

Table of contents

Acknowledgement		iii
Abstract		iv
บทคัดย่อ		vii
Table of contents		x
List of table		xii
List of illustrations		xiv
Chapter 1	Introduction	1
Chapter 2	Review of Literature	4
	2.1 Mango in General	4
	2.2 Mango Anthracnose Fruit Rot in Thailand : State of the Art and Current Export Problem	5
	2.3 Crude Extract from Thai Medicinal Plants to Control <i>Colletotrichum gloeosporioides</i> (Penz.) Sacc.	6
	2.4 Fruit Development and Harvest Maturity	7
	2.5 Ripening Processes	8
	2.6 Antifungal Compounds in Plants	20
	2.7 Method of Plant Analysis	24
	2.8 Bioassay	62
	2.9 Mode of Action	66
	2.10 Influence of Fungicides on Fungal Fine Structure	81
	2.11 Formulation	87

Chapter 3	LABORATORY STUDIES ON THE EFFICIENCY OF CRUDE EXTRACT TO INHIBIT THE GROWTH OF <i>COLLETOTRICHUM GLOEOSPORIOIDES</i> (Penz.) Sacc.	97
	3.1 Introduction	97
	3.2 Material and Methods	97
	3.3 Result and Discussion	107
	3.4 Conclusion	135
Chapter 4	EFFECT OF 1'-ACETOXYCHAVICOL ACETATE AND β-ASARONE ON RIPENING	137
	4.1 Introduction	137
	4.2 Material and Methods	137
	4.3 Result and Discussion	144
Chapter 5	FORMULATION OF 1'-ACETOXYCHAVICOL ACETATE	171
	5.1 Introduction	171
	5.2 Material and Methods	172
	5.3 Result and Discussion	173
Chapter 6	Conclusion	180
Reference		182
Curriculum vitae		207

List of table

Table	Page
2.1 Carotenoid content in the ripe pulp of some mango cultivars.	11
2.2 R_f data of flavonol 5-methyl ethers: compation of actual R_f and R_f calculated from ΔR_m	32
2.3 Spectral properties of the different classes of plant pigment.	43
2.4 Some proton nuclear magnetic resonance chemical shifts characteristic of different classes of plant products.	50
2.5 The type of criteria needed for the identification of known plant constituents.	55
2.6 Davis's HLB group Number	94
3.1 Percentage yield (w/w) of crude extract from galanga, sweetflag and <i>Rhinacanthus nasutus</i> Kurz. when using different solvent extraction.	110
3.2 Percentage inhibition of crude extracts from galanga, sweetflag, and <i>Rhinacanthus nasutus</i> Kurz. when studied with <i>Cladosporium cladosporioides</i> and poison food PDA.	111
3.3 The inhibition percentage of crude extracts from galanga, sweetflag and <i>Rhinacanthus nasutus</i> Kurz. on the colonial growth of <i>Cladosporium gloeosporioides</i> (Penz.) Sacc.	113
3.4 R_f value of clear zone from TLC-bioassay of crude extract from sweetflag and galanga.	117
3.5 Information from data file of beta-asarone.	120
3.6 Percent inhibition of active substance on mycelium growth of <i>Colletotrichum gloeosporioides</i> (Penz.) Sacc. according to Abbott's formula.	130
3.7 Percentage inhibition of spore germination of <i>Colletotrichum gloeosporioides</i> (Penz.) Sacc. as affected by active substance from sweetflag and galanga.	133

Table	Page
4.1 Concentration of β -asarone and 1'-acetoxychavicol acetate in different dipping solutions	138
4.2 Percentage of inhibition of β -asarone and 1'-acetoxychavicol acetate at different concentration	145
4.3 Disease incidence on mango fruits at 18 days after dipped in active substance solution.	148
4.5 Disease rate of mango fruits treated with β - asarone and 1' – acetoxychavicol acetate on day 21 after storage.	151
4.6 Peel firmness (kg/cm^2) of mango fruit treated with β - asarone and 1'-acetoxychavicol acetate.	153
4.7 Change in flesh firmness in different storage day as affected by β - asarone and 1'-acetoxychavicol acetate.	154
4.8 Effect of β - asarone, 1' – acetoxychavicol acetate on changes in flesh colour, flesh aroma and taste of mango fruit during of storage (Consumer's Perception).	162
4.9 Consumer's perception of the fruits treated with β - asarone, 1' – acetoxychavicol acetate.	163
5.1 Q.C. Report from the Company showing the major content of 1'-acetoxychavicol acetate formulations and the conditions achieved.	175
5.2 Poison food studies of 1'-acetoxychavicol acetate formulation to control <i>Colletotrichum gloeosporioides</i> (Penz.) Sacc.	176
5.3 Efficiency of formulation and benomyl to control postharvest anthracnose disease of mango fruits CV. Nam – Dok – Mai.	179

List of illustrations

Figure	Page
2.1 Some inhibitins from plant.	22
2.2 Representative phytoalexins.	23
2.3 A general procedure for extracting fresh plant tissues and fractionating into different classes according to polarity.	28
2.4 GLC trace of the separation of the sterol acetates present in oat seed.	36
2.5 HPLC traces of the flavonoids of two species of <i>Chondropetatum</i> , where the same compounds are present but in different amount.	39
2.6 Ultraviolet adsorption spectrum of the <i>Xanthone mangiferin</i> .	42
2.7 Infrared spectra of two alkaloids from tobacco smoke.	45
2.8 Mass spectrum of the growth regulator zeatin.	47
2.9 Proton NMR spectrum of the flavone luteolin (as the trimethylsilyl ether).	52
2.10 Carbon – 13 shifts relative to TMS (in p.p.m.) for the different carbon atoms in the molecule of the spirobenzylisquinoline alkaloid sibiricine from <i>Corydalis sibirica</i> .	53
2.11 The effect of cuprous oxide on germination of conidia of <i>Macrosporium sarcinaeforme</i> .	63
2.12 Effect of cycloheximide on the germination of <i>Cladoporium</i> sp. Conidia.	64
2.13 Investigative steps of initial mode of action Studies.	72
2.14 Comparison of enzyme substrates with inhibitor structures.	76
2.15 Natural product with antifungal activities.	77
2.16 Differences in chemical complexity of natural inhibition.	82
2.17 Site of action of systemic fungicides on fungal fine structure.	83
2.18 Hansen's solution parameter.	93
2.19 Variation of type and amount of residue emulsion	93

Figure	Page
3.1 Iodine tank with developed TLC-plate inside.	101
3.2 Spraying of <i>Cladosporium cladosporioides</i> spore suspension on TLC-plate and incubation in moist chamber for 48 hrs.	102
3.3 Active fraction on TLC-plate after sprayed with <i>Cladosporium cladosporioides</i> spores.	103
3.4 Fractional group arrangement by PTLC from a crude of galanga.	105
3.5 Confirmation PTLC-bioassay on the fractional group arrangement by <i>Cladosporium cladosporioides</i> .	105
3.6 Ring layers of <i>Colletotrichum gloeosporioides</i> (Penz.) Sacc. and black colour sporemass of <i>Cladosporium cladosporioides</i> at 15 days on PDA.	108
3.7 Acervulus, conidiophore, and conidia of <i>Colletotrichum gloeosporioides</i> (Penz.) Sacc. from mango peel and drawing.	109
3.8 Colony of <i>Colletotrichum gloeosporioides</i> (Penz.) Sacc. on poison food PDA mixed with crude extract from galanga (G), sweetflag (Sf), and <i>Rhinacanthus nasutus</i> Kurz. (Rh) at 500 ppm and control (Ct).	113
3.9 Colony of <i>Colletotrichum gloeosporioides</i> (Penz.) Sacc. on poison food PDA mixed with crude extract from galanga (G) sweetflag (Sw) and <i>Rhinacanthus nasutus</i> Kurz. (Rh) at 1,000 ppm and control (Ct).	114
3.10 Clear zones of control treatment (CT), <i>Rhinacanthus nasutus</i> Kurz. (Rh), sweetflag (Sw), and galanga (G) on TLC-bioassay plate using <i>Cladosporium cladosporioides</i> as indicator.	115
3.11 <i>R_f</i> of each fraction on TLC-plate of sweet flag (Sw) and galanga (G).	116
3.12 No spore germination and mycelium development was found on the plate treated with A13, L13, L14 and L15 but well grew in control treatment (Ct).	116
3.13 Germination of spore and growth of mycelium of <i>Cladosporium cladosporioides</i> on PDA after 14 days of treatment with A12 and A13 compared to control (Ct).	118

Figure	Page
3.14 Germination of spore and growth of mycelium of <i>Cladosporium cladosporioides</i> on PDA after 14 days of treatment with L12, L13, L14 and L15 compared to control (Ct).	118
3.15 Data file of GC-MS of beta –asarone fraction.	121
3.16 Acquisition comment of beta-asarone from IR-resonance (HITACHI R-1500)	122
3.17 Proposed fragmentation of cis-beta- asarone.	123
3.18 The data file of 1' – acetoxychavicol acetate from GC-MS.	125
3.19 The major component as 1' – acetoxychavicol acetate in the data file of GC-MS.	126
3.20 Acquisition comment of 1' – acetoxychavicol acetate from IR –resonance (HITACHI R-1500).	127
3.21 Proposed fragmentation of 1' acetoxychavicol acetate	128
3.22 Clear zone of <i>Colletotrichum cladosporioides</i> surroundy paper disc saturated with β - asarone at the concentration 70 and 1'–acetoxychavicol at 100 ppm. compared to control.	130
3.23 Dosage response curve (DR-curve) of β -asarone and 1'–acetoxychavicol acetate.	132
3.24 Germination of spore of <i>Colletotrichum gloeosporioides</i> (Penz.) Sacc. in control treatment.	134
3.25 Inhibition of spore germination in <i>Colletotrichum gloeosporioides</i> (Penz.)Sacc. when treated with 1' – acetoxychavicol acetate.	134
4.1 Incubation of mango fruit in transparent plastic bag keeping at room temperature at 7 days.	139
4.2 Positions on mango fruit use for detect the fruit firmness and change in peel and flesh colour.	141
4.3 Chamber for measure CO ₂ concentration by studying respiration rate.	142
4.4 Growth of <i>Colletotrichum gloeosporioides</i> (Penz.) Sacc. on PDA poison food mixed with 1' -acetoxychavicol acetate at different concentration compared to control.	145

Figure	Page
4.5 Growth of <i>Colletotrichum gloeosporioides</i> (Penz.) Sacc. on PDA poison food mixed with β -asarone at different concentration compared to control.	146
4.6 correlation equation showed optimum dosage (OD) with poison food technique as Minimum Fungicidal Concentration (MFC).	147
4.7 Effect of β -asarone or 1'-acetoxychavicol acetate (ACA) on control of fruit not disease compared to control.	149
4.8 Disease infested and peel colour of Nam-Dok-Mai mango fruit treated with 1'-acetoxychavicol acetate, β -asarone, benomyl, adjuvant and water.	155
4.9 Flesh colour and juiciness of mango fruit at 25 days (storage at room temperature 25 °C) after treated with 1'-acetoxychavicol acetate, β -asarone, adjuvant and water.	156
4.10 Change in respiration rate of mango fruits as affected by β -asarone and 1'-acetoxychavicol acetate.	158
4.11 Change in pH, percent titrable acid, and total soluble solid of mango fruits treated with β -asarone, 1'-acetoxychavicol acetate and benomyl.	160
4.12 Methylene blue absorbed hyphae of <i>Colletotrichum gloeosporioides</i> Penz. (Sacc.) after grown in different concentration of 1'-acetoxy chavicol acetate.	164
4.14 Ultrastructure of <i>Colletotrichum gloeosporioides</i> (Penz.) Sacc. Crosssection of hypha cell organelles and cell treated with 1'-acetoxychavicol acetate.	167
4.15 Spitzenkörper of <i>colletotrichum gloeosporioides</i> (Penz.) Sacc. showing active site of membrane and wall formation.	168
4.16 Active site of 1'-acetoxychavicol acetate.	169
5.1 Correlation equation curve between percentage inhibition and concentration of formulation.	176

5.2 Disease incidence on Num-Dok-Mai mango fruit after treated with water, 1'-acetoxychavical acetate formulation 570 ppm, and benomyl 224 ppm and kept moist at room temperature for 21 days.	178
---	------------

มหาวิทยาลัยเชียงใหม่
Chiang Mai University