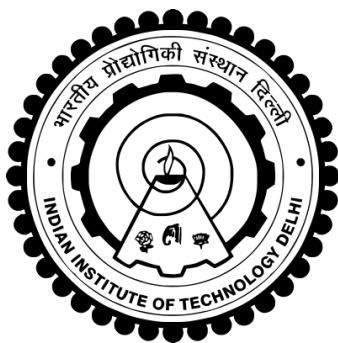


**POLYPHASIC APPROACH FOR ASSAYING
THE ROLES OF BIOCONTROL AGENTS
AGAINST *FUSARIUM* WILT IN *SOLANUM
LYCOPERSICUM***

MONIKA JANGIR



**CENTRE FOR RURAL DEVELOPMENT AND TECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY DELHI**

APRIL 2019

© Indian Institute of Technology Delhi (IITD), New Delhi, 2019

**POLYPHASIC APPROACH FOR ASSAYING
THE ROLES OF BIOCONTROL AGENTS
AGAINST *FUSARIUM* WILT IN *SOLANUM
LYCOPERSICUM***

by

MONIKA JANGIR

CENTRE FOR RURAL DEVELOPMENT AND TECHNOLOGY

Submitted

in fulfilment of the requirements of the degree of Doctor of Philosophy

to the



INDIAN INSTITUTE OF TECHNOLOGY DELHI

APRIL 2019

Dedicated

To

The Almighty

&

My family

CERTIFICATE

This is to certify that the thesis entitled “**Polyphasic approach for assaying the roles of biocontrol agents against *Fusarium wilt in *Solanum lycopersicum**” submitted by **Ms. Monika Jangir** has been prepared under our 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. Satyawati Sharma)

Professor

Centre for Rural Development and
Technology
Indian Institute of Technology Delhi
Hauz Khas New Delhi – 110016,
India

(Dr. Shilpi Sharma)

Associate professor

Department of Biochemical
Engineering and Biotechnology
Indian Institute of Technology Delhi
Hauz Khas New Delhi – 110016,
India

ACKNOWLEDGEMENTS

First and foremost I bow down to divine almighty "SAI BABA" for providing me constant strength to achieve milestones in life, which can add meaning to it. He was always there to rescue me when I was weary and emotionally distraught. It is his saying to have 'patience and faith' in him that have brought me to this accomplishment.

*It gives me immense pleasure and satisfaction to express my kind gratitude and respect to my supervisor **Prof. Satyawati Sharma** for her motherly affection, motivation, enthusiasm, productive criticism and positive support. Her academic virtuosity and constant positive guidance helped me sail through my work. Her innovative ideas and optimistic approach towards each research objective gave me absolute confidence to work in the right direction. She is a source of motivation and ideas that have helped me to accomplish my targets. Undoubtedly, it was a fortunate experience to work under her refractive guidance. I take this esteemed opportunity in expressing my sincere bouquet of gratitude to my co-supervisor **Prof. Shilpi Sharma** for her guidance and support for successful completion of this thesis. I appreciate her unwavering support of me for these four and half years. At times, when I was weak at any front of life, her support and inspiration was a driving force for me. She keeps on inspiring to be a fighter. Certainly, it is my fortunate experience to work in her group.*

I would like to extend my gratitude to the honorable members of my research committee Prof. Saroj Mishra (Chairperson, D.B.E.B.), Prof. S. K. Khare (External Expert, Department of Chemistry), Prof. S. N. Naik (Internal expert, C.R.D.T) for their valuable suggestions, time, comments, and moral support to improve my research work. I am thankful to Dr. Hariprasad P. (C.R.D.T.) and Dr. Amrishi (I.P.F.T.) for their help in few experiments.

I wish to express my cordial thanks to my seniors Dr. Monica Verma, Dr. Pratibha Yadav, Dr. Sharad Verma, Dr. Megha Pant, Dr. Ritika Pathak, Dr. Shalinee, Dr. Himanshi, Dr. Garima Tiwari, Dr. Aditi Gupta, Dr. Abhishek Sharma and Dr. Anurup Adak for their continuous support, valuable suggestions and motivation for completing my research work.

I am indebted to my friends and colleagues, Garima Singh, Mandira Kapri, Md. Aamir Khan, Abhay Tiwari, Upma Singh, Richa Sharma, Gautam Anand, Shivani, Vijay Lakshmi, Swati, Vasu, Nidhi yadav, Shivani Khatri, Swati and Shubham Dubey for their support. I express my sincere thanks to laboratory staff and office staff of C.R.D.T. I am grateful to Mr. Ramkumar and Mr. Shivkant Yadav for his endless support for conducting my field level experiments. His experience and valuable suggestions helped me in successful completion of my experimental work.

I am thankful to my friends cum lifelines, Alka yadav, Deepti Sharma, Rupali Bhakar, Shilpa Yadav, Sudesh Yadav, Varuna Chauhan, Jyoti mahapatra, Shaily Malik, Sunita, Deepali, Shruti, Anubhav, Moulshree Tripathi, Surbhi Sharan and special thanks to my roommate, Garima Gupta, for helping me out in my tough times and continuously supporting me during my entire period of my doctoral degree.

It would be incomplete if I forget to thank my papa, maa and my brother, Ankur, for always believing in me and helping me come out of the stressful situations during my research tenure. Their love, affection, blessings and motivation has helped me achieve my goals in professional as well as personal life. My heartfelt thanks to my parents-in law for their true support and love throughout my Ph.D. journey and for having full faith in my performance.

*Of course last but never ever last, my heartfelt thanks to my amazingly supportive husband **Himanshu Jangir**, who joined me half way in my doctoral journey and always wanted me to be ahead of him. Overseas distance did not hinder his support for me and I am grateful to him for his love and all sacrifices he has done for me. I thank him for being with me during hard time of Ph.D. I would never have been able to achieve such heights without his support.*

Monika Jangir

ABSTRACT

Globally tomato (*Solanum lycopersicum*), belonging to the family Solanaceae, is the second important vegetable crop after potato. *Fusarium* wilt is one of the most devastating soil-borne diseases affecting tomato crop and is responsible for major economical losses in its yield. To obtain an environment friendly alternative for wilt disease control in tomato, utilizing the potential of biocontrol agents and oil seed cakes to mitigate *Fusarium* wilt control has been explored in the present study. In an attempt to develop a method of biological control against *Fusarium oxysporum* f. sp. *lycopersici*, efficacy of *Bacillus subtilis* and *Trichoderma harzianum* was evaluated in wilt control. In addition, present work comprised an extensive study to understand the biocontrol mechanisms of both antagonistic microorganisms and their non-target effects on soil bacterial community.

Dual culture assay revealed that *B. subtilis* (MTCC 2274) and *T. harzianum* (MTCC 3928) showed highest inhibition against fungal pathogenic mycelia. Additionally, indigenous fungal (3T) and bacterial (B44) strains were isolated from rhizospheric soil of tomato exhibiting antagonistic efficacy against pathogen. Further, characterization of isolated and procured strains was done for their antifungal and plant growth promoting properties. After ribotyping, it was found that 3T showed similarity with *Aspergillus flavus* and B44 with *Bacillus* sp. As the fungal isolate was identified as a plant pathogen it was not considered further for *in planta* assay whereas the sequence of bacterial isolate (B44) was submitted to NCBI database (accession number: MG779639). Disease control efficacy of all strains was tested in *in planta* assay; it was observed that dual inoculation of *B. subtilis* and *T. harzianum*

exhibited higher efficacy with disease reduction of 56% as compared to chemical treatment (48.7%). The dual inoculation evidently enhanced the plant growth parameters. Furthermore, non-target effect of biocontrol agents on soil bacterial microbiome was assessed using denaturing gradient gel electrophoresis (DGGE). DGGE fingerprints revealed positive shifts in bacterial community of rhizospheric soil upon treatment with dual inoculation of *B. subtilis* and *T. harzianum* as compared to untreated and uninoculated control. Also, several ecologically important species were identified in the rhizospheric soil treated with dual inoculation.

In the present study, an extensive study was carried out for the identification of antifungal metabolites produced by antagonistic strains. It was observed that both biocontrol agents were capable of producing hydrolytic enzymes, volatile and non-volatile bioactive compounds. For *B. subtilis* activity of β -1,3-glucanase was found maximum ($12.69 \text{ U ml}^{-1} \text{ min}^{-1}$) on 7th day whereas for *T. harzianum* it was highest ($21.47 \text{ U ml}^{-1} \text{ min}^{-1}$) at 5th day of incubation. Protease production was maximal at 7th day of incubation (929 and $846 \text{ U ml}^{-1} \text{ min}^{-1}$) for *B. subtilis* and *T. harzianum*, respectively. For *B. subtilis*, highest value of chitinase activity ($33.69 \text{ U ml}^{-1} \text{ min}^{-1}$) was observed after 2 days of incubation. However, in case of *T. harzianum* highest activity ($154.23 \text{ U ml}^{-1} \text{ min}^{-1}$) was observed at day 5. In *in vitro* volatile assay, an inhibition of 61.7% and 47.2% against fungal pathogen was observed with *B. subtilis* and *T. harzianum* whereas culture filtrates (40%) exhibited 58.51 and 44.66% inhibition against pathogen, respectively. The volatile compounds were extracted with solid phase microextraction (SPME) and identified with gas chromatography-mass spectrophotometry (GC-MS). However, antifungal bioactive fractions of non-volatile compound were screened using direct bioautography and further UPLC-MS identified morpholine, 4-tridecanoyl and N~2~,N~4~-Bis[3-(piperidin-1-yl)propyl]-1,3,5-

triazine-2,4,6-triamine as one of the major compounds for *T. harzianum* and *B. subtilis*, respectively.

Different formulations viz., talc formulation, alginate beads and water dispersible granules (WDG) were developed for biocontrol agents. In *in planta* study, it was notable that WDG was the most efficient in disease reduction followed by talc formulation and alginate beads. Also, utilization of oil seed cakes (OSCs) was taken into consideration for disease control. Among all OSCs, mustard cake showed highest inhibition (36.8%) of fungal mycelial growth with 10% aqueous extract. Then, mustard cake was tested as a growth substrate for biocontrol agents, and evaluated its efficacy in *in planta* assay. In pot study, mustard cake formulation showed 48% disease reduction after 6 months of growth, with increased fruit yield, fresh weight, shoot length and root length over untreated control.

The findings of the present study identified a sustainable and environment friendly alternative for control of *Fusarium* wilt. The application of biocontrol agent with organic amendment could aid in mitigating disease and enhancement of plant health. Dual inoculation of biocontrol agents, potential source of bioactive volatile and non-volatile metabolites, exhibited promising effect on disease suppression and plant growth promotion. In addition, the treatment showed non-target effect by exerting an enhancement on the abundance of bacteria in rhizosphere of host plant over untreated control as compared to single inoculation. In addition, dual inoculation exhibited the presence of some species of ecological importance and reduced the deteriorating effect of fungal pathogen on bacterial community.

सार

विश्व स्तर पर टमाटर (सोलेनम लाइकोपर्सिकम), सोलनैसे परिवार से संबंधित, आलू के बाद दूसरी महत्वपूर्ण सब्जी फसल है। टमाटर की फसल को प्रभावित करने वाली सबसे अधिक विनाशकारी मृदा जनित बीमारियों में से एक है विल्ट और इसकी पैदावार में बड़े आर्थिक नुकसान के लिए जिम्मेदार है। टमाटर में इस बीमारी के नियंत्रण के लिए पर्यावरण के अनुकूल विकल्प प्राप्त करने के लिए, वर्तमान अध्ययन में बायोकंट्रोल एजेंटों और तेल बीज केक की क्षमता का उपयोग किया गया है। ऑक्सीस्पोरम एफ०एसपी० लाइकोपर्सिकी के खिलाफ जैविक नियंत्रण की एक विधि विकसित करने के प्रयास में, बेसिलस सबटिलिस और ट्राइकोडर्मा हर्जियानम का मूल्यांकन विल्ट कंट्रोल में किया गया है। इसके अलावा, वर्तमान काम में दोनों विरोधी सूक्ष्मजीवों के जैव तंत्र और मिट्टी जीवाणु समुदाय पर उनके गैर-लक्ष्य प्रभावों को समझने के लिए एक व्यापक अध्ययन शामिल है।

ड्यूल कलचर परख से पता चला कि बी० सबटिलिस (एम०टी०सी०सी० 2274) एवं टी० हर्जियानम (एम०टी०सी०सी० 3928) ने फंगल रोगजनक मायसेलिया के खिलाफ उच्चतम अवरोध दिखाया। इसके अतिरिक्त, इनडीजिनस फंगल (3T) और बैक्टीरियल (B44) उपभेदों को टमाटर की राइजोस्फेरिक मिट्टी से अलग किया गया था जो रोगजनक के खिलाफ विरोधी प्रभाव दिखा रहा था। इसके अलावा, पृथक और खरीदे गए उपभेदों के लक्षणों का वर्णन उनके एंटीफंगल और पौधों के विकास को बढ़ावा देने वाले गुणों के लिए किया गया था। राइबोटाइपिंग के बाद, यह पाया गया कि 3T ने एस्परजिलस फलेवस और B44 ने बेसिलस के साथ समानता दिखाई। चूंकि फंगल उपभेद (3T) को पौधों के लिए रोगजनक के रूप में पहचाना गया है, इसलिए इसे अध्ययन में आगे नहीं लिया गया, जबकि बैक्टीरियल (B44) आइसोलेट को अनुक्रम NCBI डेटाबेस (परिग्रहण संख्या: MG 779639) में जमा किया गया है।

पॉट अध्ययन में सभी उपभेदों के रोग नियंत्रण प्रभावकारित का परीक्षण किया गया, यह देखा गया कि बी० सबटिलिस एवं टी० हर्जियानम के ड्यूल

इनोक्यूलेशन ने रासायनिक उपचार (48.7%) की तुलना में 56% बीमारी की रोक के साथ उच्च प्रभावकारित का प्रदर्शन किया। डुयल इनोक्यूलेशन ने पौधे के विकास के मापदंडों को स्पष्ट रूप से बढ़ाया। इसके अलावा, मृदा जीवाणु माइक्रोबायोम पर बायोकंट्रोल एजेंटों के गैर – लक्षित प्रभाव का मूल्यांकन डी.जी.जी.ई. का उपयोग करके किया गया था।

डी.जी.जी.ई. फिंगर प्रिंट ने बी₀ सबटिलिस और टी₀ हर्जियानम के दोहरे इनोक्यूलेशन के साथ उपचार पर राइजोस्फेरिक मिट्टी के जीवाणु समुदाय में सकारात्मक बदलावों का पता लगाया। इसके अलावा, कोई पारिस्थितिक रूप से महत्वपूर्ण प्रजातियों की पहचान दोहरे इनोक्यूलेशन की मिट्टी में की गई थी।

वर्तमान अध्ययन में, प्रतिपक्षी जीवाणु द्वारा उत्पादित एंटीफगल मेटाबोलाइट्स की पहचान के लिए एक व्यापक अध्ययन किया गया था। यह पाया गया था कि दोनों बायोकंट्रोल एजेंट हाइड्रोलिटिक एंजाइमों, वाष्पशील और गैर-वाष्पशील अनाकर यौगिकों का उत्पादन करने में सक्षम थे। बी. सबटिलिस के लिए बीटा-1, 3-ग्लूकेनेस की गतिविधि 7 वें दिन अधिकतम ($12.64 \text{ Uml}^{-1} \text{ min}^{-1}$) पाई गई जबकि टी. हर्जियानम के लिए 5 वें दिन ($21.74 \text{ Uml}^{-1} \text{ min}^{-1}$) उच्चतम थी। बी. सबटिलिस और टी. हर्जियानम के लिए प्रोटीज का उत्पादन अधिकतम 926 एवं 846 $\text{Uml}^{-1} \text{ min}^{-1}$ था।

फाईटिनेस उत्पादन बी₀ सबटिलिस के लिए, इंक्योबेशन के 2 दिनों बाद देखा गया था ($33.69 \text{ Uml}^{-1} \text{ min}^{-1}$)। हालांकि, टी₀ हर्जियानम के लिए उच्चतम गतिविधि 5 दिन के बाद देखी गई ($154.23 \text{ Uml}^{-1} \text{ min}^{-1}$)। इन विट्रो वाष्पशील तकनीक में, बी₀ सबटिलिस और टी₀ हर्जियानम के साथ 61.7% 47.2% निषेध देखा गया, जबकि कल्चर फिल्ट्रेट्स (40%) ने 58.51 और 44.6% रोगजनक के खिलाफ क्रमशः निषेध दिखाया। वाष्पशील यौगिकों को एस.पी.एम.ई. के साथ निकाला गया और गैर क्रोमैटोग्राफी-मास स्पेक्ट्रोफोटोमेट्री (जी सी-एमएस) के साथ पहचाना

गया। हालांकि गैर-वाष्पशील यौगिक के ऐंटीफंगल अंशो को डाईरेक्ट बायोआटोग्राफी और आगे यू.पी.एल.सी.-एम.एस. द्वारा जांचा गया।

बायोकंट्रोल एजेंटो के लिए अलग-अलग फॉर्मूलेशन जैसे टॉल्क फॉर्मूलेशन, एल्जिनेट बीड्स और वाटर डिस्पर्सिबल गैन्ग्यूल्स बनाए गए थे। पॉट अध्ययन में, यह उल्लेखनीय था, कि वाटर डिस्पर्सिबल गैन्ग्यूल्स रोग के रोकथाम में सबसे कुशल था, इसके बाद टॉल्क फॉर्मूलेशन और एल्जिनेट बीड्स थे। इसके अलावा रोग नियंत्रण के लिए ऑयल सीड केक (ओ.एस.सी.) के उपयोग को ध्यान में रखा गया। सभी ओएससी के बीच, सरसों के केक में उच्चतम निषेध (36.8%) देखा गया। फिर, सरसों के केक को बायोकंट्रोल एजेंटो के लिए एक विकास सबस्ट्रेट के रूप में परीक्षण किया गया था, और पॉट अध्ययन में इसकी प्रभावकारिता का मूल्यांकन किया। पॉट अध्ययन में, सरसों के केक उपचार ने 48% रोग में कमी दिखाई, जिसमें फल की पैदावार, शूट की लंबाई और वजन अनुपचारित नियंत्रण में ज्यादा था।

वर्तमान अध्ययन के निष्कर्षों ने फ्यूजेरियम विल्ट के नियंत्रण के लिए एक स्थायी और पर्यावरण के अनुकूल विकल्प की पहचान की। जैविक संशोधन के साथ बायोकंट्रोल एजेंट के आवेदन से रोग को कम करने और पौधों के स्वास्थ्य को बढ़ाने में मदद मिल सकती है। बायोकंट्रोल एजेंटो के दोहरे इनोक्यूलेशन, बायोएक्टिव वाष्पशील और गैर-वाष्पशील सेटाबोलाइट्स के संभावित स्रोत, रोग दमन और पौधे के विकास को बढ़ावा देने वाले प्रभाव का प्रदर्शन करते हैं। इसके अलावा, एकल इनोक्यूलेशन की तुलना में दोहरे इनोक्यूलेशन में राइजोस्फेरिक जीवाणुओं की प्रचुरता को बढ़ाकर गैर-लक्ष्य प्रभाव दिखाया। इसके अलावा दोहरे इनोक्यूलेशन में पारिस्थितिक महत्व की कुछ प्रजातियों की उपस्थिति भी पाई गई और बैक्टीरिया समुदाय पर फंगल रोगजनक के बिगड़ते प्रभाव को भी कम किया।

CONTENTS

Certificate		i
Acknowledgements		ii
Abstract		iv
List of figures		xiv
List of tables		xx
Abbreviations		xxiii
Chapter 1	INTRODUCTION	1-9
1.1	Background	1
1.2	<i>Solanum lycopersicum</i> and <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>	2
1.3	Biological control	5
1.3.1	Biocontrol agents	5
1.3.2	Oil seed cake	6
1.4	Scope of work	7
1.5	Objectives of the study	9
Chapter 2	REVIEW OF LITERATURE	10-46
2.1	Model plant: <i>Solanum lycopersicum</i>	10
2.2	Economic losses caused by <i>Fusarium</i> wilt	10
2.3	<i>Fusarium</i> wilt	11
2.3.1	Causal agent	11
2.3.2	Biology of the pathogen	11
2.3.3	Host specificity and toxicity of <i>Fusarium</i>	12
2.3.4	Symptoms of wilting	13
2.3.5	Disease cycle	14
2.4	Management of <i>Fusarium</i> wilt	16
2.4.1	Physical control	17
2.4.2	Chemical control	18
2.4.3	Biological control	19
2.4.3.1	Biocontrol agents	20

	2.4.3.2 <i>Bacillus subtilis</i>	23
	2.4.3.3 <i>Trichoderma harzianum</i>	24
2.5	Dual inoculation versus mono-inoculation	25
2.6	Biocontrol mechanisms of biocontrol agents	26
2.6.1	Antifungal compounds and antibiotics	28
2.6.2	Inactivation of pathogenic proteins and enzymes	30
2.6.3	Induced resistance	30
2.6.4	Mycoparasitism and hydrolytic enzymes	31
2.6.5	Competition	32
2.6.6	Secondary metabolites	32
	2.6.6.1 Identification of secondary metabolites	34
	2.6.6.1.1 Volatile metabolites	34
	2.6.6.1.2 Non-volatile metabolites	35
	2.6.6.1.2.1 Antifungal assay: Direct bioautography	35
	2.6.6.1.2.2 Ultra performance liquid chromatography-mass spectrometry	36
2.7	Risk assessment: Non-target effect of biocontrol agents	37
2.8	Organic amendments	38
2.8.1	Biocontrol efficacy of oil seed cakes	39
2.9	Microbial formulation development	42
Chapter 3	MATERIALS AND METHODS	47-83
3.1	Selection of efficient strains of biocontrol agents for wilt control	48
3.2	Initial screening of strains for mycelial inhibition against <i>F. oxysporum</i>	49
3.3	Interaction study between selected strains	49
3.4	Characterization of strains for antifungal and plant growth promoting properties	50
3.4.1	Ammonia production	50
3.4.2	Hydrogen cyanide production	50
3.4.3	Indole acetic acid (IAA) production	50
3.4.4	Siderophore production	51
3.4.5	Qualitative assay for hydrolytic enzymes	51

3.5	Isolation of indigenous antagonistic <i>Trichoderma</i> and <i>Bacillus</i> strains from rhizospheric soil	51
3.5.1	Collection of soil	51
3.5.2	Soil analysis	52
3.5.3	Isolation of <i>Trichoderma</i> and <i>Bacillus</i> strains from soil samples	52
3.6	<i>In vitro</i> characterization of isolates for antifungal and plant growth promoting (PGP) properties	53
3.7	Identification of isolates by ribotyping	53
3.8	Development of talc formulation	54
3.9	Seed germination assay with biocontrol agents	55
3.10	Effect of chemical pesticides on biocontrol agents	55
3.11	Volatile compounds produced by biocontrol agents against pathogen	56
3.12	Activity of compounds produced by biocontrol agents in culture filtrate	56
3.12.1	Cell free culture filtrate of biocontrol agents	57
3.12.2	Poisoned food technique using culture filtrate against pathogen	57
3.12.3	Extraction of compounds from culture filtrate	57
3.12.4	Agar well diffusion assay against pathogen	58
3.12.5	GC-MS apparatus and chromatography conditions	58
3.13	Hydrolytic enzymes	59
3.13.1	Protease	59
3.13.2	Chitinase	60
3.13.3	β -1,3-glucanase	60
3.14	<i>In planta</i> assay for efficacy evaluation of biocontrol agents	61
3.14.1	Experimental design	61
3.14.2	Evaluation of plant growth parameters and disease reduction	62
3.14.2.1	Chlorophyll content	63
3.14.2.2	Total phenolic content	63
3.14.2.3	Total soluble protein	64
3.15	Non-target effect of biocontrol agents on soil bacterial community	65
3.15.1	DNA extraction from soil	65

3.15.2	PCR-denaturing gradient gel electrophoresis (PCR-DGGE)	66
3.15.3	DGGE gel analysis	66
3.15.4	Phylogenetic affiliation of bands	67
3.16	Isolation of bioactive compounds	67
3.16.1	Initial screening of solvent for extraction	67
3.16.2	Thin layer chromatography (TLC) for separation of compounds	68
3.16.3	Direct bioautography with biocontrol agents	69
3.16.4	Chromatography conditions of UPLC-MS	70
3.16.5	UPLC-MS data processing and analysis	71
3.17	Identification of compounds produced by biocontrol agents in dual inoculation with pathogen	71
3.17.1	Extraction of compounds	71
3.17.2	Identification of compounds	71
3.18	Development of different type of microbial formulations	72
3.18.1	Talc formulation	72
3.18.2	Alginate beads	72
3.18.3	Water dispersible granules	73
	3.18.3.1 Preparation of granules	73
	3.18.3.2 Optimization of components by response surface methodology (RSM)	75
3.19	<i>In planta</i> assay to evaluate efficacy of formulations in wilt control under natural conditions	76
3.20	Selection of non-traditional biomass to use as biopesticide and suitable media for cultivating selected biocontrol agent	77
3.20.1	Oil seed cakes	77
3.20.2	Screening of oil seed cakes for antifungal efficacy against <i>F. oxysporum</i>	78
	3.20.2.1 <i>In vitro</i> assay for volatile compounds	78
	3.20.2.2 <i>In vitro</i> assay for compounds in aqueous extract of oil seed cake	78
	3.20.2.2.1 Preparation of aqueous extract of oil seed cakes	78
	3.20.2.2.2 Poisoned food technique using aqueous	78

	extract	
3.20.3	Growth of biocontrol agents on selected oil seed cake	79
	3.20.3.1 Characterization of oil seed cake	79
	3.20.3.2 Growth of biocontrol agents in cake broth and solid state fermentation	79
3.20.4	Extraction and identification of bioactive compounds of cake against <i>F. oxysporum</i>	80
	3.17.4.1 Volatile compounds of oil seed cake	80
	3.17.4.2 Compounds in aqueous extract of oil seed cake	80
3.20.5	Seed germination study with cake based formulation	81
3.20.6	<i>In planta</i> assay for wilt disease control with cake based formulation	82
3.21	Statistical analysis	83
Chapter 4	RESULTS AND DISCUSSION	84-190
4.1	Antagonistic strains of biocontrol agents against <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>	84
	4.1.1 Efficiency of strains procured from culture collection	84
	4.1.2 Compatibility between both antagonistic strains	85
	4.1.2.1 Characterization for biocontrol and plant growth promoting (PGP) properties	86
	4.1.3 Soil parameters	88
	4.1.4 Efficiency of isolated bacterial and fungal strains	89
	4.1.4.1 Preliminary screening of isolates for antagonistic activity against <i>F. oxysporum</i>	89
	4.1.4.2 Characterization of shortlisted <i>Bacillus</i> isolates for antifungal and plant growth promoting properties	91
	4.1.5 Molecular identification of the isolates	93
4.2	Seed germination assay with biocontrol agents	94
4.3	Effect of chemical pesticides on biocontrol agents	96
4.4	Bioactive volatile compounds against <i>F. oxysporum</i>	99
	4.4.1 Activity of volatile compounds in <i>in vitro</i> plate assay	99

4.4.2	Compounds identified by GC-MS	102
4.5	Activity of compounds produced by biocontrol agents in culture filtrate and their identification by GC-MS	116
4.5.1	Activity of cell free culture filtrate in <i>in vitro</i> plate assay	116
4.5.2	Compounds identified in GC-MS	118
4.6	Production of hydrolytic enzymes by biocontrol agents	126
4.7	Biocontrol and plant growth promoting potential of biocontrol agents against <i>F. oxysporum</i> under <i>in vivo</i> conditions	132
4.8	Non-target effects of biocontrol agents on rhizospheric bacterial community	139
4.8.1	Denaturing gradient gel electrophoresis (DGGE) for bacterial community	139
4.8.2	Diversity index, richness and evenness of bacterial community	144
4.9	Isolation of bioactive compounds	145
4.9.1	Initial screening of solvent for extraction	145
4.9.2	Standardized solvent system for <i>Bacillus subtilis</i> 2274	146
4.9.3	Direct bioautography for <i>Bacillus subtilis</i>	146
4.9.4	Compounds identified in bioactive fraction of <i>Bacillus subtilis</i>	148
4.9.5	Standardized solvent system for <i>Trichoderma harzianum</i> 3928	152
4.9.6	Direct bioautography for <i>Trichoderma harzianum</i>	153
4.9.7	Compounds identified in bioactive fraction of <i>Trichoderma harzianum</i>	154
4.9.8	Compounds produced by <i>T. harzianum</i> and <i>B. subtilis</i> in response to <i>F. oxysporum</i>	158
4.10	Development of formulation and their efficacy evaluation	162
4.10.1	Development of water dispersible granules (WDG)	162
4.10.2	<i>In planta</i> assay for efficacy evaluation in disease control	168
4.11	Screening of oil seed cakes for antifungal activity	172
4.11.1	Antifungal activity of aqueous extract of oil seed cakes	172
4.11.2	Antifungal activity of oil seed cakes in volatile assay	174
4.11.3	Antifungal activity of oil seed cakes in solid state fermentation	176
4.11.4	Mustard cake as a growth substrate for biocontrol agents	178

4.11.4.1	Characterization of mustard cake	178
4.11.4.2	Growth curve of biocontrol agents on mustard cake	179
4.11.5	Bioactive compounds of mustard cake	180
4.11.5.1	Volatile compounds extracted using solid phase microextraction (SPME)	180
4.11.5.2	Compounds extracted from mustard cake aqueous extract	182
4.11.6	Germination assay for evaluating toxicity of mustard cake on tomato seeds	185
4.11.7	Validation of cake formulation in <i>in planta</i> assay	187
Chapter 5	SUMMARY AND CONCLUSION	191-197
	REFERENCES	198-227
	Curriculum Vitae	228-230

LIST OF FIGURES

Figure No.	Title	Page No.
1.1	Worldwide consumption of chemical pesticides	1
1.2	Global scenario of India in tomato production	3
1.3	Percentage share of tomato production in India	3
2.1	Asexual reproductive structures of <i>Fusarium oxysporum</i> (a) microconidia, (b) macroconidia and (c) chlamydospores	12
2.2	Disease cycle of <i>Fusarium oxysporum</i>	16
2.3	Different methods for management of wilting in tomato plant	17
2.4	Different modes of action of biocontrol against pathogen for their antagonistic activity	27
2.5	Solid phase microextraction of volatile compounds using headspace technique	35
3.1	Schematic diagram of the work plan	47
3.2	Morphology of (a) <i>Bacillus subtilis</i> and (b) <i>Trichoderma harzianum</i>	48
3.3	Mycelial morphology of <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>	49
3.4	Illustrations of a developed thin layer chromatography	69
3.5	Schematic diagram showing the steps for development of alginate beads	73
3.6	Pot experiment of tomato in net house at CRDT micromodel, IIT Delhi	77
4.1	Percentage inhibition of <i>Fusarium oxysporum</i> in dual culture assay with strains of <i>Bacillus subtilis</i> (MTCC 8113, 8114 and 2274) and <i>Trichoderma harzianum</i> (MTCC 792, 795 and 3928). Bars with the same letter are not significantly different at $p < 0.05$ by Duncan multiple range test, and error bars indicate \pm SD of triplicates.	84
4.2	Dual culture assay for assessment of antagonism between (a) <i>Trichoderma harzianum</i> 3928 and (b) <i>Bacillus subtilis</i> 2274 against <i>Fusarium oxysporum</i> as compared to (c) control.	85
4.3	<i>In vitro</i> dual plate assay assay for compatibility test between <i>Trichoderma harzianum</i> and <i>Bacillus subtilis</i> after 5 days of incubation	86

4.4	<i>In vitro</i> dual culture assay between (a) bacterial isolate B44 and (b) fungal isolate 3T against <i>Fusarium oxysporum</i> as compared to (c) control	93
4.5	Number of seeds germinated each day upon treatment with biocontrol agents for 15 days where TH= <i>Trichoderma harzianum</i> 3928, BS= <i>Bacillus subtilis</i> 2274, TH + BS= combination of <i>T. harzianum</i> and <i>B. subtilis</i> , B (44) = selected isolate of <i>Bacillus</i> and control= seeds without any coating.	95
4.6	Germination rate (%) of tomato seed upon treatment with biocontrol agents after 15 days where TH= <i>T. harzianum</i> 3928, BS= <i>B. subtilis</i> 2274, TH + BS= combination of <i>T. harzianum</i> and <i>B. subtilis</i> , B (44) = selected isolate of <i>Bacillus</i> and control= seeds without any coating. Bars followed by the same letter are not significantly different at $p < 0.05$ by Duncan multiple range test; error bars indicate \pm SD of $n=30$.	95
4.7	Chemical pesticide tolerance of (a) <i>Trichoderma harzianum</i> , (b) <i>Bacillus subtilis</i> and (c) <i>Bacillus</i> isolate B44 in <i>in vitro</i> plate assay where A= mancozeb, B= carbendazim, C= chlorpyrifos and $x= 1000$ ppm (recommended dose). In each figure, bars with significantly different values by Duncan multiple range test ($p < 0.05$) are marked with different lowercase letters, error bars indicate \pm SD of triplicates.	98
4.8	Percentage inhibition of <i>Fusarium oxysporum</i> by volatile compounds of (a) <i>Trichoderma harzianum</i> , (b) <i>Bacillus subtilis</i> and (c) <i>Bacillus</i> isolate B44 in <i>in vitro</i> plate assay. In each figure, bars with significantly different values by Duncan multiple range test ($p < 0.05$) are marked with different lowercase letters, error bars indicate \pm SD of triplicates.	101
4.9	GC-MS chromatograms of volatile organic compounds produced by (a) <i>Bacillus subtilis</i> , (b) <i>Trichoderma harzianum</i> and (c) <i>Bacillus</i> isolate B44 extracted using solid phase micro extraction with their retention time	102
4.10	Structure of the major compounds produced by <i>Bacillus subtilis</i> identified by GC-MS using solid phase microextraction; (a) 1,2-benzenedicarboxylic acid, dioctyl ester, (b) eicosylamine, N-N-dimethyl, (c) 4-octadecylmorpholine, (d) 6- undecylamine and (e) 1-heptadecanecarboxylic acid.	110
4.11	Structure of the major compounds produced by produced by <i>Trichoderma harzianum</i> identified by GC-MS using solid phase micro extraction; (a) santalol, (b) 9,12-octadecadienoic acid (z,z), (c) γ -butyrolactone,(d) 6-octadecenoic acid, methyl ester, (z) and (e) cis-verbenyl angelate.	110
4.12	Structure of the major compounds produced by produced by <i>Bacillus</i> isolate B44 identified by GC-MS using solid phase micro extraction; (a) 1,2-benzenedicarboxylic acid, (b) 6-undecylamine, (c) 2-methyloctacosane, (d) 9-octadecenoic acid and (e) 1-tetradecanamine, N, N-dimethyl.	111

4.13	Percentage distribution of different volatile organic compounds produced by (a) <i>Bacillus subtilis</i> (b) <i>Trichoderma harzianum</i> (c) <i>Bacillus</i> isolate B44 identified by GC-MS using solid phase micro extraction	115
4.14	GC-MS chromatogram of volatile organic compounds produced by (a) <i>Bacillus subtilis</i> , (b) <i>Trichoderma harzianum</i> and (c) <i>Bacillus</i> isolate B44 extracted with ethyl acetate and identified by GC-MS with their retention time.	119
4.15	Percentage distribution of organic compounds in culture filtrate of (a) <i>Bacillus subtilis</i> (b) <i>Trichoderma harzianum</i> (c) <i>Bacillus</i> isolate B44 identified by GC-MS in ethyl acetate extract.	124
4.16	Structures of major compounds in culture filtrate of (A) <i>Bacillus subtilis</i> , (B) <i>Trichoderma harzianum</i> and (C) <i>Bacillus</i> isolate B44 identified by GC-MS in ethyl acetate extract. For name of the compound refer to Table 4.8.	125
4.17	Production of (a) chitinase, (b) β -1, 3-glucanase and (c) protease by <i>Trichoderma harzianum</i> . In each figure, bars with significantly different values by Duncan multiple range test ($p < 0.05$) are marked with different lowercase letters. Error bars indicate standard deviation of triplicates.	128
4.18	Production of (a) chitinase, (b) β -1, 3-glucanase and (c) protease by <i>Bacillus subtilis</i> . In each figure, bars with significantly different values by Duncan multiple range test ($p < 0.05$) are marked with different lowercase letters. Error bars indicate standard deviation of triplicates.	129
4.19	Production of (a) protease, (b) chitinase and (c) β -1, 3-glucanase by <i>Bacillus</i> isolate B44. In each figure, bars with significantly different values by Duncan multiple range test ($p < 0.05$) are marked with different lowercase letters. Error bars indicate standard deviation of triplicates.	130
4.20	UPGMA tree for bacterial community fingerprints obtained from denaturing gradient gel electrophoresis of 16S rRNA gene amplified from rhizospheric samples from tomato at (a) time point-1 (30 DAS) and (b) time point-3 (90 DAS) where T1= <i>Trichoderma harzianum</i> + <i>Bacillus subtilis</i> + <i>Fusarium oxysporum</i> , T2= <i>B. subtilis</i> + <i>F. oxysporum</i> , T3= <i>T. harzianum</i> + <i>F. oxysporum</i> , T4= <i>F. oxysporum</i> , T5= carbendazim (1000 ppm) + <i>F. oxysporum</i> and T6= uninoculated control (only plant). Percentage similarity is represented on scale, and values on branch nodes depict the cophenetic correlation.	140

4.21	Denaturing gradient gel electrophoresis fingerprint of 16S rRNA gene from soil samples of tomato rhizosphere at first time point where lane T1= <i>Trichoderma harzianum</i> + <i>Bacillus subtilis</i> + <i>Fusarium oxysporum</i> , T2= <i>B. subtilis</i> + <i>F. oxysporum</i> , T3= <i>T. harzianum</i> + <i>F. oxysporum</i> , T4= <i>F. oxysporum</i> , T5= carbendazim (1000 ppm) + <i>F. oxysporum</i> , T6= uninoculated control (only plant) and L= ladder. Numerical digits refer to number of eluted band.	141
4.22	Denaturing gradient gel electrophoresis fingerprint of 16S rRNA gene from soil samples of tomato rhizosphere at third time point where lane T1= <i>Trichoderma harzianum</i> + <i>Bacillus subtilis</i> + <i>Fusarium oxysporum</i> , T2= <i>B. subtilis</i> + <i>F. oxysporum</i> , T3= <i>T. harzianum</i> + <i>F. oxysporum</i> , T4= <i>F. oxysporum</i> , T5= carbendazim (1000 ppm) + <i>F. oxysporum</i> , T6= uninoculated control (only plant) and L= ladder. Numerical digits refer to number of eluted band.	142
4.23	Heat map for variability in Shannon index (H) and richness (S) of bacterial community in rhizospheric samples from tomato at time point-1 (30 DAS) and time point-3 (90 DAS). For abbreviations, refer to legend of Fig. 4.22.	144
4.24	Antifungal activity of ethyl acetate extract (dissolved in DMSO) of <i>Bacillus subtilis</i> against <i>Fusarium oxysporum</i> in agar well diffusion assay with (a) control (blank DMSO) and (b) crude extract of ethyl acetate.	145
4.25	Thin layer chromatography for standardization of solvent system for ethyl acetate crude extract of <i>Bacillus subtilis</i> where A= methanol, B= chloroform, C= hexane, D= petroleum ether, E= acetone and F= ethyl acetate.	146
4.26	Direct bioautography for <i>Bacillus subtilis</i> with ethyl acetate as the mobile phase for developing TLC. 'a' and 'b' depicts white inhibition zone with retention factor of 0.475 and 0.625, respectively.	147
4.27	UPLC-MS chromatogram of bioactive fraction of ethyl acetate extract of <i>Bacillus subtilis</i> 2274 with retention time (min) on x-axis and area (%) on y-axis.	149
4.28	Structure of compounds identified in bioactive fraction of <i>Bacillus subtilis</i> identified by UPLC-MS. For name of the compound refer to Table 4.11.	151
4.29	Thin layer chromatography for standardization of solvent system for ethyl acetate crude extract of <i>Trichoderma harzianum</i> where A= ethyl acetate, B= petroleum ether, C= methanol, D= acetone, E= hexane and F= chloroform.	152
4.30	Direct bioautography for <i>Trichoderma harzianum</i> with mixture of chloroform and petroleum ether (8:2) as the mobile phase for developing TLC. 'a' and 'b' denotes white inhibition zone with retention factor of 0.175 and is 0.4125,	153

	respectively.	
4.31	Inhibition zone formed by extract of band ‘a’ mentioned in Fig. 4.30 against <i>Fusarium oxysporum</i> .	154
4.32	UPLC-MS chromatogram for compounds identified in bioactive fraction of ethyl acetate extract of <i>Trichoderma harzianum</i> with their retention time (min) on x-axis and area (%) on y-axis.	155
4.33	Structure of major compounds identified in bioactive fraction of <i>Trichoderma harzianum</i> identified by UPLC-MS. For name of the compound refer to Table 4.12.	156
4.34	3D response curve showing interaction between concentrations of acacia gum-A and alginic acid-B on (a) wetting time, (b) dispersing time and (c) suspensibility of water dispersible granules.	167
4.35	Dispersion of water dispersible granules (WDG) developed with optimized concentration of wetting agent and dispersing agent.	168
4.36	(a) Fresh weight and fruit yield, (b) shoot length and (c) root length of tomato plants treated with T1= water dispersible granules (WDG) (<i>B. subtilis</i>) + <i>F. oxysporum</i> , T2= WDG (<i>T. harzianum</i>) + <i>F. oxysporum</i> , T3= Talc formulation (<i>B. subtilis</i>) + <i>F. oxysporum</i> , T4= Talc formulation (<i>T. harzianum</i>) + <i>F. oxysporum</i> , T5= Alginate beads (<i>B. subtilis</i>) + <i>F. oxysporum</i> , T6= Alginate beads (<i>T. harzianum</i>) + <i>F. oxysporum</i> , T7= only <i>F. oxysporum</i> and T8= carbendazim (1000 ppm) + <i>F. oxysporum</i> . In each figure, bars with significantly different values by Duncan multiple range test ($p < 0.05$) are marked with different letters. Error bars indicate standard deviation of triplicates.	171
4.37	Comparative efficacy of aqueous extract of oil seed cakes against <i>F. oxysporum</i> in poisoned food technique. Comparison has been done among percentage inhibition of all cakes at each day of incubation. Bars with significantly different values by Duncan multiple range test ($p < 0.05$) are marked with different letters. Error bars indicate standard deviation of triplicates.	173
4.38	Growth of <i>Fusarium oxysporum</i> on media amended with aqueous extract of (A) jatropha, (B) simarouba, (C) mustard, (D) castor, (E) neem, (F) karanja oil seed cakes and (G) control (potato dextrose agar without any amendment)	174
4.39	Spore count of <i>Fusarium oxysporum</i> on different cakes in solid state fermentation. Comparison has been among spore count of all cakes at each day of incubation. Bars with significantly different values by Duncan multiple range test ($p < 0.05$) are marked with different letters. Error bars indicate standard deviation of triplicates.	177

4.40	Growth of <i>Fusarium oxysporum</i> on (a) karanja, (b) neem, (c) jatropha, (d) castor, (e) mustard, (f) simorauba oil seed cakes and (g) control (wheat) in solid state fermentation after 15 days of incubation.	177
4.41	Growth curve of (a) <i>Trichoderma harzianum</i> on solid mustard cake for 15 days with wheat as control and (b) <i>Bacillus subtilis</i> in liquid medium for 7 days with nutrient broth as control. Error bars indicate \pm SD of triplicates.	179
4.42	Morphology of (a) spores and (b) mycelium of <i>Trichoderma harzianum</i> on mustard cake under scanning electron microscope.	180
4.43	GC-MS chromatogram for volatile compounds extracted from mustard cake using solid phase microextraction with retention time.	181
4.44	Structure of major volatile compounds extracted from mustard cake identified by GC-MS. For name of the compound refer to Table 4.25.	182
4.45	GC-MS chromatogram for compounds identified in ethyl acetate fraction of mustard cake aqueous extract with their retention time.	183
4.46	Structure of major compounds identified by GC-MS in ethyl acetate fraction of mustard cake aqueous extract. For name of the compound refer to Table 4.26.	184
4.47	Effect of cake based treatments on growth parameters (a) seedling length (root + shoot) and (b) fresh weight of seedlings under controlled conditions in plant growth chamber. In each figure, bars with significantly different values by Duncan multiple range test ($p < 0.05$) are marked with different letters. Error bars indicate standard deviation of triplicates.	187
4.48	(a) Root length, (b) shoot length and (c) fresh weight and fruit yield of tomato plants treated with T1= [AI + 90% Talcum powder + 10% raw cake and 1% CMC] + <i>F. oxysporum</i> , T2= carbendazim (1000 ppm) + <i>F. oxysporum</i> , T3= <i>T. harzianum</i> + <i>F. oxysporum</i> and T4= only <i>F. oxysporum</i> . In each figure, bars with significantly different values by Duncan multiple range test ($p < 0.05$) are marked with different letters. Error bars indicate standard deviation of triplicates.	189

LIST OF TABLES

Table No.	Title	Page No.
1.1	Synthetic pesticide and global biopesticide market	2
2.1	Different biocontrol agents used against <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> for wilt disease control	21
2.2	Antifungal compounds and antibiotics produced by biocontrol agents	28
2.3	Oil seed cakes tested for biocontrol efficacy against phytopathogens	41
2.4	Commercial available formulations of <i>Bacillus</i> and <i>Trichoderma</i> sp.	45
3.1	Layout of <i>in planta</i> assay with antagonistic strains of <i>Trichoderma</i> and <i>Bacillus</i> for wilt disease control in tomato	62
3.2	Combination of different substrates, bulking agent, dispersing agent and wetting agent for the development of efficient water dispersible granules	74
3.3	Experimental design employed in the response surface methodology (RSM) using a central composite design matrix for increasing efficiency of water dispersible granules	75
3.4	Design of <i>in planta</i> assay for talc formulation, alginate beads and water dispersible granules (WDG) of <i>T. harzianum</i> and <i>B. subtilis</i> under natural conditions	76
3.5	Layout of the treatments with varying concentration of cake and talcum powder	81
3.6	Layout for <i>in planta</i> assay with cake based formulation for wilt disease control	82
4.1	Antifungal and plant growth promoting properties of <i>Trichoderma harzianum</i> 3928 and <i>Bacillus subtilis</i> 2274	87
4.2	Physico-chemical characteristics of the soil collected from tomato rhizosphere (Farrukhnagar, Haryana) for isolation of antagonistic strains against <i>Fusarium oxysporum</i>	89
4.3	Percentage inhibition of fungal strains, isolated from tomato rhizosphere, against <i>Fusarium oxysporum</i> in <i>in vitro</i> dual culture assay	90
4.4	Characterization of shortlisted <i>Bacillus</i> isolates for antifungal efficacy and plant growth promoting properties	92

4.5	Efficacy of chemical pesticides against <i>Fusarium oxysporum</i> where A= mancozeb, B= carbendazim, C= chlorpyrifos and x=1000 ppm (recommended dose). In each column, significantly different values by Duncan multiple range test ($p < 0.05$) are marked with different lowercase letters. Values are depicted with SD of $n=3$.	97
4.6	Profile of volatile organic compounds produced by <i>Bacillus subtilis</i> , <i>Trichoderma harzianum</i> and <i>Bacillus</i> isolate B44 identified by GC-MS using solid phase microextraction.	104
4.7	Percentage inhibition of culture filtrate (CF) of (a) <i>Trichoderma harzianum</i> , (b) <i>Bacillus subtilis</i> and (c) <i>Bacillus</i> isolate B44 against <i>Fusarium oxysporum</i> . In each column, significantly different values by Duncan multiple range test ($p < 0.05$) are marked by different lowercase letters. Values are depicted with standard deviation for $n=3$.	117
4.8	Major compounds in culture filtrate of (a) <i>Bacillus subtilis</i> , (b) <i>Trichoderma harzianum</i> and (c) <i>Bacillus</i> isolate B44 identified by GC-MS in ethyl acetate extract.	121
4.9	Plant growth parameters of tomato plants treated with T1= <i>Trichoderma harzianum</i> + <i>Bacillus subtilis</i> + <i>Fusarium oxysporum</i> , T2= <i>B. subtilis</i> + <i>F. oxysporum</i> , T3= <i>T. harzianum</i> + <i>F. oxysporum</i> , T4= <i>F. oxysporum</i> , T5= carbendazim (1000 ppm) + <i>F. oxysporum</i> , T6= uninoculated control (only plant) and T7= <i>Bacillus</i> isolate B44 + <i>F. oxysporum</i> at (a) time point-1 (30 days after sowing), (b) time point-2 (60 days after sowing) and (c) time point-3 (90 days after sowing). For each column, significantly different values by Duncan multiple range test ($p < 0.05$) are marked with different letters. Values are depicted with standard deviation for $n=3$.	134
4.10	Antifungal spectrum of inoculants in <i>in planta</i> assay under natural conditions to control <i>Fusarium</i> wilt in <i>Solanum lycopersicum</i> where T1= <i>Trichoderma harzianum</i> + <i>Bacillus subtilis</i> + <i>F. oxysporum</i> , T2= <i>B. subtilis</i> + <i>F. oxysporum</i> , T3= <i>T. harzianum</i> + <i>F. oxysporum</i> , T4= <i>F. oxysporum</i> , T5= carbendazim (1000 ppm) + <i>F. oxysporum</i> , T6= uninoculated control (only plant) and T7= <i>Bacillus</i> isolate B44 + <i>F. oxysporum</i> .	137
4.11	Major compounds identified by UPLC-MS in bioactive fraction of ethyl acetate extract of <i>Bacillus subtilis</i> .	149
4.12	Major compounds identified by UPLC-MS in bioactive fraction of ethyl acetate extract of <i>Trichoderma harzianum</i> .	155

4.13	Major new compounds produced on co-culturing of <i>Trichoderma harzianum</i> and <i>Fusarium oxysporum</i> .	159
4.14	Major new compounds produced on co-culturing of <i>Bacillus subtilis</i> and <i>Fusarium oxysporum</i> .	159
4.15	Profile of compounds produced by <i>Trichoderma harzianum</i> in mono inoculation and dual inoculation with <i>Fusarium oxysporum</i> .	160
4.16	Profile of compounds produced by <i>Bacillus subtilis</i> in mono inoculation and dual inoculation with <i>Fusarium oxysporum</i> .	161
4.17	Response for water dispersible granules developed with combination of different substrates, bulking agent, dispersing agent and wetting agent.	164
4.18	Experimental design and results of central composite design for the development of efficient water dispersible granules	165
4.19	ANOVA analysis for response 1 [wetting time (s)]	165
4.20	ANOVA analysis for the response 2 [dispersing time (s)]	166
4.21	ANOVA analysis for the response 3 [Suspensibility (%)]	166
4.22	Antifungal spectrum of inoculants in <i>in planta</i> assay under natural conditions to control <i>Fusarium</i> wilt in <i>Solanum lycopersicum</i> .	170
4.23	Mycelial growth inhibition in <i>in vitro</i> volatile assay of cakes against <i>F. oxysporum</i> . In each column, significantly different values by Duncan multiple range test ($p < 0.05$) are marked with different letters. Values are depicted with standard deviation for $n=3$.	175
4.24	Physico-chemical parameters of mustard cake	178
4.25	Major volatile organic compounds extracted from mustard cake using solid phase microextraction with their retention time, area, molecular weight and chemical formula.	181
4.26	Major compounds identified by GC-MS in ethyl acetate fraction of mustard cake aqueous extract with their retention time, area, molecular weight and chemical formula.	184
4.27	Germination percentage (GP) and seedling vigour index of tomato seeds upon treatment with different cake based treatments. In each column, significantly different values by Duncan multiple range test ($p < 0.05$) are marked with different letters. Values are depicted with standard deviation for $n=30$.	186
4.28	Antifungal spectrum of treatments in <i>in planta</i> assay under natural conditions to control <i>Fusarium</i> wilt in <i>Solanum lycopersicum</i> .	190

ABBREVIATIONS

°C	Degree centigrade
°E	Degree east
°N	Degree north
6-PP	6-pentyl-2H-pyran-2-one
ACC	1-aminocyclopropane-1-carboxylate
AI	Active ingredient
ANNOVA	Analysis Of Variance
BLAST	Basic local alignment search tool
bp	Base pair
BSA	Bovine serum albumin
Ca Cl ₂	Calcium chloride
CCD	Central composite design
CFU	Colony forming unit
CHCl ₃	Chloroform
CMC	Carboxymethylcellulose
DAI	Days after incubation
DAS	Days after sowing
df	Degree of freedom
DGGE	Denaturing gradient gel electrophoresis
DMRT	Duncan's new multiple range test
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNS	Dinitrosalicylic acid
DSI	Disease severity index
EC	Electrical conductivity
Fig.	Figure
g	Gram
GC	Guanine-cytosine
GC	Gas chromatography
GP	Germination percentage
h	Hour
H	Shannon-index
HCN	Hydrogen cyanide
HPLC	High performance liquid chromatography
IAA	Indole acetic acid
ITCC	Indian Type Culture Collection
l	Litre
LB	Luria-Bertani broth
M	Molar
m/z	Mass/charge

mg	Milligram
min	Minutes
ml	Millilitre
mm	Millimetre
mM	Millimolar
mRNA	Messenger RNA
MS	Mass spectrophotometry
MTCC	Microbial Type Culture Collection
MTT	3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide
N	Normal
NA	Nutrient agar
NAG	N-acetyl glucosamine
NCBI	National Center for Biotechnology Information
nm	Nanometre
OCSs	Oil seed cakes
OD	Optical density
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PDB	Potato dextrose broth
PE	Petroleum ether
PGP	Plant growth promoting
PGPR	Plant growth promoting rhizobacteria
pmole	Picomoles
PO ₄	Phosphate
ppm	Parts per million
psi	Pound-force per square inch
qpcr	Quantitative-polymerase chain reaction
Rf	Retention factor
rpm	Revolutions per minute
RSM	Response surface methodology
s	Seconds
S	Richness
SD	Standard deviation
SEM	Scanning electron microscope
SPME	Solid phase microextraction
SSF	Solid state fermentation
TAE	Tris-acetate-ethylenediaminetetraacetic acid
TE	Tris- ethylenediaminetetraacetic acid
TLC	Thin layer chromatography
TMS	Trimethylsilyl
TSA	Tryptic soy sgar
U ml ⁻¹ min ⁻¹	Units of activity per ml per minute

UPGMA	Unweighted pair group method with arithmetic mean
UPLC	Ultra performance liquid chromatography
USD	United states dollars
UV-VIS	Ultraviolet–visible
v/v	Volume/volume
VOCs	Volatile organic compounds
w/v	Weight/volume
WDG	Water dispersible granules
Zn	Zinc
μl	Microlitre
μS/cm	Microsiemens per centimetre