

C O N T E N T S

	Page
1. INTRODUCTION ..	1
2. REVIEW OF LITERATURE ..	5
3. MATERIALS AND METHODS ..	52
3.1 Plant Material ..	52
3.1.1 Selection ..	52
3.1.2 Source ..	52
3.1.3 Plantation ..	52
3.1.4 Growth and Maintenance ..	54
3.2 Fungal Culture ..	54
3.2.1 Source ..	54
3.2.2 Completion of Koch's postulate ..	54
3.2.3 Maintenance of stock culture ..	55
3.2.4 Assessment of mycelial growth in liquid media ..	55
3.3 Inoculation Technique ..	55
3.3.1 Detached leaf ..	55
3.3.2 Cut shoot ..	56
3.3.3 Whole plant ..	56
3.4 Disease Assessment ..	57
3.4.1 Detached leaf ..	57
3.4.2 Cut shoot ..	57
3.4.3 Whole plant ..	57
3.5 Composition of media ..	58
3.6 Extraction of Total Soluble Protein ..	63
3.6.1 Leaf protein ..	63
3.6.2 Mycelial protein ..	64
3.7 Estimation of Total Soluble Protein Content ..	64

(Contd.....)

	Page
3.8 SDS-Polyacrylamide Gel Electrophoresis of Soluble Protein ..	64
3.8.1 Preparation of gel solution and gel column ..	64
3.8.2 Sample preparation ..	66
3.8.3 Electrophoresis ..	67
3.8.4 Fixing ..	67
3.8.5 Staining ..	67
3.9 Total and Orthodihydroxy Phenol Contents of Healthy and <u>P. theae</u> inoculated tea leaves ..	67
3.9.1 Extraction ..	67
3.9.2 Estimation ..	68
3.10 Collection of Leaf Diffusates and Fungi-toxic Assay ..	69
3.11 Detection of Antifungal Compound from Healthy and <u>P. theae</u> inoculated tea leaves ..	69
3.11.1 Extraction ..	69
3.11.2 Chromatographic analysis ..	70
3.11.3 Bioassay ..	70
3.11.3.1 Radial growth ..	70
3.11.3.2 Chromatogram inhibition ..	71
3.11.3.3 Spore germination ..	71
3.11.4 UV-Spectrophotometric analysis ..	71
3.12 Cell Wall of <u>Pestalotiopsis theae</u> ..	72
3.12.1 Isolation ..	72
3.12.2 Preparation of mycelial wall extract..	72
3.12.3 Estimation ..	73
3.12.3.1 Carbohydrate ..	73
3.12.3.2 Protein ..	73
3.12.4 Bioassay of mycelial wall extract ..	73
3.12.5 Characterization ..	74
3.12.5.1 SDS-PAGE analysis ..	74
3.12.5.2 Binding of FITC labelled concanavalin A ..	76

	Page
3.13 Phyllosphere Microorganisms of Tea ..	76
3.13.1 Isolation ..	76
3.13.2 Identification ..	77
3.13.2.1 Fungi ..	77
3.13.2.2 Bacteria ..	77
3.13.3 <u>In vitro</u> interaction ..	83
3.13.3.1 Solid medium ..	83
3.13.3.2 Liquid medium ..	83
3.14 Culture Filtrate of Antagonistic Microorganisms ..	84
3.14.1 Preparation ..	84
3.14.2 Bioassay ..	84
3.15 Separation of Active Principle ..	85
3.15.1 Solvent Extraction ..	85
3.15.2 Thin-layer Chromatography ..	85
3.15.3 Bioassay ..	86
3.15.3.1 Agar cup ..	86
3.15.3.2 Radial growth ..	86
3.15.3.3 Spore germination ..	86
3.15.4 UV analysis ..	86
3.16 <u>In vivo</u> assay ..	87
3.16.1 Detached leaf ..	87
3.16.2 Cut shoot ..	87
4. EXPERIMENTAL ..	88
4.1 Varietal resistance test of tea against <u>Pestalotiopsis theae</u> ..	88
4.1.1 Detached leaf ..	88
4.1.2 Cut shoot ..	92
4.1.3 Whole plant ..	92
4.2 Cultural characteristics of <u>P. theae</u> ..	97

	Page
4.3 Factors influencing mycelial growth and spore germination of <u>P. theae</u> ..	100
4.3.1 Mycelial growth ..	100
4.3.1.1 Incubation period ..	100
4.3.1.2 pH ..	102
4.3.1.3 Carbon sources ..	105
4.3.1.4 Nitrogen sources ..	105
4.3.2 Spore germination ..	109
4.3.2.1 Concentration ..	109
4.3.2.2 Incubation period ..	110
4.3.2.3 pH ..	110
4.3.2.4 Age of culture ..	110
4.4 Studies on host and parasite proteins ..	113
4.4.1 Comparison of protein content of healthy and <u>P. theae</u> inoculated tea leaves ..	113
4.4.2 Mycelial protein content of <u>P. theae</u> ..	113
4.4.3 SDS-PAGE analysis of mycelial protein of <u>P. theae</u> ..	116
4.4.4 Analysis of protein pattern of healthy and <u>P. theae</u> inoculated tea leaves ..	116
4.5 Determination of levels of phenolics in healthy and <u>P. theae</u> inoculated tea leaves ..	119
4.5.1 Characterization of simple phenolics in healthy leaves ..	119
4.5.2 Levels of total phenols in healthy and <u>P. theae</u> inoculated tea leaves ..	121
4.5.3 Levels of orthodihydroxy phenols in healthy and <u>P. theae</u> inoculated tea leaves ..	121

	Page
4.6 Studies on the leaf diffusates of tea ..	126
4.6.1 Biological activities ..	126
4.6.2 UV-spectral analysis ..	126
4.7 Detection of antifungal compounds in tea leaves after challenge with <u>P. theae</u> ..	129
4.7.1 Radial growth bioassay ..	132
4.7.2 TLC plate bioassay ..	134
4.7.3 Chromogenic spray ..	135
4.7.4 Glass slide bioassay ..	135
4.7.5 UV-spectrophotometric analysis ..	136
4.8 Studies on elicitors of <u>P. theae</u> ..	140
4.8.1 Determination of chemical nature of mycelial wall extract ..	140
4.8.1.1 SDS-PAGE analysis ..	141
4.8.1.2 ConA-FITC binding ..	141
4.8.2 Determination of nature of disease reaction elicited by the elicitor ..	141
4.8.3 Bioassay of diffusible compounds elicited by the elicitor ..	145
4.9 Studies on phylloplane microorganisms of tea and their <u>in vitro</u> interaction with <u>P. theae</u> ..	145
4.9.1 Isolated microorganisms of tea phyllosphere and their identification ..	148
4.9.2 <u>In vitro</u> interaction with <u>P. theae</u> ..	148
4.9.2.1 Solid medium ..	148
4.9.2.2 Liquid medium ..	162
4.9.2.3 Replacement of culture medium ..	162

	Page
4.10 <u>In vivo</u> tests with selected antagonistic microorganisms ...	168
4.10.1 Effect of aqueous cell suspension, washed cell and cell-free culture filtrate on disease development ..	168
4.10.2 Effect of different ratios of antagonists and <u>P. theae</u> on disease development ..	181
4.11 Studies on the cell-free culture filtrates of <u>B. pumilus</u> and <u>Micrococcus</u> sp. ..	181
4.11.1 Bioassay of cell-free culture filtrates ..	183
4.11.2 Separation of active principles from cell-free culture filtrates ..	184
4.11.2.1 Bioassay of active principle ..	184
4.11.2.1.1 Spore germination ..	184
4.11.2.1.2 Radial growth ..	185
4.11.2.1.3 Agar cup ..	186
4.11.2.2 UV-spectral analysis ..	187
4.11.3 Effect of incubation period on the production of antifungal compound ..	190
4.11.4 Dose response test ..	192
4.12 Partial purification of active principle from <u>B. pumilus</u> and <u>in vivo</u> test ..	192
4.12.1 Partial purification ..	192
4.12.1.1 Preparative TLC and bioassay ..	194
4.12.1.2 UV-Spectrophotometry ..	194
4.12.2 <u>In vivo</u> test ..	197
4.12.2.1 Detached leaf ..	197
4.12.2.2 Cut shoot ..	199
4.12.2.3 Whole plant ..	201

(Contd.....)

		Page
5. DISCUSSION	...	202
6. SUMMARY	...	218
7. REFERENCES	..	223
