DEVELOPMENT OF AN INTEGRATED TECHNOLOGY PACKAGE FOR PADDY STRAW MANAGEMENT

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by

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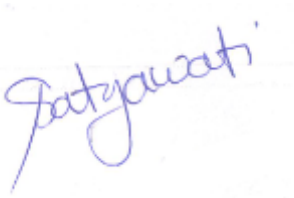
Dedicated to my

PARENTS

CERTIFICATE

This is to certify that the thesis entitled **"Development of an Integrated Technology Package for Paddy Straw Management"** being submitted by **Ms. Garima Singh** to the **Indian Institute of Technology Delhi** for the award of "**Doctor of Philosophy**" is a record of bonafide research carried by her. She has worked under our supervision and guidance and has fulfilled the requirements for the submission of the thesis. To the best of our knowledge, the results presented in this thesis have not been submitted in part or full to any other institute or university for the award of any degree or diploma.



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Garima Singh

ABSTRACT

Addressing the concern of stubble burning, the study conducted under this thesis envisages on recycling and reuse of Paddy Straw (PS) to assign a commercial utility to this under-utilized biomass. In this context, a multipronged strategy towards zero waste management of PS by developing value-added products (mushrooms, bioformulations (biopesticides/biofertilizers), nanosilica, and enzymes) was attempted.

Valorization of PS using various de-oiled cakes (DEOCs), depicted that supplementation of 5% soybean cake (SC) gave maximum yield of 1014 g kg^{-1} *P. ostreatus* fruiting bodies, furnishing an increase of 18.14 % over PS control. Principal component analysis (PCA) performed by analysing the correlation amongst nutritional parameters depicted a total variation of 78.2%. Energy Dispersive X-Ray (EDX) analysis indicated significant increase of minerals in the fruiting bodies and high content of silica (19-20%) in the spent. The cultivation of *T. asperellum* on spent divulged the fact that the fungal growth was comparatively high in spent ($14.73*10^{11}$) over raw PS ($4.2*10^{3}$). *T. asperellum* grown on spent showed 66.26% inhibition of *Fusarium oxysporum* compared to the non-supplemented PS spent (54.26%).

In the next part, the Spent Mushroom Substrate (SMS) of *P. ostreatus* was utilized for the development of microbial bioformulations, for plant nutrient and disease management in wilt (*F. oxysporum*) susceptible tomato plant F1 Hybrid King 180. SMS (PS+5% SC) supported the growth of *T. asperellum* (TA) to an extent of $12.37x10^{13}$ conidia/g substrate. GC-MS analysis of SMS detected several bioactive metabolites known for plant health management. Bioformulations were developed employing Press Mud (PM) and Talcum Powder (TP) as carrier materials. Among the different bioformulations tested in pots study; SMS (PS+5% SC) SiTAPM, collectively named as TF-I, provided improved levels of morpho-biochemical and nutritional parameters, A significant ($p<0.05$) reduction in the Disease Severity Index (84.34% to 21.23%), was also observed over the pathogen affected plant. The fruits and leaves garnered under TF-I displayed Total Polyphenol Content (TPC) of 74.5 and 126.9 mg/g gallic acid, respectively, with 83.73% DPPH and 72.25% FRAP activity, indicating the elicitation of antioxidant properties. EDX analyses showed 21.53% Silica (Si) in SMS, and plant mapping investigation indicated a substantial accumulation of Si, which is well conceded to promote growth, disease resistance, and antioxidant parameters. The developed bioformulation was delivered to farmers in villages of Uttar Pradesh and a survey study was conducted, which delivered positive outcomes and keen interest towards the implementation of the technology.

An *in-silico* approach was also employed to assess the prospective role of bioactives of *Trichoderma* spp. in combating the *Fusarium* wilt causing enzymes Polygalacturonase (PG2) and tomatinase. The findings of the study revealed that Trichodermamide B produced by *T. harzianum* and Viridin, Virone, and Trichosetin produced by *T. virens* emerged as the potential inhibitors of the phytopathogen's enzymes. MD simulations and MMPBSA confirmed the structural rigidity and stability of the docked complex.

The silica rich straw was further explored for the synthesis of nanosilica (SiNPs) employing the sol-gel method, via integrating the aqueous extract of *Sapindus mukorossi* as surfactant. EDS and FTIR spectra confirmed the predominant peaks of Si and O. FE-SEM confirmed the spheroid morphology of SiNPs with an average particle size of 20.34 ± 2.64 nm as determined by TEM. DLS studies revealed the stability of SiNPs with zeta potential of -14.37 mV and PDI of 0.198, implying its monodispersed nature. BET surface area of SiNPs was recorded as 746.32 m^2/g with a cumulative pore volume of 2.059 cm^3/g. The employment of SiNPs as a carrier material for clove oil (CO), depicted 62.64% encapsulation of CO in SiNPs under UV analysis. The antifungal efficacy of CO-SiNPs against *F. oxysporum* exhibited minimum inhibitory concentration (MIC) of 125 mg/L. The application of SiNPs in photocatalytic degradation of methylene blue reflected that 66.26 % of the dye was degraded in the first 10 mins, and the degradation reflected a first-order kinetics with a half-life of 6.79 mins.

In another objective of this study, PS was explored for lignocellulolytic enzymes under Solid State Fermentation by *Trichoderma* spp. Response Surface Methodology (RSM) optimization of PS in combination with de-oiled neem cake (NC) depicted that PS:NC in 8:3 ratio, provided maximum xylanase activity of 693.56 U/g on the 5^{th} day, with cellulase and laccase activity of 126 U/g and 28.12 U/g, respectively. OHR-LCMS study of the partially purified enzyme revealed the presence of β-xylanase and α-L-arabinofuranosidase. Enzymatic saccharification of various substrates enhanced the release of reducing sugars demonstrating its applicability in the biofuel domain. LC-MS, ICMPS, and EDX profiling of the residual spent unravelled the manifestation of bioactives, minerals, and silica, playing an essential role as biopesticide and biofertilizer. Life Cycle Assessment (LCA) for the SSF process, depicted adverse impacts due to electricity consumption (92.84%) and use of ammonium sulphate salt (6.17%). Nonetheless, employing renewable energy and reducing salt consumption could help minimize these impacts. The findings of the study asserted that PS can be substantially utilized for the development of value-added products. The study also promises new ventures for development of PS based small scale industries, as a source of revenue generation.

शोध का सार

पराली जलाने की समस्या को सम्बन्धित इस शोध के तहत किये गये अध्ययन में धान के पुआल (पीएस) के पुनर्चक्रण और पुनःउपयोग की परिकल्पना की गई है ताकि इस कम उपयोग वाले बायोमास को व्यावसायिक उपयोगिता प्रदान की जा सकें। इस संदर्भ में मूल्यवर्धित उत्पादनों जैसे मशरूम, बायोफार्मुलेशन, जैव कीटनाशक / जैव उर्वरक, नैनोसिलिका और एंजाइम विकसित करके धान के पुआल के शून्य अपशिष्ट प्रबंधन की दिशा में एक बहुआयामी रणनीति का प्रयास किया गया है।

विभिन्न डी–आयल्ड केक का उपयोग करते हुए धान के पुआल का वैलोराइजेशन दर्शाता है कि 5% सोयाबीन केक (यससी) के पूरक ने 1014 ग्राम किग्रा $^{-1}$, पी. ओस्ट्रीटस फ्रूटिंग बाडी की अधिकतम उपज दी और धान के पुआल नियंत्रण पर 15.14% की वृद्धि दर्ज की। प्रमुख घटक और पोषण संबन्धी मापदंडो के बीच सहसंबन्ध का विश्लेषण 78.2% की कुल भिन्नता दर्शाता है। एनर्जी डिस्पर्सिव एक्स–रे (ईडीएक्स) विश्लेषण ने फ्रूटिंग बाडीज में खनिजों की महत्वपूर्ण वृद्धि और खर्च में सिलिका की उच्च सामग्री (19-20%) का संकेत दिया। स्पेंट पर टी. एस्पेरेलम की खेती ने इस तथ्य को उजागर किया कि अपरिष्कृत पीएस ($14.73*10^{11}$) की तुलना में स्पेंट पीएस ($4.2*10^{3}$) में फफूंद वृद्धि तुलनात्मक रूप से अधिक थी। खर्च पर उगाए गये टी0एस्पेरेलम ने गैर–पूरक पीएस खर्च (54.26%) की तुलना में फुसैरियम आक्सीस्पोरम का 66.26% अवरोध दिखाया।

शोध के अगले भाग में, पी. ओस्ट्रीटस के स्पेंट मशरूम सबस्ट्रेट (एसएमएस) का उपयोग माइक्रोबियल बायोफार्मुलेशन के विकास के लिए किया गया है। पौधे के पोषक तत्व और विल्ट (एफ. आक्सीस्पोरम) मे रोग प्रबंधन के लिए अतिसंवेदनशील टमाटर का पौधा एफ–1 हाइब्रिड किंग 180 एसएमएस (पीएस+5% एससी) ने $12.37x10^{13}$ कोनीडिया/ग्राम सब्सट्रेट की सीमा तक टी एसपेरेलम (टीए) की वृद्धि का समर्थन किया। एसएमएस के जीसी–एमएस विश्लेषण ने पौधों के स्वास्थ्य प्रबंधन वाले कई बायोएक्टिव मेटाबोलाइट्स का पता लगाया। वाहक सामग्री के रूप में प्रेस मड और टैल्कम पाउडर का उपयोग करके बायोफार्मुलेशन विकसित किए गए थे। बर्तनों में परीक्षण किए गए विभिन्न जैव–सूत्रों के बीच अध्ययन, SMS (PS+5% SC) SiTAPM,एसएमएस (पीएस +5% यससी) SiTAPM जिसे सामूहिक रूप से टीफ–I नाम दिया गया है, मार्फो–बायोकेमिकल और पोषण संबन्धी मापदंडों का बेहतर स्तर प्रदान करता है। रोगजनक प्रभावित पौधे पर रोग गंभीरता सूचकांक (84.34% to 21.23%) में एक महत्वपूर्ण ($p<0.05$) कमी देखा गया। टीफ–I के तहत प्राप्त फलों और पत्तियों ने 83.73% (डीपीपीएच) और 72.25% (एफ आर ए पी) गतिविधि के साथ टोटल पालीफेनोल सामग्री (टीपासी) क्रमशः 74.5 और 126.9 मिली / ग्रा0 गैलिक एसिड प्रदर्शित की, जो एंटीआक्सिडेंट गुणों की प्राप्ति का संकेत देती है। ईडीएक्स विश्लेषण ने एसएमएस में 21.53% सिलिका (सी) दिखाया, और प्लांट मैपिंग जांच ने सी के पर्याप्त संचय का संकेत दिया, जो विकास, रोग प्रतिरोध और एंटीआक्सीडेंट पैरामीटर को बढ़ावा देने के लिए अच्छी तरह से माना जाता है। उत्तर प्रदेश के गांवों में विकसित बायोफार्मूलेशन किसानों तक पहुंचाया गया और एक

सर्वेक्षण अध्ययन किया गया, जिसके सकारात्मक परिणाम मिले और प्रौद्योगिकी के कार्यान्वयन में गहरी दिलचस्पी दिखाई दी।

फ्यूजेरियम विल्ट के कारण होने वाले एंजाइम पालीगैलेक्टुरोजेज (पीजी2) और टोमैटिनेज का मुकाबला करने में ट्राइकोडर्मा एसपीपी की बायोएक्टिव्स की संभावित भूमिका का आंकलन करने के लिए एक इन-सिलिको दृष्टिकोण भी नियोजित किया गया था। अध्ययन के निष्कर्षो से पता चला है कि टी. हार्जियानम और विरिडिन, विरोन और टी. वीरेन्स द्वारा उत्पादित ट्राइकोडर्माइड बी फाइटोपैथेजेन के एंजाइमों के संभावित अवरोधक के रूप में उभरा है। एमडी सिमुलेशन और एमएमपीबीयस ने डाक्ड किए गए काम्पलेक्स की संरचनात्मक कठोरता और स्थिरता की पुष्टि की।

नैनोसिलिका (SiNPs) के संश्लेषण के लिए सिलिका समृद्ध पुआल का पता लगाया गया था, जो सपिंडस मुकोरोसी के जलीय अर्क को सर्फेक्टेंट के रूप में एकीकृत करके सोल-जेल विधि को नियोजित करता है। EDS और STIR LisDVªk ने Si और O की प्रमुख चोटियों की पुष्टि की। FE-SEM ने 20-34 ± 2-64 एनएम के औसत कण आकार के साथ के SiNPs गोलाकार आकृति विज्ञान की पुष्टि की, जैसा कि TEM द्वारा निर्धारित किया गया है। डीएलएस अध्ययनों ने −14.37 एमवी की जीटा क्षमता और 0.198 के पीडीआई के साथ एसआईएनपी की सिरिता का खुलासा किया, जो इसकी मोनोडिस्पर्स्ड प्रकृति को दर्शाता है। SiNPs का बीईटी सतह क्षेत्र 2.059 सेमी0/जी की संचयी ताकना मात्रा के साथ 746.32 एम2/जी के रूप में दर्ज किया गया था लौंग के तेल (CO) के लिए वाहन सामग्री के रूप में SiNPs का रोजगार, न्ट विश्लेषण के तहत में SiNPs का CO के 62.64% एनकैप्सुलेशन को दर्शाता है। एफ आक्सीस्पोरम के खिलाफ CO- SiNPs की एंटीफंगल प्रभावकारिता ने 125 mg/L की न्यूनतम निरोधात्मक सांद्रता (MIC) प्रदर्शित की। मेथिलीन ब्लू के फोटोकैटलिटिक डिग्रेडेशन में SiNPs के अनुप्रयोग से परिलक्षित होता है कि पहले 10 मिनट में डाई का 66.26 % अवक्रमित हो गया था, और गिरावट 6.79 मिनट के आधे जीवन के साथ पहले क्रम के कैनेटीक्स को दर्शाता है।

इस अध्ययन के एक अन्य उद्देश्य में, ट्राइकोडर्मा एसपीपी0 द्वारा ठोस अवस्था किण्वन के तहत लिग्लोसेल्युलोलिटिक एंजाइमों के लिए पीएस की खोज की गई। रिस्पांस सरफेस मेथडोलाजी (RSM) आप्टिाइजेशन आफ PS इनकाम्बिनेशन विथ डी-आयल्ड नीम केक (NC) दर्शाता है कि PS:NC 8:3 अनुपात में, सेल्यूलेस और लैकेस एक्टिविटी के साथ 5वें दिन 693.56 U/g की अधिकतम गलसंदेम गतिविधि प्रदान करता है। क्रमशः 126यू/जी और 28.12 यू/जी आंशिक रूप से शुद्ध किए गए एंजाइम के OHR-LCMS अध्ययन से β-xylanase और α-L-arabinofuranosidase की उपस्थिति का पता चला। विभिन्न सब्सट्रेट्स के एंजाइमैटिक सैक्रिफिकेशन ने जैव ईधन डोमेन में इसकी प्रयोज्यता को प्रदर्शित करने वाली शर्करा को कम करने की रिहाइ को बढ़ाया। LC-MS,ICMPS, और EDX खर्च किए गए अवशिष्ट की रूपरेखा ने बायोएक्टिव्स, खनिजों और सिलिका की अभिव्यक्ति को उजागर किया, जो जैव कीटनाशक और जैव उर्वरक के रूप में एक आवश्यक भूमिका निभाते है। एसएसएफ प्रक्रिया के लिए जीवन चक्र आकलन (एलसीए), बिजली की खपत (92.84%) और अमोनियम सल्फेट नमक (6.17%) के

उपयोग के कारण प्रतिकूल प्रभाव दर्शाता है। बहरहाल, अक्षय ऊर्जा को रोजगार और नमक की खपत को कम करने से उन प्रभावों को कम करने में मदद मिल सकती है।

अध्ययन के निष्कर्षो यह दावा किया है कि मूल्यवर्धित उत्पादों के विकास के लिए पीएस का काफी हद तक उपयोग किया जा सकता है। अध्ययन राजस्व सृजन के स्रोतों के रूप में पीएस आधारित लघु उद्योगो के विकास के लिए नए उद्योगो का वादा भी करता है।

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LIST OF ABBREVIATIONS

Symbol	Full Name
%	Percentage
°C	Degree Celsius
µ	Micro
µg	Microgram
µL	Microliter
3D	Three dimensional
a.i.	Active ingredients
AFM	Atomic Force Microscopy
BCA	Biocontrol agents
BET	Brunauer–Emmett–Teller
BSA	Bovine Serum Albumin
C	Carbon
C/N	Carbon and Nitrogen
CC	Castor cake
CCD	Central Composite Design
CFU	Colony Forming Units
CHN	Carbon, Hydrogen and Nitrogen
CMC	Carboxymethyl Cellulose
CNDs	Colloidal Nano delivery systems
CO	Clove oil
CRF	Central Research Facility
CTAB	Cetyltrimethyl ammonium bromide
DCB	Dichlorobenzene
DEOCs	De-oiled cakes
DLS	Dynamic Light Scattering
DMRT	Duncan’s multiple range test
DPPH	2,2-diphenyl-1-picrylhydrazyl
DSI	Disease Severity Index
dw	Dry weight
EDS	Energy Dispersive X-Ray Spectroscopy
EDTA	Ethylenediaminetetraacetic acid
EO	Essential Oil
EU	European Union
f.w.	Fresh weight
Fe	Iron
FE-SEM	Field Emission Scanning Electron Microscopy
Fig.	Figure

Symbol	Full Name
FOL	*Fusarium oxysporum lycopersici*
FPLC	Fast Protein Liquid Chromatography
FRAP	Ferric Reducing Antioxidant Power
FTIR	Fourier Transform Infrared spectrophotometry
g	Grams
GA	Gallic acid
GAE	Gallic acid equivalent
GC	Groundnut cake
GCMS	Gas chromatography–mass spectrometry
GHGs	Greenhouse gases
GOI	Government of India
GP	Germination Percentage
h	Hour
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
IAA	Indole Acetic Acid
ICPMS	Inductively Couple Plasma Mass Spectrometry
IPM	Integrated Pest Management
ISR	Induced Systemic Response
JC	Jatropha cake
kDa	Kilodaltons
KOH	Potassium hydroxide
LCA	Life Cycle Assessment
LCMS	Liquid chromatography–mass spectrometry
m/z	Mass/Charge
m/z	Mass/charge
MB	Methylene Blue
MC	Mustard cake
mg	Milligrams
MIC	Minimum Inhibitory Concentration
min	Minutes
min	Minutes
mL	Milliliter
mm	Millimeter
mM	Millimolar
MSNs	Mesoporous silica nanoparticles
Mt	Million tons
NAG	N-acetyl glucosamine
NaOH	Sodium hydroxide

Symbol	Full Name
NC	Neem cake
nm	nanometers
NPK	Nitrogen Phosphorus Potassium
OECD	Organization for Economic Cooperation and Development
OHR-LCMS	Orbitrap High Resolution Liquid chromatography–mass spectrometry
P. ostreatus	*Pleurotus ostreatus*
PCA	Principal Component Analysis
PDA	Potato Dextrose Agar
PDI	Polydispersity Index
PE	Pickering Emulsions
PG2	Polygalacturonase
PGP	Plant Growth Promoting
PGPF	Plant Growth Promoting Fungi
PM	Particulate matters
PM	Press mud
ppm	Parts per million
PR	Pathogenesis Related
PS	Paddy straw
PSA	Paddy straw ash
psi	Pounds per square inch
ROS	Reactive oxygen species
rpm	Revolutions per minute
Rs.	Rupees
RSM	Response Surface Methodology
RT	Retention Time
SAIF	Sophisticated Analytical Instrument Facility
SAR	Systemic Acquired Resistance
SC	Soybean cake
SD	Standard deviation
SDGs	Sustainable Development Goals
SDS	Sodium Dodecyl Sulphate
sec	Seconds
Si	Silica
SiNPs	Nanosilica particles
SmF	Submerged State Fermentation
SMS	Spent Mushroom Substrate
SSF	Solid State Fermentation
TA	*Trichoderma asperellum*
TEM	Transmission Electron Microscopy

Symbol	Full Name
Temp.	Temperature
TGA	Thermogravimetric Analysis
TP	Talcum powder
TPC	Total Phenolic Content
TPTZ	2,4,6-tripyridyl-2-triazine
TSP	Total Soluble Protein
TSS	Total Soluble Sugars
U	Enzymatic Unit
U/g	Enzymatic units per gram substrate
UV-vis	Ultraviolent visible
v/v	Volume/volume
w/w	Weight/weight
XRD	X-ray diffraction
AQI	Air Quality Index
CRB	Crop Residue Burning
FAO	Food and Agriculture Organization
MNRE	Ministry of New and Renewable Energy
VIRRS	Visible Infrared Imaging Radiometer Suite
OECD	Organization for Economic Cooperation and Development
IPM	Integrated Pest Management
PDB	Protein Data Bank
CWDEs	Cell wall degrading enzymes
SPC	Simple Point Charge
RMSD	Root Mean Square Deviation
RMSF	Root Mean Square Fluctuation
SASA	Solvent Accessible Surface Area
STL	Standard Template Library
Rg	Radius of Gyration
SDF	Spatial Data File