Chapter 6

Conclusion

Formulation of active substance from galanga, sweet flag and *Rhinacanthus nasutus* Kurz. were studied to control anthracnose postharvest fruit rot in mango, which caused by fungus *Colletotrichum gloeosporioides* (Penz.) Sacc.. The results revealed that.

- 6.1 This fungus has the identify characteristics of growth pattern. Hypha spread out on PDA in many ring layer, which compiled of conidiomata. Colony consisted of aerial mycelium, the colour varied from grey to dark grey. Sporemass was orange in colour. Conidia was rod shape form with the average size of $3.23 \times 13.45 \,\mu m$ and acervulus size $39.5 \times 41.2 \,\mu m$.
- 6.2 Among the solvents studied only ethanol, methanol and dichloromethane crude extract from galanga and sweetflag showed a positive efficiency to inhibit spore germination and growth of Colletotrichum gloeosporioides (Penz.) Sacc.. Dichloromethane crude extracted from Rhinacanthus nasutus Kurz. had no antifungal efficiency to control Colletotrichum gloeosporioides (Penz.) Sacc.. The dicloromethane crude extracted was however better than alcoholic extracted, due to less water residue in the crude products.
- 6.3 The TLC studied showed the R_f value of active ingredient from galanga at 0.24-0.33, 0.38-0.45, 0.50-0.83, 0.87-0.97 and from sweetflag at 0.20-0.27, 0.42-0.53, 0.67-0.96. Structural elucidation by GC-MS and IR resonance confirmed the active ingredient in galanga and sweet flag to be 1' acetoxychavicol acetate and cis β asarone, respectively.

- 6.4 The optimum dosage (or minimum fungicidal concentration, MFC) of β -asarone and 1' acetoxychavicol acetate to inhibit growth and development of *Colletotrichum gloeosporioides* (Penz.) Sacc. were 375 ppm and 670 ppm, receptively. Both substances gave the similar best result as benomyl. Therefore a high potential to replace benomyl in postharvest mango treatment.
- 6.5 β -asarone showed comparatively better result to inhibit *Colletotrichum gloeosporioides* (Penz.) Sacc. than 1' acetoxychavicol acetate but a disadvantage in enhancing respiration rate of mango fruit, which consequently caused a lower fruit firmness and increased fruit weight loss. Together with a risk to be banned of β -asarone due to its carsinogenic activity in animal studies, 1' acetoxychavicol acetate is therefore the highest potential.
- 6.6 For mode of action studies under transmission electron micorscope, 1' acetoxychavicol acetate affected the fungus growth and development by interfere with membrane development in especially tonoplast and septate formation. Cytoplasm of the fungus cell are more condensed and darker in colour when studied under light microscope.
- 6.7 Since active ingredient from galanga, 1'acetoxychavical acetate, is directly combined with essential oil; the formulation of the substance must be produced in the form of emulsifier concentrate (EC.). Successful mixer were Triton X-100 (primary emulsifier), Agrisol P-135 (secondary emulsifier) and Xylene (solvent) in the proportion of 23.8 % active ingredient plus 3.5% Triton X 100, 3.2% Agrisol P 135 and 69.5% Xylene, respectively. Minimum fungicidal concentration study confirmed the efficiency of the formulation to control *Colletotrichum gloeosporioides* (Penz.) Sacc. At the concentration of 570 ppm, was the disease incident even less than the fruit treated with benomyl.