STUDIES ON SPECIALITY MUSHROOM SHIITAKE

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INDIAN INSTITUTE OF TECHNOLOGY DELHI

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STUDIES ON SPECIALITY MUSHROOM SHIITAKE

by

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Submitted

in fulfilment of the requirements for the degree of Doctor of Philosophy

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DEDICATED

to

MY PARENTS

CERTIFICATE

*This is to certify that the thesis entitled '**Studies on specialty mushroom Shiitake'** submitted by **Ms. Shalinee** for the degree of **DOCTOR OF PHILOSOPHY** has been prepared under my guidance with the rules and regulations of Indian Institute of Technology Delhi, India. The research report and results presented in the thesis have not been submitted for any degree or diploma in any other institute or university.*

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(Shalinee)

ABSTRACT

The present scenario of rising population, malnutrition, depleting agricultural land, rise in environmental pollution and need for quality food production in our country is quite challenging. In this context, mushrooms emerge as a promising substitute that can resolve all these issues upto a certain extent. They are higher fungi, rich in protein (providing all the essential amino acids), low in fat, and contains relatively higher amounts of carbohydrates and fiber. Although India is endowed with favorable natural agro-climatic conditions and plenty of agro-wastes that could be exploited for cultivation of diverse mushroom species, yet our country does not have any significant status either as a mushroom producer or as a consumer. Cultivation of only three mushroom species is popular in the country i.e *Agaricus*, *Pleurotus* and *Volvariella*. Shiitake (*Lentinus edodes*), the most popular and third largest cultivated mushroom species in world is known for its immunomodulatory actions and therapeutic implications but has only limited popularity in our country. Despite sporadic research efforts to standardize its cultivation technology in India, it could not reach the commercial level, as available technology is not viable and henceforth it's cultivated and consumption is restricted. The present study was centered on designing a unified approach for the popularization of *L.edodes* by conducting extensive R & D in the area of its cultivation, nutraceutical and nutritional properties, value added product development and consequent awareness generation among people for its wider dissemination and acceptability.

Locally available saw dust substrate was initially screened for its potential to support the growth of shiitake mushroom. Out of all the selected substrates, mixed saw dust substrate supported maximum growth. The saw dust was supplemented with nitrogen sources namely

wheat bran, mustard cake and cottonseed cake at 0, 2, 3, 5, 10, 20%. The analysis of the substrates revealed good amount of nutrients that increased the mushroom yield. Maximum yield of 60.87% was attained in case of 2% cottonseed cake supplementation giving an average increase of 25% over the control. Mushroom fruit bodies obtained from supplemented substrates were found to be rich in proteins, amino acids and minerals (N, P, K, Fe, Zn, Mn) but low in fat and sugar content. Significant degradation of celluloses, hemicelluloses and lignin (quantitatively estimated) was found in spent (having 2% cotton seed cake supplementation) with good manurial values. The supercritical CO_2 extraction revealed increase in the extract yield upon addition of 5% methanol as co-solvent. Also, more no of compounds were present in the supercritical extract as compared to the solvent extract with the predominance of Linoleic acid (41.94%). Extraction and quantification of β-glucans in the fruit bodies was carried out by megazyme kit assay, HPLC, FTIR and NMR studies. Quantification by megazyme conceded highest β-glucan content i.e. 56.71 g/100g. FTIR, HPLC and NMR studies confirmed the presence of β-glucan in the isolated polysaccharides. Nutraceutically enriched fruit bodies were successfully utilized to make value added products like cookies and pickles. Cookies made with 10% shiitake powder were found to be highly acceptable with increased quantity of protein, minerals and β-glucan. Out of the two varieties of pickles developed i.e. sweet and sour and salty, the salty pikles were highly acceptable on sensory parameters. The mushroom based value added products were highly acceptable at the rural villages especially among children over the control cookies. The profit analysis indicated that processing of shiitake into value added products might offer great investment returns over fresh shiitake mushroom and will also augment farmer's income. Field surveys, group discussions, workshops and practical demonstrations were carried out in order to popularize shiitake mushroom. It was observed that there was a lot of interest among people to

adopt mushroom cultivation as an entrepreneurship. An integrated strategy for continual development through mushroom technology for sustainable rural development and to ensure nutritional security is thus presented here.

सार

वर्तमान परिदृश्य में बढ़ती आबादी, कुपोषण, कृषि भूमि की कमी, पर्यावरण प्रदूषण में वृद्धि और गुणवत्ता वाले खाद्य पदार्थों की उत्पादन की आवश्यकता काफी चुनौतीपूर्ण है। इस संदर्भ में, मशरूम एक आशाजनक विकल्प के रूप में उभरते हैं जो इन सभी मुद्दों को कुछ हद तक हल कर सकता है। ये उच्च कवक हैं जो की प्रोटीन में समृद्ध (सभी आवश्यक अमीनो एसिड से युक्त हैं), वसा में कम, और अधिक मात्रा में कार्बोहाइड्रेट और फाइबर से परिपूर्ण हैं । भारत देश में अनुकूल प्राकृतिक कृषि-जलवायु स्थितियों का होना और अनेक प्रकार के कृषि-अपशिष्टों की सम्पन्नता, विविध मशरूम प्रजातियों की खेती के लिए प्रयाप्त है । यह दुर्भाग्य पूर्ण है की बावजूद हर तरह की उपलब्धता के, हमारे देश का अब तक कोई भी स्थान न तो मशरुम की खेती के आधार पर और न ही उसकी खपत के आधार पर बन सका। देश में केवल तीन मशरूम प्रजातियों की खेती लोकप्रिय है। शिटाके (लेंटिनस एडोड्स) दुनिया में सबसे लोकप्रिय और तीसरी सबसे मशहूर मशरूम प्रजातियों में से एक है। यह मशरूम अपने इम्यूनोमॉड्यूलेटरी कार्यों और चिकित्सकीय गुणों के लिए जाना जाता है। किन्तु हमारे देश में इसकी बहुत ही सीमित उत्पादन है। साथ ही इसकी खपत भी अधिक नहीं है। वर्तमान अध्ययन शिटाके की लोकप्रियता को बढ़ाने के लिए केंद्रित था, जिसमें इसकी खेती, न्यूट्रास्यूटिकल और पौष्टिक गुणों की वृद्धि और इसके व्यापक प्रसार के लिए, लोगो के बीच जागरूकता पैदा करने के क्षेत्र में व्यापक अनुसंधान किया गया । स्थानीय लकड़ी के बुरादों का चयन किया गया था । यह पाया गया की सभी चयनित सब्सट्रेट्स में से, मिश्रित लकड़ी का बुरादा सबसे अधिक शिटाके उत्पादन का समर्थन करता है। मिश्रित बुरादे को अलग अलग प्रकार के नाइट्रोजन स्रोत जैसे की गेहूं का चोकर, सरसों का केक और कपास केक के साथ 0, 2, 3, 5, 10, 20% स्तर पर डाला गया ।सब्सट्रेट्स के विश्लेषण ने पोषक तत्वों की अच्छी मात्रा का खुलासा किया जो मशरूम की उपज में वृद्धि करने में सहायक साबित हुआ। सबसे ज़्यादा 60.87% उपज 2% कपास केक के साथ पाया गया जो की कंट्रोल (जिसमे किसी भी नाइट्रोजन स्रोत का उपयोग नहीं किया गया था) उससे औसतन 25% से ज़्यादा था। मशरूमों (जो तीनो तरह के पूरक के प्रयोग से उगाया गया) में यह पाया गया की वे प्रोटीन, एमिनो एसिड और खनिजों (एन, पी, के, आयरन, जिंक मेंगनेसियम) में समृद्ध है जबकि वसा और चीनी में

कम है । सेल्युलोज़, हेमिसेल्यूलोज़ और लिग्निन (मात्रात्मक रूप से अनुमानित) में महत्वपूर्ण गिरावट अच्छे मॅन्यूरियल (खाद बनाने के लिए) वैल्यू के होने की संभावना की पुष्टि हुई। सुपरक्रिटिकल CO_2 निष्कर्षण में 5% मेथनॉल को सह-विलायक के रूप में सबसे अच्छा पाया गया क्यूंकि उसके उपयोग ने यील्ड की मात्रा को बढ़ा दिया। सुपरक्रिटिकल निष्कर्षण तकनीकी में सामान्य सालवेन्ट निष्कर्षण की तुलना में ज़्यादा मात्रा में बायोएक्टिव (जैवसक्रिय) कंपाउंड्स पाए गए जिसमे कि सबसे अधिक मात्रा में लिनोलिक एसिड (41.94%) पाया गया। β- ग्लुकान के निष्कर्षण और उसकी मात्रा की परख, मेगाज़ाइम किट, एच.पी.एल.सी (HPLC) ऍफ़. टी. आई. आर (FTIR), और एन. एम. आर (NMR) अध्ययनों द्वारा किया गया। सबसे ज़्यादा β- ग्लुकान की मात्रा मुशरूमो में 56.71 ग्राम / 100 ग्राम पाया गया।

सभी अध्ययन तरीकों ने β- ग्लुकान पालिसैकराइड की उपस्थिति की पुष्टि की। इन उगाये हुए मशरूमों से अचार और कुकीज़ बनाये गए। 10% शिटाके पाउडर से बने कुकीज़ प्रोटीन, खनिज और β- ग्लुकान की मात्रा में अत्यधिक स्वीकार्य पाए गए थे। अचार की दो किस्मों में से विकसित खट्टा-मीठा और नमकीन में से , नमकीन को ज़्यादा स्वीकार्य पाया गया। गावों में भी सर्वेक्षण में बच्चों के बीच में भी शिटाके निर्मित मशरूम कुकीज़ अधिक पसंद किये गए। लाभ विश्लेषण से यह संकेत मिलता है कि मूल्यवर्धित उत्पादों में शिटाके मशरूम की प्रसंस्करण ताजा शिटाके की तुलना में अधिक निवेश रिटर्न कर सकती है और किसानों कि आय भी बढ़ेगी। शिटाके मशरूम को लोकप्रिय बनाने के लिए क्षेत्र सर्वेक्षण, समूह चर्चा, कार्यशालाएं और व्यावहारिक प्रदर्शन किए गए और इस दौरान यह पाया गया कि मशरूम की खेती को उद्यमिता के रूप में अपनाने के लिए लोगों में बहुत रुचि थी। दीर्घकालिक (सस्टेनेबल) ग्रामीण विकास के लिए शिटाके मशरूम की खेती में निरंतर विकास एवं पोषण सुरक्षा सुनिश्चित करने के लिए यह इस शोध में ठोस रणनीति प्रस्तुत की गई है ।

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ABBREVIATIONS

%	percent
ºC	Degree Celsius
h	hour
min	minutes
s	seconds
SD	Saw dust
kJ	Kilo Joules
PDA	Potato Dextrose Agar
g	gram
mg	milligram
µg	microgram
L	Litre
mL	milliliter
ppm	Parts per millions
rpm	Revolutions per minute
/	per
cm	centimeter
m	meter
nm	nanometer
mm	millimeter
BE	Biological Efficiency
fw	Fresh weight
dw	Dry weight
LP	*Lentinus* Powder
WF	Wheat Flour
^{1}H	Proton
^{13}C	Carbon-13

N	Nitrogen
C	Carbon
K	Potassium
P	Phosphorus
Fe	Iron
Zn	Zinc
Mn	Manganese
CSC	Cotton seed cake
WB	Wheat bran
MC	Mustard cake
NMR	Nuclear Magnetic Resonance
FTIR	Fourier Transform Infrared Spectroscopy
HPLC	High Performance Liquid Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
MTCC	Microbial Type Culture Collection and Gene Bank
kg	kilogram
Rs	Rupees
ANOVA	Analysis of Variance
DMRT	Duncan's Multiple Range Test
@	at
SCE	Supercritical Extraction
EtAc	Ethyl Acetate
MeOH	Methanol

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HPLC	High Performance Liquid Chromatography
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