. Rice straw and rice straw + saw dust had non significant difference in He-03. Similarly, in He-05 saw dust and wheat straw, wheat straw and rice straw, rice straw and

PLATE-2

Mycelium run on cropping substrates alone and in combination with supplements

rice straw + saw dust, wheat straw + saw dust and rice straw + wheat straw showed non significant difference.

4.3.2 Number of fruit bodies of *Hericium erinaceus*

The data presented in Table 4.4 reveals that in case of strain He-03, rice straw and rice straw + saw dust showed the non significant difference where as in He-05, saw dust + wheat straw, rice straw + saw dust, rice straw + saw dust and rice straw + wheat straw were non significant from each other. In He-05, saw dust and wheat straw, rice straw and rice straw + saw dust, rice straw + saw dust and wheat straw + saw dust were non significant between the substrates. For He-03, maximum number of fruit bodies (15.50) was recorded in saw dust substrate followed by 14.30 in wheat straw; 13.00 in wheat straw + saw dust; 12.80 in rice straw and 12.30 in rice straw + saw dust, whereas minimum number of fruit bodies (10.60) were recorded in rice straw + wheat straw. In case of He-05 strain, maximum number of fruit bodies (14.90) were observed in saw dust followed by 14.20 in wheat straw; 12.80 in wheat straw + saw dust; 12.50 in rice straw and 11.90 with rice straw + sawdust whereas minimum number of fruit bodies (10.10) was observed in rice straw + wheat straw.

4.3.3 Average fruit body weight of *Hericium erinaceus* cultivated on different substrates

Data presented in Table 4.4 reveals that in case of strain He-03, maximum average weight of fruit bodies (33.84 g) was observed on wheat straw + saw dust followed by saw dust (32.19 g); 31.22 g on wheat straw + rice straw; 29.72 g on wheat straw and 28.04 g on rice straw + saw dust while minimum average weight of 24.60 g was obtained on rice straw. In case of strain He-05, maximum average weight of fruit bodies (34.76 g) was obtained on wheat straw + saw dust followed by 33.86 g on wheat straw + rice straw; 33.69 g on saw dust; 30.42 g on wheat straw and 29.74 g on rice straw + saw dust while the minimum average weight of 25.60 g was obtained on rice straw. In the strains, He-03 and He-05, there was non significant difference in saw dust and rice straw + wheat straw.

Table 4.4. Effect of various substrates on cropping physiology of *Hericium erinaceus*

Substrate	Diameter of clump of spines (cm)		Number of fruit bodies		Average weight of fruit body(g)	
	Strain		Strain		Strain	
	He-03	He-05	He-03	He-05	He-03	He-05
Saw Dust	8.20	7.60	15.50	14.90	32.19	33.69
Wheat Straw	7.20	7.00	14.30	14.20	29.72	30.42
Rice Straw	6.50	6.40	12.80	12.50	24.60	25.60
Rice Straw + Saw Dust	6.60	6.80	12.30	11.90	28.04	29.74
Wheat Straw + Saw Dust	5.40	5.20	13.00	12.80	33.84	34.76
Rice Straw + Wheat Straw	4.70	5.00	10.60	10.10	31.22	33.86
C.D ($p < 0.05$)	0.66	0.96	0.71	1.07	1.18	0.70

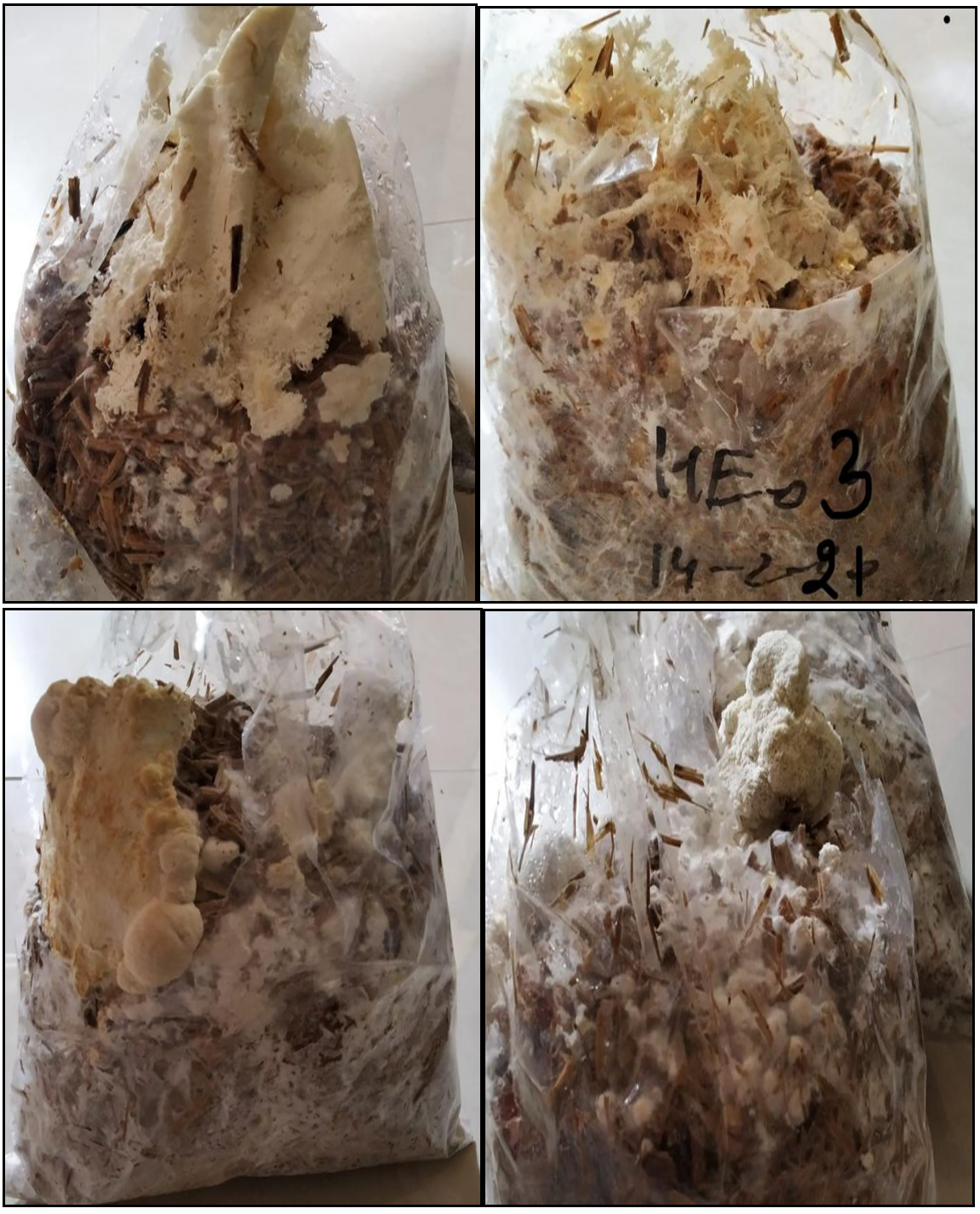
4.4 Evaluation of cropping substrates for yield pattern of *Hericium erinaceus*

Cropping substrates were evaluated for their yield pattern *viz*., emerging of fruit bodies on substrates in terms of number of flushes and the yield per flush.

4.4.1 Yield of *Hericium erinaceus* during first flush

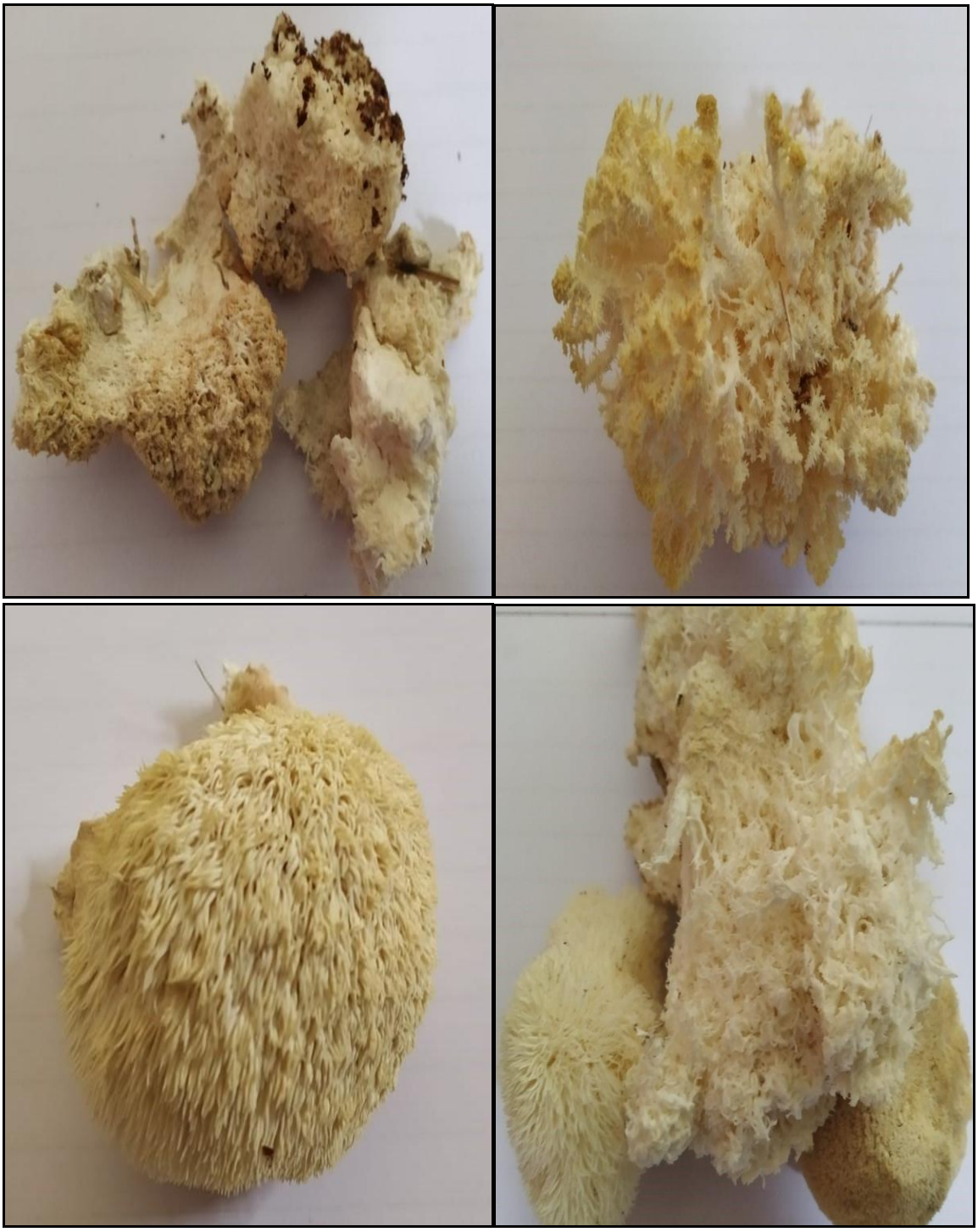
It is evident from Table 4.5 that in He-03, there was non significant difference in rice straw + saw dust and rice straw + wheat straw, whereas for He-05 rice straw and rice straw + saw dust, rice straw + saw dust and rice straw + wheat straw were non significant to each other. In strain He-03, maximum yield of 174.33 g was recorded in saw dust followed by 153.00 g in wheat straw + saw dust and 145.00 g, 125.66 g and 119.33 g in wheat straw; rice straw + saw dust; and rice straw + wheat straw respectively. Minimum yield of 105.33 g per 1000 g of dry substrate was recorded in rice straw alone. In case of He-05 strain, maximum yield (178.00 g per 1000 g of dry substrates) was recorded in saw dust followed by 160.33 g in wheat straw + saw dust whereas 141.33 g, 124.00 g, and 119.00 g in wheat straw, rice straw + wheat straw and rice straw + saw dust, respectively. Minimum yield of 117.66 g per 1000 g of dry substrates was recorded in rice straw.

PLATE-3



Growth of fruiting bodies on cropping substrates and in combination with supplements

PLATE-4



Fruiting bodies of *Hericium erinaceus* grown on cropping substrate alone and in combination with supplements

4.4.2 Yield of *Hericium erinaceus* during second flush

The data recorded on yield of fruit bodies of *Hericium erinaceus* obtained per 1000 g of dry substrates during second flush presented in Table 4.5 reveals that in strain He-03, there was no significant difference showed in rice straw + wheat straw and rice straw + saw dust, rice straw and rice straw + saw dust, rice straw and rice straw + wheat straw, wheat straw and wheat straw + saw dust. Similarly, in He-05 strain wheat straw and wheat straw + saw dust, rice straw and rice straw + wheat straw also showed non significant difference. For strain He-03, maximum yield (165.33 g) was recorded in saw dust which was followed by 142.33 g, 140.00 g, 115.66 g and 115.33 g on wheat straw, wheat straw + saw dust, rice straw and rice straw + saw dust respectively. Minimum yield of 114.66 g per 1000 g of dry substrates was recorded in rice straw + wheat straw. In case of He-05, maximum yield of 167.33 g per 1000 g of dry substrate was recorded in saw dust, whereas 146.00 g, 145.66 g, 125.33 g and 112.66 g was recorded in wheat straw + saw dust, wheat straw, rice straw + saw dust and rice straw + wheat straw per 1000 g of dry substrates respectively. However, minimum yield (103.00 g) was recorded in rice straw substrate.

4.4.3 Yield of *Hericium erinaceus* during third flush

Data in the Table 4.5 exhibit that in He-03 strain there was no significant difference showed in rice straw and rice straw + wheat straw, rice straw and rice straw + saw dust, rice straw + saw dust and rice straw + wheat straw, wheat straw and wheat straw + saw dust while in He-05 strain, rice straw and rice straw + saw dust, rice straw and rice straw + wheat straw, wheat straw and wheat straw + saw dust showed non significant difference. In He-03, maximum yield of 160.00 g per 1000 g of dry substrates was recorded in saw dust followed by 147.33, 138.33, 105.00 and 98.66 g in wheat straw + saw dust, wheat straw, rice straw + sawdust and rice straw + wheat straw, respectively. Minimum yield (95.66 g per 1000 g of dry substrates) was recorded in rice straw. Similar trend was observed in case of He-05 strain, as the maximum yield (157.66 g) was recorded in saw dust which was followed by 146.00 g wheat straw, 139.33 in wheat straw + saw dust, 110.00 g in rice straw + saw dust and 106.33 g in rice straw + wheat straw. Minimum yield (100.33 g per 1000 g of dry substrates) was recorded in rice straw.

Table 4.5. Effect of cropping substrates on yield pattern of *Hericium erinaceus* strains

Substrate	Yield/ 1000 g substrate					
	Ist flush		2nd flush		3rd flush	
	Strain		Strain		Strain	
	He-03	He -05	He-03	He-05	He-03	He-05
Saw Dust	174.33	178.00	165.33	167.33	160.00	157.66
Wheat Straw	145.00	141.33	142.33	145.66	138.33	146.00
Rice Straw	105.33	117.66	115.66	103.00	95.66	100.33
Rice Straw + Saw Dust	125.66	119.00	115.33	125.33	105.00	110.00
Wheat Straw + Saw Dust	153.00	160.33	140.00	146.00	147.33	139.33
Rice Straw + Wheat Straw	119.33	124.00	114.66	112.66	98.66	106.33
C.D ($p < 0.05$)	12.86	14.14	8.46	11.49	14.23	14.01

4.4.4 Total yield of *Hericium erinaceus* on substrates

Data on the effect of different substrates on the total yield of *H. erinaceus* strains are presented in Table 4.6. Among the various substrates, saw dust proved to be the best producing maximum total yield of 499.66 g /1000 g of dry substrates for He-03 strain, followed by wheat straw + saw dust with total yield of 440.33 g, wheat straw having yield of 425.66 g, rice straw + saw dust (345.99 g) and rice straw + wheat straw giving fruit body yield of 332.65 g. The least effective substrate proved to be rice straw with minimum total yield of 316.65 g/1000 g of dry substrates. Similar trend was observed in case of He-05 strain in which saw dust proved to be best with maximum total yield of 502.99 g/1000 g of dry substrates whereas total yield of 445.66 g, 432.99 g, 354.30 g and 342.99 g were recorded in wheat straw + saw dust, wheat straw, rice straw + saw dust and rice straw + wheat straw, respectively. The least effective substrate was rice straw with minimum total yield of 320.99 g/1000 g of dry substrates. In He-03 strain wheat straw and wheat straw + saw dust, rice straw + saw dust and rice straw + wheat straw had non significant difference while in He-05 strain rice straw + saw dust and rice straw + wheat straw had non significant difference.

4.4.5 Biological efficiency of *Hericium erinaceus* strains

Data in the Table 4.6 reveals that the biological efficiency of *H. erinaceus* strains was significantly affected by the selected substrates. In He-03, maximum biological efficiency of 49.96 per cent was recorded on saw dust, followed by 44.03, 42.56, 34.59 and 33.26 per cent by wheat straw + saw dust, wheat straw, rice straw + saw dust and rice straw + wheat straw, respectively. Minimum biological efficiency of 31.66 per cent was recorded in rice straw. In case of He-05, maximum biological efficiency of 50.29 per cent was recorded in saw dust which was followed by 44.56 per cent in wheat straw + saw dust, 43.29 per cent in wheat straw, 35.43 per cent in rice straw + sawdust, and 34.29 per cent in rice straw + wheat straw. Minimum biological efficiency of 32.09 per cent was recorded in rice straw.

Table 4.6. Total yield and biological efficiency of the *Hericium erinaceus* on different substrates

Treatments	Total yield/1000 g of substrates		Biological efficiency (%)	
	He-03	He-05	He-03	He-05
Saw Dust	499.66	502.99	49.96	50.29
Wheat Straw	425.66	432.99	42.56	43.29
Rice Straw	316.65	320.99	31.66	32.09
Rice Straw + Saw Dust	345.99	354.33	34.59	35.43
Wheat Straw + Saw Dust	440.33	445.66	44.03	44.56
Rice Straw + Wheat Straw	332.65	342.99	33.26	34.29
C.D ($p < 0.05$)	15.14	15.66		

4.4.6 Cost: Benefit ratio

To find out the substrate which has maximum economic feasibility, Cost: Benefit ratio of different treatments was calculated and data presented in Table 4.7 shows that maximum cost: benefit ratio for He-03 was observed in wheat straw + saw dust (1:3.99) followed by wheat straw (1:3.97), saw dust (1:3.32), rice straw + saw dust (1:3.03), rice straw + wheat straw (1:3.00) and minimum cost: benefit ratio was found in rice straw

(1:2.92). In case of He-05 strain, maximum cost: benefit ratio was found in wheat straw + saw dust and wheat straw (1:4.05) followed by saw dust (1:3.34), rice straw + saw dust (1:3.13) and rice straw + wheat straw (1:3.12) and minimum cost: benefit ratio was found in rice straw (1:2.97).

Table 4.7. Cost: Benefit ratio of cultivation of *Hericium erinaceus* on different substrates

Substrate	Total Expenditure incurred per bag (Rs)		Total income generated per bag (Rs)		Benefit per bag (Rs)		Cost: Benefit ratio	
	He-03	He-05	He-03	He-05	He-03	He -05	He-03	He-05
Saw Dust	37.00	37.00	159.87	160.92	122.87	123.92	1:3.32	1:3.34
Wheat Straw	27.40	27.40	136.19	138.52	108.79	111.12	1:3.97	1:4.05
Rice Straw	25.80	25.80	101.31	102.68	75.51	76.88	1:2.92	1:2.97
Rice Straw + Saw Dust	27.40	27.40	110.68	113.37	83.28	85.97	1:3.03	1:3.13
Wheat Straw + Saw Dust	28.20	28.20	140.89	142.59	112.69	114.39	1:3.99	1:4.05
Rice Straw + Wheat Straw	26.60	26.60	106.43	109.72	79.83	83.12	1:3.00	1:3.12

4.5 Biochemical composition of fruiting bodies of *Hericium erinaceus* grown on different substrates

Fruit bodies grown on different substrates were analysed for various biochemical components. Results of various biochemical components of *Hericium erinaceus* grown on different substrates has been discussed in Table 4.8.

4.5.1 Moisture content

The per cent moisture content of the fruiting bodies of *H. erinaceus* (He-03) harvested from different substrates ranged from 87.66 to 91.66 per cent (Table 4.8). Maximum moisture content (91.66 %) was observed in fruiting bodies obtained from rice straw + wheat straw, followed by 91.33, 90.33, 89.66 and 89.00 per cent in wheat straw +

saw dust, wheat straw, rice straw and rice straw + sawdust, respectively. Minimum moisture content (87.66 %) was recorded in fruiting bodies obtained from saw dust. Similar trend was observed with He-05 strain grown on different substrates in which moisture content ranged from 90.66 to 88.66 per cent. Maximum moisture content (90.66 %) was observed in fruit bodies obtained from rice straw + wheat straw, followed by 90.33, 89.66, 89.50 and 89.33 per cent in wheat straw + saw dust, wheat straw, rice straw and rice straw + saw dust respectively. Minimum moisture content of 88.66 per cent was observed in fruit bodies obtained from sawdust. In He-03 there were non significant difference showed in wheat straw + saw dust, rice straw + wheat straw, rice straw and rice straw + saw dust, wheat straw and rice straw while in He-05 strain wheat straw and rice straw, wheat straw + saw dust and rice straw + wheat straw, rice straw and rice straw + saw dust substrates were non significant to each other.

4.5.2 Crude protein content

Crude protein content in fruiting bodies of He-03 strain harvested from different substrates ranged from 24.83 to 27.16 per cent. The highest crude protein content of 27.16 per cent was observed in fruiting bodies grown in wheat straw followed by 26.33 per cent in saw dust and rice straw + wheat straw, 26.16 per cent from wheat straw + saw dust and 25.33 per cent in rice straw + sawdust substrates. Minimum crude protein content of 24.83 per cent was observed in the fruiting bodies obtained from rice straw. In He-05, similar trend was observed in which crude protein content in fruit bodies ranged from 25.00 to 27.50 per cent harvested from different substrates. Maximum crude protein content of 27.50 per cent was observed in fruiting bodies obtained from wheat straw followed by 26.66 per cent in rice straw + saw dust, 26.50 per cent in rice straw + wheat straw, 26.00 per cent in saw dust and 25.16 per cent in wheat straw + saw dust. Minimum crude protein content of 25.00 per cent was observed in fruit bodies obtained from rice straw. In the strains, He-03 and He-05 wheat straw + saw dust and rice straw + wheat straw, rice straw and rice straw + saw dust substrates were non significant to each other.

4.5.3 Crude fat content

Crude fat content (per cent) in fruiting bodies of He-03 strain grown on different substrates ranged from 3.16 to 4.83 per cent (Table 4.8). Maximum crude fat content of

4.83 per cent was observed in fruiting bodies obtained from rice straw followed by 4.50, 3.83, 3.50 and 3.33 per cent in rice straw + saw dust, wheat straw + saw dust, wheat straw and rice straw + wheat straw, respectively. Minimum crude fat content (3.16 %) was observed in fruiting bodies cultivated on saw dust substrate. In case of He-05 strain, crude fat content varied from 3.50 to 4.66 per cent. Maximum crude fat content (4.66 %) was observed in fruiting bodies obtained from rice straw followed by 4.33 per cent in rice straw + saw dust, 4.16 per cent in wheat straw + saw dust, 3.83 per cent in wheat straw and 3.66 per cent in sawdust. Minimum crude fat content (3.50 %) was observed in fruiting bodies cultivated on rice straw + wheat straw. The crude fat content of fruiting bodies of *H. erinaceus* harvested from different substrates was significantly different from each other. In He-03 strain wheat straw + saw dust and rice straw + wheat straw, rice straw and rice straw + saw dust, saw dust and wheat straw substrates were non significant from each other. Similarly, in case of He-05 rice straw and rice straw + saw dust, saw dust + wheat straw, rice straw and wheat straw + saw dust substrates were non significant to each other.

4.5.4 Crude fibre content

Crude fiber content (%) in fruiting bodies of *H. erinaceus* strains harvested from different substrates was analysed and the crude fibre content in fruiting bodies of He-03 was observed to range from 7.00 to 7.90 per cent (Table 4.8). Maximum crude fibre (7.90 %) was observed in fruit bodies grown on saw dust followed by 7.60, 7.40, 7.23 and 7.10 per cent from rice straw + saw dust, rice straw, wheat straw + saw dust and wheat straw respectively. Minimum crude fibre (7.00 %) was observed in fruit bodies cultivated on rice straw + wheat straw combination substrates. Similarly, in He-05 crude fibre content of fruit bodies ranged from 7.26 to 8.10 per cent with maximum crude fibre content (8.10 %) observed in fruit bodies obtained from saw dust followed by 7.60 per cent in wheat straw + saw dust, 7.53 per cent in rice straw + saw dust, 7.46 per cent in wheat straw and 7.36 per cent in rice straw + wheat straw. Minimum crude fibre (7.26 %) was observed in fruiting bodies harvested from rice straw substrate. In He-03 strain rice straw + saw dust and wheat straw + saw dust, rice straw and rice straw + saw dust, wheat straw and rice straw substrates were non significant to each other while in He-05 strain wheat straw and

rice straw, rice straw and rice straw + saw dust, wheat straw + saw dust and rice straw + wheat straw substrates had non significant differences.

4.5.5 Total ash content

Total ash content (%) in fruiting bodies of He-03 strain cultivated on different substrates ranged from 10.20 to 12.86 per cent. Higher total ash content (12.86 %) was observed in fruit bodies obtained from rice straw followed by 12.30 per cent in rice straw + saw dust, 11.96 per cent in wheat straw, 11.56 per cent in wheat straw + saw dust and 11.20 per cent in sawdust. Lower total ash content (10.23 %) was observed in fruiting bodies cultivated on rice straw + saw dust. In case of He-05 strain that was cultivated on different substrates, total ash content of fruit bodies ranged from 10.60 to 12.66 per cent. Higher total ash content of 12.66 per cent was observed in fruit bodies harvested from rice straw followed by rice straw + saw dust (12.03 %), saw dust (11.73 %), wheat straw (11.53 %) and wheat straw + saw dust (11.30 %). Lower total ash content (10.60 %) was observed in fruit bodies cultivated on rice straw + wheat straw. In He-03 strain wheat straw and wheat straw + saw dust had non significant difference to each other. Similarly, wheat straw and saw dust, wheat straw and wheat straw + saw dust were also non significant to each other in He-05 strain.

4.5.6 Total carbohydrates content

Total carbohydrates content (%) in the fruit bodies of He-03 strain grown on different substrates ranged from 59.66 to 61.83 per cent. Maximum total carbohydrates (61.83 %) was observed in fruit bodies grown on rice straw + wheat straw followed by 61.56, 61.46, 61.43 and 61.26 per cent in rice straw, rice straw + saw dust, wheat straw + saw dust and saw dust respectively. Minimum total carbohydrate (59.66 %) was observed in fruit bodies grown on wheat straw. In case of He-05 strain, total carbohydrates content of fruit bodies varied from 58.50 to 61.92 per cent. Maximum total carbohydrates (61.92 %) was observed in fruiting bodies of sawdust followed by 61.76 per cent in wheat straw + sawdust, 61.46 per cent in rice straw + wheat straw, 60.66 per cent in rice straw and 59.86 in rice straw + wheat straw. Minimum total carbohydrates (58.50 %) were observed in fruiting bodies grown on wheat straw. There was a significant variation in

carbohydrates content of fruit bodies of *Hericium erinaceus* harvested from different substrates. In He-05 wheat straw + saw dust and rice straw + wheat straw, rice straw and wheat straw, were highly significant different. Rice straw + wheat straw and sawdust were non significant to each other.

Table 4.8. Chemical composition of fruiting bodies of *Hericum erinaceus* grown on different substrates

Substrate	Moisture (%)		Crude Protein (%)		Crude Fat (%)		Crude Fibre (%)		Total ash (%)		Total Carbohydrates (%)	
	He-03	He-05	He-03	He -05	He-03	He -05	He-03	He-05	He-03	He-05	He-03	He-05
Saw Dust	87.66	88.66	26.33	26.00	3.16	3.66	7.90	8.10	11.20	11.73	61.26	61.92
Wheat Straw	90.33	89.66	27.16	27.50	3.50	3.83	7.10	7.46	11.96	11.53	59.66	58.50
Rice Straw	89.66	89.50	24.83	25.00	4.83	4.66	7.40	7.26	12.86	12.66	61.56	60.66
Rice Straw + Saw Dust	89.00	89.33	25.33	26.66	4.50	4.33	7.60	7.53	12.30	12.03	60.46	59.86
Wheat Straw + Saw Dust	91.33	90.33	26.16	25.16	3.83	4.16	7.23	7.60	11.56	11.30	61.43	61.76
Rice Straw+ Wheat Straw	91.66	90.66	26.33	26.50	3.33	3.50	7.00	7.36	10.23	10.60	61.83	61.46
C.D ($p < 0.05$)	0.84	0.74	0.63	0.72	1.12	0.23	0.39	0.48	0.42	0.37	0.86	0.96

Chapter-V

Discussion

CHAPTER-V

DISCUSSION

The result of the present investigation on **"Evaluation of Different Strains and Substrates for Cultivation of Lion's Mane Mushroom"** are hereby discussed as under:

5.1 Evaluation for mycelia growth of *Hericium erinaceus* strains on PDA

In the present study, the different *Hericium erinaceus* strains were evaluated for mycelial growth on Potato Dextrose Agar (PDA) media. It was observed that after 18 days of incubation, the strain He-01 grew 22.43 mm, He-02 strain grew 28.33 mm, He-03 strain grew 86.86 mm**,** He-04 strain grew 70.63 mm and He-05 grew 78.06 mm in PDA medium. Maximum mycelial growth was observed in He-03 strain. In terms of average mycelia growth, it was observed that He-03 showed the maximum average mycelial growth rate of 4.23 mm per day followed by 3.91 mm per day in He-05. However, the minimum average growth (1.30 mm) was observed in He-01. Imtiaj *et al*. (2008) showed that PDA, YM, Hennerberg and Glucose peptone are the most suitable for mycelial growth of strains of *H. erinaceus.*

5.2 Evaluation of different substrates alone and in combination for the cultivation of *Hericium erinaceus*

The use of different substrates (wheat straw, saw dust, rice straw, rice straw + saw dust, wheat straw + saw dust and rice straw + wheat straw) was used for the cultivation of *H. erinaceus* strains. Among five strains only two strains were evaluated for cultivation because these two strains showed maximum mycelial growth on media. Spawn run was observed quite well only in all substrates. It was observed that He-03 strain observed minimum (38.66 days) taken for complete spawn run and minimum days (51.33) in He-05 for fruit initiation in rice straw + wheat straw. He-03 observed maximum 48.66 days for complete spawn run and maximum 65.00 days for fruit initiation on saw dust. The results are in agreement with the findings of Hassan (2007), who found that incubation time of different media formulae ranged from 37 – 44 days in the first season. While it ranged between 38 – 46 days in the second season and from 40 – 43 days in the third

season. Sawdust seems to have the longest spawn run time followed by rice straw and wheat straw. Sawdust + wheat straw and rice straw + wheat straw had the shortest spawn run time and differ significantly. Our results also corroborate with Han *et al*. (2005) who carried out an experiment by using several agricultural by-products as supplements of sawdust substrate for the production of *Hericium erinaceus*. The oak sawdust substrate with 20 per cent rice bran supplement showed 26–70 per cent biological efficiency. Similarly, Siwulski and Sobieralski (2005) cultivated two *H. erinaceus* strains on beech sawdust substrate enriched with wheat bran (20 %) and reported that saw dust substrate supplemented with wheat bran was better substrate for cultivation of Lion's mane mushroom. The yields of carpophores on these substrates were more than 52 g/100 g dry matter of substrat**e**. In other experiments of Ehlers and Schnitzler (2000) wherein he grew six strains of *H. erinaceus* on coarse and fine beech and ash saw dust with wheat bran and obtained highest yields (254.3 kg / kg substrate) on fine beech saw dust with 20 per cent wheat bran. Eisenhut and Fritz (1995) also reported sawdust to be an effective and economic substrate for *H. erinaceus.*

5.2.2 Diameter of clumps of spines, Number of fruit bodies and average weight of fruit body of *Hericium erinaceus* strains grown on different substrates

Maximum diameter of clumps of spines (8.20 cm), number of fruit bodies (15.50) and average weight of fruit body (33.84 g) was observed with saw dust and wheat straw + sawdust while minimum diameter of clumps of spines of 4.70 cm, number of fruit bodies of 10.60 and average weight of fruit body of 24.60 g was observed with rice straw + wheat straw and rice straw respectively for He-03. Similar results were observed in case of He-05, where maximum diameter of clumps of spines (7.60 cm), number of fruit bodies (14.90) and average weight of fruit body (34.76 g) was observed with saw dust and wheat straw + sawdust respectively, while minimum diameter of clumps of spines (5.00 cm), number of fruit bodies (10.10) and average weight of fruit body (25.60 g) was observed with rice straw + wheat straw and rice straw respectively. These findings are in accordance with Hassan (2007) who also reported that average diameter of clumps of spines ranged from 5-9 cm and average weight of fruit body of *H. erinaceus* ranged from 30 to 45 g.

5.3 Evaluation of cropping substrates on yield and yield related parameters.

5.3.1 Yield of *Hericium erinaceus* per 1000 g of dry substrate during different flushes

Yield is the economic parameters for mushroom cultivation. For strain He-03, the maximum yield of 174.33 g/1000 g dry substrate during first flush, 165.33 g for second flush and 160.00 g for third flush was recorded on saw dust, whereas, minimum yield of 105.33 g for first flush, 115.33 g for second flush and 95.66 g for third flush was observed on rice straw, rice straw + saw dust and rice straw substrate. Similar results were observed in case of strain He-05 in which highest yield of 178.00 g/1000 g dry substrate was observed during first flush, 167.33 g for second flush and 157.66 g for third flush was obtained on saw dust substrate. While minimum yield of 117.66 g, 103.00 g and 100.33 g was recorded for first, second and third flush on rice straw substrate. These results are in line with the findings of Atila and Tuzel (2016) who reported that saw dust gave significantly higher yield among all the substrates evaluated.

5.3.2 Total yield and biological efficiency of *Hericium erinaceus*

Saw dust exhibited maximum total yield of 499.66 g with highest biological efficiency of 49.96 per cent and lowest total yield of 316.65 g with biological efficiency of 31.66 per cent on rice straw substrate for He-03 strain. Similar result was observed in case of He-05 in which saw dust proved to be ideal substrate by producing highest total yield of 502.99 g and corresponding highest biological efficiency of 50.29 per cent, while lowest total yield (320.99 g/1000 g of dry substrate) and corresponding biological efficiency (32.09 %) was observed on rice straw substrate. The results are in conformity with the findings of Hassan (2007) who also reported that saw dust substrate was the best substrate for the cultivation of *H. erinaceus* strain with highest yield of 184/1000 g of dry substrate and the corresponding highest biological efficiency of 50.3 per cent, whereas the highest yield of 165 g/1000 g of dry substrate and the corresponding highest biological efficiency of 46.5 per cent was observed on wheat straw + saw dust substrate. Chang *et al.* (1999)[a] also reported that higher biological yield of 356 g/850 ml and biological efficiency (147.8 %) of *H. erinaceus* in pot culture.

5.4 Chemical composition of fruiting bodies of *Hericium erinaceus* strains grown on different substrates

In the present studies, significant variations were observed in the chemical composition (crude fibre, total ash and total carbohydrates) of fruiting bodies of *Hericium erinaceus* grown from different substrate. However, there was non-significant difference in the composition of crude fat, crude protein and moisture content of the fruiting bodies. Moisture per cent in fruiting bodies harvested from different substrates ranged from 87.66 to 91.66 per cent in He-03. Maximum moisture content (91.66 %) was observed in wheat straw + rice straw and the minimum of 87.66 per cent was observed with fruiting bodies grown on sawdust. Similar result was observed with He-05 strain grown on different substrates in which moisture content ranged from to 88.66 to 90.66 per cent. Maximum moisture content (90.66 %) was observed from wheat straw + rice straw substrates; however, minimum (88.66 %) was from sawdust. Hassan (2007) also reported maximum moisture content (91.05) of fruiting bodies harvested from saw dust + wheat straw and minimum moisture content (89.63) of fruiting bodies from saw dust substrates. Kwon *et al.* (2008) reported that the moisture content of AK and BAK extracts were 94.36 per cent and 97.36 per cent, respectively however, those of extracts of *H. erinaceus* cultured in AK and BAK decreased to 90.95 per cent and 94.20 per cent, respectively.

The highest crude protein content (27.16 %) was observed in fruiting bodies harvested from wheat straw, however lowest crude protein content of 24.83 per cent was observed on rice straw of He-03 whereas in case of He-05, highest crude protein content (27.50 %) harvested from wheat straw and lowest crude protein content of 25.00 per cent was observed on rice straw. Our results are in concurrence with the finding of Hassan (2007) who reported that maximum crude protein content of 26.80 per cent harvested from wheat straw substrate whereas minimum 24.07 per cent harvested from rice straw substrate**.** Mau *et al*. (2001) also reported average protein content of fruit bodies of *H. erinaceus* to be 22.3 per cent. Sokół *et al*. (2016) reported that fruit bodies of Lion's mane Mushroom contain 22.3 per cent protein on dry matter (d.m) basis. Maximum ash content (12.86 %), crude fat (4.83 %) and crude fibre (7.90 %) were observed in fruiting bodies of He-03 grown on rice straw and saw dust while minimum ash content (10.23 %),

crude fat (3.16 %) and crude fibre (7.00 %) were observed in fruiting bodies harvested from rice straw + wheat straw, saw dust and rice straw + wheat straw substrate respectively. Similar trend was observed in case of He-05 strain with maximum ash content (12.66 %) and crude fat (4.66 %) from rice straw whereas crude fibre (8.10 %) was observed in fruit bodies obtained from saw dust substrate, while minimum ash content (10.60 %) and crude fat (3.50 %) were observed from rice straw + wheat straw whereas crude fibre (7.26 %) was observed in fruiting bodies harvested from rice straw substrate. In studies conducted by Sokół *et al*. (2016) they reported that fruit bodies of Lion's mane Mushroom contain, 3.52 per cent fats, 7.81 per cent fiber and 9.35 per cent ash per dry matter (d.m).

Maximum total carbohydrates (61.83 %) of *H. erinaceus* strain He-03 was observed in fruiting bodies grown on rice straw + wheat straw substrate and minimum of 59.66 per cent were observed on wheat straw substrate. Similar trend was observed in case of He-05 strain where maximum total carbohydrates (61.92 %) was observed in fruiting bodies grown on saw dust substrates and minimum total carbohydrates (58.50 %) was observed in fruiting bodies grown on wheat straw substrate. These results are in accordance with the results of Hassan (2007) who reported that ash content range from 9.69 to 11.27 per cent, crude fat content range from 3.95 to 4.21 per cent and total carbohydrates range from (60.95 - 58.92 %) wheat straw substrate. Sokół *et al*. (2016) also reported that fruit bodies of Lion's mane Mushroom contain 57 per cent carbohydrates per dry matter (d.m). Mau *et al*. (2001) reported similar results for crude fat, crude fibre, carbohydrate and ash content of *H. erinaceus* strains.

Chapter-VI

Summary And Conclusions

CHAPTER-VI

SUMMARY AND CONCLUSIONS

The findings of the present investigation on **"Evaluation of Different Strains and Substrates for Cultivation of Lion's Mane Mushroom''**are summarized as under:

Under *in vitro* conditions He-03 strain showed maximum mycelial growth of 86.86 mm on potato Dextrose Agar after 18 days of incubation with average mycelial growth rate of 4.23 mm per day.

He-03 strain observed minimum days (38.66) for complete spawn run and He-05 observed minimum days (51.33) for fruit initiation on a substrate combination of rice straw + wheat straw and wheat straw + saw dust, respectively.

He-03 strain recorded maximum diameter of clumps of spines (8.20 cm) and maximum number of fruit bodies (15.50) on saw dust whereas maximum average weight of fruit body (34.76) were observed in wheat straw + saw dust in He-05 while minimum diameter of clumps of spines (4.70 cm) was recorded in He-03 strain.

Minimum number of fruit bodies (10.10) was recorded on rice straw + wheat straw in He-05 strain whereas minimum average weight of fruit body (24.60 g) was observed in rice straw. Maximum yield for first flush (178.00 g), second flush (167.33 g) was recorded on saw dust in He-05 strain while maximum yield for third flush (160.00 g) was recorded on saw dust in He-03 strain.

In He-05 strain, saw dust substrate gave highest total yield of 502.99 g/1000 g of dry substrate with corresponding 50.29 per cent biological efficiency while the least effective substrate was rice straw substrate which produced a total yield of 316.65 g/1000 g of dry substrate in He-03 strain.

Maximum cost: benefit ratio (1:4.05) was recorded in wheat straw + saw dust in He-05 strain while minimum cost: benefit ratio (1:2.92) was observed in rice straw substrate in He-03 strain.

Biochemical analysis of fruit bodies of *Hericium erinaceus* strain He-03 showed maximum moisture content (91.66 %) on rice straw + wheat straw while maximum crude fat content (4.83 %) and maximum ash content (12.86 %) was observed in fruit bodies grown on rice straw while maximum crude protein content (27.50 %) and crude fibre (8.10 %) were recorded in fruit bodies grown on wheat straw and saw dust in He-05 strain while minimum moisture content (87.66 %), crude protein content (24.83 %), crude fat content (3.16 %), crude fibre (7.00 %) and ash content (10.23 %) were observed in fruit bodies grown on saw dust, rice straw, saw dust, rice straw + wheat straw and rice straw + wheat straw, respectively in He-03 strain. Maximum carbohydrates (61.92 %) were recorded in fruit bodies of He-05 strain cultivated on saw dust while minimum carbohydrates (58.50 %) were observed in fruit bodies of He-05 strain grown on wheat straw.

The results of present study may be concluded in the following salient points:

- Among the *Hericium erinaceus* strains tested for culture studies, He-03 strain proved to be the most fast growing strain followed by He-05 strain.
- *Hericium erinaceus* strain He-05 proved to be the best strain for commercial production.
- Among the different substrates used for the cultivation of Lion's mane mushroom, saw dust proved to be the best substrate followed by wheat straw + rice straw.
- Chemical components of fruit bodies varied according to the substrates on which they grew and fruit bodies of *H. erinaceus* grown on saw dust were found to be rich in fibre and carbohydrates, while fruit bodies grown on wheat straw were rich in protein, whereas the fruit bodies grown on rice straw were observed to have more crude fat and ash while, maximum moisture content was found in fruit bodies grown on wheat straw + saw dust substrates.

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Vita

VITA

Name of Student	**:**	Renu Devi
Father's Name	**:**	Sh. Kumb Karan
Mother's Name	**:**	Smt. Shello Devi
Nationality	**:**	Indian
Date of Birth	**:**	10-03-1995
Permanent Home Address	**:**	R/o Bhullari, Tehsil Bani, Dist. Kathua, Jammu and Kashmir**.**

EDUCATIONAL QUALIFICATION

For Master's Degree Students

Bachelor's Degree	**:**	B.Sc. Agriculture (Hons.)
University and Year of Award	**:**	SKUAST-J, Chatha, 2018.
OGPA	**:**	7.30/10.00
Master's Degree OGPA	**:**	7.53/10.00

CERTIFICATE-IV

Certified that all necessary corrections as suggested by the external examiner and advisory committee have been duly incorporated in the thesis entitled **"Evaluation of Different Strains and Substrates for Cultivation of Lion's Mane Mushroom"**, submitted by **Ms. Renu Devi,** Registration No. **J-18-M-576.**

Dr. Sachin Gupta
Major Advisor

Place: Jammu

Dated: 15-09-2021

Head of Division