

**ENHANCEMENT OF NUTRACEUTICAL POTENTIAL OF
CALOCYBE INDICA MUSHROOM**

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CENTRE FOR RURAL DEVELOPMENT AND TECHNOLOGY

INDIAN INSTITUTE OF TECHNOLOGY DELHI

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by

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CENTRE FOR RURAL DEVELOPMENT AND TECHNOLOGY

Submitted

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

to the



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DEDICATED TO MY PARENTS

CERTIFICATE

This is to certify that the thesis entitled 'Enhancement of nutraceutical potential of Calocybe indica mushroom' submitted by Ms. Himanshi 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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ABSTRACT

In the era of increasing world population, shrinking lands and limited agricultural resources, edible mushrooms offers the promising substitute providing tenacity towards the problems of waste generation, food security, malnutrition and poverty. Edible mushrooms such as *Agaricus bisporus*, *Pleurotus* spp., *Lentinus edodes* etc. have been successfully explored for their production on various kinds of wastes including their nutraceutical enhancement, value addition and spent utilization for the compost preparation. Also, these mushroom species are well known for their unique significant pharmacological properties. Indigenous edible mushroom *Calocybe indica* grown in tropical and sub-tropical regions has also been emerged as one of the culinary mushroom providing great nutritional properties along with the marvelous therapeutic values. In spite having easy cultivation technology, good biological efficiency, great nutritional properties and huge revenues, the mushroom species has not been explored. The present study aimed to enhance the nutritional and nutraceutical properties of the *C. indica* mushroom by substrate enrichment using novel organic and inorganic supplements, post harvest treatments, their utilization for development of functional food product and propagation of the complete technology package developed to the selected rural areas.

Waste nitrogenous tree biomasses viz. *Bauhinia variegata* (BVL), *Syzygium cumini* (SCL) and *Cassia fistula* (CFL) were identified, collected and used as the co-substrate in varying proportions (0, 25, 50 and 75%) with the wheat straw (WS) for the production of *C. indica*. Results indicated that 25% BVL was the best co-substrate for producing highest yield (622g) and biological efficiency (82.93%) of *C. indica*. High contents of nitrogen presented in the CFL hindered the growth of *C. indica* and yielded poorly. Supplementation significantly ($p < 0.05$) improved the protein, ash, fibers and minerals contents of the fruit bodies. Additionally, among

the ten treatments, 25 BVL produced the fruiting bodies with high antioxidant properties followed by 25 CFL and 25 SCL. Significant degradation of complex material such as cellulose, hemicelluloses and lignin was scrutinized (quantitative and qualitative by FTIR) in all mushroom spent and found to support the growth of *E. fetida* earthworms, juveniles and cocoons. The vermi-compost produced after 70 days possessed good manurial values.

Inorganic supplements viz. essential mineral Fe, Zn and Se were utilized to improve the quality and quantity of *C. indica*. Preliminary studies identified the optimum doses for the supplementation i.e. 6.25 ppm for Fe, 125ppm for Zn and 5 & 10 ppm for Se for producing higher mycelial yield, which were then further carried out in the field for the production of mushroom fruit bodies. Bio-accumulation of the minerals studied using SEM –EDX was found to be in well correlation with the minerals estimated *via* ICP-MS. Further, antioxidant profiling estimated signified a negative correlation with the Fe enrichment, whereas Zn was found to increase the contents of TPC, DPPH and FRAP assays. Interestingly, Se was found to enhance the protein contents of the fruit bodies and hence evaluated for their amino acid profiling, HPLC graphs revealed increased chemical scores of glutamic acid (4.73 g/100g) followed by aspartic acid (1.80 g/100g), glycine (1.61 g/100g) and leucine (1.39 g/100g) in the 10 ppm Se treated mushrooms. Also, antioxidant studies examined showed a significant ($p < 0.05$) increment in the TPC contents along with noticeable scavenging effects and ferric reducing capacities.

The fruit bodies harvested were further studied for their vitamin D₂ contents by experimenting with natural sunlight and artificial UVB light. The kinetic performed at different time intervals (0, 15, 30, 45, 60 and 90 minutes) revealed a linear increment of the vitamin D₂. Both the sunlight and UVB radiation were able to improve the vitamin D levels, but significant high contents were recorded with the UVB treated mushrooms after 60 min of irradiations.

Surprisingly appreciable amount of β -glucan (44.5 g/100g) was recorded with the fruit bodies irradiated under UVB source for 60 min. Further, these rays positively augmented the TPC, TFC FRAP and DPPH radical scavenging activities along with the altered scores for all the seventeen amino acids analyzed of the treated fruit bodies. Nutraceutically enriched fruit bodies were further processed into the dried powder and studied for their hydration properties (WHC, OHC & WSI) and found appropriate for developing bakery product. *C. indica* powder (CIP) mushroom cookies were successfully formulated with a high acceptance of 10% fortification. Cookies were found rich in protein, fibre, minerals (Fe, Zn and K), and β -glucan contents as compared to the wheat flour cookies. Also, CIP incorporation increased the levels of TPC, TFC, DPPH and FRAP of the cookies and reduced the glycemic index of the same.

Propagation of the technology designed for *C. indica* mushroom was successfully carried out in the selected nine villages of Haryana. The program effectively resolved the problems of low income and availability of quality food from the respective areas. The farmers implemented the technique transferred and are willing to continue the activity for their future wellbeing.

सार

विश्व में बढ़ती आबादी, घटती भूमि और सीमित कृषि संसाधनों के युग में, कचरे से उत्पादित खाद्य मशरूम की खेति कुपोषण, खाद्य सुरक्षा, और गरीबी की समस्याओं में दृढ़ता प्रदान करने वाला एक आशाजनक विकल्प हैं। खाद्य मशरूम जैसे की बटन मशरूम, प्लीरोटस की किस्मे, लेंटिनस एडोडस इत्यादि को विभिन्न प्रकार के अपशिष्टों पर उनके उत्पादन के लिए सफलतापूर्वक खोजा गया है, जिनमें न्यूट्रस्यूटिकल पावर को बढ़ाना, उनसे वैल्यू एडेड प्रोडक्ट बनाना और कंपोस्ट तैयार करना शामिल है। इसके अलावा, इन मशरूम प्रजातियों को उनके अद्वितीय महत्वपूर्ण औषधीय गुणों के लिए भी जाना जाता है। स्वदेशी खाद्य मशरूम 'कैलोसिबे इंडिका' जोकि उष्णकटिबंधीय और उप उष्णकटिबंधीय क्षेत्रों में उगाई जाती है, भी एक अद्भुत मशरूम के रूप में उभर रही है। यह मशरूम भी अद्भुत चिकित्सकीय मूल्यों के साथ साथ महान पौष्टिक गुण प्रदान करता है। आसान खेती तकनीक, अच्छी जैविक दक्षता, अच्छे पौष्टिक गुणों और विशाल राजस्व के बावजूद भी इस मशरूम प्रजाती की खोज नहीं की गई है। वर्तमान अध्ययन का उद्देश्य कैलोसिबे इंडिका मशरूम के औषाधिये (न्यूट्रास्यूटिकल) एवं पोषण गुणों को बढ़ाने हेतु किया गया है, जिसमें आर्गेनिक एवं नॉन-आर्गेनिक सप्लीमेंट का खेती के समय इस्तेमाल, फसल तोड़ने के बाद उपचार, फसल का गुणात्मक आहार में प्रयोग, एवं चुने गए पूर्ण प्रौद्योगिकी पैकेज को ग्रामीण क्षेत्रों में प्रचार करना शामिल है।

नाइट्रोजेन से भरपूर पेड़ों की पत्तिया जैसे की बोहिनिया वेरिगाटा (कचनार), सिजीजियम कमिनी (जामुन) और कैसिया फिस्टुला (अमलतास) शोध के लिए चुने गए। इन सभी पत्तियों को गेहूं के भूसे (डब्ल्यूएस) के साथ अलग-अलग अनुपात (0, 25, 50 और 75%) में सह-सबस्ट्रेट के रूप में सी इंडिका के उत्पादन के लिए मिलाया गया। परिणाम बताते हैं कि 25% कचनार उच्चतम उपज (622 ग्राम) और जैविक दक्षता (82.93%) के उत्पादन के लिए सबसे अच्छा सह-सबस्ट्रेट था। अमलतास में प्रस्तुत नाइट्रोजन की उच्च मात्रा के कारण, सी इंडिका की उपज एवं विकास में बाधित और खराब साबित हुई। तुड़ाई उपरांत पूरक मशरूम प्रोटीन, राख, फाइबर और खनिज सामग्री में सर्वश्रेष्ठ पाए गये। इसके अतिरिक्त, दस उपचारों में से, 25% कचनार से उच्च एंटीऑक्सीडेंट गुण भी पाए गए। सेलूलोज़, हेमिसेलूलोज़ और लिग्निन जैसी जटिल सामग्री के महत्वपूर्ण गिरावट की जांच भी की गई (एफटीआईआर द्वारा मात्रात्मक और गुणात्मक)। सभी उपचार इ. फेतिदा केचुओ, किशोर और कोकून के विकास का समर्थन करने के लिए उत्तम पाया गए। 70 दिनों के बाद उत्पादित वर्मी-कंपोस्ट में अच्छे मानदंड मूल्य थे।

अकार्बनिक पूरक जैसे आवश्यक खनिज Fe, Zn और Se का उपयोग सी इंडिका की गुणवत्ता और मात्रा में सुधार के लिए किया गया था। प्रारंभिक अध्ययनों ने पूरक के लिए इष्टतम खुराक की पहचान की, जिसमें 62.5 ppm Fe के लिए, 125 ppm Zn और 5 ppm Se उच्च और माइक्रोलियल उपज के लिए उत्तम पाए गए। SEM-EDX का उपयोग करके अध्ययन किए गए खनिजों का बायो-संचय आईसीपी-एमएस के माध्यम से अनुमानित खनिजों के साथ अच्छी तरह से सहसंबंध में पाया गया था। इसके अलावा, एंटीऑक्सीडेंट प्रोफाइलिंग ने अनुमानित रूप से Fe संवर्द्धन के साथ एक नकारात्मक सहसंबंध को इंगित किया, जबकि Zn को टीपीसी, डीपीपीएच और फ्रैप assays में वृद्धि

हुई। दिलचस्प बात यह है कि Se से सप्लीमेंट करे गये मुशरूम के प्रोटीन में वृद्धि पाई गई और इसलिए उनके एमिनो एसिड प्रोफाइलिंग (HPLC) के लिए मूल्यांकन किया गया, ग्लूटामिक एसिड (4.73 ग्राम / 100 ग्राम), एस्पार्टिक एसिड (1.80 ग्राम / 100 ग्राम), ग्लिसिन (1.61 ग्राम / 100 ग्राम) और ल्यूसिन (1.39 ग्राम / 100 ग्राम) के रासायनिक स्कोर 10 पीपीएम सप्लीमेंटेड मशरूम में अधिक पाए गए।

फसल निकायों को प्राकृतिक सूरज की रोशनी और कृत्रिम UVB प्रकाश के साथ प्रयोग करके विटामिन डी की मात्रा बढ़ाने हेतु अध्ययन किया गया था। विभिन्न समय अंतराल (0, 15, 30, 45, 60 और 90 मिनट) में किए गए गतिशीलता ने विटामिन डी की रैखिक वृद्धि का खुलासा किया। सूरज की रोशनी और UVB विकिरण दोनों विटामिन डी के स्तर में सुधार करने में सक्षम थे, लेकिन 60 मिनट विकिरण के बाद UVB से ट्रीट की गए मशरूम के साथ महत्वपूर्ण उच्च मात्रा दर्ज की गई। UVB स्रोत के तहत 60 मिनट के लिए विकिरणित फल निकायों के साथ β -glucan (44.5 ग्राम / 100 ग्राम) की आश्चर्यजनक रूप से सराहनीय मात्रा दर्ज की गई। इसके अलावा, इन किरणों ने टीपीसी, टीएफसी एफआरपी और डीपीपीएच कट्टरपंथी स्कावेजिंग गतिविधियों को सकारात्मक रूप से बढ़ाया, साथ ही इलाज किए गए फल निकायों में एमिनो एसिड के बढ़े हुए स्कोर भी दर्ज किये गए। न्यूट्रस्यूटिकली समृद्ध फल निकायों को सूखे पाउडर में बदलकर उन्हें आगे संसाधित किया गया और उनके हाइड्रेशन गुणों (डब्ल्यूएचसी, ओएचसी और डब्ल्यूएसआई) के लिए अध्ययन किया गया था और बेकरी उत्पाद विकसित करने के लिए उपयुक्त पाया गया था। सी इंडिका पाउडर (सीआईपी) मशरूम कुकीज़ सफलतापूर्वक 10% की उच्च स्वीकृति के साथ तैयार की गई थी। गेहूं के आटे की कुकीज़ की तुलना में मशरूम कुकीज़ प्रोटीन, फाइबर, खनिज (Fe, Zn और K), और β -glucan में समृद्ध पाए गए थे। इसके अलावा, सीआईपी निगमन ने कुकीज़ के टीपीसी, टीएफसी, डीपीपीएच और एफआरपी के स्तर में वृद्धि की और इसके ग्लाइसेमिक इंडेक्स को कम कर दिया।

सी इंडिका मशरूम के लिए डिजाइन की गई तकनीक का प्रचार हरियाणा के चयनित नौ गांवों में सफलतापूर्वक किया गया था। इस कार्यक्रम ने कम आय की समस्याओं और संबंधित क्षेत्रों से गुणवत्ता वाले भोजन की उपलब्धता को प्रभावी ढंग से हल किया। किसानों ने तकनीक को स्थानांतरित कर दिया और भविष्य के कल्याण के लिए गतिविधि को जारी रखने के प्रति इच्छा भी जताई।

CONTENTS

Acknowledgments	i-ii
Abstract	iii
Contents	vi-xiv
List of Figures	xv-xviii
List of Tables	xix-xxi
List of Plates	xxii
Abbreviations	xxiii-xxiv
CHAPTER 1: INTRODUCTION	1-12
1.1. Background	1
1.2. Food security scenario: status, problems and alternatives	1
1.3. Mushrooms: Current scenario	3
1.4. Mushroom production	6
1.5. Nutritional composition of mushrooms	7
1.6. Medicinal mushrooms	9
1.7. Mushroom based value added products	12
1.8. Objectives of the present work	13
CHAPTER 2: REVIEW OF LITERATURE	14-41
2.1. Mushroom diversity in India	14

2.2. Mushrooms: types and cultivation	16
2.3. Integrated mushroom diseases	18
2.4. Genus <i>Calocybe</i>	21
2.4.1. Taxonomy of <i>C. indica</i>	22
2.4.2. Substrates for cultivation of <i>C. indica</i>	23
2.5. Cultivation method	27
2.5.1 Chemical and nutritional composition of <i>C. indica</i>	29
2.5.2. Antioxidant properties of <i>C. indica</i>	31
2.5.3. Nutraceutical importance of <i>C. indica</i>	33
2.6. Value addition of mushrooms	34
2.7. Scope of the work	39
2.7.1. Exploration of various non-traditional biomasses for the cultivation of <i>C. indica</i>	39
2.7.2. Quality improvement by substrate supplementation of the <i>C. indica</i>	40
2.7.3. Post harvest management of the <i>C. indica</i>	40
2.7.4. Value added products of the <i>C. indica</i>	40
2.7.5. Propagation of <i>C. indica</i> in the selected villages of Haryana	41
CHAPTER 3: MATERIAL AND METHODS	42-65
3.1. Mushroom cultivation	43
3.1.1. Utilization of nitrogenous tree wastes for the cultivation of <i>Calocybe indica</i>	43
3.1.1.1. Microorganism and spawn production	43
3.1.1.2. Substrates and casing soil preparation	43
3.1.1.3. Experimental set up	43
3.1.1.4. Harvesting of fruit bodies and determination of biological	44

<i>efficiency</i>	45
3.1.1.5 <i>Chemical analysis of substrate and mushroom fruit bodies</i>	45
3.1.1.5.1. <i>Mineral contents of the substrates and mushroom fruit bodies</i>	45
3.1.1.6. <i>Antioxidant activities of the mushroom fruit bodies</i>	47
3.1.1.6.1. <i>Preparation of methanolic extracts</i>	47
3.1.1.6.2. <i>Free radical scavenging activity</i>	47
3.1.1.6.3. <i>Ferric reducing antioxidant power (FRAP)</i>	47
3.1.1.6.4. <i>Total phenol content (TPC)</i>	48
3.1.1.7. <i>Fourier transform infrared (FTIR) spectroscopy</i>	48
3.1.2. <i>Vermi-culturing of Eisenia fetida using mushroom spent</i>	48
3.1.2.1. <i>Procurement and rearing of E. fetida</i>	48
3.1.2.2. <i>Preparation of different mushroom spent substrates for vermi-culturing of E. fetida</i>	49
3.2. Quality improvement by substrate supplementation	51
3.2.1. <i>Iron, Zinc and selenium supplementation</i>	51
3.2.1.1. <i>Enrichment of inorganic iron, zinc and selenium for culturing of C. indica mycelium</i>	51
3.2.1.2. <i>Scanning Electron Microscopy and Energy-dispersive X-ray spectroscopy (SEM-EDX) of C. indica mycelium cultured on iron, zinc and selenium enriched media</i>	51
3.2.1.3. <i>Cultivation of C. indica mushroom on iron, zinc and selenium amended wheat straw substrate</i>	51
3.2.1.4. <i>Chemical analysis of C. indica</i>	52
3.2.1.4.1. <i>Determination of mineral content in mycelium and mushroom fruit bodies</i>	52

3.2.1.5. Antioxidant profiling of the enriched fruit bodies of the <i>C. indica</i>	52
3.2.1.5.1. Methanolic extracts of mushroom fruit bodies	52
3.2.1.5.2. Free radical scavenging activity	52
3.2.1.5.3. Ferric reducing antioxidant power	52
3.2.1.5.4. Total phenol content	52
3.2.2. Chemical analysis of selenium enriched fruit bodies of <i>C. indica</i> (SE-CI)	52
3.2.2.1. Extraction of Nucleic Acid from Se-CI	52
3.2.2.2. Extraction of Polysaccharide from Se-CI	53
3.2.2.3. Extraction of protein from Se-CI	53
3.2.2.4. Determination of protein content and amino acid content of SE-CI fruit bodies	53
3.3. Post harvest treatments of <i>C. indica</i> in order to enhance their vitamin D contents	54
3.3.1. Vitamin D enhancement of <i>C. indica</i> by UVB radiation	54
3.3.1.1. Experimental setup for sunlight and artificial UVB irradiation	54
3.3.1.2. Ergosterol assay	54
3.3.1.3. Estimation of ergosterol by High pressure and liquid chromatography (HPLC)	55
3.3.2. Determination of β -glucan contents in irradiated mushrooms	55
3.3.3. Antioxidant analysis of the irradiated mushrooms	55
3.3.3.1. Preparation of methanolic extracts	55
3.3.3.2. Free radical scavenging activity	55
3.3.3.3. Ferric reducing antioxidant power	55
3.3.3.4. Total phenol content	56
3.3.3.5. Total flavonoid content	56
3.3.4. Amino Acid profiling of irradiated mushrooms	56
3.4. Value added food product	56
3.4.1 Procurement of mushroom fruit bodies	56

3.4.2. Ingredient collection and preparation	56
3.4.3. Hydration properties	57
3.4.4. Development of mushroom cookies	57
3.4.5. Physical characteristics of mushroom cookies	59
3.4.5.1. <i>Physical properties of cookies</i>	59
3.4.5.2. <i>Texture</i>	60
3.4.5.3. <i>Microstructure</i>	61
3.4.6. Nutritional composition	61
3.4.6.1. <i>Chemical composition of the mushroom powder and baked cookies</i>	61
3.4.6.2. <i>Qualitative and quantitative analysis of β-glucan in cookies</i>	61
3.4.6.3. <i>In vitro starch digestibility test</i>	62
3.4.6.3.1. <i>Total starch estimation</i>	62
3.4.6.3.2. <i>Kinetics of hydrolysis index</i>	62
3.4.7. Antioxidant profiling of cookies	63
3.4.7.1. <i>Preparation of methanolic extracts</i>	63
3.4.7.2. <i>Free radical scavenging activity</i>	63
3.4.7.3. <i>Ferric reducing antioxidant power</i>	63
3.4.7.4. <i>Total phenol content</i>	63
3.4.7.5. <i>Total Flavonoid contents</i>	63
3.4.8. Fuzzy logic analysis	63
3.4.9. Determination of free fatty acids	65
3.5. Statistical analysis	65
CHAPTER 4: RESULTS AND DISCUSSION	66-92
4.1 Cultivation and yield of <i>C. indica</i>	66
4.1.1 Characterisation of substrates	67

4.1.2. Effect of different substrates on yield of <i>C. indica</i>	69
4.1.3. Biological efficiency of <i>C. indica</i>	72
4.1.4 Effect of different substrates on nutritional composition of <i>C. indica</i>	81
4.1.5 Effect of different substrates on mineral contents of <i>C. indica</i>	82
4.1.6 Effect of different substrates on antioxidant properties of methanolic extracts of <i>C. Indica</i>	84
4.1.7. Degradation of complex molecules in the mushroom spent	87
4.1.8. Vermi-culturing of <i>Eisenia fetida</i> using mushrooms spent	87
4.1.8.1. Effect of different leafy biomass substrates on the number of mature earthworms, juveniles and cocoons of <i>E. Fetida</i>	87
4.1.8.2 Manurial value of vermi-compost produced from different substrate combination	91
4.2. Quality improvement of <i>C. indica</i> by substrate supplementation	93-127
4.2.1. Enrichment of <i>C. indica</i> mycelium and fruit bodies with inorganic mineral iron	93
4.2.1.1. Effect of Fe enrichment on the morphology and its accumulation in the <i>C. indica</i> mycelium	95
4.2.1.2. Bioaccumulation of Fe in the mycelium and fruit bodies of mushrooms	98
4.2.1.3. Effect of Fe enrichment on the antioxidant profiling of <i>C. indica</i> fruit bodies	100
4.2.1.4. Correlation effect of Fe enrichment on yield, Fe content and antioxidant activity of <i>C. indica</i> fruit bodies	102
4.2.2. Zinc (Zn) enrichment of <i>C. indica</i> mycelium and fruit bodies	103
4.2.2.1. Effect of Zn enrichment on the morphology and its accumulation in the <i>C. indica</i> mycelium	105
4.2.2.2. Bioaccumulation of Zn into the mycelium and fruit bodies of the <i>C. indica</i>	107
4.2.2.2. Effect of Zn enrichment on the antioxidant profiling of <i>C.</i>	109

<i>indica</i> fruit bodies	
4.2.2.4. Correlation effect of Zn enrichment on yield, Zn content and antioxidant activity of <i>C. indica</i> fruit bodies	111
4.2.3. Effect of Se supplementation on mycelia growth and mushroom production	113
4.2.3.1. Effect of Se supplementation on the morphology and its accumulation in the <i>C. indica</i> mycelium	115
4.2.3.2 Bioaccumulation of Se in mycelium and fruit bodies of mushrooms and its distribution in the Se-enriched <i>C. indica</i> fruit bodies	118
4.2.3.3. Total protein content and amino acid profiling of the fruit bodies	121
4.2.3.4. Effect of Se enrichment on the antioxidant profiling of <i>C. indica</i> fruit bodies	123
4.2.3.5. Correlation effect of selenium supplementation on yield, BE, selenium content and antioxidant activity of <i>C. indica</i> fruit bodies	125
4.3. Post-harvest treatments of <i>C. indica</i> in order to enhance their Vitamin D contents	128-139
4.3.1. Kinetics model parameters of vitamin D ₂ in <i>C. indica</i>	128
4.3.2. Vitamin D ₂ concentration of <i>C. indica</i> exposed under natural sunlight and UVB light	130
4.3.3. Effect of sunlight and UVB processing on the β -glucan contents of the <i>C. indica</i> fruit bodies	132
4.3.4. Effect of sunlight and UVB processing on the total phenol, flavonoid and anti-oxidative properties of <i>C. indica</i> fruit bodies	133
4.3.5. Amino acid profiling of the irradiated <i>C. indica</i> fruit bodies	137
4.4. Value addition of <i>C. indica</i> mushroom	140-157
4.4.1. Hydration properties of the different mushroom flour blends	140

4.4.2. Physical characterization of CIP cookies	142-144
4.4.2.1 <i>Physical parameters of the CIP cookies</i>	142
4.4.2.2 <i>Texture of the CIP cookies</i>	143
4.4.2.3 <i>Microstructure of the CIP cookies</i>	144
4.4.3. Chemical profiling of the CIP and cookies	146-147
4.4.3.1 <i>Proximate composition and mineral contents</i>	146
4.4.3.2 <i>FTIR spectra of CIP cookies</i>	147
4.4.4. Starch hydrolysis and glycemic index of the mushroom cookies	149
4.4.5. Antioxidant profiling of the mushroom cookies	151
4.4.6. Sensory analysis of mushroom cookies using fuzzy logic	153
4.4.7. Shelf life study of CIP cookie	157
4.5. Propagation of <i>C. indica</i> mushroom in the villages of Haryana	158
4.5.1. Area profile of Haryana	158
4.5.2. Selection of farmers/ beneficiaries	159
4.5.3. Establishment of the mushroom centres	160
4.5.4. Training programs on mushroom (<i>C. indica</i>) cultivation conducted during 2015-2017	164
4.5.4.1 <i>Training programs at HAIC, Murthal</i>	164
4.5.4.2 <i>Training programs conducted at different mushroom centers established in different villages</i>	166
4.5.4.3 <i>Training programs for preparation of casing soil</i>	168
4.5.4.4 <i>Spawn production training given to Self Help Group at village Hari Nagar</i>	170
4.5.4.5 <i>Training programs on harvesting, packaging and marketing of fruit bodies</i>	172
4.5.5. Marketing of the <i>C. indica</i> mushroom	172
4.5.6. Value addition of the <i>C. indica</i> mushroom	175

4.5.7. Demonstration cum awareness programmes (dissemination) conducted in the villages including schools	176
4.5.8. Workshops conducted for the beneficiaries	181
4.5.9. Problems faced during the propagation of <i>C. indica</i> in block Farrukh Nagar, Haryana	186
4.5.10. Sustainability of the technology	187
CHAPTER 5. Summary, conclusion and future prospective	188-201
References	202-219
Appendices	220-227
Publication & Curriculum vitae	228-233

LIST OF FIGURES

Fig No.	Title	Page No.
1.1	Contribution of different mushrooms to the world mushroom production	4
1.2	Total world mushroom production and world population in last few decades	5
1.3	Mushroom production in India	6
2.1	Major mushroom species being cultivated in India and dominating areas for their cultivation	15
2.2	Morphology of a mushroom	17
2.3	Different species of <i>Calocybe</i> genus	22
2.4	State wise cultivation of <i>Calocybe indica</i> in India (in tones)	24
2.5	Medicinal properties of <i>C. indica</i> mushroom	34
3.1	The flow chart of the study	42
3.2	(a) Control cookie formulated using wheat flour (b) 5% (c) 10% (d) 15% and (e) 20% of <i>C. indica</i> incorporated cookies	59
3.3	Texture analyser used for assessing cookie fructrability	60
3.4	Representation of fuzzy logic analysis by a triangular membership function of the sensory scales	64
4.1	Biological efficiencies obtained for different treatments (%)	72
4.2	Principal component analysis of (A) different nutritional variables (fats, crude protein, dry matter, carbohydrates, moisture and ash) and (B) different treatments observations.	77
4.3	Principal component analysis of (A) different mineral variables (K, Mg, Mn, Na, Fe, Zn and Se) and (B) different treatments observations.	78
4.4	FTIR spectra showing peaks for cellulose, hemicelluloses and lignin in 25CFL (A), 25SCL (B), WS (C), and 25BVL (D)	85
4.5	(A-C). Scanning Electron Microscopy images of Fe	96

4.6	supplemented mycelium A) untreated B) Fe enriched mycelium at 125 ppm C) Fe enriched mycelium at 500 ppm EDX graphs of Fe supplemented mycelium A) untreated B) Fe enriched mycelium at 125 ppm C) Fe enriched mycelium at 500 ppm	97-98
4.7	Bioaccumulation of Fe in the mycelium and fruit bodies of <i>C. indica</i>	100
4.8	Principle component analysis (PCA) biplot of yield, Fe content, total phenol content (TPC) and radical scavenging activity (DPPH) of Fe treated <i>C. indica</i> fruit bodies	103
4.9	Scanning Electron Microscopy images of Zn supplemented mycelium A) untreated B) Zn enriched mycelium at 125 ppm C) Zn enriched mycelium at 500 ppm	106
4.10	EDX graphs A) 62.5ppm Zn enriched and B) 250ppm Zn enriched mycelium	107
4.11	Zinc bioaccumulation in the mycelium and fruit bodies of <i>C. indica</i>	109
4.12	Principle component analysis (PCA) biplot of yield, Fe content, total phenol content (TPC) and radical scavenging activity (DPPH) of Fe treated <i>C. indica</i> fruit bodies	112
4.13	<i>C. indica</i> fruit bodies enriched with A) control (0 ppm), B) 5 ppm Se and C) 10ppm Se	114-115
4.14	Scanning Electron Microscopy images of Se supplemented mycelium (A) untreated (Se 0), (B) Se-enriched at 5ppm, (C) Se-enriched at 20ppm	116
4.15	EDX graphs of Se supplemented mycelium A) untreated B) Se enriched mycelium at 5 ppm C) Se enriched mycelium at 20 ppm	117
4.16	Schematic diagram of Se transportation mechanism	119
4.17	Representative chromatogram of amino acids present in the	123

	Se enriched (10 ppm) fruit bodies of <i>C. indica</i>	
4.18	Principle component analysis (PCA) biplot of yield, selenium, protein, total phenol content (TPC), radical scavenging activity (DPPH) and ferric reducing property (FRAP) of selenium treated mushroom fruit bodies	126
4.19	Effect of time of sunlight and UVB irradiation on the conversion of ergosterol into vitamin D ₂ in <i>C. indica</i> mushrooms	129
4.20	HPLC chromatographs representing vitamin D peak in 60 min UVB irradiated <i>C. indica</i> mushroom.	131
4.21	β-glucan contents in the sunlight and UVB irradiated <i>C. indica</i> fruit bodies at different time interval	133
4.22	HPLC chromatographs representing the amino acids present in <i>C. indica</i> fruit bodies irradiated under UVB light	138
4.23	SEM images of cookies A. Control, B 5% cookie, C 10% cookie, D 15%% cookie & D 20% cookie	145
4.24	Representative FTIR spectra of all the treated cookies, A. 0% CIP, B. 5% CIP cookie, C. 10% CIP cookie, D. 15% CIP cookie & D. 20% CIP cookie	148
4.25	A) Average cumulative curves (AUC) of time course starch hydrolysis B) Hydrolysis index values C) Glycemic index values of CIP cookies at different percentages. In particular: 0 % CIP (control cookie), 5%, 10%, 15% and 20% CIP fortified cookies	150-151
4.26	Acidity of the fat extracted from the 10% CIP cookie in percentage by mass	157
4.27	Map of Haryana showing 22 districts	158
4.28	Selected villages of block Farrukh Nagar for the propagation of <i>C. indica</i>	160
4.29	Survey conducted pertaining to the mushroom cultivation and related activities	161

4.30	Wooden laminar hood for spawn preparation designed at IITD	170
4.31	A & B Sensory evaluation of biscuits in Farrukh Nagar village	175

LIST OF TABLES

Table No.	Title	Page No.
1.1	Per capita dietary energy supply and prevalence of under nutrition in total population	2
1.2	Commonly used substrate for mushroom species and their biological efficiencies	7
1.3	Bioactive compounds isolated from mushrooms	10-11
2.1	Summary of studies published on the utilization of the non-traditional biomass for the cultivation of edible mushrooms	18
2.2	Major fungal and bacterial diseases of mushrooms	21
2.3	Biological efficiency of <i>C. indica</i> on some non-traditional substrates	25-26
2.4	The comparative information about the proximate composition (g/Kg) and energy value (kcal/Kg) of some edible mushrooms.	30
2.5	Mineral composition of the <i>C. indica</i>	31
2.6	Antioxidant properties of <i>C. indica</i>	32
2.7	Some of the latest reported mushroom based value added products	37-38
3.1	Different substrate treatments used for <i>C. indica</i> cultivation (% by dry weight)	44
3.2	Ingredients for preparation of cookies	58
4.1	Physico-chemical properties of WS, BVL, SCL and CFL	67
4.2	Physico- chemical properties of combinations designed by replacing wheat straw	68
4.3	Comparison of yield of <i>C. indica</i> on different substrates	70
4.4	Nutritional parameters (%) of the fruit bodies of <i>C. indica</i>	76

harvested from WS supplemented with nitrogenous tree leaves.

4.5	Correlation matrix of nutritional parameters (%) of the fruit bodies of <i>C. indica</i> harvested from WS supplemented with nitrogenous tree leaves	79
4.6	Mineral content (mg/100g) of the <i>C. indica</i> grown on WS supplemented with nitrogenous tree leaves	80
4.7	Correlation matrix of mineral content (mg/100g) of the <i>C. indica</i> grown on WS supplemented with nitrogenous tree leaves	82
4.8	Antioxidant activities of the methanolic extracts of <i>C. indica</i> harvested from different treatments	84
4.9	Degradation of complex material in mushroom spent (%)	86
4.10	Substrate composition of mushroom spents selected for vermi-culturing	89
4.11	Effect of substrate on mean number of mature earthworms (E), juveniles (J), and cocoons (C) of <i>E. fetida</i>	90
4.12	Manurial value of vermi compost after 70 days of culturing <i>E. fetida</i> in different mushroom spent	91
4.13	Enrichment of <i>C. indica</i> fungi with Fe	95
4.14	Total phenol content, DPPH and FRAP IC ₅₀ values of methanolic extracts of Fe enriched <i>C. indica</i>	101
4.15	Correlation matrix of yield, Fe contents and antioxidant properties of <i>C. indica</i> fruit bodies enriched with Fe	102
4.16	Enrichment of <i>C. indica</i> fungi with Zn	105
4.17	Total phenol content, DPPH and FRAP IC ₅₀ values of methanolic extracts of Zn enriched <i>C. indica</i>	110
4.18	Correlation matrix of yield, Zn contents and antioxidant properties of <i>C. indica</i> fruit bodies enriched with Zn	112
4.19	Effect of Se supplementation on the mycelia growth and fruit body production of <i>C. indica</i>	114
4.20	Bioaccumulation of Se by the mycelium and fruit bodies of <i>C. indica</i>	119
4.21	Distribution of Se (µg/g) in different components nucleic acids, proteins and polysaccharides of mushroom fruit	120

	bodies	
4.22	Amino acid contents of the Se enriched fruit bodies	122
4.23	Total phenol content, DPPH and FRAP values of methanolic extracts of Se enriched fruit bodies	125
4.24	Correlation matrix of the Se accumulation, yield, total protein and antioxidant properties of Se enriched <i>C. indica</i> fruit bodies	127
4.25	Content of vitamin D in <i>C. indica</i> exposed under natural sunlight and artificial UVB light and rate of conversion of ergosterol R_s at different time interval (min^{-1})	132
4.26	Total phenol, flavonoids and antioxidant activities (DPPH & FRAP) of sunlight and UVB exposed <i>C. indica</i>	136
4.27	Amino acid contents of the sunlight and UVB irradiated <i>C. indica</i> fruit bodies	139
4.28	Functional characteristics of different CIP-flour blends	140
4.29	Mean values of weight, thickness, diameter, spread ratio and hardness of mushroom cookies	143
4.30	Chemical composition of the <i>C. indica</i> powder (CIP) and mushroom cookies	147
4.31	Mean and SD values of total phenol, total flavonoids, radical scavenging activity and ferric reducing power of the mushroom cookies	153
4.32	A Overall score and defuzzified score of mushroom cookies calculated using fuzzy logic approach B. Sum of the different preferences of the judges with triplet relative weightage for quality attributes for selecting cookies in general	155-156
4.33	A.B.C. Yield of the <i>C. indica</i> obtained by the benefeceries from self made and ready to harvest packets provided by IITD (2015,2016 & 2017)	173-174
4.34	List of training- cum –awareness programs conducted on <i>C. indica</i> in different villages	176-177
4.35	Economics of the <i>C. indica</i> cultivation per farmer per year	188

LIST OF PLATES

Plate No.	Title	Page No.
3.1	Steps in <i>C. indica</i> cultivation: (A) Substrate filling in bags (B) Autoclaving of the bags (C) Spawn run in bags (D) Application of casing soil (E) Pin head appearance (F) Harvested <i>C. indica</i>	46
3.2	(A) Spent of wheat straw (WS) (B) Spent of 25BVL:75WS (C) Spent of 25CFL:75WS (D) Spent of 25SCL:75WS (E) Ready vermi-compost	50
4.1	Mushroom centers established in different villages A) Hari Nagar, B) Joniwas, C) Farrukh Nagar D) Mushedpur E) Mubarikpur	162
4.2	(A-F). Mushroom centers established in village Khurrampur and Khera	163
4.3	(A-I) Glimpses of training programs conducted at HAIC Murthal Haryana	165
4.4	(A-F) Glimpses of various visits made at different mushroom centers in villages	167
4.5	(A-F) Training programs on preparation and application of casing soil	169
4.6	(A-C) Glimpses of spawn training programmes conducted at village Hari Nagar	171
4.7	(A-D). Dissemination of milky mushroom in different villages	178
4.8	(E-H). Some glimpses of Awareness programmes conducted in different schools by IIT Delhi	179-180
4.9	(A-D). Glimpses of one day sanghosti organized at village Farrukh Nagar	183
4.10	(A-C). Glimpses of workshop conducted at KVK Sikhohpur on 30th March 2017	184
4.11	(A-F). Glimpses of workshop conducted at village Khurrampur on 9th August 2017	185

ABBREVIATIONS

ANNOVA	Analysis of Variance
DMRT	Duncan's Multiple Range Test
%	Percentage
°C	Degree centigrade
h	Hour
min	Minutes
s	Seconds
g	Gram
mg	Milligram
µg	Microgram
L	Litre
mL	Milliliter
ppm	Parts per millions
rpm	Revolutions per minute
/	Per
cm	Centimeter
m	Meters
nm	Nanometer
mm	Millimeter
BE	Biological Efficiency

fw	Fresh weight
dw	Dry weight
KV	Kilovolts
lbs	Pound
W	Watt
N1	Newton
N	Normality
Ha	Hectare
Kg	Kilogram
Rs	Rupees
@	At