APPENDICES

Appendix 1 . Chemical Reagents used in this study

1. 5 % solution of potassium hydroxide (KOH) or sodium hydroxide (NaOH)

1) Composition

KOH or NaOH	5 grams
Distill water	100 ml

2) Procedure to prepare this solution

These two kinds of chemicals may be prepared by dissolving 5 grams of KOH or NaOH pellets in 100 milliliters of distilled water. The 5 percent solution is the usual mounting medium for examining spores or dried tissue because it restores their natural plumpness and helps to eliminate bubbles of air trapped in the mount and also clearing opaque material (Thompson and Lim, 1965). The solution is also useful for identification because it stains certain species distinctive colors (Horn *et al.*, 1993).

2. Melzer's reagent

1) Composition

Iodine	2.5 grams
Potassium iodide	7.5 grams
Chloral hydrate	100 grams
Distilled water	100 ml

2) Procedure to prepare this solution

This is another good mounting medium that is also used for certain tests. The preparation of Melzer's is straightforward: Dissolve 2.5 grams of iodine, 7.5 grams of potassium iodide, and 100 grams of chloral hydrate in 100 milliliters of disstilled water.

Melzer's solution is necessary to determine the characteristic color change in spores in many genera (Horn *et al.*, 1993).

3. 70 % solution of ethyl alcohol (ethanol)

This is another good wetting agent for preparing microscope slides. It is especially useful for premounting the hydrophobic spores. Place a drop of alcohol on the spores, and when it is almost dry, add a drop of mounting medium. Ethyl alcohol at this concentration is also a good preservative if one wishes to preserve a mushroom in a jar instead of drying it for posterity. Several changes of alcohol may be necessary to clear the liquid of debris and leached-out pigments (Horn *et al.*, 1993).

4. Lactophenol solution

1) Composition

Phenol crystals	20	grams
Lactic acid	20	ml
Glycerin	40	ml
Distilled water	20	ml

2) Procedure to prepare this solution

Dissolve the phenol in cold water and add the lactic acid and glycerin. Place in small bottie fitted with a rubber teat and glass dropping tube (Thompson and Lim, 1965).

5. Lactophenol/cotton blue stain

Add 0.05 percent cotton blue to lactophenol solution; this is a dilute stain which stains protoplasm. For quicker and deeper staining add 0.1 percent cotton blue (Thompson and Lim, 1965).

6. Alcoholic solution of guaiacum

1) Composition

Gum guaiac	0.5 grams
90% ethyl alcohol	30 ml

2) Procedure to prepare this solution

Dissolve 0.5 grams gum guaiac in 30 ml of 95 percent ethyl alcohol. This solution dropped on the mycilial growth of *Ganoderma* to test the formation of extracellular oxidase. The rapid appearance of blue color indicates the occurrence of extracellulase; no change, or tardy appearance of a pale blue color, indicates its absence (Nobles, 1965; Zoberi, 1972).

Appendix 2 : Preparation of culture media

1. Potato-dextrose agar (PDA)

(1) Composition

Potato	200	grams
Distilled water	1	liter
Dextrose	20	grams
Agar	15	grams

(2) Preparation

Peel some tubers of potato (*Solanum tuberosum*), cut into small cubes and weigh 200 grams. Place in a beaker (2 litters) or double saucepan with one litter of distilled water. Boil for 20 minutes and add water to replace loss in cooking. Filter through muslin cloth and squeeze the cloth to expel into the liquid most of the semi-liquid mush. Add 20 grams dextrose and 15 grams agar powder per litter and boil. Suitable for culture of fungi and bacteria (Thompson and Lim, 1965).

2. Malt-extract agar

(1) Composition

Malt extract	25	grams
Peptone	5	grams
Agar	15	grams
Distilled water	1	liter

(2) Preparation

Boil 1 litters of distilled water in a 2 litters beaker, weigh 25 grams of Malt extract and 5 grams of peptone in a beaker (150 ml), add some of the hot water to the beaker, stir and pour the mixture into the large beaker; add 15 grams agar powder, heat and stir until the agar is dissolved. This medium is suitable for obtaining the sexual

(zygospore) stages of the Zygomycetes and for the growth of members of the Polyporaceae (Thompson and Lim, 1965).

3. Tannic Acid Agar

(1) Composition

Difco Bacto malt extract	15	grams
Difco Bacto Agar	5	grams
Distilled water	1,000	ml
Tannic acid	5	grams

(2) Preparation

Boil 1 litters of distilled water in a 2 litters beaker, weigh 15 grams of Difco Bacto malt extract, add 15 grams Difco Bacto agar powder, heat and stir until the agar is dissolved. Autoclave this medium and add 5 grams tannic acid, then mix these composition together before pouring plate (Nobles, 1965).

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