3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Field apparatus

(1) Altimeter

(2) Thermometer

(3) Soil moisture meter

(4) Wet/Dry thermometer

(5) Light intensity meter

(6) Paper and plastic bags

(7) Plastic box

(8) Trowel

(9) Materials for labelling purpose

(10) Camera with macrolens and colour slides

(11) Ruler

3.1.2 Laboratory apparatus

(1) Stereo microscope

(2) Compound microscope

(3) Micrometer

(4) Petridish 9 cm diameter

(5) Forceps

(6) Filter paper

(7) Tissue paper

(8) Lighter

(9) Needles

(10) Razor blade

(11) Gas lamp

(12) Laminar flow or transfer chamber

3.1.3 Chemical reagents

(1) 5%KOH

(2) Melzer's reagent

(3) Lactophenol

(4) Phloxine

(5) Alcoholic solution of gum guaiac.

(6) Sodium hypochlorite or Chlorox 10%

Composition and preparation are in Appendix 1

3.1.4 Culture media

(1) Potato Dextrose Agar (PDA)

(2) Malt Extract Agar (MEA) and

(3) Tannic Acid Agar (TCA)

Composition and preparation are in Appendix 2

3.2 Methods

3.2.1 The selection of study sites and sampling points

Three study areas were selected according to the altitude. They were site 1 with the altitude lower than 1,000 masl (Fig.2), consisted of three study points i.e. Huay Kaew waterfall, Wang Bua Baan and Monthatarn waterfall; site 2 was the area between 1,000 and 1,500 masl (Fig.3), composed of two study points i.e. Huay Kok Mah and the forest area near National Park Headquarters; and site 3 was the summit of Doi Suthep-Pui with the altitude higher than 1,500 masl (Fig.4), consisted of two study points i.e. San Koo and Laem Son.

3.2.2 Field investigation

Each study site was investigated at least once a month from October 1996 to January 1997. The following information were recorded: species and numbers of macrofungi found in each site; brief description of the characteristics, structures, colors of cap and stipe of macrofungi; date, substratum, habitat, relative humidity, soil moisture, air temperature, altitude, location, soil pH and light intensity at the ground level. Photos of the macrofungi were taken on site. Basidiocarps of some macrofungi in the family Ganodermataceae were also collected for laboratory examination.

3.2.3 Laboratory examination

(1) Pure cultures preparation

Small pieces of basidiocarp were cut aseptically by using razor blade and surface sterilized by putting in 10 percent Chlorox for 10 minutes, then rinsed in

Fig.2 Study site1; Monthatarn waterfall, altitude lower than 1,000 masl, December 1996.

Fig.3 Study site 2; the forest area near National Park Headquarter, altitude between 1,000 and 1,500 masl, December 1996.

Fig.4 Study site 3; Laem Son, altitude higher than 1,500 masl, December 1996.

sterile distilled water 3 times and blotted dry on the sterile tissue paper. After that, cultures were made by placing 5 pieces of sterile tissues on the potato dextrose agar or malt extract agar in 9 cm-petridish aseptically. The plates were incubated at room temperature (25 °C). The mycelial growth appeared in 3-7 days.

(2) Collection, preparation, and examination of basidiospores

Basidiospores were obtained in the laboratory by free-hand sectioning of the fruiting bodies from recently collected specimens. Basidiospores were examined by compound (bright-field) microscope. For bright-field examination, spores were rehydrated using Melzer's Reagent (MR) or 5% potassium hydroxide. Approximately twenty-five spores from each specimen were measured and calculated for the average spore size. Photos of the spores were taken at 400x using 35 mm Kodak Ektachrome film (Adaskaveg and Gilbertson, 1988).

(3) Macroscopic cultural study

A piece of mycelial culture grown in (1) was taken by a sterile (0.5 cm diameter) cork borer and placed on 2% MEA plate. The plate was incubated at room temperature (approximately 25 °C). Cultural morphology was then studied according to Nobles (1948, cited from Adaskaveg and Gilbertson, 1986).

(4) Identification of the samples

The collected macrofungi were classified by the macroscopic and microscopic features according to Ainsworth *et al.*(1973), Tosco (1973), Nilsson and Persson (1978), Pacioni (1981, 1985), Dickinson and Lucas (1982), Phillips (1983), Bessette and Sundberg (1987), Ellis and Ellis (1990), Horn *et al.* (1993) and Schalkwijk-Barendsen (1994).

(5) Measurement of growth rate of mycelium on MEA

One piece (0.5 cm diameter) of pure culture of fungi from (1) was aseptically taken and placed at the edge of 9 cm MEA plate. The plate was incubated in the dark at room temperature. The mycelial growth on each plate was measured every 7 days until the mycelial growth covered the surface of the medium.

(6) Chemical examination for the extracellular oxidase

One piece (0.5 cm diameter) of the pure culture of fungi was taken from (1) aseptically and placed at the edge of 9 cm MEA plate for one week at room temperature. The growing mycelium was tested by placing a drop of alcoholic solution of gum guaiac on the mycelium. No change in the color of mycelium indicated no extracellular oxidase formation (negative). The green or blue color change of the mycelium indicated the formation of extracellular oxidase (positive). Another way to

examine the extracellular oxidase formation was using tannic acid agar. The positive reaction, i.e. the extracellular oxidase formation, was indicated when the medium around the fungal colony changed to dark brown. No color change indicated no extracellular oxidase formation.

(7) Basidiocarp preparation

Specimens were air-dried and placed in the dry plastic boxes containing the silica gel to prevent the specimens from the deterioration (Thompson and Lim, 1965).

3.2.4 Analysis of data

(1) Data processing

The filed and laboratory data were organized according to study sites and kinds of samples. The number of individuals, density and distribution of each species and the whole family were computed. The composition of species at each site and the whole area were calculated. The mean environmental parameters including altitude, temperature, relative humidity, light intensity at the ground level, soil moisture and soil pH at each site were calculated. The ecological indices for each study site were computed from specific computer software--- ECOSTAT ®.

(2) Statistical analysis

The following statistical methods were used:

(2.1) Descriptive statistics

(2.2) Correlation statistics

All statistical data came from SPSS ® (Statistical Package for Social Sciences) for MS WINDOW ® Release 6.0.