ENGINEERING OF THE RHIZOSPHERIC BACTERIAL COMMUNITY STRUCTURE AND FUNCTION BY AGRI-MANAGEMENT PRACTICES – A METAORGANISM BASED APPROACH

UPMA SINGH

DEPARTMENT OF BIOCHEMICAL ENGINEERING
AND BIOTECHNOLOGY

INDIAN INSTITUTE OF TECHNOLOGY DELHI
NOVEMBER 2021

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by

UPMA SINGH

DEPARTMENT OF BIOCHEMICAL ENGINEERING AND BIOTECHNOLOGY

Submitted

In fulfilment of the requirements of the degree of Doctor Of Philosophy

to the

INDIAN INSTITUTE OF TECHNOLOGY DELHI
NOVEMBER 2021

DEDICATED
TO
THE ALMIGHTY
&
MY FAMILY

CERTIFICATE

This is to certify that the thesis entitled "Engineering of the rhizospheric bacterial community structure and function by agri-management practices – A metaorganism based approach" being submitted by Ms Upma Singh is worthy of consideration for the award of the degree of the Doctor of Philosophy. The thesis has been prepared under my supervision and guidance in conformity with the rules and regulation of Indian Institute of Technology Delhi and is a record of the original bonafide research work. The results presented in this thesis have not been submitted in part or full to any other universities or institutes for the award of any degree or diploma.

Dr. Shilpi Sharma
Professor
Department of Biochemical Engineering and Biotechnology
Indian Institute of Technology Delhi
New Delhi 110016
India

ACKNOWLEDGEMENTS

At the very outset, I bow down to ***"GOD divine almighty"*** *for granting me constant strength, inspiration, wisdom and blessing to achieve the completion of my research task, together with several other milestones in life, which add meaning to it. He was always there to rescue me when I was emotionally troubled and weary.*

It gives me immense satisfaction and pleasure to express my deep sense of respect and gratitude to my supervisor, **Dr. Shilpi Sharma,** *for her constant support, advice and tenacity over the past five and half years. She took a chance on me in 2015, which I consider myself blessed, that enabled me to complete my Ph.D. It has been an immense privilege to work under such a genuine and ideal person, who leads by example to complete and excel in any task. Because of her constant support, I have been able to present this research contribution in the area of "Environmental Genomics". There would not be sufficient words to describe her continuous guidance and love in my overall development as a researcher and a good human being. Her constant dedication and enthusiasm for science, excellent guidance, time management, to provide the best of facilities, freedom to pursue ideas, deep intellect and perception, brainstorming scientific discussions and positive criticism have been responsible for the completion of this thesis work and would be an encouragement for my upcoming research journey. She was always there for us for any kind of discussion (personal or professional), which encouraged all her students to share their opinions. In moments of great turmoil, she has been a big support and saviour, and keeps on inspiring me to be a fighter. In my career, she is the one and only supervisor I have ever seen whose doors are always open for her students in any circumstances. I always say to Ma'am that if I can attain even a portion of her dedication and perseverance in me, it will be enough for me for my personal and professional growth; she has always pushed me to grow. Finally, I would like to say that my Ph.D. journey*

has been a pleasant, adventurous and exciting experience, all very thanks to my dearest supervisor.

I would like to extend my gratitude to the members of my research committee (SRC members) ***Dr. Preeti Srivastava, Dr. D. Sundar (DBEB)****, and* ***Dr. Dileep Kumar Singh*** *(Dept. of Zoology, University of Delhi) for their valuable suggestion, time and intense scientific discussion during my research task that helped me improve my thesis work.*

I am highly grateful to **Dr. Anil Kumar Choudhary** *(former affiliation: ICAR-IARI) for extending knowledge, support, and help with the field experiments.*

*I would like to thank the present (***Dr. D. Sundar***) and the previous (***Dr. Atul Narang***) heads of the Department for the support and kind help they have extended in all possible ways.*

I would like to acknowledge the fellowship received from **University Grants Commission (UGC)***, India, during my doctoral work. The funding for the project was received from* ***Department of Biotechnology****, Government of India, (grant no BT/PR27680/BCE/8/1434/2018). I also wish to acknowledge* **IIT Delhi** *for providing me opportunity and support for my Ph.D. work.*

The most enjoyable part of my Ph.D. has been meeting an outstanding and fantastic group of fellow researchers from all over India. The journey could not be pleasant and exciting without the unwavering help and support by my lab mates ***Jeny, Varsha, Gautam, Annapurna Ma'am, Richa, Vijal, Shivani, Shubham, Swati, Priyanka, Vasu, Nidhi, Sakshi Ma'am.*** *It was wonderful to have such generous and kind people as lab mates and very good friends. I especially thank* **Gautam, Jeny** *and* **Monika** *for always being with me during the early stages of my Ph.D. and providing me constant support, motivation, unconditional help, and mental strength. I want to express my gratitude towards* ***Annapurna Ma'am*** *and* **Richa** *for always being like sisters to me during the latter part of my thesis work and providing continuous support, motivation, and valuable suggestions. I want to thank my lovely lab mates* ***Vijal,***

Shubham, Shivani *and* ***Swati*** *for always being incredibly helpful and equivalently making me laugh and offering me constant support. I am also grateful for the help and support by lab technical staff Late* ***Shiv bhaiya****, who helped me during my field experiments. I will remember our group that withstands all the difficulties and challenges together as a unified entity, and is always there for each other.*

I would like to thank my friends ***Vidhi, Sonia*** *and* ***Shalakha*** *for the support and wonderful time that we have together. Vidhi and Shalakha were pillars during my Ph.D. journey and beyond. They provide me constant support, love, motivation and guidance. I would like to mention my friends* ***Gargi, Anvi, Anu, Kunal, Sakshi*** *and* ***Sonam*** *for the wonderful time that we have spent together.*

It would be incomplete if I forgot to thank my loving parents, ***my papa, maa, sister, brother*** *and* ***brother-in-law****.* ***Papa and maa****, their love, blessings, affection, confidence, encouragement and motivation has helped me to achieve my goals in personal and professional journey. I would like to dedicate my achievements to my dear parents who are always there for me, my elder sister* ***Aparna*** *and younger brother* ***Anurag*** *for always believing in me, and their great emotional support and unconditional help to come out of stressful situations. My brother-in-law,* ***Sanjay****, gave me the confidence and encouragement to start on and continue this doctoral journey. My deep heartfelt gratitude to my* ***parents-in-law*** *for their true love and support during my Ph.D. journey and for having full belief in my capabilities. I am very grateful to my sisters-in-law,* ***Sushila*** *and* ***Renu****, for their emotional support, motivation and booster doses at times.*

Last, but never ever least, my heartfelt and massive thanks to my amazingly supportive husband ***Devendra Bhamu*** *for his love, affection, care, motivation, and for putting up with my mood swings and grumpiness at times. I am very thankful for his patience and encouragement, which* *upheld me, especially in times when I spent almost all my time with my laptop. I thank him for being my rock-solid support through all turns and twists.*

Upma Singh

ABSTRACT

Agriculture, one of the major factors of the uprising Indian economy, is under immense pressure to fulfil the necessities of increasing population. Due to rapid industrialization and urbanization, deleterious impacts have been seen on the ecosystem. Moreover, there is a continuous threat to the ecosystem and environmental health with the practice of conventional agriculture (viz., application of agrochemicals, heavy tillage, use of high-yielding crop varieties, and irrigation). This calls for an urgent requirement for sustainable agricultural practices that can boost crop production and prevent environmental deterioration. The alternative agri-management practices focus on the application of green and animal manure, integrated pest management, reduced tillage, crop rotation and diversification. Such practices like conservation agriculture (centres on minimum or zero-tillage, crop rotation, and inclusion of dead organic soil covers, especially the inclusion of legume) and organic agriculture (concentrates on the omission of synthetic and chemical inputs) propagate the use of eco-friendly alternatives to improve crop production, and soil health and fertility along with the elimination of conventional farming methods.

The present work focused on the impacts of different agri-management practices (conventional agriculture, conservation agriculture and organic agriculture) on soil health, which drives rhizospheric soil bacterial communities both structurally (in terms of bacterial composition) and functionally (bacterial communities involved in important biogeochemical cycles such as N and P cycles). This, in turn, was correlated to the impact on plant growth and crop yield under the three agri-management practices. The study was performed to assess crop-specific differences (pigeonpea and soybean), seasonal variations [two consecutive years (2017 and 2018) for soybean and three consecutive years for pigeonpea (2017, 2018, and 2019)], plant stage-specific differences (flowering and harvest stages), and different crop management approaches (tillage, beds, fertilizers, wheat crop residues, and bio-fertilizers). Subsequently, for the mitigation of stress induced by agrochemicals such as biopesticides on resident soil bacterial communities, bacterial inoculants were applied as microbe-based approach. To meet the research goal, traditional microbiology tools together with advanced molecular microbiology techniques were employed. The responses of different agricultural practices on rhizospheric soil bacterial communities were studied quantitatively and qualitatively by implementing molecular methods such as, quantitative polymerase chain reaction (qPCR), denaturing gradient gel electrophoresis (DGGE), and amplicon sequencing of 16S rRNA gene (MiSeq Illumina sequencing). Abundances of specific bacterial groups dominant in the rhizosphere (*α-Proteobacteria, β-Proteobacteria, Acidobacteria, Firmicutes,* and *Actinobacteria*) were targeted by qPCR. Additionally, to assess the functional potential of bacterial communities, bacterial guilds involved in nitrogen and phosphorus cycles were targeted. This was achieved by quantification of genes involved in major steps of the nitrogen cycle [nitrogen fixation (*nifH*), nitrification (*amoA*) and denitrification *(narG* and *nirK*)], and phosphorus cycle [solubilization (*pqqC*), mineralization (*phoD*)] by qPCR. The effect of azadirachtin biopesticide in the presence of bacterial inoculants was assessed on total bacterial community and specific bacterial groups by employing DGGE and qPCR of 16S rRNA gene. High-performance liquid chromatography (HPLC) was used to estimate the residual concentration of azadirachtin in the soil.

It was concluded that the alternative agri-management practice, conservation agriculture, competed well with conventional agriculture in terms of improving plant growth attributes and crop yield at field level. The strong positive impact of conservation agriculture was visible on the resident rhizospheric bacterial community, and bacterial guilds involved in nitrogen and phosphorus cycles. It was also inferred that shifts in rhizospheric bacterial community structure and function were dependent on agri-management practices and crop systems, whereas, cropping seasons had minimal impact. Also, the strategically selected bacterial inoculants proved to be efficient in reducing residual concentration of azadirachtin biopesticide from soil, thereby improving plant growth, mitigating biopesticide stress and enriching rhizospheric soil bacterial community structure. The study enhanced the understanding that better manipulation of rhizospheric microbiome under different agri-management practices is not only beneficial for plant growth promotion but also helpful in mitigation of stress induced by conventional methods and in improvement of soil fertility.

सार

कृषि, भारतीय अर्थव्यवस्था के उत्थान के प्रमुख कारकों में से एक है और बढ़ती जनसंख्या की आवश्यकताओं को पूरा करने के लिए अत्यधिक दबाव में है। तेजी से औद्योगीकरण और शहरीकरण के कारण पारिस्थितिकी तंत्र पर हानिकारक प्रभाव देखा गया है। इसके अलावा, पारंपरिक कृषि (जैसे कृषि रसायनों का उपयोग, भारी जुताई, अधिक उपज देने वाली फसल की किस्मों का उपयोग और सिंचाई) के अभ्यास से पारिस्थितिकी तंत्र और पर्यावरणीय स्वास्थ्य के लिए एक निरंतर खतरा है। यह स्थायी कृषि पद्धतियों की तत्काल आवश्यकता का आह्वान करता है जो फसल उत्पादन को बढ़ावा दे सकती हैं और पर्यावरणीय गिरावट को रोक सकती हैं। वैकल्पिक कृषि-प्रबंधन प्रथाएं जैसे कि संरक्षण कृषि और जैविक कृषि, पारंपरिक कृषि विधियों के उन्मूलन के साथ-साथ फसल उत्पादन और मिट्टी के स्वास्थ्य और उर्वरता में सुधार के लिए पर्यावरण के अनुकूल विकल्पों के उपयोग का प्रचार करती हैं।

वर्तमान कार्य, मृदा स्वास्थ्य पर विभिन्न कृषि प्रबंधन प्रथाओं के प्रभावो पर केंद्रित है, जो राइजोस्फेरिक मृदा जीवाणु समुदायों को सरंचनात्मक (जीवाणु संरचना के संदर्भ में) और कार्यात्मक (एन और पी चक्र जैसे महत्वपूर्ण जैव-भू-रासायनिक चक्रों में शामिल जीवाणु समुदाय) रूप से प्रभावित करता है। यह बदले में, तीन कृषि-प्रबंधन प्रथाओं के तहत पौधों की वृद्धि और फसल की उपज पर प्रभाव से संबंधित था। अध्ययन फसल-विशिष्ट अंतर (अरहर और सोयाबीन), मौसमी विविधता [सोयाबीन के लिए लगातार दो साल (2017 और 2018) और अरहर के लिए लगातार तीन साल (2017, 2018, और 2019)], पौधे के चरण-विशिष्ट अंतर (फूल और कटाई के चरण) और विभिन्न फसल प्रबंधन दृष्टिकोण (जुताई, क्यारी, गेहूं की फसल के अवशेष और जैव-उर्वरक) का आकलन करने के लिए किया गया था। निवासी मृदा जीवाणु समुदायों पर जैव कीटनाशकों जैसे कृषि रसायनों से प्रेरित तनाव के शमन के लिए सूक्ष्म जीव-आधारित दृष्टिकोण के तहत जीवाणु इनोकुलेंट्स लागू किए गए थे।

अनुसंधान लक्ष्य को पूरा करने के लिए आणविक तकनीकों के साथ पारंपरिक सूक्ष्म जीव विज्ञान उपकरणों को नियोजित किया गया था। राइजोस्फेरिक मृदा जीवाणु समुदायों पर विभिन्न कृषि पद्धतियों की प्रतिक्रियाओं का मात्रात्मक और गुणात्मक रूप से आणविक विधियों जैसे मात्रात्मक पोलीमरेज़ चेन रिएक्शन (qPCR), डिनाट्यूरिंग ग्रेडिएंट जेल वैद्युतकणसंचलन (DGGE) और 16S rRNA जीन की एम्प्लिकॉन अनुक्रमण (MiSeq Illumina) को लागू करके अध्ययन किया गया था। राइजोस्फेर में प्रमुख विशिष्ट जीवाणु समूहों (*α-प्रोटीओबैक्टीरिया, β-प्रोटीओबैक्टीरिया, एसिडोबैक्टीरिया, फर्मिक्यूट्स* और *एक्टिनोबैक्टीरिया*) की प्रचुरता को qPCR द्वारा लक्षित किया गया था। इसके अतिरिक्त, जीवाणु समुदायों की कार्यात्मक क्षमता का आंकलन करने के लिए नाइट्रोजन और फास्फोरस चक्रों में शामिल जीवाणु संघों को लक्षित किया गया था। यह qPCR के उपयोग करके नाइट्रोजन चक्र [नाइट्रोजन निर्धारण (*nifH*), नाइट्रिफिकेशन (*amoA*) और डिनाइट्रिफिकेशन (*narG* और *nirK*)] और फास्फोरस चक्र [घुलनशीलता (*pqqC*), खनिजकरण (*phoD*)] के प्रमुख चरणों में शामिल जीनों की की मात्राओ का आंकलन करके किया गया था । 16S rRNA जीन के DGGE और qPCR को नियोजित करके बैक्टीरियल इनोकुलेंट्स की उपस्थिति में अज़ादिराक्टिन बायोपेस्टीसाइड के प्रभावों का मूल्यांकन कुल जीवाणु समुदाय और विशिष्ट जीवाणु समूहों पर किया गया था। उच्च प्रदर्शन तरल क्रोमैटोग्राफी (एचपीएलसी) का उपयोग मिट्टी में अज़ादिराक्टिन की अवशिष्ट सांद्रता का अनुमान लगाने के लिए किया गया था।

यह निष्कर्ष निकाला गया कि वैकल्पिक कृषि-प्रबंधन अभ्यास यानी संरक्षण कृषि पौधे की वृद्धि विशेषताओं और क्षेत्र स्तर पर फसल की उपज में सुधार के मामले में पारंपरिक कृषि के साथ अच्छी तरह से प्रतिस्पर्धा करती है। संरक्षण कृषि का मजबूत सकारात्मक प्रभाव निवासी राइजोस्फेरिक जीवाणु समुदाय और नाइट्रोजन और फास्फोरस चक्रों में शामिल जीवाणु गिल्ड पर दिखाई दे रहा था। यह भी अनुमान लगाया गया था कि राइजोस्फेरिक जीवाणु समुदाय संरचना और कार्य में बदलाव कृषि-प्रबंधन प्रथाओं और फसल प्रणालियों पर निर्भर थे जबकि फसल के मौसम का न्यूनतम प्रभाव था। इसके अलावा

रणनीतिक रूप से चुने गए बैक्टीरियल इनोकुलेंट्स मिट्टी से अजादिराच्टिन बायोपेस्टीसाइड की अवशिष्ट सांद्रता को कम करने में कुशल साबित हुए जिससे पौधों की वृद्धि में सुधार हुआ, बायोपेस्टीसाइड तनाव में कमी हुई और राइजोस्फेरिक मिट्टी जीवाणु समुदाय संरचना में समृद्धि हुई । अध्ययन ने इस समझ को बढ़ाया कि विभिन्न कृषि-प्रबंधन प्रथाओं के तहत राइजोस्फेरिक माइक्रोबायोम का बेहतर बदलाव न केवल पौधों की वृद्धि को बढ़ावा देने के लिए फायदेमंद है बल्कि पारंपरिक तरीकों से प्रेरित तनाव को कम करने और मिट्टी की उर्वरता में सुधार करने में भी सहायक है।

LIST OF CONTENTS

ACKNOWLEDGEMENT i-iv
ABSTRACT v-x
LIST OF FIGURES xvi-xxv
LIST OF TABLES xxvi-xxvii
ABBREVIATIONS xxviii-xxx

1. INTRODUCTION AND OBJECTIVES 1-10
1.1. Introduction 2
1.1.1. Sustainable agricultural practices 3
1.1.2. Metaorganism 4
1.1.3. Rhizosphere as a microbial hotspot 4
1.1.4. Microbe-mediated soil functions: nitrogen and phosphorus cycles 5
1.1.5. Non-target effects of biopesticides 6
1.1.6. Crop systems under study 7
1.2. Objectives 7

2. REVIEW OF LITERATURE 11-40
2.1. Agriculture: Current challenges 11
2.2. Rhizospheric microbiome as an indicator of disturbances 11
2.3. Impacts of agricultural practices on rhizospheric microbiome 14
2.3.1. Effect of different crop management approaches 14
2.3.2. Effect of conventional agriculture 16
2.3.3. Effect of alternative agri-management practices 17
  2.3.3.1.Conservation agriculture 18
  2.3.3.2.Organic agriculture 19
2.4. Effect of agricultural practices on microbe-mediated soil functions 26
2.4.1. Nitrogen cycle 26
2.4.2. Phosphorus cycle 27
2.5. Impact of biopesticides on soil microbiome 28
2.5.1. Application of bioinoculants for mitigation of non-target impacts of agrochemicals 30
2.6. Plant model systems used in this study 38

3. MATERIALS AND METHODS 41-69

3.1. Field experiments under three farming practices 45

3.1.1. Model crops 45

3.1.2. Experimental site 45

3.1.3. Treatments 45

3.1.3.1. Tillage practices 46

3.1.3.2. Crop residue retention 46

3.1.3.3. Crop establishment methods (crop bedding patterns) 46

3.1.3.4. Nutrient application 47

3.1.4. Period of growth of model crops 49

3.1.5. Sowing of seed and planting geometry 49

3.1.6. Measurement of plant attributes 49

3.1.6.1. Plant height 50

3.1.6.2. Dry matter accumulation 50

3.1.6.3. Root parameters 50

3.1.6.4. Seed yield and biological yield 50

3.1.7. Soil sampling 50

3.1.8. Nutrient analysis 51

3.2. Procurement of bacterial strains for the mitigation of non-target impacts of azadirachtin 51

3.2.1. Model crop 51

3.2.2. Azadirachtin treatment 51

3.2.3. Isolation of bacterial strains 51

3.2.3.1. Bacterial isolates procured from culture collections 51

3.2.3.2. Enrichment, isolation and screening of pesticide-tolerant bacterial strain 52

3.2.4. Plant growth promoting (PGP) properties of isolates 53

3.2.4.1. NH_3 production 53

3.2.4.2. Phosphate solubilization 53

3.2.4.3. HCN production 54

3.2.4.4. IAA production 54

3.2.4.5. Nitrogen fixation 54

3.2.4.6. Nitrification 55

3.2.5. Pathogenicity test of selected bacterial isolates 55

3.2.5.1.Gelatinase test 55
3.2.5.2.Coagulase test 55
3.2.6. Assessment of bacterial isolates for tolerance of azadirachtin 55
3.2.7. Extraction of genomic DNA from bacteria 56
3.2.8. Ribotyping of bacterial strains 57
3.2.9. HPLC analysis to estimate residual concentration of azadirachtin in soil 57
3.2.10. Plant growth experiments 57
3.2.10.1.Tube assay 57
3.2.10.2.Pot study 59
3.2.10.3. Collection of plant and soil samples 59
3.3. DNA extraction from soil samples **60**
3.4. Denaturing gradient gel electrophoresis (DGGE) **61**
3.5. Analysis of DGGE gels **62**
3.6. Real-time PCR (qPCR) assay **62**
3.6.1. Spiking to detect inhibition, if any 62
3.6.2. Construction of standards for qPCR 63
3.6.3. qPCR analysis 64
3.7. Amplicon sequencing **67**
3.7.1. Extraction of DNA from soil, and 16S rRNA amplicon sequencing 67
3.7.2. Bioinformatic analysis 67
3.8. Statistical analysis **68**

4. RESULTS **70-155**
4.1.Cumulative effects of agricultural modules on plant attributes and dynamics of rhizospheric bacterial community **74**
4.1.1. Effect of agri-management practices on plant growth attributes 74
4.1.2. Response of the rhizospheric bacterial community (structural diversity) to different agri-management practices 78
4.1.2.1.Shifts in resident rhizospheric bacterial community 78
4.1.2.2.Abundance of specific bacterial groups 91
α-Proteobacteria
β-Proteobacteria
Acidobacteria
Firmicutes

Actinobacteria

4.1.2.3. Composition of rhizospheric bacterial community 102

4.1.3. Impact of agri-management practices on the functional potential of rhizospheric bacterial community 112

4.1.3.1. Bacterial guilds involved in nitrogen cycle 112

Nitrogen fixers

Nitrifiers

Denitrifiers

4.1.3.2. Bacterial guilds involved in phosphorus cycle 119

Phosphate solubilization

Phosphate mineralization

4.2. Effect of individual variables on plant growth attributes and rhizospheric bacterial diversity **122**

4.2.1. Plant development stage 122

4.2.2. Seasonal variability 129

4.2.3. Crop-specific differences 132

4.2.4. Crop management approaches 135

4.2.4.1. Tillage 135

4.2.4.2. Crop residue retention 136

4.2.4.3. Crop establishment method 138

4.2.4.4. Nutrient management 140

4.3. Microbe-based strategy to mitigate impact of azadirachtin applications on growth and rhizospheric bacterial community in mungbean **142**

4.3.1. Screening of bacterial strains for utilisation of azadirachtin 142

4.3.2. Quantification of azadirachtin in culture broths 142

4.3.3. Phylogenetic affiliation of selected potent isolates 145

4.3.4. Effect of bacterial strains on plant growth parameters in the presence of azadirachtin 145

4.3.5. Impact of amendment with bacterial strains on the residual concentration of azadirachtin 146

4.3.6. Response of the total rhizospheric bacterial community to amendment with bacterial isolates 148

4.3.6.1. Fingerprinting of bacterial community to estimate shifts 149

4.3.6.2. Abundance of total bacteria and specific groups in the rhizosphere 153

5. DISCUSSION **156-187**

5.1. Impacts of agri-management practices on plant attributes and rhizospheric bacterial community dynamics **160**

5.1.1. Plant attributes 160

5.1.2. Structural diversity of bacterial community in the rhizosphere 162

5.1.2.1. Diversity and richness of rhizospheric bacterial communities 162

5.1.2.2. Abundance of dominant bacterial groups in the rhizosphere 165

5.1.2.3. Composition of rhizospheric bacterial communities 168

5.1.3. Functional potential of rhizospheric bacterial communities 171

5.2. Influence of individual variables on resident structural and functional bacterial guilds **174**

5.2.1. Plant developmental stages and seasonal variability 174

5.2.2. Crop-specific variance 175

5.2.3. Variation due to crop management approaches 176

5.3. Microbe-based strategy to mitigate impacts of azadirachtin **180**

5.3.1. Impact of plant growth promoting bacterial strains on plant attributes in the presence of azadirachtin 181

5.3.2. Bacteria-mediated mitigation of non-target effects of azadirachtin in rhizospheric soil 182

5.3.3. Effect on total rhizospheric bacterial community 183

6. SUMMARY AND CONCLUSIONS **188-195**

Bibliography **196-226**

Appendix **227-231**

CURRICULUM VITAE **232-236**

LIST OF FIGURES

Figure no.	Legend	Page no.
3.1	Experimental layout for assessment of the impact of three farming practices on plant attributes and rhizospheric bacterial communities of two legumes (Objective 1).	42
3.2	Experimental layout for application of the microbe-based method for mitigation of impact of azadirachtin in model crop mungbean (*Vigna radiata*) (Objective 2).	43
3.3	Field experiment with pigeonpea at ICAR-IARI , New Delhi.	47
3.4	Setup of plant growth experiment in tubes with mungbean and selected isolates.	58
3.5	Setup of pot experiment with mungbean at IIT Delhi nursery, (a) after sowing, (b) 30 days after sowing, and (c) 60 days after sowing.	59
3.6	Agarose gel electrophoresis of DNA extracted from soil samples. Ladder : 1 Kbp ladder, lanes S1-S5: DNA extracted from rhizospheric soil samples from pigeonpea field.	61
4.1	Representative DGGE fingerprints from 16S rRNA gene amplicons of replicate rhizospheric soil samples of soybean crop from different agri-management practices (a). Lane L: ladder, S1, S2, S3: replicates of CT, CA module and OA practice, respectively. Cluster analysis obtained from DGGE profiles (b); scale shows % similarity amongst soil samples from different modules. The cophenetic correlation values with 1% tolerance have been presented at branch nodes of the dendrogram.	79
4.2	Rhizospheric bacterial community profiles of pigeonpea generated by DGGE using 16S rRNA gene at flowering (a), and harvest (c) stages of 2017 cropping season. Lane Bulk: bulk soil, lane L: ladder, lanes ICM1-ICM4: CT modules, lanes ICM5-ICM8: CA modules and lane ICM9: OA module. Cluster analysis of DGGE profiles from flowering (b), and harvest (d) stages; scale shows % similarity amongst soil samples from different ICM modules and bulk soil. The cophenetic correlation values with 1% tolerance have been presented at branch nodes of the dendrogram.	80
4.3	Rhizospheric bacterial community profiles of pigeonpea generated by DGGE using 16S rRNA gene at flowering (a), and harvest (c) stages of 2018 cropping season. Lane Bulk: bulk soil, lane L: ladder, lanes ICM1-ICM4: CT modules, lanes ICM5-ICM8: CA modules and lane ICM9: OA module. Cluster analysis obtained from DGGE profile at flowering (b), and harvest (d) stages; scale	81

shows % similarity amongst soil samples from different ICM modules and bulk soil. The cophenetic correlation values with 1% tolerance have been presented at branch nodes of the dendrogram.

4.4 Rhizospheric bacterial community profiles of pigeonpea generated by DGGE using 16S rRNA gene at flowering (a), and harvest (c) stages of 2019 cropping season. Lane L: ladder, lane Bulk: bulk soil, lanes ICM1-ICM4: CT modules, lanes ICM5-ICM8: CA modules and lane ICM9: OA module. Cluster analysis obtained from DGGE profile at flowering (b), and harvest (d) stages; scale shows % similarity amongst soil samples from different ICM modules and bulk soil. The cophenetic correlation values with 1% tolerance have been presented at branch nodes of the dendrogram. 83

4.5 Rhizospheric bacterial community profiles of soybean generated by DGGE using 16S rRNA gene at flowering (a), and harvest (c) stages of 2017 cropping season. Lane L: ladder, lane Bulk: bulk soil, lanes ICM1-ICM4: CT modules, lanes ICM5-ICM8: CA modules and lane ICM9: OA module. Cluster analysis obtained from DGGE profile at flowering (b), and harvest (d) stages; scale shows % similarity amongst soil samples from different ICM modules and bulk soil. The cophenetic correlation values with 1% tolerance have been presented at branch nodes of the dendrogram. 86

4.6 Rhizospheric bacterial community profiles of soybean generated by DGGE using 16S rRNA gene at flowering (a), and harvest (c) stages of 2018 cropping season. Lane Bulk: bulk soil, lane L: ladder, lane ICM9: OA module, lanes ICM5-ICM8: CA modules and lanes ICM1-ICM4: CT modules. Cluster analysis obtained from DGGE profile at flowering (b), and harvest (d) stages; scale shows % similarity amongst soil samples from different ICM modules and bulk soil. The cophenetic correlation values with 1% tolerance have been presented at branch nodes of the dendrogram. 88

4.7 Correlation between treatments as represented by PCA using Margalef Species richness (a), and Shannon-Wiener (b) indices obtained from the DGGE profiles generated from the pigeonpea rhizospheric samples at flowering (F), and harvest (H) stages of consecutive cropping seasons (2017, 2018, and 2019). The x- and y-axis indicate PC1 and PC2, respectively. The percentages in the parentheses indicate to the proportion variation due to each ordination axis. Red arrows represent the correlation between plant stages and PCA axes. ICM1–ICM4: conventional agriculture, ICM5-ICM8: conservation agriculture, ICM9: organic agriculture, and Bulk: bulk soil. 90

4.8 Correlation between treatments as represented by PCA using Margalef Species richness (a), and Shannon-Wiener (b) indices obtained from the DGGE profiles generated from the soybean rhizospheric samples at flowering (F), and harvest (H) stages of consecutive cropping seasons (2017 and 2018). The x- and y-axis 91 indicate PC1 and PC2, respectively. The percentages in the parentheses indicate to the proportion variation due to each ordination axis. Red arrows represent the correlation between plant stages and PCA axes. ICM1–ICM4: conventional agriculture, ICM5-ICM8: conservation agriculture, ICM9: organic agriculture, and Bulk: bulk soil.

4.9 Effect of agri-management practices on the abundance of *α-Proteobacteria* in the rhizosphere of pigeonpea for three consecutive seasons [2017 (a), 2018 (b), and 2019 (c)] and soybean during two consecutive seasons [2017 (d), and 2018 (e)] at flowering and harvest stages. ICM1–ICM4: conventional agriculture, ICM5-ICM8: conservation agriculture, ICM9: organic agriculture, and BULK: bulk soil. Error bars represented standard deviations (*n*=3). The lowercase letters (a-e) indicate a significant difference (P ≤ 0.05) between the treatments for the abundance of *α-Proteobacteria* for a particular sampling point in each cropping season. For abbreviations, refer to the legend for Figure 4.8. 93

4.10 Effect of agri-management practices on the abundance of *β-Proteobacteria* in the rhizosphere of pigeonpea for three consecutive seasons [2017 (a), 2018 (b), and 2019 (c)] and soybean during two consecutive seasons [2017 (d), and 2018 (e)] at flowering and harvest stages. Error bars represent standard deviations (*n*=3). The lowercase letters (a-f) indicate a significant difference (P ≤ 0.05) between the treatments for the abundance of *β-Proteobacteria* for a particular sampling point in each cropping season. For abbreviations, refer to the legend for Figure 4.8. 94

4.11 Effect of agri-management practices on the abundance of *Acidobacteria* in the rhizosphere of pigeonpea for three consecutive seasons [2017 (a), 2018 (b), and 2019 (c)] and soybean during two consecutive seasons [2017 (d), and 2018 (e)] at flowering and harvest stages. Error bars represent standard deviations (*n*=3). The lowercase letters (a-g) indicate a significant difference (P ≤ 0.05) between the treatments for the abundance of *Acidobacteria* for a particular sampling point in each cropping season. For abbreviations, refer to the legend for Figure 4.8. 96

4.12 Effect of agri-management practices on the abundance of *Firmicutes* in the rhizosphere of pigeonpea for three consecutive seasons [2017 (a), 2018 (b), and 2019 (c)] and soybean during the two consecutive seasons [2017 (d), and 2018 (e)] at flowering and harvest stages. Error bars represent standard deviations (*n*=3). The lowercase letters (a-f) indicate a significant difference (P ≤ 0.05) between the treatments for the abundance of *Firmicutes* for a particular sampling point in each cropping season. For abbreviations, refer to the legend for Figure 4.8. 97

4.13 Effect of agri-management practices on the abundance of *Actinobacteria* in the rhizosphere of pigeonpea for three consecutive seasons [2017 (a), 2018 (b), and 2019 (c)] and soybean during two consecutive seasons [2017 (d), and 2018 (e)] at flowering and harvest stage. Error bars represent standard deviations (*n*=3). The lowercase letters (a-g) indicate a significant difference ($P \leq 0.05$) between the treatments for the abundance of *Actinobacteria* for a particular sampling point in each cropping season. For abbreviations, refer to the legend for Figure 4.8. 99

4.14 Rarefaction curve of the normalized OTUs at similarity of 97%. The treatments are CT: conventional agriculture, CA: conservation agriculture and OA: organic agriculture. C1, C2, C3: replicates of CT, CA module, and OA practice, respectively. 103

4.15 Effect of agri-management practices on the relative abundance of different bacterial phyla in the pigeonpea rhizosphere. The relative abundances of bacterial phyla (a), major phyla (b), and minor phyla (c). ICM3: conventional agriculture, ICM7: conservation agriculture, and ICM9: organic agriculture. Error bars depict standard deviations (*n*=3). 104

4.16 Principal component analysis biplots for analysing the effect of agri-management practices on bacterial community composition at different taxonomic levels in the pigeonpea rhizosphere, phyla (a), classes (b), orders (c), families (d), and genera (e). The x- and y-axis indicate component 1 and component 2, respectively. Red arrows represent the correlation between different bacterial groups and PC axes. ICM3: conventional agriculture, ICM7: conservation agriculture, and ICM9: organic agriculture. 107

4.17 Bacterial community diversity among different agri-management practices at phyla level (a), genera level (b). α-diversity was measured by Shannon and Simpson indices. ICM3: conventional agriculture, ICM7: conservation agriculture, and ICM9: organic agriculture. 108

4.18 Heatmaps of the relative abundances of the rhizospheric bacterial community at different taxonomic levels, phyla (a), class (b), order (c), family (d), and genera (e) under different agri-management practices. The colour scale shows the relative abundance values, Colour key: yellow (low abundance), red (medium abundance), and green (high abundance). ICM3: conventional agriculture, ICM7: conservation agriculture, and ICM9: organic agriculture. 111

4.19 Effect of agri-management practices on the abundance of *nifH* gene copies in the rhizosphere of pigeonpea during three consecutive seasons [2017 (a), 2018 (b), and 2019 (c)] and soybean during two consecutive seasons [2017 (d), and 2018 (e)] at flowering and harvest stages. ICM1–ICM4: conventional 114 agriculture, ICM5-ICM8: conservation agriculture, ICM9: organic agriculture, and Bulk: bulk soil. Error bars represent standard deviations (n=3). The lowercase letters (a-f) indicate a significant difference ($P \leq 0.05$) between the treatments for the abundance of nitrifiers for a particular sampling point in each cropping season. For abbreviations, refer to the legend for Figure 4.18.

4.20 Effect of agri-management practices on the abundance of *amoA* gene copies in the rhizosphere of pigeonpea during three consecutive seasons [2017 (a), 2018 (b), and 2019 (c)] and soybean during two consecutive seasons [2017 (d), and 2018 (e)] at flowering and harvest stages. Error bars represent standard deviations (n=3). The lowercase letters (a-f) indicate a significant difference ($P \leq 0.05$) between the treatments for the abundance of nitrifiers for a particular sampling point in each cropping season. For abbreviations, refer to the legend for Figure 4.18. 115

4.21 Effect of agri-management practices on the abundance of *narG* gene copies in the rhizosphere of pigeonpea during three consecutive seasons [2017 (a), 2018 (b), and 2019 (c)] and soybean during two consecutive seasons [2017 (d), and 2018 (e)] at flowering and harvest stages. Error bars represented standard deviations (n=3). The lowercase letters (a-f) indicate a significant difference ($P \leq 0.05$) between the treatments for the abundance of denitrifiers for a particular sampling point in each cropping season. For abbreviations, refer to the legend for figure 4.18. 117

4.22 Effect of agri-management practices on the abundance of *nirK* gene copies in the rhizosphere of pigeonpea during three consecutive seasons [2017 (a), 2018 (b), and 2019 (c)] and soybean during two consecutive seasons [2017 (d), and 2018 (e)] at flowering and harvest stages. Error bars represented standard deviations (n=3). The lowercase letters (a-f) indicate a significant difference ($P \leq 0.05$) between the treatments for the abundance of denitrifiers for a particular sampling point in each cropping season. For abbreviations, refer to the legend for Figure 4.18. 118

4.23 Effect of agri-management practices on the abundance of *pqqC* gene copies in the rhizosphere of pigeonpea during three consecutive seasons [2017 (a), 2018 (b), and 2019 (c)] and soybean during two consecutive seasons [2017 (d), and 2018 (e)] at flowering and harvest stages. Error bars represented standard deviations (n=3). The lowercase letters (a-g) indicate a significant difference ($P \leq 0.05$) between the treatments for the abundance of P solubilizing bacterial community for a particular sampling point in each cropping season. For abbreviations, refer to the legend for Figure 4.18. 120

4.24 Effect of agri-management practices on the abundance of *phoD* gene copies in the rhizosphere of pigeonpea during three consecutive seasons [2017 (a), 2018 122 (b), and 2019 (c)] and soybean during two consecutive seasons [2017 (d), and 2018 (e)] at flowering and harvest stages. Error bars represented standard deviations (*n*=3). The lowercase letters (a-f) indicate a significant difference (P ≤ 0.05) between the treatments for the abundance of P mineralizing bacterial community for a particular sampling point in each cropping season. For abbreviations, refer to the legend for Figure 4.18.

4.25 PCA for the impact of agri-management practices (CA, CT and OA) on the plant parameters of pigeonpea crop at flowering (a), and harvest (b) stages and soybean crop at flowering (c), and harvest (d) stages of consecutive cropping seasons (2017, 2018, and 2019). The x- and y-axis indicate PC1 and PC2, respectively. The percentages in the parentheses indicate to the proportion variation due to each ordination axis. Red arrows represent the correlation between plant parameters and PCA axes. CT: conventional agriculture, CA: conservation agriculture, and OA: organic agriculture; F: flowering stage, and H: harvest stage. 125

4.26 PCA for the impact of agri-management practices (CA, CT and OA) on the structure of bacterial community in the rhizosphere of pigeonpea at flowering (a), and harvest (b) stages, and soybean at flowering (c), and harvest (d) stages of consecutive cropping seasons (2017, 2018, and 2019). Beta: *β-Proteobacteria*, Alpha: *α-Proteobacteria*, Firmi: *Firmicutes*, Acido: *Acidobacteria*, Actino: *Actinobacteria.* The x- and y-axis indicate PC1 and PC2, respectively. The percentages in the parentheses indicate to the proportion variation due to each ordination axis. Red arrows represent the correlation between bacterial groups and PCA axes. CT: conventional agriculture, CA: conservation and OA: organic agriculture. F: flowering stage, and H: harvest stage. 127

4.27 PCA biplots on the impact of agri-management practices (CA, CT and OA) on the functional markers of bacterial communities involved in nitrogen cycle (*nifH, amoA, narG*, and *nirK*) and phosphorus cycle (*phoD* and *pqqC*) in the rhizospheres of pigeonpea at flowering (a), and harvest (b) stages, and soybean at flowering (c), and harvest (d) stages of consecutive cropping seasons (2017, 2018 and 2019). The x- and y-axis indicate PC1 and PC2, respectively. The percentages in the parentheses indicate to the proportion variation due to each ordination axis. Red arrows represent the correlation between gene markers of bacterial guilds involved in N and P cycles and PCA axes. CT: conventional agriculture, CA: conservation agriculture, and OA: organic agriculture. F: flowering stage, and H: harvest stage. 129

4.28 Box-Whisker plots illustrating the impact of agri-management practices (CT, CA, and OA) on specific bacterial groups at three cropping seasons [2017 (a), 2018 (b), and 2019 (c)] and functional markers of bacterial guilds involved in N 131 and P cycles across three cropping seasons [2017(d), 2018 (e), and 2019 (f)] in the pigeonpea rhizosphere. Boxes represent the interquartile ranges (Q1-Q3), thick line represents median, and lines extending from the boxes (whiskers) represent non-outlier range.

4.29 Box-Whisker plots illustrating the impact of agri-management practices (CT, CA, and OA) on specific bacterial groups at two cropping seasons [2017 (a), and 2018 (b)] and functional markers of bacterial guilds involved in N and P cycles at two cropping seasons [2017(c), and 2018 (d)] in the soybean rhizosphere. Boxes represent the interquartile ranges (Q1-Q3), thick line represents median, and lines extending from the boxes (whiskers) represent non-outlier range. 131

4.30 NMDS illustrating differences in plant growth attributes and bacterial community structure and functional potential of rhizospheric bacterial communities of pigeonpea and soybean crops according to Bray-Curtis distance. Pigeonpea in planting seasons 2018 (a), and 2019 (b); and soybean in planting seasons 2017 (c), and 2018 (d). Lines in the NMDS plot demonstrated the distance of each module to its group (agri-management practice) centroids in the multivariate space. The x- and y-axis indicate NMDS1 and NMDS2, respectively. Different color arrows represent the correlation between different variables and NMDS axes. Blue dots and ellipse: CT, green dots and ellipse: CA, and orange dot: OA. Light red arrow: plant growth attributes, Brown arrow: dominant bacterial phyla, and yellow arrow: functional genes of guilds involved in N cycle (*nifH, amoA, narG* and *nirK*) and P cycle (*phoD* and *pqqC*). Beta: *β-Proteobacteria*, Alpha: *α-Proteobacteria*, Firmi: *Firmicutes*, Acido: *Acidobacteria*, Actino: *Actinobacteria.* 134

4.31 Box-Whisker plots illustrating the impact of tillage on specific bacterial groups at two cropping seasons [2017 (a), and 2018 (b)] and functional markers of bacterial communities involved in N and P cycles at two cropping seasons [2017(c), and 2018 (d)] in the soybean rhizosphere. ZT: zero tillage, CtT: conventional tillage. Boxes represent the interquartile range (Q1-Q3), thick line represents median, and lines extending from the boxes (whiskers) represent non-outliers range. 135

4.32 Box-Whisker plots illustrating the impact of tillage on specific bacterial groups at three cropping seasons [2017 (a), 2018 (b), and 2019 (c)] and functional markers of bacterial communities involved in N and P cycles at three cropping seasons [2017(d), 2018 (e), and 2019 (f)] in the pigeonpea rhizosphere. ZT: zero tillage, CtT: conventional tillage. Boxes represent the interquartile ranges (Q1-Q3), thick line represents median, and lines extending from the boxes (whiskers) represent non-outlier range. 136

4.33 Box-Whisker plots illustrating the impact of crop residue retention (CRR) on specific bacterial groups at two cropping seasons [2017 (a), and 2018 (b)] and functional markers of bacterial guilds involved in N and P cycles at two cropping seasons [2017(c), and 2018 (d)] in the soybean rhizosphere. Boxes represent the interquartile ranges (Q1-Q3), thick line represents median, and lines extending from the boxes (whiskers) represent non-outlier range. 137

4.34 Box-Whisker plots illustrating the impact of crop residue retention (CRR) on specific bacterial groups at three cropping season [2017 (a), 2018 (b), and 2019 (c)] and functional markers of bacterial guilds involved in N and P cycle at three cropping seasons [2017(d), 2018 (e), and 2019 (f)] in the pigeonpea rhizosphere. Boxes represent the interquartile ranges (Q1- Q3), thick line represents median, and lines extending from the boxes (whiskers) represent non-outlier range. 138

4.35 Box-Whisker plots illustrating the impact of crop bedding patterns on specific bacterial groups at two cropping seasons [2017 (a), and 2018 (b)] and functional markers of bacterial guilds involved in N and P cycle at two cropping seasons [2017(c), and 2018 (d)] in the soybean. FB: flat-bed, RB: raised-bed and PRB: permanent raised-bed. Boxes represent the interquartile ranges (Q1-Q3), thick line represents median, and lines extending from the boxes (whiskers) represent non-outlier range. 139

4.36 Box-Whisker plots illustrating the impact of crop bedding pattern on specific bacterial groups at three cropping seasons [2017 (a), 2018 (b), and 2019 (c)] and functional markers of bacterial guilds involved in N and P cycle at three cropping seasons [2017(d), 2018 (e), and 2019 (f)] in the pigeonpea rhizosphere. FB: flat-bed, RB: raised-bed and PRB: permanent raised-bed. Boxes represent the interquartile ranges (Q1-Q3), thick line represents median, and lines extending from the boxes (whiskers) represent non-outlier range. 140

4.37 Box-Whisker plots illustrating the impact of nutrient management on specific bacterial groups at two cropping seasons [2017 (a), and 2018 (b)] and functional markers of bacterial guilds involved in N and P cycle at two cropping seasons [2017(c), and 2018 (d)] in the soybean rhizosphere. RDF: 100% recommended dose of fertilizers, RDF/NPK*mf*: 75% RDF and NPK microbial formulation, and AM*f*/NPK*mf*: AM fungi and NPK microbial formulation. Boxes represent the interquartile ranges (Q1-Q3), thick line represents median, and lines extending from the boxes (whiskers) represent non-outlier range. 141

4.38 Box-Whisker plots illustrating the impact of nutrient management on specific bacterial groups at three cropping seasons [2017 (a), 2018 (b), and 2019 (c)] and functional markers of bacterial guilds involved in N and P cycle at three cropping seasons [2017(d), 2018 (e), and 2019 (f)] in the pigeonpea rhizosphere. RDF: 100% recommended dose of fertilizers, RDF/NPK*mf*: 75% RDF and NPK 141 microbial formulation, and AM*f*/NPK*mf*: AM fungi and NPK microbial formulation. Boxes represent the interquartile ranges (Q1-Q3), thick line represents median, and lines extending from the boxes (whiskers) represent non-outlier range.

4.39 Quantification of residual concentration of azadirachtin in azadirachtin supplemented bacterial culture by HPLC. Error bars represent standard deviations (*n*=3). 143

4.40 Effect of bacterial strains on plant growth parameters; shoot length (a), dry weight (b), root length (c), and pod numbers (d), under azadirachtin application at 40 DAS and 65 DAS. Error bars represented standard deviations (*n*=3). The lowercase letters indicate a significant difference ($P \leq 0.05$) between the treatments for each plant attribute for a particular sampling point. Abbreviations: P: plant, A9 and A11: bacterial strains, Az: 300 ppm, and 3xAz: 900 ppm of azadirachtin. 147

4.41 Quantification of residual concentration of azadirachtin in azadirachtin treated soil in the presence of bacterial strains A9 and A11, with 300 ppm of azadirachtin (a), and 900 ppm of azadirachtin (b). Error bars represent standard deviations (*n*=3). Abbreviations: C+Az, A9+Az, A11+Az and A9+A11+Az: control (soil without bacterial strains inoculation) soil, soils with mono inoculation of A9, A11, and soil with dual inoculation A9 and A11, respectively, with the recommended dose of azadirachtin (300 ppm) application; C+3xAz, A9+3xAz, A11+3xAz and A9+A11+3xAz: control soil, soil having mono inoculation of A9, A11, and soil having dual inoculation A9 and A11, respectively, with 3x of the recommended dose of azadirachtin. 148

4.42 Bacterial community profiles generated by DGGE using 16S rRNA gene of mungbean rhizospheric soil samples under the application of azadirachtin and bulk soil at 40 DAS (a), and 65 DAS (c). Lane L: ladder, lane Bulk: bulk soil, lane P: plant soil, lane P+Az: plant having 300ppm of azadirachtin, lane P+3xAz: plant having 900 ppm of azadirachtin, Lane A9 and A11: single inoculation of bacterial isolates, Lane A9+A11: dual inoculation of bacterial isolates, Lanes A9+Az, A11+Az and A9+A11+Az: single and dual inoculations of bacterial isolates with azadirachtin application (300 ppm), lanes A9+3xAz, A11+3xAz and A9+A11+3xAz: single and dual inoculations of bacterial isolates with 900 ppm of azadirachtin application. (c) and (d) Clustering analyses for DGGE profiles obtained in part (a) and (b), respectively. Scale shows % similarity among soil samples from different treatments and bulk soil. The cophenetic correlation values with 1% tolerance have been presented at branch nodes of the dendrogram. 151

4.43 Heatmap depicting Margalef Species richness (a), and Shannon-Wiener (b) indices obtained from DGGE profiles generated from the mungbean 152 rhizospheric soil samples at 40 DAS and 65 DAS. For abbreviations, refer to the legends for figure 4.42.

4.44 Abundance of 16S rRNA gene copies within various treatments for the total bacterial community at two time points, 40 DAS and 65 DAS. Error bars represent standard deviations (*n*=3). The lowercase letters (a-g) indicate a significant difference ($P \leq 0.05$) between the treatments for a particular sampling point. For abbreviations, refer to the legend for Figure 4.42. 153

4.45 Effects of bacterial strains with azadirachtin applications on the abundances of dominant bacterial groups, viz., *α-Proteobacteria* (a), *β-Proteobacteria* (b), *Firmicutes* (c), *Acidobacteria* (d), and *Actinobacteria* (e) in the rhizosphere of mungbean at 40 DAS and 65 DAS. Error bars represent standard deviations (*n*=3). The lowercase letters (a-h) indicate a significant difference ($P \leq 0.05$) between the treatments for a particular sampling point. For abbreviations, refer to the legend for Figure 4.42. 155

6.1 Summary and Conclusions 194

LIST OF TABLES

Table no.	Legend	Page no.
2.1	Effect of different farming practices on microbial community	21
2.2	Impacts of biological pesticides on soil microbiota	32
3.1	Modules in the field experiments of soybean and pigeonpea cropping systems	48
3.2	Sampling stages of field experiments	49
3.3	Plant growth promoting (PGP) properties of bacterial isolates	52
3.4	Layout of plant growth experiment for the performance of bacterial strains (A9 and A11) with mungbean under the application of azadirachtin pesticide	58
3.5	Molecular markers used in this study for the quantitative analysis of structural diversity and functional bacterial guilds	65
4.1	Impact of agri-management practices on plant height (average values, in cm) of pigeonpea and soybean crops over two consecutive seasons	75
4.2	Impact of agri-management practices on dry weight (average values, in g plant^{-1}) of pigeonpea and soybean crops over two consecutive seasons.	76
4.3	Impact of agri-management practices on root nodules (average values, root nodules plant^{-1}) of pigeonpea and soybean crops over two consecutive seasons (2018 and 2019 for pigeonpea; 2017 and 2018 for soybean)	77
4.4	Impact of agri-management practices on pod numbers (average values, pod numbers plant^{-1}) of pigeonpea over three cropping seasons (2017, 2018, and 2019) and soybean over two cropping seasons (2017 and 2019)	78
4.5	Margalef Species richness index (R), and Shannon-Wiener diversity index (H') of the pigeonpea rhizospheric bacterial community (Figures 4.2-4.4) under agri-management practices at the flowering and harvest stages of three consecutive years	84
4.6	Margalef Species richness index (R), and Shannon Wiener diversity index (H') of the soybean rhizospheric bacterial community (Figures 4.5-4.6) under agri-management practices at the flowering and harvest stages of two consecutive years	87
4.7a	Overall impact of agri-management practices on abundance of different bacterial groups in the pigeonpea rhizosphere	100

4.7b Overall impact of agri-management practices on abundance of different bacterial groups in the soybean rhizosphere 101

4.8 Plant growth promoting (PGP) properties assayed for the selection of bacterial strains 144

ABBREVIATIONS

Molecules

HCN	Hydrogen cyanide
N_2	Dinitrogen gas
NH_3	Ammonia
NH_4^+	Ammonium
NO_2^-	Nitrite
NO_3^-	Nitrate

Functional genes as markers for various steps in the nitrogen and phosphorus cycles

amoA	Gene encoding the α-subunit of the ammonia monooxygenase
narG	Gene encoding the membrane-bound nitrate reductase
nifH	Gene encoding the nitrogenase reductase
nirK	Gene encoding the copper-containing nitrite reductase
nirS	Gene encoding the cytochrome *cd*1 nitrite reductase
Bacterial *amoA*	Gene encoding subunit A of ammonia monooxygenase in bacteria
pqqC	Gene encoding quinoprotein glucose dehydrogenase (PQQGDH)
phoD	Gene encoding alkaline phosphatase

Terminology related to agricultural practices

Az	Azadirachtin
CA	Conservation agriculture
CRR	Crop residue retention
CT	Conventional agriculture
CtT	Conventional tillage
FB	Flat bed
ICM	Integrated crop management
OA	Organic agriculture
PRB	Permanent raised bed
RB	Raised bed
ZT	Zero tillage

Elements

N	Nitrogen
P	Phosphorus
K	Potassium
C	Carbon

Important terminology

ANOVA	Analysis of variance
ARISA	Automated Ribosomal Intergenic Spacer Analysis
AM*f*	Arbuscular mycorrhizal fungi
bp	Base pair
bf	Biofertilizers
BLAST	Basic Local Alignment Search Tool
cDNA	Complementary DNA
cfu	Colony forming unit

CLPP	Community-level physiological profiling
Ct	Cycle threshold
CTAB	Cetyl-trimethyl ammonium bromide
DAS	Days after sowing
DEPC	Diethyl pyrocarbonate
DGGE	Denaturing gradient gel electrophoresis
DNA	Deoxyribonucleic acid
EC	Electrical conductivity
EDTA	Ethylene diamine tetra acetic acid
F	Flowering stage
g	Gram
H'	Shannon-Wiener index
H	Harvest stage
h	Hour
ha	Hectare
HPLC	High performance liquid chromatography
IAA	Indole acetic acid
IPTG	Isopropyl β-D-1-thiogalactopyranoside
kg	Kilogram
LB	Luria Bertani
L	Litre
°C	Degree centigrade
min	Minutes
mL	Milliliter
MCT	Microcentrifuge tube
MTCC	Microbial type culture collection
NA	Nutrient agar
NBRIP	National Botanical Research Institute's phosphate growth medium
NGS	Next generation sequencing
NMDS	Non-metric multidimensional scaling
NPK*mf*	Nitrogen-phosphorus-potassium microbial formulation
NTC	No template control
OTU	Operational taxonomic unit
PERMANOVA	Permutational multivariate analysis of variance
PBS	Phosphate buffered saline
PC	Principal component
PCA	Principal component analysis
PCR	Polymerase chain reaction
PGP	Plant growth promoting
PGPR	Plant growth promoting rhizobacteria
ppm	Parts per million
QIIME	Quantitative insight into microbial ecology
qPCR	Real-time quantitative PCR
R	Margalef Species richness
R^2/r	Coefficient of correlation
RAPD	Random amplified polymorphic DNA
RBD	Randomized block design
RDF	Recommended dose of fertilizer
RNA	Ribonucleic acid

rpm	Rotations per minute
SDG	Sustainable development goal
s	Second
TGY	Tryptone glucose yeast extract
T-RFLP	Terminal restriction fragment length polymorphism
TAE	Tris-acetate-EDTA
TSA	Tryptone soy agar
Tukey’s HSD	Tukey's Honestly Significant Difference
UPGMA	Unweighted pair group method with arithmetic mean
UV	Ultraviolet
viz.	Videlicet
X-Gal	5- bromo-4-chloro-3-indolyl-β-D-galactopyranoside
α	Alpha
β	Beta
γ	Gamma
δ	Delta
μ	Micro