Assessing the impact of organic amendments on the structural and functional diversity of rhizospheric bacteria and soil health

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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 **“Assessing the impact of organic amendments on the structural and functional diversity of rhizospheric bacteria and soil health”** submitted by **Vijay Laxmi Shrivas** to the Indian Institute of Technology, Delhi, for the award of the degree of **Doctor of Philosophy**, is a record of original bona-fide research work carried out by her under my supervision.

In my opinion, the thesis has reached the requisite standard. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma.

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Vijay Laxmi Shrivas

Abstract

Agriculture, a key contributor to the growing Indian economy, is facing significant challenges in meeting the needs of a rapidly expanding population. Synthetic fertilizers have been extensively used in modern agriculture to overcome this issue. While conventional agriculture has increased agricultural productivity, it has been at a high cost to the environment due to the indiscriminate use of agrochemicals, and intense practices. The detrimental effects of conventional agriculture have encouraged a shift towards more sustainable options, such as organic farming. Organic farming practices prioritize reduced agrochemical dependency, biodiversity enhancement, and climate resilience. Organic amendments have been found to promote microbial activity, improve soil quality, and boost plant development by nourishing microorganisms and boosting nutrient release. With a focus on ecological equilibrium, disease resilience, and incorporating organic inputs, organic farming embodies a more sustainable agricultural system, aligning with the principles of UN's Sustainable Development Goals (SDGs), effectively addressing diverse challenges.

Microorganisms, particularly bacteria, present in the soil are crucial in nutrient recycling and soil fertility. The rhizosphere, and its associated microorganisms, are extremely important for plant health and ecological function. To ensure sustainable agriculture, it is vital to comprehend how organic and conventional farming impact crop yield and soil health, including the diversity and composition of rhizospheric bacterial communities, which are excellent indicators of soil health and plant fitness. However, concerns persist in the safe use of organic amendments due to the presence of antibiotic resistance in manure. India's large livestock population and insufficient antimicrobial resistance (AMR) data raise serious concerns.

The current study evaluated three cycles of the pigeonpea-wheat cropping system (PWCS) under conventional and organic farming systems in field experiments (over a period of four years). The study aimed to determine the impact of agricultural management practices on plant

growth and soil health. The rhizospheric bacterial community was thoroughly investigated by employing a range of molecular markers targeting the structural diversity and functional potential of bacterial communities and antibiotic resistant markers. This was attained by employing a polyphasic approach, including assessment of the physicochemical parameters of plants and soil, adoption of traditional microbiological techniques and advanced molecular tools. The polyphasic analysis of a range of attributes of plants and soil provided a multifaceted understanding of the effects of several factors (agri-management practices, amendments, crops, cropping cycle) on the PWCS system.

Initially, manure samples were screened across seven states in India, to comprehend the patterns of antibiotic resistance in animal manure and their possible correlation, if any, with other environmental factors. This step aimed to identify suitable manure samples (exhibiting antibiotic resistance, and devoid of it) for subsequent field experiments. Soil samples from conventional and organic fields were investigated for their physicochemical parameters, and microbiological attributes using culture-dependent and culture-independent techniques. Plant growth attributes were recorded at the harvest stage of pigeonpea and wheat during three successive cropping seasons. Quantitative insight into the responses of total bacteria and specific bacterial groups (*α-Proteobacteria, β-Proteobacteria, Acidobacteria, Firmicutes*, and *Actinobacteria*) in the rhizosphere was gained through qPCR. Additionally, the functional potential of these bacterial communities was assessed by targeting bacterial guilds involved in nitrogen (N) and phosphorus (P) cycles. This entailed quantifying the genetic markers associated with pivotal steps in the nitrogen cycle (nitrogen fixation-*nifH*, nitrification- *amoA*, denitrification-*narG*) and the phosphorus cycle (solubilization-*pqqC*, mineralization- *phoD*) through qPCR. To comprehend the influence of farming practices on the spread of antibiotic resistance, different markers for antibiotic resistance (aminoglycoside-*aacA*, β-lactam-*blaTEM*, chloramphenicol-*chl1*, macrolide-*ermB*, sulfonamides-*sul1, sul2* and tetracycline-

tetO) and integrons-*int1* and *int2* were targeted in the rhizosphere soil using qPCR. The bacterial diversity at the harvest stage of the pigeonpea crop after the completion of the three year-field experiment, was characterized using 16S rRNA gene-based amplicon sequencing. The current study deciphered a correlation between bacterial abundance, nutrient concentrations, and antibiotic resistance in the manure samples suggesting a potential link between intensive farming practices, antibiotic usage, and the prevalence of antibiotic resistance in manure. Two manure samples were shortlisted for the setting up of field experiments, one with a higher abundance of antibiotic resistant bacteria (ARB) and another with a lower abundance of ARB. Results from the three-year (2018-19, 2019-20, and 2020-21) field experiments with PWCS exhibited a distinct impact of the agriculture practices. Conventional treatments showed the best response in terms of plant growth attributes, yield parameters, and NPK acquisition. However, organic farming practices boosted total bacterial abundance and improved both the structural diversity and functional potential of rhizospheric bacterial communities for both crops. By the end of the third cropping season, the bacterial diversity in the organic treatments had significantly diverged from the initial bacterial community composition of the applied amendments. It was also noticed that manure amendments exhibited a higher abundance of copies of antibiotic resistant genes (ARGs) than the rhizospheric soil under organic and conventional treatments. Three-year field experiment with the pigeonpea-wheat cropping system revealed a complex interaction between agricultural practices, bacterial community dynamics, and crop productivity. The notable prevalence of ARGs linked to manure amendments serves as a cautionary note, demanding responsible management practices. The complex interplay between agricultural practices, plant and soil health, rhizospheric bacterial communities, and antibiotic resistance, suggest the need for a comprehensive approach to sustainable and effective crop management.

सारांश

कृषि, जो भारत की बढ़ती अर्थव्यवस्था में एक प्रमुख योगदानकर्ता है, तेजी से बढ़ती आबादी की जरूरतों को पूरा करने में महत्वपूर्ण चुनौतियों का सामना कर रही है। इस समस्या को दूर करने के लिए आधुनिक कृषि में कृत्रिम उर्वरकों का बड़े पैमाने पर उपयोग किया गया है। जबकि पारंपरिक कृषि ने उत्पादकता में वृद्धि की है, कृषि रसायनों के अंधाधुंध उपयोग और गहन प्रथाओं के कारण पर्यावरण को इसकी भारी कीमत चुकानी पड़ी है। पारंपरिक कृषि के हानिकारक प्रभावों ने जैविक खेती जैसे अधिक संपोषणीय विकल्पों की ओर बदलाव को प्रोत्साहित किया है। जैविक खेती पद्धतियाँ कम कृषि रसायन निर्भरता, जैव विविधता वृद्धि, और जलवायु प्रतिरोध को प्राथमिकता देती हैं। सूक्ष्मजीव गतिविधि को बढ़ावा देने, मिट्टी की गुणवत्ता में सुधार करने, और सूक्ष्मजीवों को पोषण देकर और पोषक तत्वों निर्गमन को बढ़ावा देकर पौधों के विकास को बढ़ावा देने के लिए जैविक संशोधन पाए गए हैं। जलवायु संतुलन, रोग प्रतिरोधक क्षमता और जैविक आदानों को शामिल करने पर ध्यान देने के साथ, जैविक खेती एक अधिक संपोषणीय कृषि प्रणाली का प्रतीक है, जो संयुक्त राष्ट्र के सतत विकास लक्ष्यों (एसडीजी / SDGs) के सिद्धांतों के साथ संरेखित होती है, और विभिन्न चुनौतियों का प्रभावी ढंग से समाधान करती है।

मिट्टी में मौजूद सूक्ष्मजीव, विशेष रूप से बैक्टीरिया (bacteria), पोषक तत्वों के पुनर्चक्रण और मिट्टी की उर्वरता में महत्वपूर्ण हैं। राइजोस्फीयर (rhizosphere), और उससे जुड़े सूक्ष्मजीव, पौधों के स्वास्थ्य और समुचित कार्य के लिए बेहद महत्वपूर्ण हैं। संपोषणीय कृषि सुनिश्चित करने के लिए, यह समझना महत्वपूर्ण है कि जैविक और पारंपरिक खेती फसल की उपज और मिट्टी के स्वास्थ्य को कैसे प्रभावित करती है, जिसमें राइजोस्फेरिक (rhizospheric) जीवाणु समुदायों की विविधता और संरचना भी शामिल है, जो मिट्टी के स्वास्थ्य और पौधों की फिटनेस (fitness) के उत्कृष्ट संकेतक हैं। हालाँकि, जीवांश खाद में एंटीबायोटिक (antibiotic) प्रतिरोध की उपस्थिति के कारण जैविक संशोधनों के सुरक्षित उपयोग को लेकर चिंताएँ बनी हुई हैं। भारत की बड़ी पशुधन आबादी और अपर्याप्त रोगाणुरोधी प्रतिरोध (antimicrobial resistance, AMR) डेटा गंभीर चिंताएं पैदा करते हैं।

वर्तमान अध्ययन में क्षेत्रीय प्रयोगों में पारंपरिक और जैविक खेती प्रणालियों के तहत अरहर-गेहूं फसल प्रणाली (पीडब्ल्यूसीएस / PWCS) के तीन चक्रों का मूल्यांकन किया गया। अध्ययन का उद्देश्य पौधों की वृद्धि और मिट्टी के स्वास्थ्य पर कृषि प्रबंधन प्रथाओं के प्रभाव को निर्धारित करना था। जीवाणु समुदायों और एंटीबायोटिक

(antibiotic)-प्रतिरोधी मार्करों की संरचनात्मक विविधता और कार्यात्मक क्षमता को लक्षित करने वाले आणविक मार्करों की एक श्रृंखला को नियोजित करके राइजोस्फेरिक (rhizospheric) जीवाणु समुदाय की पूरी तरह से जांच की गई थी। यह पौधों और मिट्टी के भौतिक-रासायनिक मापदंडों के मूल्यांकन, पारंपरिक सूक्ष्मजीवविज्ञानी तकनीकों और उन्नत आणविक उपकरणों को अपनाने सहित, एक पॉलीफ़ेज़िक (polyphasic) दृष्टिकोण को नियोजित करके प्राप्त किया गया। पौधों और मिट्टी के विभिन्न पहलुओं के विश्लेषण ने पीडब्ल्यूसीएस (PWCS) प्रणाली पर कई कारकों (कृषि-प्रबंधन प्रथाओं, संशोधनों, फसलों) के प्रभावों की बहुमुखी समझ प्रदान की गई।

प्रारंभ में, भारत के सात राज्यों में जीवांश खाद के नमूनों की जांच की गई, ताकि पशु जीवांश खाद में एंटीबायोटिक प्रतिरोध के स्वरूप और अन्य पर्यावरणीय कारकों के साथ उनके संभावित सहसंबंध को समझा जा सके। इस कदम का उद्देश्य बाद के क्षेत्र प्रयोगों के लिए उपयुक्त जीवांश खाद के नमूनों (एंटीबायोटिक प्रतिरोध प्रदर्शित करने वाले, और इससे रहित) की पहचान करना था। पारंपरिक और जैविक क्षेत्रों से मिट्टी के नमूनों की जांच उनके भौतिक-रासायनिक मापदंडों और जीवाणु अवलम्बित (culture-dependent) और जीवाणु निरालम्ब (culture-independent) तकनीकों का उपयोग करके सूक्ष्मजीवविज्ञानी विशेषताओं के लिए की गई। लगातार तीन फसल मौसमों के दौरान अरहर और गेहूं की कटाई के चरण में पौधों की वृद्धि विशेषताओं को दर्ज किया गया। मात्रात्मक पोलीमरेज़ श्रृंखला प्रतिक्रिया (qPCR) के माध्यम से राइजोस्फीयर (rhizosphere) में कुल बैक्टीरिया (bacteria) बहुतायत और विशिष्ट बैक्टीरिया (bacteria) समूहों जैसे α-प्रोटियोबैक्टीरिया (*α-Proteobacteria*), β-प्रोटीयोबैक्टीरिया (β*-Proteobacteria*), एसिडोबैक्टीरिया (*Acidobacteria*), फर्मिक्यूट्स*(Firmicutes*), और एक्टिनोबैक्टीरिया (*Actinobacteria*) की प्रतिक्रियाओं में मात्रात्मक अंतर्दृष्टि प्राप्त की गई थी। इसके अतिरिक्त, नाइट्रोजन (nitrogen) और फास्फोरस (phosphorus) चक्रों में शामिल जीवाणु संघों को लक्षित करके इन जीवाणु समुदायों की कार्यात्मक क्षमता का आकलन किया गया था। इसमें qPCR के माध्यम से नाइट्रोजन (Nitrogen)चक्र जैसे नाइट्रोजन स्थिरीकरण (nitrogen fixation)-*nifH*, नाइट्रिफिकेशन (nitrification)-*amoA*, डिनाइट्रिफिकेशन (denitrification)-*narG*) और फॉस्फोरस (Phosphorus) चक्र [सॉल्वलाइजेशन (solubilization)-*pqqC*, खनिजकरण (mineralization)-*phoD*] में महत्वपूर्ण चरणों से जुड़े आनुवंशिक मार्करों की मात्रा निर्धारित करना शामिल था। एंटीबायोटिक (antibiotic) प्रतिरोध के प्रसार पर कृषि पद्धतियों के प्रभाव को समझने के लिए, एंटीबायोटिक (antibiotic) प्रतिरोध के लिए अलग-अलग मार्कर [एमिनोग्लाइकोसाइड

(aminoglycoside)-*aacA*, β-लैक्टम (β-lactam)-*blaTEM*, क्लोरैम्फेनिकॉल (chloramphenicol)-*chl1*, मैक्रोलाइड (macrolides)-*ermB*, सल्फोनामाइड्स (sulphonamides)-*sul1*, *sul2* और टेट्रासाइक्लिन (tetracycline)-*tetO*) और इंटीग्रोन्स (integrons)- *int1* और int2] को qPCR का उपयोग करके राइजोस्फीयर (rhizosphere) में लक्षित किया गया था। तीन साल के क्षेत्र प्रयोग के पूरा होने के बाद अरहर की फसल की कटाई के चरण में बैक्टीरिया (bacteria) की विविधता को 16S rRNA जीन (gene) आधारित एम्प्लिकॉन (amplicon) अनुक्रमण का उपयोग करके चित्रित किया गया था।

वर्तमान अध्ययन ने जीवांश खाद के नमूनों में बैक्टीरिया (bacteria) की प्रचुरता, पोषक तत्वों की सांद्रता और एंटीबायोटिक (antibiotic) प्रतिरोध के बीच संबंध को समझा, जो कि गहन कृषि पद्धतियों, एंटीबायोटिक (antibiotic) के उपयोग और जीवांश खाद में एंटीबायोटिक (antibiotic) प्रतिरोध की व्यापकता के बीच एक संभावित संबंध का सुझाव देता है। खेत प्रयोगों की स्थापना के लिए दो जीवांश खाद नमूनों को शॉर्टलिस्ट (shortlist) किया गया था, एक में एंटीबायोटिक (antibiotic)-प्रतिरोधी बैक्टीरिया (bacteria) की अधिक मात्रा थी और दूसरे में एआरबी (ARB) की कम बहुतायत थी। पीडब्ल्यूसीएस (PWCS) के साथ तीन-वर्षीय (2018-19, 2019-20 और 2020-21) क्षेत्र प्रयोगों के परिणामों ने कृषि प्रथाओं का एक विशिष्ट प्रभाव प्रदर्शित किया। पारंपरिक उपचारों ने पौधों की वृद्धि विशेषताओं, उपज मापदंडों और NPK अधिग्रहण के मामले में सबसे अच्छी प्रतिक्रिया दिखाई। हालाँकि, जैविक खेती प्रथाओं ने कुल बैक्टीरिया (bacteria) बहुतायत को बढ़ावा दिया और दोनों फसलों के लिए राइजोस्फेरिक बैक्टीरिया (rhizospheric bacteria) समुदायों की संरचना विविधता और कार्य क्षमता दोनों में सुधार किया। तीसरे फसल के अंत तक, जैविक उपचारों में जीवाणु विविधता लागू संशोधनों की प्रारंभिक जीवाणु समुदाय संरचना से काफी भिन्न हो गई थी। यह भी देखा गया कि जीवांश खाद संशोधनों ने जैविक और पारंपरिक उपचारों की तुलना में एंटीबायोटिक (antibiotic) प्रतिरोधी जीनस (genes) की प्रतियों की अधिक प्रचुरता प्रदर्शित की। अरहर गेहूं फसल प्रणाली के साथ तीन साल के क्षेत्र प्रयोग से कृषि प्रथाओं, जीवाणु समुदाय की गतिशीलता और फसल उत्पादकता के बीच एक जटिल बातचीत का पता चला। जीवांश खाद संशोधनों से जुड़े एंटीबायोटिक (antibiotic) प्रतिरोधी जीनों का उल्लेखनीय प्रसार एक चेतावनी के रूप में कार्य करता है, जो जिम्मेदार प्रबंधन प्रथाओं की मांग करता है। कृषि पद्धतियों, पौधों और मिट्टी के स्वास्थ्य, राइजोस्फेरिक (rhizospheric) जीवाणु

समुदायों और एंटीबायोटिक (antibiotic) प्रतिरोध के बीच जटिल परस्पर क्रिया, संपोषणीय और प्रभावी फसल प्रबंधन के लिए एक व्यापक दृष्टिकोण की आवश्यकता का सुझाव देती है।

LIST OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

Elements

AI	Aluminium
C	Carbon
Ca	Calcium
Fe	Carbon
K	Potassium
Mg	Magnesium
N	Nitrogen
Na	Sodium
P	Phosphorus
Pb	Lead
Se	Selenium
Zn	Zinc

Molecules and compounds

NH_3	Ammonia
CTAB	Cetyl Trimethyl Ammonium Bromide
K_2O	Potassium oxide
NO_2	Nitrite
NO_3	Nitrate
PVP	Polyvinylpyrrolidone
P_2O_5	Phosphorus pentoxide
PO_4^{3-}	Phosphate

Units

%	Percent
bp	Base Pairs
cm	Centimetre
g	Gram
mL	Millilitre
µL	Microliter
ºC	Degrees Celsius
µg	Microgram
µS	Micro Seimens
l	Litre
kg	Kilogram
m	Metre
mg	Microgram
min	Minutes
rpm	Revolutions Per Minute
mm	Millimetre
ng	Nanogram
ha	Hectare
s	Seconds
h	Hour

Important abbreviations and terminologies used in the study

α	Alpha
β	Beta
AMF	Arbuscular Mycorrhizal Fungus
AMP	Ampicillin
AMR	Antimicrobial Resistance
ANOVA	Analysis Of Variance
AP	Arunachal Pradesh
ARB	Antibiotic Resistant Bacteria
ARGs	Antibiotic Resistant Genes
AS	Assam
AZM	Azithromycin
BIOC	Biocompost
BUU	Biomass Utilization Unit
CDDEP	Center For Disease Dynamics, Economics & Policy
CHL	Chloramphenicol
CLI	Clindamycin
CLPP	Community-Level Physiological Profiling
CON	Conventional
DA&FW	Department Of Agriculture & Farmers Welfare
DAS	Days After Sowing
DGGE	Denaturing Gradient Gel Electrophoresis
DL	Delhi
DNA	Deoxyribonucleic Acid
DOX	Doxycycline
EC	Electrical Conductivity
FAME	Fatty Acid Methyl Ester
FAO	Food And Agriculture Organization
FiBL	Forschungs Institut Für Biologischen Landbau
FYM 1	Farmyard Manure with ARB
FYM 2	Farmyard Manure with minimal /less ARB
GY	Grain Yield
H	Plant Height
HGT	Horizontal Gene Transfer
HI	Harvest Index
HSD	Honestly Significant Difference
HT-qPCR	High Throughput qPCR
IARI	Indian Agricultural Research Institute
ICAR	Indian Council of Agricultural Research
IITD	Indian Institute of Technology Delhi
INM	Integrated Nutrient Management
MGEs	Mobile Genetic Elements
MIC	Minimal Inhibitory Concentration
MLSB	Macrolide-Lincosamide-Streptogramin B
NAL	Nalidixic Acid

NBRIP	National Botanical Research Institute's Phosphate Growth Medium
NCR	National Capital Region
NMDS	Nonmetric Multidimensional Scaling
NOR	Norfloxacin
NSC	National Seed Corporation
ORG	Organic
OTU	Operational Taxonomic Unit
OXY	Oxytetracycline
PC	Principal Component
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PEN	Penicillin
PLFA	Phospholipid Fatty Acid Analysis
PGPR	Plant Growth Promoting Rhizobacteria
PQQC	Pyrroloquinoline Quinone
PQQGDH	Pyrroloquinoline Protein Glucose Dehydrogenase
PWCS	Pigeonpea-Wheat Cropping System
QIIME	Quantitative Insights Into Microbial Ecology
qPCR	Quantitative Polymerase Chain Reaction
r	Pearson Correlation Coefficient
RDF	Recommended Dose of Fertilizer
RIF	Rifampicin
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
SDGs	Sustainable Development Goals
SOC	Soil Organic Carbon
SPSS	Statistical Package for Social Sciences
SSP	Single Superphosphate
STP	Streptomycin
SUL	Sulphadiazine
TET	Tetracycline
TOC	Total Organic Carbon
TPF	Triphenyl Formazan
TSS	Total Soluble Solids
UN	United Nations
USD	United States Dollars
USDA	United States Department of Agriculture
VFGs	Virulence Factor Genes