III. COLLECTION, ISOLATION AND CULTURAL PROCEDURES TAKEN FOR SPOROCRAPS OF GYMNOPILOUS CHRYSONYCES, LEUCOCOPRINUS BIRNBAUMII AND LEUCOCOPRINUS CPACESTIPES.

A. Collection and isolation of mushrooms.

The basidioecarps were collected from different parts of west Bengal. These were washed with sterile distilled water and then with 0.05% HgCl₂ solution followed by three successive washings with sterile distilled water for surface sterilization. After this, with the help of a sharp sterile scalpel the internal tissue (ca 1-2 cm³) was aseptically transferred to a sterile 3% malt extract agar slant, supplemented with 10 µg per litre of streptomycin sulphate to prevent bacterial contamination. These inoculated slants were incubated at 30°C (± 0.5°C) in complete darkness. Tissue cultures thus obtained were maintained by subculturing in 3% malt extract agar medium at regular interval of 15 days at 30°C (± 0.5°C) in complete darkness.

The identification of the test-fungi was confirmed by Dr. D.N. Pegler, Royal Botanic Garden, Kew, Surrey, England.
B. Cultural Procedures:

i) Growth medium: 3% malt-extract medium of Booth (1971) was used to maintain the tissue cultures and glucose-asparagine medium of Lilly and Barnett (1951) was used as liquid basal synthetic medium to study the physiological and biochemical aspects of the test-fungi. The composition of the media was as follows:

3% malt-extract agar medium:

- Malt extract: 30 g
- Agar: 25 g
- Distilled water: 1000 ml

Glucose asparagine medium:

- Glucose: 30 g
- Asparagine: 2 g
- MgSO₄·7H₂O: 500 mg
- KH₂PO₄: 1 g
- FeSO₄: 0.2 mg
- ZnSO₄: 0.2 mg
- MnSO₄: 0.1 mg
- Thiamine hydrochloride: 100 μg
- Biotin: 5 μg

Distilled water to make the volume 1000 ml.

ii) Preparation of inoculum: A small proportion of actively growing mycelium from mushroom culture from agar slants of each test-fungus was
aseptically transferred to a sterile 250 ml. Erlenmeyer flask containing 50 ml. of basal liquid synthetic medium and was incubated on a shaking incubator (120 r.p.m.) at 30°C (±0.5°C) for 7 days in complete darkness. After the incubation period, the mycelial mat was aseptically fragmented into small pieces with the help of a Waring blender. Fragmented mycelial mass was then washed several times with distilled water to remove any trace of medium and suspended in a phosphate buffer medium (pH 5.5) for 24 hrs. to overcome the shock encountered during blending. 1 ml. of the mycelial cell suspension was then used as inoculum.

iii) Growth conditions:

50 ml. of the liquid synthetic glucose-asparagine medium was taken in each of 250 ml. Erlenmeyer flasks. The pH of the medium was adjusted to 5.5 by 0.2 M phosphate buffer. The flasks were then plugged and sterilized at 10 p.s.i. for 20 minutes. The sterilized flasks were inoculated with 1 ml. of inoculum of each test-fungus separately and incubated in a shaking incubator (120 r.p.m.) in complete darkness for 15 days. At the end of incubation the medium and mycelium were separated by filtration through a tared sintered funnel (Jena IG-3). The filtered mycelium was washed repeatedly with distilled water to make it free from any trace of adherent medium and dried to constant weight at 60°C in an oven. Dry weight of the mycelium thus obtained was taken as an index of growth. The mean data of five flasks
were taken and several such flasks were inoculated for each treatment.

REFERENCES


IV. SCREENING TEST FOR TOXINS TO CONFIRM EDIBILITY OF GYMNOPILUS CHRYSONYCES, LEUCOCOPRINUS BIRNBAUMII AND LEUCOCOPRINUS CEPAESTIPDES.

INTRODUCTION

Among the toxins so far known, the cyclopeptides containing amanitin are deadly poisonous (Lincoff and Mitchel, 1977). Recently thin layer chromatography has given even better separation with smaller amounts of material (Palyza, 1970). Though the test-fungi are reported to be consumed by the local people, yet a screening test for cyclopeptide toxins has been done following the method of Block et al (1955).

MATERIALS AND METHODS

Test Organisms:

The fresh basidiocarps of Gymnopilus chrysomyces (Berk.) Sacc., Leucocoprins birnbaumii (Corda) SIng. and Leucocoprins cepae-
tipes (Sow. ex Fr.) Pat. were used.

Procedure: The tissue of sporocarp of each test-fungi was covered with several volumes of methanol in a beaker and extracted by boiling for a few minutes. During heating, the tissue was stirred with a rod. The extract was filtered and centrifuged and tissues were discarded. Extract was then evaporated and the residue was dissolved in a few drops of methanol. This extract was used for running in a chromatogram. A strip of Whatman No-1 filter paper (1" x 10") was used for running (spot was less than
1/16th inches in diameter. The solvent used was 20:6:5:1 parts of methyl ethyl ketone: acetone: water: butanol respectively. After 40 minutes run the paper strip was dried, sprayed lightly with a solution of 1% cinnamaldehyde in methanol, dried and suspended in a stoppered tube above conc. HCl for colour spot development.

The experiment was repeated substituting the solvents mentioned above by a mixture of butanone, acetone and water (30:3:5 v/v) which was reported to allow separation of nearly all of the phytotoxins (Wieland and Wieland, 1972).

RESULTS

No violet or blue colour found and thereby amanitin toxin could be said to be absent. The phytotoxins test results obtained was found to be negative suggesting the absence of toxins in the test-fungi.

REFERENCES

