## CHAPTER 3 MATERIALS AND METHODS

## Survey and collecting of Tar spot disease

Surveying can collection were conducted in Chiang Mai province from 2014-2016. Specimen collection was carried out by observing Tar spot symptoms on the leaves surface. The symptoms are characterized by shiny blackened leaf spots on upper side of leaf with dome- or clypeus-like stroma covering the immersed fructification of the fungal pathogens. The specimens that showed the tar spot symptoms were stored in the plastic bags. Collecting bags are sealed and labeled some information including name of host plants, collecting site, collector and collection date.

## Study morphology and of fungi cause Tar spot disease

The symptom on the leaves were examined directly under a stereo microscope to observe and clarify symptom. Samples were sectioned using a stereo and examined with a compound microscope. Squash mounts were made using water and Shear's reagent. Slides were examined with a compound microscope, and measurements of microscopic features, including stroma, ascomata, pedicel (a slender stalk or support of spores), asci, paraphysis and ascospore. Grosseries for fungal description were used as following the

Figure 4-7 **adans บหาวิทยาลัยเชียงไหม** Copyright<sup>©</sup> by Chiang Mai University All rights reserved



**Figure 4.** Shapes for fruitingbody of Phyllachorales (Wikimedia commons, 2017). Fungal ascomata characteristic A. Immersed perithecia of *Phyllchora*, B. Superficial perithecia of *Coccodielia*. C. Superficial perithecium of *Camarotella*, D. Superficial perithecia of *Trabutia*, E. Superficial perihecium of *Ophiodothella*.

MAI UNIV

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright<sup>©</sup> by Chiang Mai University All rights reserved



**Figure 5.** Conidiomatal types. A-F. Pycnidial, B. Dehiscence by a central circular ostiolae, C. dehiscence by a longitudinal ostiole (rephe), D. Superfical, E. Semiimmersed, F. Immersed, G-M, Pycnothyrial, G. With upper wall only, H. With upper and lower walls, I. With a central supporting column, J. Multilocular with several supporting columnns, K. Dehiscence from the margin, L. Dehiscence by a central ostiole, M. Dehiscence by irregular fissures, N. Acervular, O. Cupulate. P. Convoluted immersed, Q. Multilocular immersed, R. Multilocular superficial, S. Pseudostromatic (Kirk *et al.*,2008).



**Figure 6.** Shapes of Hamathecium subdivided in to interascal. A. Paraphysoids (trabeculae), B. Paraphyses, C. Periphyses and its development, D. Pseudoparaphyses and its development, E. Periphyses, F. Periphysoids (Modified from Prarce and Hyde, 2006).



**Figure 7.** Shapes based on the sphere and ellipsoids. 1. Globose or spherical, 2. Subglobose or prolate spheroidal, 3. Broadly ellipsoidal (sub-prolate) to ellipsoidal (prolate), 4. Oval (pre-prolate), 5. Fusifrom.



**Figure 8.** Shapes of ascospores and ascus. 2. Filiform, 3. Acerose, 4. Cylindrical, restricted to shapes where length to width is 2:1 to 3:2, (4a. bacilliform, 4b. with rounded apices, 4c. with truncated apices), 5. Discoid or lenticular (5a. in surface view, 5b. discoid in side view, 5c. lenticular in side view), 6. Sigmoid, 7. Reniform (faboform), 8. Allantoid, 9. Lunate (crescentic), 10. Falcate, 11. Ovoid, 12. Obovoid, 13. Lecythiform, 14. Pyrifrom, 15. Obpyriform, 16. Clavate, 17. Obclavate, 18. Capitate, 19. Spathulate (Kirk *et al.*, 2001).

All rights reserved