UNDERSTANDING THE ROLE OF SOIL MICROBIOTA IN DISEASE SUPPRESSION UNDER DIFFERENT FARMING PRACTICES

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by

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DEDICATED TO THE ALMIGHTY AND MY PARENTS

CERTIFICATE

This is to certify that the thesis entitled "**Understanding the role of soil microbiota in disease suppression under different farming practices**" submitted by **Ms. Shivani Khatri** has been prepared under my guidance with the rules and regulations of Indian Institute of Technology Delhi India. The results presented in this thesis have not been submitted for any degree or diploma in any other institute or university.

(Dr. Shilpi Sharma)

Professor

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Shivani Khatri

ABSTRACT

Controlling soil-borne plant diseases by harnessing the natural potential of soil microbial communities has several benefits, including environmental sustainability and economic advantages for farmers. Disease-suppressive soils can naturally inhibit the development of phytopathogens, reducing the incidence of plant diseases. Organic agriculture relies on natural products and helps maintain ecosystem functionality by enriching soil biodiversity and chemical composition. It is well known that the higher soil microbiome activity and diversity in organic agriculture gives the soil a disease-suppressing potential against soil-borne phytopathogens. Therefore, adopting organic farming to protect crops from soil-borne diseases can curb the increasing use of pesticides and fungicides since the green revolution to improve crop quality. However, the mechanisms underlying disease suppression in organic agriculture are poorly understood. Few studies have attempted to determine the effects of organic and conventional agriculture on the structural and functional potential of the soil microbiome in relation to "general disease suppression".

In the present study, **a comparative analysis of the effects of long-term conventional and organic farming practices (field experiment ongoing since 2004) was done in terms of identifying characteristic disease-suppressive microbial, molecular, and metabolic markers.** The soil samples from fields under conventional and organic agriculture were screened for their associated physicochemical properties and disease-suppressive potential towards a wide range of fungal and bacterial plant pathogens using dual-culture assays. The plant biometric parameters were also recorded at the harvest stage of wheat and rice grown in conventional and organic farming fields over three cropping seasons, i.e., 2018, 2019, and 2020. Once the higher disease-suppressive ability of organic farming was confirmed, its microbial basis was investigated by understanding the changes in the structural diversity of soil microbiome in conventional and organic agriculture. The bacterial and fungal diversity was

analyzed using 16S rRNA and ITS-based amplicon sequencing. Higher bacterial and lower fungal diversity was observed in soil from the field under organic farming than in conventional farming. The organic field soil harboured a higher abundance of known antagonistic bacterial and fungal genera such as *Bacillus*, *Pseudomonas*, *Burkholderia*, *Mortierella*, *Acremonium*, and *Chaetomium*. Due to the known association of *Pseudomonas* with disease-suppressive soils, its diversity was then determined in conventional and organic farming. A higher *Pseudomonas* diversity, along with increased abundance of antagonistic *Pseudomonas* species, were detected in organic farming.

The alterations in the structural diversity of the soil microbiome were then correlated with the changes in the functional potential of the soil microbiome in the context of disease suppressiveness in organic and conventional farming. Direct estimation of hydrolytic enzymes activities and quantification of disease-suppressive markers in soil, including for pyrrolnitrin, fengycin, bacillibactin, and chitinase, revealed their higher abundance in soil treated with organic fertilizers than inorganic fertilizers. A robust culture bank of biocontrol strains was generated from organic field soil, and functional characterization was done using whole-genome sequencing and untargeted metabolomics. Several antimicrobial metabolic gene clusters related to non-ribosomal peptide synthetases were found in the genomes of biocontrol isolates. The common metabolites produced by biocontrol strains in the presence of pathogenic stress were pyrrolnitrin, phenazine, obafluorin, resorcinol, etc. The bacterial isolates with good root colonization ability showed disease-suppressive ability towards *Fusarium oxysporum* and *Verticillium dahliae* with *in planta* wheat growth experiment under controlled conditions. *In planta* disease-suppressive assays were also conducted to test and compare the suppressive effects of conventional and organic field soil against *Fusarium oxysporum* and *Rhizoctonia solani* in a plant growth chamber with wheat and rice plant systems. The higher disease-suppressive ability of organic field soil was observed against both pathogens and increased

expression of biotic stress markers in plants grown in soil from conventional farming field. Based on the knowledge gained by identifying the structural and functional markers of disease-suppressiveness in organic farming, an attempt to transform a conducive soil into suppressive soil was made by a soil transplantation experiment conducted under field conditions. The transferability of disease-suppressive potential of organic field soil to the conventional field soil was confirmed by determining the changes in the structural and functional abundances of disease-suppressive markers.

Conclusively, soil under organic farming showed higher general disease-suppressive potential than that under conventional farming. The higher disease suppressiveness in organic farming could be correlated with significant changes in the structural and functional composition of soil microbiome. The microbial taxa identified in this study can be used to generate synthetic biocontrol microbial communities that mimic natural communities in organic farming to induce overall disease suppression in conducive soils. Detection of molecular and metabolic markers that are more abundant in organic agriculture can be used to characterize any agricultural soil for its disease-suppressive potential. Developing a soil transplantation assay to convert conducive soils into disease-suppressive soils offers an approach to environment-friendly plant disease control.

सार

मृदा सूक्ष्मजीव समुदायों की प्राकृतिक क्षमता का दोहन करके मृदा जनित पौधों की बीमारियों को नियंत्रित करने के कई लाभ हैं, जिनमें पर्यावरणीय स्थिरता और किसानों के लिए आर्थिक लाभ शामिल हैं। रोग-दमनकारी मिट्टी स्वाभाविक रूप से संयंत्र रोगजनकों के विकास को रोक सकती है, जिससे पौधों की बीमारियों की घटनाओं में कमी आती है। जैविक कृषि प्राकृतिक उत्पादों पर निर्भर करती है और मिट्टी की जैव विविधता और रासायनिक संरचना को समृद्ध करके पारिस्थितिकी तंत्र की कार्यक्षमता को बनाए रखने में मदद करती है। यह सर्वविदित है कि उच्च मृदा सूक्ष्मकाय गतिविधि और जैविक कृषि में विविधता मिट्टी को मृदा जनित संयंत्र रोगजनकों के खिलाफ रोग-दबाने की क्षमता प्रदान करती है। इसलिए, फसलों को मिट्टी से होने वाली बीमारियों से बचाने के लिए जैविक खेती को अपनाने से फसल की गुणवत्ता में सुधार के लिए हरित क्रांति के बाद से कीटनाशकों और फफूंदनाशकों के बढ़ते उपयोग पर अंकुश लगाया जा सकता है। हालांकि, जैविक कृषि में रोग दमन के तंत्र को कम समझा जाता है। कुछ अध्ययनों ने "सामान्य रोग दमन" (general disease suppressiveness) के संबंध में मिट्टी के सूक्ष्मकाय की संरचनात्मक और कार्यात्मक क्षमता पर जैविक और पारंपरिक कृषि के प्रभावों को निर्धारित करने का प्रयास किया है।

वर्तमान अध्ययन में, विशिष्ट रोग-दमनकारी सूक्ष्मजैविक, आणविक और चयापचय अंकनों की पहचान करने के संदर्भ में दीर्घकालिक पारंपरिक और जैविक खेती प्रथाओं (2004 से चल रहे खेती प्रयोग) के प्रभावों का तुलनात्मक विश्लेषण किया गया था। पारंपरिक और जैविक कृषि के तहत खेतों से मिट्टी के नमूनों को उनके संबंधित भौतिक-रासायनिक गुणों और रोग-दमनकारी क्षमता के लिए dual-culture assays का उपयोग करके फफूंद और जीवाणु पादप रोगजनकों की एक विस्तृत श्रृंखला के लिए जांचा गया। तीन फसली मौसमों, यानी 2018, 2019 और 2020 में पारंपरिक और जैविक खेती के खेतों में उगाए गए गेहूं और चावल की कटाई के चरण में फसल उत्पादकता में वृद्धि मानक भी दर्ज किए गए थे। जैविक खेती की उच्च रोग-दमनकारी क्षमता की पुष्टि हो जाने के बाद, इसकी पारंपरिक और जैविक कृषि में मृदा

सूक्ष्मकाय की संरचनात्मक विविधता में परिवर्तन को समझकर सूक्ष्मजैविक आधार की जांच की गई। 16S rRNA- और ITS- आधारित amplicon अनुक्रमण का उपयोग करके जीवाणु और कवक विविधता का विश्लेषण किया गया था। पारंपरिक खेती की तुलना में जैविक खेती के तहत खेत की मिट्टी में उच्च जीवाणु और कम कवक विविधता देखी गई। जैविक खेती की मिट्टी में *Bacillus*, *Pseudomonas*, *Burkholderia*, *Mortierella*, *Acremonium*, और *Chaetomium* जैसे ज्ञात प्रतिपक्षी जीवाणु और कवक जनन की अधिकता पाई जाती है। रोग-दमनकारी मिट्टी के साथ *Pseudomonas* के ज्ञात जुड़ाव के कारण, इसकी विविधता तब पारंपरिक और जैविक खेती में निर्धारित की गई थी। जैविक खेती में प्रतिपक्षी *Pseudomonas* प्रजातियों की बहुतायत में वृद्धि के साथ एक उच्च *Pseudomonas* विविधता का पता चला।

मृदा सूक्ष्मकाय की संरचनात्मक विविधता में परिवर्तन तब जैविक और पारंपरिक खेती में रोग दमन के संदर्भ में मृदा सूक्ष्मकाय की कार्यात्मक क्षमता में परिवर्तन के साथ सहसंबद्ध थे। Hydrolytic enzymes की गतिविधियों का प्रत्यक्ष अनुमान और मिट्टी में रोग दमनकारी अंकनों की मात्रा का ठहराव, जिसमें pyrrolnitrin, fengycin, bacillibactin और chitinase शामिल हैं, ये अकार्बनिक उर्वरकों की तुलना में जैविक उर्वरकों के साथ उपचारित मिट्टी में उनकी उच्च बहुतायत का खुलासा किया। जैविक खेती की मिट्टी से जैविक नियंत्रक जीवाणुओ का एक मजबूत culture बैंक तैयार किया गया था, और पूरे-जीनोम अनुक्रमण और अलक्षित metabolomics का उपयोग करके कार्यात्मक लक्षण वर्णन किया गया था। जैविक नियंत्रक जीवाणुओ के genome में non-ribosomal peptide synthetase से संबंधित कई रोगाणुरोधी चयापचय जीन क्लस्टर पाए गए। रोगजनक तनाव की उपस्थिति में जैविक नियंत्रक जीवाणु द्वारा उत्पादित सामान्य metabolites pyrrolnitrin, fengycin, obafluorin, resorcinol आदि थे। अच्छी जड़ उपनिवेशण क्षमता वाले जीवाण ने फसल गेहूं विकास प्रयोग में नियंत्रित गेहूं के विकास प्रयोग के साथ *Fusarium oxysporum* और *Verticillium dahliae* के प्रति रोग दमनकारी क्षमता दिखाई। स्थितियाँ। फसल रोग में गेहूं और चावल के पौधे प्रणालियों के साथ एक पौधे के विकास कक्ष में *Fusarium oxysporum* और *Rhizoctonia solani* के खिलाफ पारंपरिक और जैविक खेती की मिट्टी के दमनकारी

प्रभावों का परीक्षण और तुलना करने के लिए दमनकारी परीक्षण भी किए गए थे। पारंपरिक कृषि खेती से मिट्टी में उगाए गए पौधों में रोगजनकों और जैविक तनाव अंकनों की अभिव्यक्ति दोनों के खिलाफ जैविक खेती की मिट्टी की उच्च रोग-दमनकारी क्षमता देखी गई। जैविक खेती में रोग दमनकारीता के संरचनात्मक और कार्यात्मक अंकनों की पहचान करके प्राप्त ज्ञान के आधार पर, एक अनुकूल मिट्टी को दमनकारी मिट्टी में बदलने का प्रयास खेती की परिस्थितियों में किए गए मिट्टी के प्रत्यारोपण प्रयोग द्वारा किया गया था। रोग-दमनकारी मार्करों के संरचनात्मक और कार्यात्मक बहुतायत में परिवर्तन का निर्धारण करके पारंपरिक खेती की मिट्टी में जैविक खेती की मिट्टी की रोग दमनकारी क्षमता की हस्तांतरणीयता की पुष्टि की गई थी।

निष्कर्ष रूप से, जैविक खेती के तहत मिट्टी ने पारंपरिक खेती की तुलना में अधिक सामान्य रोग-दमनकारी क्षमता दिखाई। जैविक खेती में उच्च रोग दमनात्मकता को मृदा सूक्ष्मकाय की संरचनात्मक और कार्यात्मक संरचना में महत्वपूर्ण परिवर्तनों के साथ सहसंबद्ध किया जा सकता है। इस अध्ययन में पहचाने गए सूक्ष्मजैविक taxa का उपयोग कृत्रिम जैविक नियंत्रक जीवाणुओ के समुदायों को उत्पन्न करने के लिए किया जा सकता है जो अनुकूल मिट्टी में समग्र रोग दमन को प्रेरित करने के लिए जैविक खेती में प्राकृतिक समुदायों की नकल करते हैं। कार्बनिक कृषि में अधिक प्रचुर मात्रा में आणविक और चयापचय अंकनों का पता लगाने का उपयोग किसी भी कृषि मिट्टी को रोग-दमनकारी क्षमता के लिए चिह्नित करने के लिए किया जा सकता है। अनुकूल मिट्टी को रोग-दमनकारी मिट्टी में बदलने के लिए मिट्टी के प्रत्यारोपण परख का विकास करना पर्यावरण के अनुकूल पौधों के रोग नियंत्रण के लिए एक दृष्टिकोण प्रदान करता है।

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ABBREVIATIONS

Molecules and Chemicals

$(NH_4)_2SO_4$	Ammonium sulfate
CAS	Chrome azurol S
CTAB	Cetyl trimethyl ammonium Bromide
DAPG	2,4-diacetylphloroglucinol
DNS	3,5-dinitro-salicylic acid
EDTA	Ethylene diamine tetra acetic Acid
$FeCl_3$	Ferric chloride
$FeSO_4$	Ferrous sulphate
H_2O_2	Hydrogen peroxide
$HClO_3$	Chloric acid
HCN	Hydrogen cyanide
IAA	Indole acetic acid
KH_2PO_4	Dihydrogen potassium phosphate
MeCN	Acetonitrile
$MgSO_4.7H_2O$	Magnesium sulfate heptahydrate
NaCl	Sodium chloride
NAG	N-acetyl-glucosamine
SDS	Sodium dodecyl sulfate

Units

%	Percent
bp	Base pairs
cm	Centimetre
dS m^{-1}	Deci Siemens per metre
g	Gram
g cc^{-1}	Gram per cubic metre
kg	Kilogram
°C	Degrees Celsius
Ng	Nanogram
L	Litre
m	Metre
M	Molar
mL	Millilitre
mm	Millimetre
min	Minutes
rpm	Revolutions per minute
S	Seconds
v/v	Volume by volume
t_R	Retention time
µL	Microliter
µM	Micromolar
µg mL^{-1}	Microgram per milliliter
tonnes h^{-1}	Tonnes per Hectare

Elements

N	Nitrogen
P	Phosphorus
K	Potassium
C	Carbon

Important terminology used in the study

AMF	Arbuscular Mycorrhizal Fungus
BGA	Blue Green Algae
BGCs	Biosynthetic Gene Clusters
CFU	Colony Forming Unit
CLPP	Community Level Physiological Profiling
DAS	Days After Sowing
*dhb*A	Bacillibactin
DNA	Deoxyribonucleic Acid
EC	Electrical Conductivity
FAO	Food and Agriculture Organization
*Fen*A	Fengycin
FYM	Farmyard Manure
*GA*1	Chitinase
ISR	Induced Systemic Resistance
ITCC	Indian Type Culture Collection
ITS	Internal Transcribed Spacer
LC/MS	Liquid Chromatography-Mass Spectrometry
LGLM	*Leucaena* Green Manure
MCT	Microcentrifuge Tube
MLSA	Multilocus Sequence Analysis
MTCC	Microbial Type Culture Collection
NCBI	National Center for Biotechnology Information
NMDS	Nonmetric Multidimensional Scaling
NMSA	National Mission for Sustainable Agriculture
ODW	Oven Dry Weight
OTU	Operational Taxonomic Unit
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
PERMANOVA	Permutational Multivariate Analysis of Variance
PGPB	Plant Growth Promoting Bacteria
PO	Peroxidase
PPO	Polyphenol Oxidase
PPO	Phenol peroxidase
*prn*D	Pyrrolnitrin
PVC	Polyvinyl Chloride
QIIME	Quantitative Insights into Microbial Ecology

qPCR	quantitative Polymerase Chain Reaction
*rpo*D	RNA polymerase sigma factor D
rRNA	Ribosomal Ribonucleic Acid
SEM	Scanning Electron Microscopy
SGM	*Sesbania* Green Manure
SOC	Soil Organic Carbon
SRA	Sequence Read Archive
SSP	Single Superphosphate
T-RFLP	Terminal-Restriction Fragment Length Polymorphism
TAD	Take All Decline
tRNA	Transfer Ribonucleic Acid
UHPLC-MS	Ultra-High-Performance Liquid Chromatography-High Resolution Mass Spectrometry
UN	United Nations
USD	United States Dollars