

# Contents

<b>1. Introduction.....</b>	<b>1 -9</b>
<b>2. Literature review .....</b>	<b>10 – 50</b>
<b>3. Materials and Methods .....</b>	<b>51 - 91</b>
3.1. Collection of soil sample .....	51
3.2 Primary Screening.....	51
3.2.1 Isolation of microorganism from tea rhizosphere...	51
3.2.2. Isolation of phosphate solubilizing microorganism from tea rhizosphere.....	51
3.2.3. Quantitative measurement of phosphate solubilization in culture medium .....	52
3.2.4. Isolation and Identification of AM spores from rhizosphere soil.....	52
3.2.4.1 Screening of root for VAM infection.....	52
3.2.5. Isolation and identification of fungal pathogens.....	53
3.3. <i>In vitro</i> testing for antagonism to pathogens.....	53
3.3.1. Fungus.....	53
3.3.1.1 Solid medium .....	53
3.3.1.2. Liquid media.....	54
3.3.2. Bacteria.....	54
3.3.2.1 Solid medium.....	54
3.3.2.2. Liquid medium .....	54
3.4. Selection and identification of antagonistic microorganism.....	55
3.4.1 Microscopic observation for bacteria .....	55
3.4.1.1. Biochemical tests .....	55
3.4.2. Microscopic observation for fungus.....	56
3.5. Plant material .....	57
3.5.1. Tea.....	57
3.5.1.1. Propagation of tea .....	57
3.5.1.2. Plantation.....	59
3.5.2. Marigold.....	59
3.5.3. Soybean.....	59
3.6. Test organisms.....	60
3.6.1. Fungal Culture.....	60
3.6.2. Source of culture.....	60
3.7. Assessment of mycelial growth.....	60
3.7.1. Solid media .....	60
3.7.2. Liquid media.....	61
3.8. Bioinoculants.....	62
3.8.1. Application procedure.....	62
3.8.1.1. Kalisena .....	62
3.8.1.2. Josh.....	62
3.9. Assessment of bacterial growth.....	62
3.10. Inoculation techniques and disease assessment.....	63
3.10.1. Preparation of inoculum.....	63
3.10.1.1. Sand maize meal medium.....	63

3.10.1.2. Tea root pieces.....	64
3.10.2. Inoculation of healthy tea plants.....	64
3.10.3 Disease assessment.....	64
3.11. Joint inoculation of Biocontrol agents.....	65
3.12. <i>In vitro</i> characterization of selected fungi and bacteria for Plant growth Promoting activity.....	65
3.12.1. Phosphate solubilization.....	65
3.12.1.1. Quantification of P solubilization .....	65
3.12.2. Chitinase production .....	66
3.12.3. Siderophore production .....	66
3.12.4. HCN production .....	67
3.12.5. IAA production.....	67
3.12.6. Volatile production.....	67
3.13. Extraction of antifungal compounds from PGPR.....	68
3.13.1. Cell free culture filtrate .....	68
3.13. 2. Solvent extraction.....	68
3.13.3. Bacterial cell.....	68
3.14. Bioassay of active principle.....	69
3.14.1. Spore germination .....	69
3.14.2. Sclerotial germination.....	69
3.14.3. Radial growth: .....	69
3.14.4. Agar cup bioassay.....	70
3.14.5. Liquid media .....	70
3.15. Application of bacteria.....	70
3.15.1. Soil drench.....	70
3.15.2. Foliar spray.....	70
3.15.3. Seed bacterization .....	70
3.16. <i>In vivo</i> tests of plant growth promoting activity of selected fungus and bacteria.....	71
3.16.1. Tea.....	71
3.16.2. Soybean.....	71
3.16.3. Marigold.....	72
3.17. Extraction and estimation of Chlorophyll .....	72
3.17.1. Extraction .....	72
3.17.2. Estimation.....	72
3.18. Extraction of phenol contents from leaves and roots .....	72
3.18.1. Estimation of phenols contents.....	73
3.18.1.1. Total phenol.....	73
3.18.1.2. Ortho-dihydroxyl phenol.....	73
3.19 Extraction of Catechin.....	73
3.19.1. HPLC analysis of catechins.....	74
3.20. Extraction of enzyme from leaves.....	74
3.20.1. $\beta$ -1, 3- glucanase, .....	74
3.20.2. Chitinase.....	74
3.20.3. Phenylalanine ammonia lyase (PAL).....	74
3.20.4. Peroxidase.....	74
3.20.5. Polyphenol oxidase.....	75
3.21. Assay of enzyme activities .....	75

3.21.1. $\beta$ -1, 3- glucanase .....	75
3.21.2. Chitinase.....	75
3.21.3. Phenylalanine ammonia lyase.....	76
3.21.4. Peroxidase.....	76
3.21.4. Peroxidase.....	76
3.21.5. Polyphenol oxidase.....	76
3.22. Mass multiplication of AM fungi.....	77
3. 22.1 VAM spore germination.....	77
3.22.2. VAM root staining.....	77
3.23. Modified Morgan Extraction for Phosphorous from soil.....	77
3.24. Extraction of proteins .....	79
3.24.1 Leaves .....	79
3.24.2 Mycelia.....	79
3. 25. Immunological studies.....	80
3.25.1 Preparation of antigen.....	80
.25.1.1. Bacterial antigens.....	80
3.25.1 2. Fungal antigen.....	80
3.25.1. 3. Soil antigen.....	80
3.25.2 Estimation of protein content and SDS-PAGE analysis of antigenic proteins.....	80
3.25.2.1 Estimation of protein content: .....	80
3.25.2.3. SDS-PAGE analysis of soluble proteins..	81
3.25.2.4 Preparation of stock solution.....	81
3.25.2.5 Preparation of gel.....	82
3.25.2.6 Sample preparation .....	83
3.25.2.7 Electrophoresis .....	83
3.25.2.8 Fixing and staining.....	83
3.26. Raising of polyclonal antibodies .....	83
3.26.1. Rabbits and their maintenance .....	83
3.26.2. Immunization .....	84
3.26.3. Bleeding .....	84
3.27. Purification of IgG .....	84
3.27.1 Precipitation'.....	84
3.27.2 Column preparation.....	85
3.27.3 Fraction collection.....	85
3.28. Immunological assays.....	85
3.28.1 Preparation of agarose slides.....	85
3.28.2. Diffusion.....	86
3.28.3. Washing, staining and drying of slides.....	86
3.29. Determination of bacterial sustainability in soil.....	86
3.29.1. Enzyme linked Immunosorbent assay ( ELISA)....	86
3.29.2. Plate trapped antigen coated (PTA) ELISA.....	87
3.29.3. Dot blot.....	88
3.30. Determination of pathogen in soil by immunological method.....	89
3.30.1. ELISA.....	89
3.30.2. Dot- blot.....	89
3.30.3. Colony-blot .....	89
3.31. Fluorescence antibody staining and microscopy.....	90

3.31.1. Fungal mycelia .....	90
3.31.2. Cross section of tea .....	90
3.31.3. Immunocytochemical staining.....	91
3.31.4. Substrate stain solution.....	91
<b>4. Experimental .....</b>	<b>92 - 180</b>
4.1 Charcoal stump rot disease.....	92
4.1.1 Disease incidence in Sikkim hills.....	93
4.1.2 Varietal resistance test against <i>U.zonata</i> .....	95
4.2 Cultural characteristics of the pathogen ( <i>U.zonata</i> ).....	96
4.2.1. Media.....	96
4.2.2 Temperature.....	97
4.2.3 Incubation period.....	97
4.2.4 pH.....	99
4.2.5 Carbon sources.....	99
4.2.6 Nitrogen sources.....	100
4.3 Analyses of soil samples of temi tea Estate.....	102
4.4 Isolation of microorganism from tea rhizosphere and their identification.....	103
4.4.1 Isolation of microorganism.....	103
4.4.2 Identification of fungal isolates.....	104
4.5 Rhizosphere microorganisms of tea and their in vitro interaction with root pathogens.....	108
4.6 Screening of Phosphorous solubilizing fungi and bacteria from tea rhizosphere.....	111
4.6.1 Phosphorous solubilization efficiency on PVK plates.....	113
4.6.2 Evaluation of phosphorous solubilization by fungal isolates in PVK broth amended with tricalcium phosphate (TCP) and rock phosphate (RP).....	113
4.7 Searching for Arbuscular mycorrhizal fungi from tea plants grown in Sikkim hills and plains.....	117
4.8 Biochemical changes in tea plants following inoculation with <i>U. zonata</i> .....	123
4.8.1 Estimation of phenol content.....	123
4.8.1.1 Total phenol.....	125
4.8.1.2 Ortho-dihydroxy phenol.....	125
4.8.2 Determination of enzyme activities.....	126
4.8.2.1. Phenyl alanine ammonia lyase.....	126
4.8.2.2. Peroxidase.....	127
4.8.2.3. Polyphenol oxidase.....	127
4.9 Cultures Characteristics of PSB ( <i>B. pumilus</i> ) and PSF ( <i>A. niger</i> ) isolates.....	128
4.9.1 PSB isolates ( <i>B. pumilus</i> ).....	129
4.9.1.1 pH.....	129
4.9.1.2 Temperature.....	129
4.9.1.3 Media.....	129
4.9.1.4 Incubation period.....	129
4.9.1.5 Antibiotic sensitivity.....	129
4.9.2 PSF isolates ( <i>A. niger</i> ) .....	130

4.9.2.1 pH.....	130
4.9.2.2 Temperature .....	130
4.9.2.3 Media.....	130
4.10 in vitro studies of selected PSF and PSB isolates	
against test pathogen.....	130
4.10.1 Phosphate solubilizing fungus.....	130
4.10.1.1 Solid medium.....	131
4.10.1.2 Liquid medium.....	131
4.10.2 Phosphate solubilizing bacterium.....	132
4.10.2.1 Solid medium.....	132
4.10.2.2 Liquid medium .....	132
4.11. Effects PSF application on growth of tea plants.....	133
4.12. Effects of PSF application on Charcoal stump rot disease	
development of tea.....	133
4.13. Biochemical changes in PSF treated tea plants following	
inoculation with <i>U. zonata</i> .....	134
4.14. Effect of PGPR ( <i>Bacillus pumilus</i> ) application on the growth	
of tea plants.....	136
4.15. Effects of <i>B. pumilus</i> application on disease development in tea.....	139
4.16. Biochemical changes in tea following application of <i>B. pumilus</i> .....	140
4.16.1. Phenol content.....	140
4.16.2. Chlorophyll.....	145
4.16.3. Enzymes activities.....	145
4.17. <i>In vitro</i> determination of mechanism of action of <i>B. pumilus</i> .....	147
4.17.1. Phosphate solubilization.....	147
4.17.2 IAA production.....	147
4.17.3. Siderophore production.....	147
4.17.4. HCN production.....	147
4.17.5. Chitinase production.....	148
4.17.6. Volatile production.....	148
4.18. Bioassay of active principle from <i>B. pumilus</i> against test fungi.....	149
4.19. Application of Bioformulations on growth of tea plants.....	149
4.20. Disease assessment following treatments .....	161
4.21. Biochemical changes in tea plants following application of	
bioformulations.....	161
4.22. Field application of <i>G. mosseae</i> and <i>B. pumilus</i> and plant growth.....	167
4.22.1 Catechins.....	167
4.23 Immudetection of <i>U. zonata</i> and <i>B. pumilus</i> .....	171
4.23.1. Immunodetection of <i>U. zonata</i> in soil .....	176
4.23.2. Detection of <i>U. zonata</i> in infected tea root tissues.....	178
4.23.3 Dectection of <i>B. pumilus</i> in soil.....	179
<b>5. Discussion.....</b>	<b>181 - 200</b>
<b>6. Summary.....</b>	<b>201 - 203</b>
<b>7. References.....</b>	<b>204 - 232</b>
<b>8. Corrigendum.....</b>	<b>233 - 234</b>