IV. MATERIALS AND METHODS

Culture

The tissue cultures of *Volvariella diplasia*, *V. volvacea*, *V. esculenta*, *Pleurotus ostreatus*, *P. flabellatus*, *P. sajor-caju* and strains of *A. bisporus* were obtained from the Head of the Division of Mycology and Plant Pathology. Indian Agricultural Research Institute, New Delhi and were maintained in PDA agar and oat agar slants. The cultures were subcultured regularly at interval of 45 days and stored at 20°C ± 1°C in complete darkness.

Spawn Production

Grain spawns were prepared by using unruptured boiled whole grains of different materials. These grains were filled in a wide mouth bottle or polypropylene bag to its 2/3rd capacity, plugged and sterilized. The sterilized bottle or bag was then inoculated with the pure culture of the test-fungi separately and incubated at 35°C ± 1°C for *Volvariella* and 25°C ± 1°C for *Pleurotus* and *Agaricus*. The spawns became ready for use in 10-12 days time.

Volvariella cultivation practice

The beds were prepared on a raised platform made up of bamboo sticks in an open shaddy place in order to avoid direct sunlight, rains and hot winds. The size of the bed was 50 x 50 x 100 cm.

Paddy straw bundles (preferably hand threshed and one year old) of 1.0 to 1.5 kg. were soaked in clean water for 24 hrs. and the excess water was drained off. Then four of these bundles were placed side by side on the raised platform with butt ends on one side. Then another set of four bundles were placed over them in a similar way but with butt ends on the opposite. These eight bundles together and so arranged formed the first layer. In a similar way second layer was placed over the first layer but with butt ends at right angles to the butt ends of the first layer. Then the third layer was placed over the second layer but which was just similar to the first layer. The fourth or topmost layer was placed over the third layer but in a similar way to the second layer. All the sides of the layers in stack were trimmed with sharp by spade.

Spawnning was made in between two successive layers along the margin covering 5 cm areas apart on the surface of the layer and along the margin of the layer. Gram seed powder was sprinked over the spawnning surfaces. After spawnning the layers were

pressed together to make a compact heap to reduce the air space within the heap. The beds were covered with polythene sheet to raise the temperature and also to protect the bed from direct sunlight, rain, and hot winds. However, extreme care was taken so that the polythene sheet never touched the beds in order to present the accumulation of moisture vapour in the form of water vapour. The temperature and relative humidity were more or less mentioned at 35°C - 28°C and 85-95% respectively.

Watering (sprinkling) of the bed was done immediately after spawnning to keep the bed always under moist condition. The beds were watered once a day or alternate day in the morning when environmental moisture was high to or as and when required.

The mushroom started to appear in cluster on all sides of the beds after 9-10 days of spawnning. The cropping was continued for 15 to 20 days in 2-4 flushes. After this the beds were abundoned.

The mushrooms were harvested by gently picking them by twisting the root by hand in the morning time. Picking was done on every 2 to 3 days intervals depending on the cropping patterns.

Pleurotus

The paddy straw was chopped manually into pieces of 3-5 cms in length. The chopped straw was then soaked for

10-12 hours in clean water in a big earthen pot or a clean pond. After soaking the excess water from the straw was allowed to run off. Then the chopped soaked straw was filled in a nylon net ba 5 cm thick and spawn was sprinkled over the whole surface. Then another layer of soaked straw (5 cm thick) was placed over the first layer and again spawned similarly. Similarly third and fourth layer were similarly to filled in the net bag and spawned also. The top most layer was a covering layer of soaked paddy straw. Now the entire amount of soaked straw was compressed from the top with the help of flat wooden small board. The net bag was covered with a polythene sheet and hung on a wooden stand. The temperature of the cultivation area was found to be 25°C to 18°C. After 18-20 days in the plains and 8-10 days in the hills of spawn running the polythene sheet was removed and regular watering was done in order to maintain a relative humidity of 85 to 95%. Now 5-7 days the crop started coming out in flushes. The mushrooms were picked by twisting the roots of the fructification so that no broken portions were left behind. The cropping continued for 15-20 days in 5-7 days intervals and after three or four flushes the net bag was abundonded.

Agaricus

The compost for Agaricus was prepared according to the long day composting method comprising of 28 days schedule. The compost formulae was as follows.

The compost was stacked and turned at regular intervals according to the following schedule.

0 day	stack
6th day	1st turn (50% add N fertilizer)
10th day	2nd turn (add molasses)
13th day	3rd turn (add gypsum)
16th day	4th turn
19th day	5th turn
22nd day	6th turn
25th day	7th turn
28th day	8th turn

Lindane 1% was sprayed in the compost and covered with polythene sheet for 24 hours.

The trays were having an individual area of 0.5 sq.m and compost Ca. 30 kg.) was seeded with two bottles of spawn (200 g each). Seeded trays were covered with moist newspaper soaked 2% Dithene Z-78 and incubated in covered space. The relative humidity was maintained at 75-80% for spawn running and 85-90% during cropping. Casing was done with sand and soil (1:1). The pH of the casing soil was maintained around 6.5 to 7.8.

The other experimental procedures if any are given alongwith the experimental results.