Button mushroom has tremendous scope for its cultivation in our country. This is because of the fact that this species is more attractive, palatable and delicious fetching high market prices. In such situation, constant efforts are now being made to increase the yield and improve the quality of crop by selecting highly nutritious media for preparation of culture and good quality spawn with high vigour, by making trials in respect of supplementation of the substrate with organic materials and inorganic chemicals before and after composting, by proper sanitary managements and efficient temperature control devices applied in cultivation room and management in respect of pathological operations and monitoring of over all procedure. The entire activities and operations related with the cultivation of button mushroom (*Agaricus bisporus*) was brought about by the following maner from October to March in three cropping seasons (from 1998-2001).

**Preparation of pure culture**

Pure culture of *Agaricus bisporus* was obtained from mushroom section of Plant Pathology Department, C. S. Azad University of Agriculture and Technology, Kanpur (U.P.). This was maintained by subculturing at every fort night on Potato dextrose agar (P.D.A.), Malt extract-yeast-extract agar and Nutrient agar media (N.A.) at our convenience, in culture tubes and plates.
Materials

1- **Potato dextrose agar**

- Potato (Peeled and sliced) 200 g
- Dextrose 20 g
- Agar 20 g
- Distilled water 1000 ml

2- **Malt extract-yeast-extract agar**

- Maltose 10 g
- Peptone 8.0 g
- Yeast extract 2.5 g
- Agar 20 g
- Distilled water 1000 ml

3. **Nutrient agar**

- Beef extract 3.0 g
- Peptone 5.0 g
- Agar 15 g
- Distilled water 1000 ml

**Preparation of Culture media**

**Method**: The solid ingredients of culture media were accurately weighed in a conical flasks, dissolved in distilled water, made up the volume and then plugged with cotton. These were then autoclaved at 126°C temperature and 20 lbs pressure for 45 minutes and culture plates
were prepared by pouring 15-20 ml of molten agar medium into sterilized Petriplates. From previously grown culture plates, 7 mm size agar blocks were transferred to fresh culture plates at laminar flow work station. These plates were then incubated at 25±°C in B.O.D. incubator to obtain pure culture.

**Raising of pure culture**

**Materials**: Healthy fruit body of *A. bisporus*, knife, cotton, 50% ethanol, 0.1% mercuric chloride solution, nutrient medium.

**Method**: Pure culture was obtained from mushroom section of Plant Pathology Department, C. S. Azad University of Agriculture and Technology, Kanpur (U.P.). Where ever necessary, this was also prepared from a fresh and healthy fruit body procured from and nearest mushroom house and a fresh inoculum was obtained by subculturing as stated earlier. The lower portion of the stipe was cut at the soil level with the help of a pre-sterilized knife and fruit body was cleaned with a bit of cotton moistened in 50% ethanol to remove the soil particles and finally dipped in 0.1 per cent mercuric chloride solution for 30-60 seconds to avoid any chance of contamination. A bit of tissue from inner part of basidiocarp was taken out under sterile condition and inoculated on a nutrient medium. These tissues when develop, can provide the starting point for subsequent spawn manufacture.

**Preparation of mother spawn**

**Materials**: Pure culture, wheat grains, CaCO₃, Gypsum, Newspaper, Glucose bottles, cotton etc.
**Method**: Wheat grains were boiled in water for 10-15 minutes and allowed to remain soaked in the same hot water for about 15-25 minutes without boiling. Water was then drained off over a wire netting to make them slightly dry. 4 per cent gypsum (CaSO$_4$ 2H$_2$O) and 2 per cent lime (CaCO$_3$) were mixed with the boiled grains. Gypsum prevented sticking of grains together and lime adjusted the pH between 6.5-6.7. The grains were filled into glucose bottles to two-third of their capacities. These were then plugged with non-absorbent cotton and sterilized at 20 lbs pressure at 126°C temperature for 1½ to 2 hrs. Sterilized bottles were then taken out from the autoclave while still hot and shaken to avoid clump formation of the grains. These bottles were immediately transferred to inoculation room and allowed to cool down over night. On the next day, bottles were inoculated with a bit of agar medium colonized with the mycelium of pure culture, raised by tissue culture. About 2-3 days after inoculation, bottle were shaken vigorously to avoid clumping of grains due to heavy growth of mycelium. 7-10 days after inoculation the bottles were ready as stock culture for further multiplication of spawn. The incubation of bottle was done in B.O.D. incubator at 13-14°C temperature when one bottle of stock culture was sufficient to multiply into 30-40 bottles.

**Preparation of planting spawn**: Substrate used in mother spawn was the same in planting spawn preparation. The technique employed and containers used the same which were incubated at 13-14°C in B.O.D. incubator which took one week time in preparation for seeding the compost.
Cultivation of button mushroom (*Agaricus bisporus*) in climatic conditions prevailing at Faizabad

**Geographical Situation:** The district Faizabad is situated in the east of Faizabad division of Uttar Pradesh. It lies in between the parallels of 26° 9" to 29°5" north latitudes and 81°4" to 83°5" east longitude. The boundary of district is covered by Gonda and Basti in north separated by the Ghaghra river, Ambedkar Nagar in the east, Sultanpur in south and Barabanki in the west. It has five tahsilis viz., Faizabad (Sadar), Bikapur, Milkipur, Sohawal and Rudauli. The district is a level plain of generally uniform character. It can be divided into two natural divisions i.e. (i) Low land or Manjha and Upland or Uparhar. The low land lies in the bed of Ghaghra while upland is a level plain cut up by a few streams. The vast expense of cultivation in this tract is interspersed by Jhils, usarlands and plains.

**Climatic conditions:** Climatically, Faizabad falls within sub-tropical zone. It is characterized by extreme fluctuation of temperature and rainfall. On the basis of climatic conditions a year may be divided into three seasons. Winter season starts in late November and lasts till February. It is characterized by low temperature. There is gradual increase in temperature from mid winter onwards, occasionally casual rains are encountered due to winter monsoon. The summer season starts in March and continues till the middle of June. The increase in temperature and decreased humidity is maintained throughout the summer. In this season rain fall is very scanty. Rainy season starts from

24
middle of June and lasts till the end of September. It is characterized by the heavy rainfall. The rainy season is followed by post monsoon months of October and November which shows transition from rainy season to winter the too winter. Based on rainfall and soil characteristics Faizabad is placed under the eastern plain region of Uttar Pradesh. The average range of temperature rainfall and humidity of the district is indicated in (Table-1; Fig-1).

**Availability of Agro wastes**: Wheat and paddy straw occupy first rank amongst priorities of substrate for mushroom cultivation. Being lignocellulosic in chemical composition these are sole source of nutrition for the larger fungi. As per study of The Department of Agriculture, Uttar Pradesh the total production of wheat and paddy in state during 1990-91 was estimated 180 MT and 82.0 MT respectively. The ratio of grain and straw in high yielding varities of wheat and paddy is approximately 1:1. Thus the raw materials required in compost making for button mushroom cultivation is available in enough quantity throughout the year. To ensure the all time availability and economic feasibility, the straw were procured at the threshing time of the crop and were used when ever required. The cultivation of button mushroom included several steps starting from compost preparation to spawn making, spawning, casing, caring, cropping and harvesting which was performed in phases.
Table - 1: Weekly meteorological data in three cropping periods  
(Oct. 1998-March 2001)

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26
Fig. 1 - Weekly meteorological data in three cropping periods (Oct. 98-Mar. 2001)
PREPARATION OF COMPOST

The substrate in its half decomposed form is required for vegetative growth and fruitbody formation in cultivation of button mushroom (*Agaricus bisporus*). This is technically known as compost and constitutes an extremely important step in cultivation of mushroom because the chance of getting a good yield are remote if the compost is of poor quality. Maximum attention needs to be devoted to each aspect while mixing, turning and watering of compost. Some other factors like quality of spawn and casing soil, watering of bed and proper care in respect of hygiene and sanitation also have some influence on growth and production. Peak heating of the compost is last step in compost preparation which makes it germ free and ensures a good crop.

The compost mixture is prepared by mixing wheat or paddy straw with a number of organic and inorganic manures in a fixed proportions. The composting involves the killing of all the harmful organisms present there in and transformation of matters in the forms being used for suitable growth of mushroom. (Plate-2)

In present investigation, wheat straw was used as a basic ingredient in compost preparation. This was mixed with decided proportion of inorganic fertilizers before and during composting. The quantity and appropriate proportion of ingredients used in compost making have better impact on production of good crop with high yield. These ingredients are:
Plate 1: Landscape photograph of cultivation unit

Plate 2: Preparation of compost
(i) **Wheat straw**

The wheat straw used for composting is known as "Bhusha" in Northern India. This is obtained as a by-product during threshing of wheat crop. The straw to be used for this purpose should be fresh, yellow in colour, obtained from the recent wheat crop. This should be 2-3 inches long, free from sand and dust and unexposed to rain. Thick and long straw makes better compost than very thin and small one.

(ii) **Supplements**

Since base material, the wheat straw does not have adequate amount of nitrogen and other components required for its fermentation, the composting mixture is supplemented with nitrogen and carbohydrate materials to start and speed up the process.

(a) **Carbohydrate nutrients**

Molasses is employed as carbohydrate source. Such nutrients are needed to correct C/N ratio and also for the establishment of bacterial flora in the compost.

(b) **Nitrogenous fertilizers**

In fertilizers of this category, ammonium sulphate, Calcium ammonium nitrate and urea are generally used. The nitrogen content in them is generally very high (25-46%) which is released quickly and results in rapid growth of micro-organism.
(c) Concentrated Meal

In this category of supplements, animal feeds are generally used which include wheat or rice bran, dried brewers grain, seed meals of cotton, soya, caster, mustard and linseed. They supply carbohydrate and nitrogen both but these are released at a slower rate.

Materials for rectifying the mineral deficiencies

Besides carbon and nitrogen sources, *A. bisporus* requires other fertilizers such as murate of Potas, Superphosphate and other trace elements for its good growth and production. In addition to above, gypsum and calcium carbonate can also be kept in the category of such fertilizers which are used in the compost to precipitate suspended colloidal materials and to remove greasiness.

Formulations

Different compost formulations have been suggested by different workers, which depend largely upon the basic raw materials used for this purpose. The horse manure based compost is known as natural compost and wheat straw based compost is synthetic compost.

The availability and cost are criteria for substrate selection. In this part of Eastern Uttar Pradesh wheat straw is available in plenty, therefore, this has been selected for compost preparation which is based primarily on C.M.U. solan formulation. The long method was applied in compost preparation which took 10 days more time than short method and did not require a separate step of pasteurization. The constituents of compost included:
Wheat straw  300 kg
Ammonium sulphate  9 kg
Urea  3.6 kg
Potassium sulphate  3.0 kg
superphosphate  3.0 kg
wheat bran  30 kg
Gypsum  30 kg
Furadon 3G or Nemagon  150 gm
Linden  250 gm
Molasses  5.0 kg

**Composting yard**

The compost was prepared on a smooth, clean, concrete floor of a huge dimension on which all the composting operations were carried out properly. Smooth concrete floor may favour its thorough cleaning while making compost stacks and also after sowing. This compost plateform was elevated in centre and inclined towards the sides to make slope for proper drainage of extra water from the compost. The space requirement for various operations related with compost preparation would be exactly in proportion of amount of straw. A 2½ tonnes wheat straw when spread over compost floor with its one ft. depth, at the time of prewetting, it covered an area of 35 ft × 35 ft. The stack prepared from this material was 30 ft long, 5 ft wide and 5 ft high and after 6th turning the size of the stack reduced to 25ft × 5ft × 5 ft.
In this study Kasseri roof was erected over compost pit which protected the compost from drying in sunlight and drain away of nutrients from it during rain. This is so because the loss of moisture affects the microbial activity and drain away of nutrients from compost reduces its quality as a result the compost is left with soggy lumps of wheat straw which fails to produce the desirable quantity of mushroom crop. When ever required, the compost heap was covered with plastic sheets in such a manner that it did not come in direct contact of compost stack. For this purpose, prop of bamboo poles of suitable length were placed in between compost stack and polythene cover. This cover was removed when not needed.

**Method**

The composting yard was thoroughly cleaned by scrubbing and washing, and 2 per cent formalin was sprayed over it before starting the process of compost formation. Dry and fresh wheat straw was spread on the floor of compost yard to a depth of not more than 9 inches and frequently spraying of water was given keeping the regular turning over the straw till it was thoroughly drenched. When dry straw absorbed water to its maximum limit and every single straw become wet, this was considered ideal for mixing the organic and inorganic fertilizers in a fixed proportion. Much care was taken while moisturizing the straw which is an important step for better compost preparation. The moist straw was kept as such for 24 hours. This was then mixed with half quantity of wheat bran which was moist but not wet. To it 2/3 quantity of
Ammonium sulphate and Urea and full amount of potassium sulphate and super phosphate was added, mixed thoroughly and covered with gunny bags. The day when straw was moistened and fertilizers were mixed with moist wheat bran, this was considered minus 1 day.

'O' day: The sprinkled wheat bran which had been mixed with fertilizers was spread over moist wheat straw in such a manner that it covered the complete surface of the straw. These were then mixed thoroughly and filled in a rectangular block by means of forks or basket. The sides of piles were trampled while the central portion was filled loosely. The wooden boards were used from the sides to make pile which were then removed to get a smooth stack of compost mixture. Thus the heap prepared from straw and different other nutrient mixture measured 15'×5'×5'. From the next day onwards till first turning, the sprinkling of water was necessarily done since the top and sides of heap were exposed to air where water loss was inevitable. A day earlier (5th day) to the first turning, remaining amount of fertilizers was mixed with rest amount of moist wheat bran kept in stock and covered with jute bags.

6th day; 1st turning: The dry upper surface layer of stacks was removed and moistened, and then stacks were broken to make them exposed. These were then mixed with remaining 1/3 amount of fertilizers and half of wheat bran (prepared on 5th day). 5 kg of molasses and 40 ml of Nemagon mixed in half a bucket of water was also mixed with compost mixture. In case Nemagon was not available Furadon 3G was
used at the rate of $\frac{1}{2}$ g per kg of compost. After thorough mixing of above mixture the new stack was made as before.

10\textsuperscript{th} day; 2\textsuperscript{nd} turning: Stack was reopened and remade, when ever necessary the dry upper surface was watered and mixed with above mixture.

13\textsuperscript{th} day; 3\textsuperscript{rd} turning: stack was broken and added with 30kg of Gypsum, which was then restacked.

16\textsuperscript{th} day; 4\textsuperscript{th} turning: The stack was broken, watered if necessary, then restacked.

19\textsuperscript{th} day; 5\textsuperscript{th} turning: Reported as above.

22\textsuperscript{th} day; 6\textsuperscript{th} turning: Reported as above.

25\textsuperscript{th} day; 7\textsuperscript{th} turning: Reported as above but added with 250gm of Lindane.

28\textsuperscript{th} day; 8\textsuperscript{th} turning: The compost was checked for presence of ammonia in it. If ammonia did not smell only then the same was subjected to spawning, other wise extra turnings were given at 3 days intervals till smell of ammonia disappeared. This method of compost preparation is known as long method which undergoes pasteurization simultaneously with composting and does not need any additional equipment like boilers and pasteurization room for pasteurization of compost. There are some important aspects which must be taken into the consideration while going through compost preparation.
Turning

Turning is an important aspect of compost preparation which was carried out with forks. The main objective of turning was to provide heat, moisture and aeration for fermentation to each and every part of compost stack. The central portion of stack got ideal condition for fermentation while top surface lacked moisture and bottom portion lacked aeration. It was suggestive, therefore, to check the position of the stack components of compost to avoid uneveness in conditions which were necessary for composting. Thus, correct method employed in turning of compost suggested the ideal place for central portion was bottom, those for sides and top was centre and that for bottom were top and sides, while restacking the compost.

Watering

While turning was going on, watering was done to make pile moist in an even manner. So that it was neither too dry nor too wet. Watering was performed by foot sprayer when the portions which appeared dry were very gently spread with water.

Temperature monitoring

Temperature raised upto 80°C within 48hrs of composting particularly in the middle of the stack. The temperature reading taken at different parts of heap at various intervals determined the process of fermentation of the substrate.
Composting:

Composting was strictly done as per schedule. It was not permissible to leave compost in pile for long time which would cause over composting of the substrate as a result this would be poor in nutrients and vitamins. Under composted compost used for spawning may become too hot because decomposition of substrate was still continuing, which would kill the spawn. A good compost was judged by hand and nose. There was no smell of ammonia in a perfectly prepared compost. For moisture determination, when compost was squeezed in hand no water trickled through fingers, however. It should be wet enough to moisten the palms. This should be free of greasiness. pH of ready compost was checked by pH paper which should be neutral, in between 6.5-7.5.

LAYOUT PLAN OF CULTIVATION ROOM

There is no definite design and materials required for construction of a mushroom house and any covered accommodation may be suitable for this purpose. Mushroom can be grown satisfactorily in a motor garage, a varandah closed temporarily with tarpulins or in cold storage laying empty during the winter months. Mud hut with windows and a thatched roof or a wooden frame house covered with sheets of polythene would be equally workable. The modern mushroom houses are having insulated walls, sophisticated temperature and humidity controlling devices. However, keeping in view the commercial viability of the investigation and other practical considerations the hut with thatched
roof had been erected in which multtiiered racks made up of bamboo chips were fabricated. The situation of mushroom cultivation house was significantly important because cultivation was done at commercial level. It was raised very closed to metalled road and railway station to bring the raw materials and despatch the products. There was constant and continuous source of water and power supply.

The farm house utilized for the proposed work was a hut of (20×20) size (Plate-2) with thatched roof and extra accommodation for storage of raw materials and tools for irrigation, humification and sterilization. The cultivation room was fabricated with racks made up of bamboo chips and poles which were set in three tires for commercial purpose. The bed were made directly on these racks while different set of experiments were planned by making the bed in polythene bags. Mushroom house was provided with windows and ventilators for natural air circulations. The floor of farm house was cemented and smooth to make it easy to wash and to make it clean after each and every operation.

**THE GROWING SYSTEM EMPLOYED**

Two systems were employed for raising the mushroom crop. These were rack system and pocket system. The rack system involved a crude method of mushroom production which was managed with less investment of money and as such this method was recommendable for the people of lower income group of society. In pocket and bag system the polythene bags of 18"×22" and 18"×24" size were used for sowing. These were preferred on account of the better yield potentials, its easy availability and better faesibility in planing the experimental sets.
1. **Rack system**: Racks were made from bamboo chips where these were held together making racks of 8'-10' × 3'-4' size. At every 3-4 ft intervals the rack was supported by bamboo poles to make it straight and uniform at surface level. Usually 3-4 tiers of racks were made in row with working space of 1½–2 ft. A gangway about 2 ft width between each row was felt necessary to facilitate operations like watering picking and casing. The number of row in a room depended upon the width of the room.

2. **Polythene Bags**: Keeping in mind the high price of wooden tray, conventionally used for mushroom cultivation, the cheapest and easily available polythene bags were choosen. These bags were discarded at the end of each crop. The cost of each 18"×24" size bag is approximately Rs. 1.00. These were filled with 15 kg of compost. Experiences showed that compost filled to a height of 8-9" in each bag gave best result. Each bag took an area of 1½ sq. ft of floor space. The bags were placed either on bamboo chips racks constructed one above the other to utilize maximum room space or on cemented floor.

The final choice of materials and the system adopted depended upon the facilities available in our laboratory.

**SPAWNING OR SOWING**

The process of introducing spawn (seed) into the compost is known as spawning. For this grain spawn was used. The spawn used as seed in spawning should be healthy with vigour and entirely free from disease, competitor moulds and pests.
**Grains**: Different types of grains are used for spawn preparation. These are cereals like wheat, barley, oats, and millets viz., Jowar, Bajra, and Kakun. The spawn produced on Bajra grains gives greater yield because in the same weight there are more grains, therefore, comparatively more surface area is available for mycelial running. However, on account of easy availability in this area, wheat grain was used in present investigation.

**Containers**: Spawn are generally supplied in glass bottles (milk or glucose bottles) each of which containing 250 g of wheat grain. The spawn is removed from the bottles just before it is to be spawned. This has to be done carefully to avoid breaking of bottles. Smaller bottles with narrow necks (Glucose bottles) are also used by some manufactures due to shortage of milk bottles but now a days bottles are not much popular due to their shortage. Spawn is now being prepared in polypropylene bags which are much cheaper, lighter and easier to handle. In present study, normally glucose bottles were used in spawn preparation but polypropylene bags were also employed on greater demand at a time.

**Quantity of spawn**: The quantity of spawn to be used per unit area of bed plays an important role in the final outcome of the crop. If it is too little in amount the mycelium will not impregnate the compost sufficiently to give maximum yield. On the other hand too much of it would mean the unnecessary extra expense without any effect on yield. The normal rate of spawning is to be one bottle for one sq. meter of
bed area or for the three bags of (20"×26") size. This is a rough and ready method of working out the total quantity of spawn required in mushroom industry but the correct way of determining the spawn requirement is to relate the quantity of spawn to the weight of compost. The recommended percentage of spawn which was also followed in this study was 0.45 per cent of the compost and accordingly worked out to 4.5 kg of spawn for one tonn of compost.

**Technique of spawning**: Fresh spawn was used as early as possible because its storage in worm weather was likely to damage the mycelium. If storage was unavoidable the spawn was kept in the bottom shelf of refrigerator or in the cold storage for a minimum possible period. A day before spawning the compost heap was "soaked up" to allow it to cool down. The spawn was introduced into the compost when temperature at three inches below the surface of compost stack dropped to 23.9°C. Although the higher temperature damages the mycelium but growth may be very slow if temperature was allowed to fall below 15.5°C before spawning. To avoid such situation, a careful check by inserting thermometer in the compost at various places were carried out before taking a decision for spawning.

Spawning was done in layer wise manner in polythene bags and racks. 3" thick compost was filled in bags, packed tightly by hand and made its surface smooth. The spawn grains were spread on the surface of compost with their larger amounts toward sides than in centre. This was covered with another layer of compost of same thickness and sown
again with spawn. In this way sowing was done in 3-4 layers, when last layer of spawn was covered with 1" thick layer of compost. (Plate-3)

During spawning it should be remembered that the more compressed and greater depth of compost may cause greater danger of temperature rise particularly when weather was slightly warm. After spawning had been completed, polythene bags were made with folds outside. These were then arranged in growing room and covered with newspaper sheets sprayed with 2 per cent of formalin on both the sides. The spawn running room was kept clean.

The temperature of the room was maintained at 21°C to 24°C and the surface of newspaper was moistened by watering with gentle showers of foot sprayer. Care was taken that water did not percolate through the newspaper to the compost. If this happens there will be danger for mycelium which may accordingly be killed. After 6-7 days of spawning, the running of mycelium was observed which at first was in form of faint greyish circle growing out of the grain. With further growth the entire compost was impregnated within 15-20 days and the colour of compost was changed from dark brown to whitish cottony mass.

At this stage of mycelial running there was greater need for CO₂, therefore, the doors and windows of cultivation room were kept close during this period.
CASING

Casing is the last major operation of mushroom cultivation which mean covering of the spawned compost with a suitable material known as casing soil. This is indispensable step in button mushroom cultivation and no fruiting body are formed without casing of spawned compost, since it triggers the fruiting. For this purpose, decomposed cow dung and garden soil was used. A fully decomposed cow dung manure (at least 18 months old) was mixed with loam soil in ratio of 1:1. The pH of mixture, if necessary, was adjusted in between 7-8 by adding lime. It has been estimated that usually about 5 kg of lime was sufficient for 1 cubic meter of soil.

**Sterilization of casing soil**: Casing soil was sterilized by chemical means with 2% formalin solution. For ½ cubic meter of casing soil 1½ litres of formalin mixed in 2 buckets of water was used. This treatment was done 21 days before its use in casing. The treated soil was covered with polythene sheet for 48 hrs to make the disinfection effective. Casing heap was then spread over the polythene sheet to evaporate the gas. This was then packed in bags for use in future.

An ideal casing mixture has a good water holding capacity, sufficient porocity, free from organisms like insects and pests, a moderate pH (7.0-7.5) and with fairly open texture and not pan when water is applied.

**Casing operation**: Casing was done 17-18 days after spawning, yield were reduced if the casing was done too early or too late. It was
realized that casing of bed should be done as soon as the mycelium completely colonised and covered the beds from all sides (Plate-4). It is advisable to case late rather than early. During casing the newspaper covering the compost was removed and a uniform layer of sterilized casing soil was applied over the bed to depth of 1-1½ inch. Special attention was given to the edge and corners which are generally left out or covered with only a thin layer. The casing layer was not to be pressed after it has spread and levelled. After casing, the cultivation room was cleaned thoroughly with Benlet for control of verticillium by using 50g of Benlet in 40 litres of water. All the window and ventilators were kept closed and fresh air was permitted to introduce in cultivation room for 1-2 hrs through the ventilators, regularly for the next seven days. Humidity of the cultivation room was maintained at 85% and air temperature at about 20-21°C which is normal temperature indoors towards the end of October and early November in the eastern Uttar Pradesh. After casing, the bed was moistend regularly by spraying water.

**WATERING**

(1) **Watering during composting**: While turning the compost, watering was done to make the pile moist in an even manner so that it was neither to dry nor too wet. It was desirable to have a man holding pipe of foot sprayer, spraying water on the portions of compost which appeared dry. Almost invariably fire fangs (white flecks) were noticed when about 12 inches of the sides and the top of the compost was removed and these "fire fanged" areas were watered and thrown in the
centre on the new heap. The maximum amount of water running from
the pile was noticed as an indication that probably no more water was
needed. If no water appeared that meant the amount of water added
was just right. Another method employed by growers to test the amount
of water in the compost was to squeeze it. When it was applied the
water oozed out through the fingers.

**Watering during spawn run** : The surface of the compost was
kept moist by watering the sheet of the newspaper covering the bed.
The frequency of watering was once a day in climatic condition prevailing
in this region. At this stage, since much ventilation was not required, it
was easier to maintain a moist atmosphere by keeping the floors and
walls wet and windows closed. This accordingly reduced the water loss.

**Watering after casing** : The casing layer was applied in a fairly
wet condition which was kept moist throughout its depth. The bed was
watered daily either once or twice according to the need. Dry patch on
the bed was not ignored and it was watered to keep that properly
moist. The excess watering made the compost too wet in early stage
which harmed the mycelium resulting into loss in crop.

**Watering during cropping** : No watering was done when pinheads
were being formed as they were extremely delicate and could get damaged.
When they had grown to the size of a pea, regular watering was applied
to keep the surface moist. The frequency of watering was according to
the atmospheric conditions. At certain times it became necessary to
water twice a day where as at other, once a day was sufficient. Several
waterings were always preferable than one heavy application. Watering was
 carried out after picking, simultaneously maintaining air circulation so
 that there was a constant movement of cold air over the bed. The bags or
 beds near the doors and windows loose moisture quicker than those in
 the middle of the room. The top most bed also tends to dry sooner than
 the lower. Each bed was, therefore, examined and watered according to
 its requirements.

AERATION AND VENTILATIONS

The requirement of fresh air in mushroom cultivation is measured in
term of CO₂ concentration in growing room which is difference of the carbon
dioxide produced and its amount removed through ventilation. The production
of carbon dioxide depends on amount of compost in growing room and rise in
temperature accordingly increases the CO₂ production. During spawn run, high
concentration of carbon dioxide was desirable when ventilation was kept at
minimum. Mushroom mycelium as a matter of fact, tolerates carbon dioxide level
as high as 30 per cent. As a rule, about 20 m² fresh air is required per tonn of
compost per hour during spawn run.

Aeration after casing: The casing layer acts as blanket and conserves heat
produced in compost due to mycelium activity during the first week after
 casing. As as result there is a slight rise in temperature and CO₂
concentration especially when compost supplementation has practised. To
remove the accumulated heat and CO₂, and to maintain the optimum
conditions, a combination of ventilation and recirculation
of air are needed. It was observed that the high grade of CO₂ concentration in compost and low in cropping room triggers sporophore initiation. If the CO₂ concentration was higher the mycelium continued to grow vegetatively to form stroma. After the pin head formation, this condition caused the slower growth of developing fruit bodies. In severe cases development may also beyond pinhead formation and mushroom produced have typical long stipe. The requirement of fresh air at this stage depends directly on temperature differences between bed and air. The requirement of fresh air thus calculated was 1 m² fresh air per kg of mushrooms produced at 16°C. A one degree rise in temperature increased the fresh air requirement by 20 per cent. Too much fresh air introduced in cropping room on the other hand resulted in drying of fruit body. Ventilation is, therefore, required to be kept at an optimum level that maintained proper O₂/CO₂ concentration in the cropping room. During pining, the ventilation was increased four times as compared to ventilation given at spawn run stage.

SANITATION

Generally it was observed that there was fall in average yield of mushroom from year to year. The logical reason for this decline in production was that some form of contaminants were carried from crop to crop through racks, shelves, tools and the composting yards which certainly had adverse effect on mushroom production. Certain insects and pests like nematodes sciarids etc. harbouring in crevices in the wood work and wall constructions remained dormant for a year or more and were capable of causing extensive damage to succeeding crops.
This loss was stated in respect of mushroom crop production which was saved better by applying principle "Prevention is better than cure". Such measures which were adopted to discourage disease and prevented insects and pests to harbour the bed and house included:

(i) The composting yard made up of cemented concrete was washed carefully with 2 per cent formalin before and after composting.

(ii) Windows and ventilators were properly protected with wire gauze screens to prevent the entry of insects into the cultivation room.

(iii) The compost and casing soil were sterilized by chemical means using 2% formalin solution.

(iv) Workers engaged in spawning and casing were advised to clean their hands and wears properly.

(v) Pickers were told to wash their hands thoroughly before they start the picking of crop. Picking was started from the bed which had come into crop last i.e. the newest bed.

(vi) Upon the occurrence of any abnormality what so ever that was, appropriate action was taken immediately.

(vii) Spent compost was not dumped within a mile distance from the farm. A large number of diseases and pests were found in the spent compost which could readily infect subsequent crop.

(viii) The floor of mushroom house was kept wet during spawning, casing and cropping. Occurrence of any dry patch on the bed during these stages of mushroom production was tackled at once.
a- After each crop, the containers, racks, shelves and all implements were sterilized by fumigation with formalin. This was done by vaporising it by means of heat generated when mixed with potassium permangnates.

**PICKING, GRADING AND PACKING**

Mushroom like all the fleshy fruits and vegetables are highly perishable because of their high moisture contents and delicate nature, and can not be stored for more than 24 hours at ambient temperature. Once the furiting body matures, degradation process starts and it becomes unconsumable after some times. Development of brown colour is the first sign of deterioration and is a major factor contributing to quality losses. The enzyme Polyphenol oxidase in the presence of oxygen and the substrate catalyses the oxidation of colourless phenolic compounds into quionones which combines with amino acid derivatives to form highly coloured complexes, thus making them highly unacceptable whose disposal has been advised as soon as possible.

Mushrooms are divided into three commercial grades which in fact are three stages of their development. These are buttons, cups and opens or flates.

(i) Buttons : The cap in between one and two inches in diameter with the membrane fully intact.

(ii) Cups : Where the membrane connecting cap and stipe has already been broken.

(iii) Flats or opens: Where as the gills are fully visible and the cap forms the letter 'T' with the stalk.
Mushrooms were picked up while still in the first stage i.e., Button stage. (Plate 5-6). The diameter of cap should not extend beyond one inch diameter when that was to be sold to a canning unit. Otherwise for the purpose of sell a fresh, the diameter of the cap would not be more than two inches size. The cap must be closed otherwise they would not be accepted in the market. It has been experienced that a majority of the buyers in our country are under the impression that open mushroom are not fit for consumption. The circumstances which led people to believe in such a nation are not known but it is unfortunate for the growers that people could not believe in a thing which is not correct.

In foreign countries where people have been growing mushroom for over 200 years use button for canning but for normal daily use cups and opens are preferred. The point which is not generally understood by buyers in this country is that small mushrooms are immature and the real flavaour lies in the spores which darken the gills of the open mushrooms. The real "Mushroomy tests" of course, depends on how well they are cooked. Special boxes were preferred for picking mushrooms from the growing rooms. But the polythene bags were employed in this purpose because of their low prices.

The picking and packing in the course of present study was done in early hours of the morning and pickers were trained in the correct method of picking. One mushroom was picked at a time with the left hand by giving it a gentle twist holding the mushroom between the thumb and
Plate 5: Fruitings of early stage

Plate 6: Fruitings of harvesting stage
the first two fingers. These were not pulled out with Jerk which could have caused damage of some pin heads and mycelium. The root portion were cut at soil level by a sharp knife and trimmed button were kept in small polythene packets of 200 g capacity. This process was continued till all the mushroom of desired size in the house have been picked and packed in desired containers.

The roots were thrown away or buried to make a good manure for vegetable and flower bed. Extreme care was taken in picking weighing and despatching the mushroom because these were delicate, highly perishable and get bruised easily. Picking, packing and despatch were therefore, as important as the other operations in mushroom cultivation. If necessary, mushrooms were washed before packing depending on the preference of the buyers. After they had been washed they were spread on a cotton sheet for at least one hour to make them dry before being packed. Washed mushroom had two advantages.

Firstly, they were more presentable and look cleaner than one that had not been washed. Secondly, washing in cold water helped in removing latent heat produced in mushroom when they were packed. Between the time of picking and despatch, the mushroom was kept in a cool place. They were packed just at the time of despatch. If they had to be stored, they were kept in a refrigerator up to about 4-5 days without any loss of the texture or food value except their appearance when they were a little brown in colour.

Grading and packing was done according to the requirement of the retailer. Usual packing of 200 g net, packed in polythene bags with
punch hole to allow for aeration was preferred. It was experienced that there was a certain amount of loss in weight during transit due to dehydration. The extra mushroom packed in each bag would take care of that loss.

Packets of 200g were packed again in ordinary wooden boxes for dispatch over a short distance where the transit period was not more than 8 hours. Cheep second hand wooden boxes were obtained from a fruit shops during the apple season. For long distance transit thermocol packing was advised in which ice was kept to keep it cool during the Journey. These boxes were marked with certain commercial indications and their despatch by the right trains was ensured.

**USES OF INSECTICIDES AND FUNGICIDES**

Some of the insecticides and fungicides used for the control of mushroom disease and pests are very poisonous and it is essential that they should be used strictly according to the manufacturers instructions. A good spraying machine was used for automising and spraying. The quantity was properly measured and mixed thoroughly and any kind of carelessness in application and use of these chemicals was avoided. The chemical for the purpose were:

(i) **Lindane 0.65 per cent** : It is also called gamma BHC, it is widely used against mushroom pests like sciarid flies, springtails and mites. 883g per tonn of compost was used during the seventh turning. If this was not available at that time BHC 5 per cent at the same dose was applied. As per need it was also used for casing materials at the rate of 1 kg/tonn of casing material.

51
(ii) Malathion 50 EC: 5-7 ml of malathion 50 EC was mixed in 10 litres of water for 100 sq meter bed area. This was sprayed two days after spawning. An addition two days before casing, this was used against phorid and sciarid flies. This is a safe insecticide and it is toxic to insects but not to the growing mycelium.

(iii) Nuvan: 6 ml of Nuvan was mixed in 10 litres water for 100 sq meter bed area at the spawning time to kill adults of phorid and sciarid flies. This was alternated with malathion to avoid insect becoming resistant to the spray. At cropping time Nuvan was mixed at the rate of 1 ml in 10 litres of water for 100 sq metre bed area and applied after a complete flush was harvested. No mushroom could be picked for the next 48-72 hours. The room was completely shut to get best results. Simultaneously the windows, doors and floors were also sprayed with this insecticide solution.

(iv) Diazinon: It is similar to malathion in its effects against pests but less safe to handle. Rubber gloves and a face shield have been advised to worn when this was handled. The prolonged breathing during the spary mist was avoided. It is effective against phorid, flies, spring tail and mites. 20 per cent emulsion at the rate of 57 ml in 24 litres of water per tonn of compost at the time of spawning was used. If 40% wettable powder was used it was applied at the rate of 14 g in 24 litres of water per tonn of compost sprayed on the compost during spawning.

(v) Dithane Z-78: This chemical was applied as a protectant against several moulds and other competitors. The spraying was done 2-3 times with 0.25 per cent mixture.
(vi) **Benlate**: In case Diathane Z-78 was not available Benlet was applied with good results against moulds and competitors like dry bubble, green mould, *Dectylum mycogone*, *Trichoderma* and *Verticilium*. 50g of Benlate was used in 40 litres of water and applied as a spray on the bed surface at the rate of 500 ml per sq metre. The first application was immediately performed after casing and then repeated at pinhead formation. No application was done two days before the harvest. This was also used for canning purpose where 2000 bags of mushroom was treated with 62.5 g fungicide mixed in 50 litres of water.

7- **Nemagon** (60 per cent): This chemical has shown promising results against nematodes (eel worms). Nemagon was applied at the rate of 133 ml per tonn of compost.

8- **Formaldehyde**: 150-200 ml of formalin mixed in 6-8 litres of water was sprayed on news paper covering the spawned bed and polythene bags, mushroom house, wall, windows, ventilators and other appliances.
**Precomposting organic supplementation and its effect on productivity of *A. bisporus***

Poultry manure has been used for over long times in growing mushrooms and is considered as the basic material for compost supplementation. However, it is not easily available everywhere and all the times when needed. Now a days other animal manure, such as sheep manure, pig manure and cow dung manure are extensively being used to increase the yield of mushroom. Still the efforts are continuing to explore other cheap substances which can increase the yield when supplemented to the compost. While supplementing the compost, three main considerations viz., supplementation should not increase the composting period, the supplement should be available everywhere and all the times when needed and that should definitely increase the yield of mushroom crop, must be kept in mind. Looking towards above considerations, certain locally available substances were chosen which were used as organic supplement while preparing the compost for cultivation of *A. bisporus*. These experiments were conducted at mushroom research lab, K. S. S. P. G. College, Ayodhya, Uttar Pradesh to find out the best possible compost mixture giving higher yield. These were:

(1) **Cassia foliage**: Cassia is a leguminous weed, widely growing on waste land in this area which is easily available without any cost for the purpose of compost supplementation. The foliage of this weed were chopped in small sizes, mixed with compost mixture in equal amounts (5kg + 5 kg) which was obtained on 6th day of composting (Plate-9). This mixture was kept in nylon cages and subjected to composting with the routine compost mixture. This supplement not only enriched the
compost to increase the growth of microorganisms for satisfactory composting but also increased the level of nitrogen in compost, which improved the quality and texture of the compost-increasing yield of mushroom crop.

2-Horse manure: Horse manure was added to compost on 6th day of composting in equal amounts (5+5 kg) Plate-11. This compost mixture was kept in nylon cages and subjected to composting with routine compost. This manure provided nitrogen to the composting mixture. A little amount of carbohydrate was also released slowly during composting. In addition to the supply of nutrients to compost horse manure greatly increased the bulk of the compost which was considered an important factor for good yield in conditions prevailing in this region. Keeping in view the cost of wheat straw and other nutrient materials added in compost preparation, horse manure supplement of compost was undoubtedly considered as the best material for this purpose. Horse manure along with bedding and urine has also been tried and found suitable for compost preparation, which did not require any further supplementation.

(iii) Mustard cake: Mustard cake is also a good supplement of compost used in small proportion. To 7.5 kg of compost obtained on 6th day of composting 2½ kg of mustard cake was mixed (Plate-10) and kept in nylon cages, which then subjected to composting with routine compost. This provided both carbohydrate and nitrogen to the compost, which were released slowly resulting into increase in yield of mushroom crop. Supplemented above compost mixtures were spawned in each one polythene bag of 18"×24" size.
Plate 9: Supplementation of compost mixtures with Cassia foliage

Plate 10: Supplementation of compost mixtures with Mustard cake
Post composting inorganic supplementation and its effect on productivity of *A. bisporus*

Mushroom can take up chemicals or metal ions both from the substrate as well as from the surrounding environment. The need for such micro-nutrients during the cultivation of *A. bisporus* have got relatively little importance in the past and very little is known regarding the mechanisms involving the mode of action of chemicals, their preferential uptake, site of accumulation, molecular basis of tolerance and toxicity. The study of these micro-nutrients is further complexed due to the fact that these nutrients very often do not help the mushroom directly but only have an indirect effect through the enhancement in the microfloral population of the substrate intervening enzymatic reactions without being taken up by the mushroom. Researches have suggested that pre-harvest treatments involving addition of chemicals to irrigation water, either separately or in combination, results in production of better quality fresh mushroom. The study pertaining to the use of microelements in compost supplementation has been under taken due to obvious reasons which possibly has better impact on the yield potential of a cultivable mushroom species like *A. bisporus*.

*A. bisporus* was grown in winter months when three successive crops were obtained. The cultivation trials were laid indoor under natural climatic conditions at an ideal temperature (14°-20°C) and optimum relative humidity (75-90%) in mushroom research form at K. S. Saket Post Graduate College, Ayodhya, Faizabad. Wheat straw based compost, prepared in 28 days by long method using standard formulation was subjected to inorganic supplementation prior to spawning. Three inorganic salts viz., Ferrus sulphate, Copper sulphate and Zinc sulphate
in three different concentrations (0.5%, 1.0%, 1.5%) were added to each 20 kg of ready compost (Plate-15). The aqueous solution of inorganic salts were prepared in each 500 ml of sterilized distilled water in glucose bottles. For this purpose 2.5g, 5.0g and 7.5g of each salt was dissolved in 500 ml of distilled water in triplicate. The bottles were then shaken vigorously to dissolve the chemicals. Each concentration of salt solutions was added to ready 20kg of compost prior to spawning by spraying over the compost. These were then gently mixed by hand and then spawned separately at each concentration gradient in polythene bags. Proper care of bed in respect of casing, irrigation, ventilation and sanitation was done during mycelial running and cropping stages. The flushes of crop was noted and production in each concentration of inorganic salt supplementation was calculated. The bed in bags not supplemented with inorganic salts were considered as control. The supplemented compost mixtures were spawned in to each polythene bag 18"×24" size.

**Mycoflora of compost**

The mycoflora of compost was determined on the 5th, 12th, 22nd and 28th day of composting. For this purpose, at each sampling date the samples were taken at random from the different places of compost pile and these were mixed together. 1.0 g of this compost mixture was then suspended in 100 ml of sterilized distilled water and shaken vigorously to release the spores and mycelia of fungi from the substrate. 1.0 ml of this suspension was then poured over PDA containing culture plates with the help of pipette and spread over the surface of medium. These inoculated Petridishes were then incubated at room temperature.
25± 1°C for 10 days when colonies of fungi developed on the surface of culture plate. A bit of fungal mycelium was taken out from culture plates with the help of sterilized forceps, put on to the slide and mounted in lactophenol. These were then observed regularly under high power lens and identified on the basis of the morphological characteristics of the organism. The characters of fungi were compared with authentic literatures to establish the exact identity of the isolate.

**Casing soil**

Casing is the last major operation of mushroom cultivation which mean covering of the spawned compost with a suitable material known as casing soil. This is indispensable step in mushroom cultivation and no fruiting body are formed with out casing of spawned compost since it triggers the fruiting in button mushroom. Keeping in view the physical properties of different soil used for this purpose which base particularly on moisture holding capacity, pore space and pH, different types of soil mixtures have been used. Among different such soil mixtures sphagnum peat and chalk mixture gave good results. But the peat is not available all over India except Kashmir valley so it becomes necessary to find out substitute of those materials which are available in local areas. In present study, emphasis has been given on the formation of substrate mixture, method of its sterilization and also pertaining to some important factors which are related with casing research.

(a) **Collection and Preparation of casing soil**

Various casing materials viz., garden soil, sand, loam soil decomposed cow dung, tree bark and one year old spent compost were collected from different localities and sources and stored in cement
bags at room temperature for further use. The different casing mixtures prepared for this purpose were:

(i) **Garden soil and sand mixture**: The garden soil to be used for this purpose was loam or clayey loam containing leaf residues. This was fairly stable for watering. At the time of sampling 2 inch top layer of soil was removed which supposed to harbour the hyphae of disease causing fungi, and larvae and ova of the pests. The fresh soil was dug out and mixed with sand in ratio of 4:1. The chemical sterilization of this casing mixture was done with 2% formalin solution. This was thoroughly mixed with soil mixture, made into the heap and carefully covered with polythene for 48 hrs. For pH adjustment limestone was added but only after the sterilization of casing mixtures. The pH of casing mixture was adjusted between 7 to 8. It has been estimated that usually about 5 kg lime was sufficient for one cubic meter of soil.

(ii) **Cow dung manure and garden soil**: A fully decomposed cow dung manure (at least 18 months old) was mixed with garden soil in ratio of 1:1. The pH of mixture, if necessary, was adjusted in the same manner as mentioned above.

(iii) **Spent compost and sand**: The spent compost obtained from button mushroom cultivation was used in preparation of casing mixture. The spent compost and sand were mixed together in ratio of 3:1. This mixture was then added with lime in proportion of 4:1 and then sprayed with 100 ml of nemagon. The heap thus prepared from this mixture was subjected to turning at every 3 months and watering was done at a regular interval to keep it moist. After 24 months of this operation the mixture became ready as casing soil. The pH of this mixture was adjusted as stated earlier.
(iv) **Tree bark and Farmyard manure**: The tree bark of Acacia and Tamarindus were mixed with farmyard manure in 1:1 ratio. The pH of mixture was adjusted, if necessary, in the manner as stated above.

(b) **Sterilization of casing soil**

Casing soil may carry propagules of disease causing competitor moulds and insect pests, which may multiply further because conditions for growth of button mushroom favour their growth also. The casing soil was therefore, pretreated to eliminate these undesirable microorganisms.

(i) **Chemical treatment of casing mixture**: The best material employed for treatment of casing mixture was formaldehyde used at 2 per cent concentration (2 litres of commercial grade formalin in 40 liters of water). The casing mixture was made free from gravels and thoroughly drenched with 2 per cent formaldehyde solution. The piles were then made from this mixture and covered with polythene sheet. The treatment was given at least two weeks before its use. Later on the polythene sheet was removed and casing mixture was turned with fork to evaporate the traces of formalin. High formalin content in casing soil may result in partial killing of mushroom mycelium and may delay the cropping.

(ii) **Pasteurization**: Moist casing soil was filled in trays which in turn stacked in pasteurized room. Live steam was introduced to raise the temperature of room to 60-70°C and maintained it for 7-8 hours. This treatment has been found to give effective killing to all harm full microorganisms including nematodes.

(iii) **Steam Sterilization**: This was complete sterilization of casing medium done by the steam under pressure in autoclave. In this method besides the killing of harm full organism also killed the beneficial bacteria
and cropping in such casing media was delayed; yield was also adversely affected in a given periods of time. However, bacterial flora in casing may slowly build up again giving yields in later flushes.

(c) **Method of Casing**

Casing was done three weeks after spawning. Yield reduced when casing was done too early or too late than the schedule time.

The newspaper covering the compost was removed and a uniform layer of sterilized casing soil was applied over the bed to a depth of 1-1½ inch. Special attention was given to edges and corners which are generally left out or covered with only a thin layer. The casing layer was not to be pressed after it has spread and labelled. After casing, the cultivation room was cleaned thoroughly with Benlet for the control of verticillium by using 50gm of Benlet in 40 litres of water. All the windows and ventilators were closed and fresh air was permitted to introduce for 1-2 hours daily from the next seven days. Humidity was maintained at 85% by spraying water on the bed to let that moist and also on the floor and walls of cultivation room. As such the occurrence of any dry patches at the surface of bed was prevented.

(d) **Physical characteristic** : The accuracy was observed in pH determination performed as per method suggested by Shandilya (1982) on the 7th, 14th, 21st, 28th, 35th, 42nd, 49th, 56th day after casing. The pore space and water holding capacity was found out according the method of Flegg (1951) employed at different stages of cropping.

(e) **Yield** : The compost obtained after composting was spawned in polythene bags (18”×24” size) in the laboratory with the strain U3 of *A. bisporus* at the rate of 2% by weight of fresh compost and these
were incubated at 25°C in cabinet until the compost was completely colonised by mushroom mycelium. The polythene bags were cased with all the four test materials to an even depth and chalk was added to give a pH of 7.8-8.0. The temperature in the cabinet was again set at 25°C. After 8 days when mycelium had grown through casing to the surface, the temperature was lowered by passing cool air into cabinet. Pin heads were seen after 16-18 days of casing and first mushroom were generally harvested at the cup stage. Dry matter of the fruit bodies from casing media was calculated by drying the known fresh weight of fruit bodies to a constant weight at 100-105°C.

(f) **Quality assessment**: White ness of fruit bodies was determined by method suggested by Mac Canna and Gormley (1969) while light percentage reflected by cap surface of fruit bodies was measured using a mark III reflectometer.
**Ratio of substrate utilization and mushroom production**

For determination of per cent production of mushroom on ready compost, the experiment was performed in polythene bags of known capacities. The dry weight of compost was found out to fill the fresh compost in a fixed capacity level of the polythene bag and then got that, out of the bag to make it dry in sunlight. The dry compost was then weighed to find out its weight in a fixed volume of the bag. Four replicates of bags filled with fresh compost at a fixed capacity level were spawned in a usual manner. On 30th, 45th, 60th and 75th day of casing, the crop was harvested. When fruit body formation ceased to occur the spent compost was dried and weighed to find out the amount of compost utilized by the mushroom. The amount of substrate utilized by the mushroom was found out by deducting the amount of spent compost from the ready unutilized one. The correlation between the substrate utilization and fruit body production in a particular cropping period was determined in terms of per cent production of mushroom to divide the amount of mushroom produced by the amount of substrate utilized multiplied by hundred:

\[
\text{percent production of mushroom} = \frac{\text{Dry wt. of mushroom produced}}{\text{Amount of substrate utilized}} \times 100
\]

For the determination of bio-conversion of substrate by mushroom mycelium, the chemical analysis of ready and spent compost in respect of total Nitrogen, Cellulose, Hemicellulose, Lignin and Soluble sugars was done and the amount of these components utilized by mushroom was determined by deducting the value of these component from spent compost divided by the value of ready compost. The ratio of bioconversion was estimated by the following formula:

\[
\text{Bioconversion} = \frac{\text{Percent production of mushroom}}{\text{Percent value of compost component used by mushroom}}
\]
Chemical analysis of compost

In order to determine the ratio of substrate utilization and fruit body formation in button mushroom, Chemical analysis of compost was done at four stages of mushroom cultivation. These were ‘Ist’ stage (prepared compost obtained just before spawning) and ‘II’ stage or ceaseation stage (when production has terminated or virtually from spent compost). For chemical analysis the compost samples were taken from the two stages as mentioned above. These were oven dried at 80°C for 48 hours and ground into powder. These were packed in polythene bags and then analysed for Total soluble sugars, Total protein, Hemicellulose, Cellulose and Lignin by the following methods:

(i) **Total soluble sugars**: Total soluble sugar were determined by Anthron sulphuric acid Method of Loewus (1952) as given by Paech and Tracey (1955).

0.05 gm of dry compost powder was dissolved in 25 ml of distilled water. This suspension was subjected to centrifugation at 2000rpm for 15 minutes and then decanted. The residue obtained was discarded and decant was kept for further analysis. 2.0 ml of decant was taken and to it 0.5 ml of Anthrone reagent solution (2% anthrone solution in A.R. ethanol acetate) was added. 5.0ml of conc. H₂SO₄ A. R. was carefully layered into the tube which was then swirled gently until the ethyl acetate had hydrolysed as indicated by appearance of a “floc” of anthrone. After at least 10 minutes, the developed blue green colour was read in absorptiophotometer in 620 mµ filter against distilled water and corrected for a blank containing only water and reagent.
To calculate the amount of sugars in unknown solution 0.1 per cent solution of glucose was prepared as standard and different dilutions were made from it. These were then treated with anthron reagent solution and conc. \( \text{H}_2\text{SO}_4 \) on above pattern. The intensity of colour was read in absorptio photometer in the same filter (620 m\( \mu \)). Total sugars were plotted between \( \log \left( \frac{I}{I_o} \right) \) And c

where, \( I \) = Reading of absorptiophotometer for solution.

\( I_o \) = Reading of absorptiophotometer for blank.

C = Molar concentration of solution

(ii) **Total nitrogen**: Total nitrogen was estimated by the method of Cotton (1945). 0.05 gm of dried crushed sample was taken and digested with 1.0 ml of salicylo sulphuric acid (1:2). Two crystals of sodium thiosulphate were simultaneously added (for quick digestion) and heated till fumes appeared. It was then cooled and mixed with 1.0 ml of perchloric acid followed by gentle heating till the solution become colourless. The solution was then made to 25 ml by adding distilled water. To 2.0 ml of this solution, 8.0 ml of distilled water and 1.0 ml of Nesseler's reagent were added and kept in water bath (80°C) for 10 minutes. It was then cooled and the colour intensity of solution was measured in obsoptiophotometer using 420 m\( \mu \) filter. Blank reading was taken by adding 1.0 ml of Nessler's reagent to 10ml of distilled water subjecting it to absorptiophotometer.

To calculate the amount of total nitrogen, 0.1 per cent solution of \( (\text{NH}_4)_2\text{SO}_4 \) was prepared as standard and different dillutions were

65
made. These were then treated with 1.0 ml of Nessler's reagent. The intensity of colour developed was read in absorptiophotometer using the same filter (420 μm). Total nitrogen was calculated from the graph plotted between $\log \left( \frac{I}{I_0} \right)$ and C

where $c = \text{Molar concentration of solution.}$

(iii) **Hemicelluloses and cellulosies**: Hemicelluloses and cellulosies were determined by the method of wise, Murphy and D addieco (1946) as given by Paech and Tracey (1955). 5.0 g of dried crushed sample was treated with varying strength of KOH (5, 10, 15 and 24 per cent) for 15 minutes with occasional shaking. Different insoluble fractions obtained were bulked together and considered as cellulose fraction of holocellulose. The filtered alkaline extract was neutralized with excess amount of glacial acetic acid and then precipitated with ethanol. Precipitated hemicellulose was quickly separated, dried and weighed to avoid degeneration.

(iv) **Lignins**: Determination of Lignins was done by the method of Klason (Paech and Tracey, 1955). 5.0 gm of dried crushed sample was treated with 20 ml of 72% $\text{H}_2\text{SO}_4$. The mixture was left to stand for 48 hours at room temperature. It was then diluted with 20 ml of distilled water and subjected to centrifugation at 2000 rpm for 15 minutes. The solution was discarded and residue obtained was centrifuged three times after adding 50 ml of distilled water. The water washed residue was mixed with 50 ml of 0.5% $\text{HCl}$ and heated at 100°C for 12 hours. The residues obtained was again washed with water to remove the acid, dried at 100°C and weighed.
Effect of temperature on fruit body formation of *A. bisporus*

Experiment to investigate the effect of temperature manipulation during the cropping cycle was done by using the commercially available U₃ strain of *A. bisporus*.

In this experiment, by temperature manipulation good quality of fruit body of similar size and maturity were harvested in fewer picking days than that not achieved using the standard procedure of growing where only one temperature is used throughout the cropping cycle.

Two experiments were performed to determine the effect of periods at elevated temperature on sporophores at varying stages of development:

**Experiment-I** : In the first trial, 20 polythene bags of (18"×22") size, each accommodating 6 Kg of spawn run compost were cased with garden soil and cow dung manure mixture in ratio of 1:1 and placed in two cabinets adopted for mushroom cultivation. During the prepinning stage, environmental conditions in the cabinets was maintained, relative humidity was controlled at 85-90% and the temperature was set at 19-20°C. When mushroom mycelium become visible at the casing soil surface the carbon dioxide level was reduced below 0.1% by ventilation. The casing soil surface of all polythene bags was watered and temperature in cabinet I was reduced to 16.5-17.5°C to induce sporophore development. The temperature in second cabinet (II) was then increased and maintained at 24°C. Four experimental treatments (a,b,c,d) were allocated to 20 polythene bags in cabinet I to give a randomised block of 5 replicates. Four type of treatments were given in two steps. In treatment (a), polythene bags were maintained at 16.5-17.5°C in cabinet I throughout the experimental period to represent
“normal” cropping. In treatment (b), polythene bags were transferred from I to the higher temperature cabinet (II) when at least one sporophore per polythene bag attained 1 cm cap diameter, similarly (c), polythene bags attained 2 cm and (d), developed to 3 cm size. Each polythene bag was then replaced from the corresponding position in cabinet II. Mushroom continuing to develop at the higher temperature were harvested at the “cup” or veil breaking stage. Once individual polythene bag had been cleared off matured fruit bodies, they were returned to their original position in cabinet I until the appearance of the second flush. When the procedure was repeated, polythene bags remaining in cabinet II throughout the experimental periods were monitored to observe on sporophore formation and subsequent fruit body development. Number and weight of mushrooms were recorded daily from individual polythene bag. In experiment I the harvesting was performed over a period of 26 days.

**Experiment-2**: Experiment II was done in similar manner as it was performed in experiment I but the only difference was temperature shift in the letter trial which was operated from the appearance of the second flush on words. Weight and number of mushroom were recorded daily from individual polythene bag.
Effect of humidity on fruit body formation of *A. bisporus*

Water content of substrate and relative humidity of its surrounding play important role in growth and reproduction of fungi. The role of water in metabolism of fungi is significant because they absorb their food in form of solution. Sexual reproductive phase leading to the fructification in general requires greater quantity of water in the substrate and higher relative humidity in atmosphere than the vegetative phase in larger fungi. In present study, investigation was carried to work out the role of humidity in initiation and maturation of fruit bodies in commercial cultivation of *A. bisporus*.

In order to study the effect of humidity on fruit body formation of *A. bisporus* the polythene bags containing spawned substrate were traditionally kept under the chamber which was fabricated with pasteurized wet gunny bags made up of chopped paddy straw hanged on doors and windows to increase the humidity of cultivation room but the present study humidifiers of different capacities were employed for obtaining the differential per centage of relative humidity in cultivation room and their impact on fruit body formation was noted in respect of mushroom production.
Food value determination of *A. bisporus*

Since mushrooms have been accepted as an important food article for human consumption, the determination of their chemical constituents is necessary to highlight their dietary importance. Very little information on the biochemical parameters of mushrooms, grown under the agroclimatic conditions of Uttar Pradesh is available. Therefore, this is our present time demand to undertake such study, particularly in respect of biochemical diagnosis of button mushroom (*Agaricus bisporus*) grown under defined conditions in the atmosphere at Faizabad.

Button mushroom, cultivated at the mushroom growing farm of K. S. S. P. G. College, Ayodhya was taken for this experimental study. Fresh, well development, clean and healthy fruit bodies at five different stages of its development viz., Stage-I: when fruit body initials appeared, Stage-II: Fruit body initials swelled and stipe become differentiated from the pileus, Stage-III: Margin of pileus slightly came upto breaking stage, Stage-IV: Margin of the pileus starts turning and Stage-V: Fruiting body expended and attained full maturity. Fruit bodies (ten in number) were selected and dried at 60 ± 5°C) to a constant weight in order to determine its various contents. Chemical analysis was done from a composite sample of ten mushroom fruitings as per methods suggested below:

(i) **Nitrogen and crude protein**: Total nitrogen content was estimated by the method of Cotton (1945). Since average value of N in biological materials is 16% on dry matter (DM) basis, a factor 6.25 ($\frac{100}{16}$) was used for the determination of crude protein content.
0.05 g of dried crushed sample was taken and digested with 1.0 ml of salicylo-sulphuric acid (1:2). Two crystals of sodium thiosulphate were simultaneously added (For quick digestion) and heated till fumes appeared. It was then cooled and mixed with 1.0 ml of perchloric acid followed by gentle heating till the solution become colourless. The solution was then made to 25 ml by adding distilled water. To 2.0 ml of this solution, 8.0 ml of distilled water and 1.0 ml of Nessler’s reagent were added and kept in water bath at 80°C for 10 minutes. This was cooled and the colour intensity of solution was measured in absorptiophotometer using 420 μm filter. Blank reading was taken by adding 1.0 ml of Nessler’s reagents to 10 ml of distilled water and subjecting it to absorptiophotometer.

To calculate the amount of total nitrogen, 0.1 per cent solution of \((\text{NH}_2)_2\text{SO}_4\) was prepared as standard and different dilutions were made. These were then treated with 1.0 ml of Nessler’s reagent. The intensity of colour developed was read in absorptiophotometer using the same filter (420 mm). Total nitrogen calculated by graph was plotted between \(\log \left( \frac{1}{T} \right)\) and \(C\) as stated earlier. The total crude protein was determined by multiplying the amount of total nitrogen with 6.25.

(ii) **Crude Fat**: Crude fat was estimated by the method Pathak et al., 1996. Crude fat in this sample was extracted by refluxing a known quantity of material with petroleum ether. This ether extract included triglycerides, sterols, resins, waxes, essential oils and fat soluble pigments etc.

1.0 g of sample was weighed correctly in a stainless scoop and transferred quantitatively into a numbered extraction thimble and then
plugged with cotton wool. The thimble with sample was gently placed in the extractor keeping the open (Plugged) end upwards. A clean oil flasks dried in hot air oven was cooled to room temperature in a desiccator and then weighed accurately. After this, oil flask was attached with the lower leak proof joint of extractor which was ultimately connected with the condensor at the top. Now complete assembly was placed on a water bath. Petroleum ether was poured with the help of funnel where its amount was 1.5 times to that of the sample. Heating was regulated to facilitate the condensation of 4-6 drops per second. Complete extraction of fat and fat solubles took about 6-8 hours. At the end of extraction the petroleum ether was condensed in the extractor and removed by 3-4 extractions from the oil flask. A little amount of petroleum ether left in the oil flask was evaporated in a hot air oven at 100±5°C for 30 minutes. The oil flask with ether extract was removed from the oven and cooled to room temperature in the anhydrous atmosphere of a desiccator and then weighed on an analytical balance. The amount of crude fat was calculated according to the formula given below:

Weight of dry empty oil flask = Ag

Weight of oil flask with EE = Bg

Weight of sample in thimble (DM is sample) = Cg

EE in sample = (B−A)g

EE% or DM in sample = \( \frac{B-A}{C} \times 100 \)

(iii) **Crude Fibre**: Crude fibre was estimated by the method Pathak et al., 1996, pp. 22. The residue left after the digestion of fat extracted

72
material with a weak solution of acid followed by a weak solution of alkali in a controlled system is crude fibre. It includes cellulose, some part of hemicellulose and major portion of lignin.

The residue left in thimble after extraction of Fat with petroleum ether was quantitatively transferred in to a spoutless beaker of 1 litre capacity marked at 200 ml from the bottom. After this, 25 ml of 10% \( \text{H}_2\text{SO}_4 \) was added and then diluted with water upto 200 ml mark to make the final strength of acid 1.25%. The beaker was covered with a round bottom condensor and then placed on a stand bath or hot plate and boiled exactly for 30 minutes under reflux to maintain the volume. The content of beaker was shaken 4-5 times during boiling. After 30 minutes of boiling the content in beaker was diluted with hot water and filtered through a muslin cloth using suction. The acid digested residues in beaker was quantitatively transferred on the muslin cloth with a hot water jet and 5-6 washings were given with hot water to make it acid free. The washed residues on muslin cloth was quantitatively transferred back into the beaker with the help of the hot water jet. Then 25 ml of 10% \( \text{NaOH} \) was poured into the beaker and diluted with water to 200 ml mark to make the strength of alkali 1.25%. It was refluxed as earlier during boiling for exactly 30 minutes from the onset of boiling. After this the beaker was removed from the water bath and content was diluted with hot water and then filtered through the same muslin cloth and 6-7 washings with hot boiling water were given to make the residue alkali free.

The alkali free washed residues on muslin cloth was quantitatively transferred into a clean silica basin with the help of a spatula along with
few drops of water. The silica basin with digested residues was dried at 100 ±5°C then cooled in a desiccator and weighed to a constant weight. After this it was put in a muffle furnace at 550°C–600°C for 2-3 hours to complete burning of organic mater (which is CF). This was then cooled in a desiccator and weighed as usual to a constant weight. The crude fibre was calculated according to the formula given below:

\[
\text{Weight of initial sample} = a \text{g}
\]

\[
\text{DM\% in sample} = 90\%
\]

\[
\text{Weight of Silica basin + Finally digested residues after drying in hot air oven}=b \text{g}
\]

\[
\text{Weight of Silica basin with Ash} = b \text{g}
\]

\[
\text{Weight of crude fibre (CF) in sample (b–c)} = c \text{g}
\]

\[
\text{CF\% on DM basis} = \frac{b-c}{0.9a} \times 100
\]

(iv) **Total Ash content**: Total Ash content was estimated by the method Pathak et al., 1996. pp. 13. The residue left after complete ignition of a biological material is called total Ash and this represents the inorganic matter contents of the sample.

A ground dry sample, as usual, was spread in a tray for 2-3h and then 2g sample was weighed in a dry moisture cup of constant weight and dried at 100±5°C in hot air oven for the determination of DM content (A). Another 3g sample was weighed in a previously weighed and numbered dry silica basin of constant weight. The weighed sample in crucible was heated over a burner to make it smoke free and then placed in a muffle furnace at 550-600°C for 2-3 hrs for complete ignition. The
silica basin with ash was removed from the muffle furnace, cooled in a desiccater to room temperature and weighed to constant weight. The oxidised residue left in the silica basin was total ash in the sample which was calculated by the formula given below:

- Weight of dry moisture cup = $A_g$
- Weight of dry moisture cup + sample = $B_g$
- Weight of air dry sample = $(B-A)$ g
- Weight of moisture cup + sample after oven drying = $D_g$
- $DM\%$ in sample = $\frac{D-A}{B-A} \times 100$
- Weight of dry silica basin = $E_g$
- Weight of silica basin + air dry sample = $F_g$
- Weight of air dry sample = $(F-E)$ g
- Weight of silica basin + total Ash = $G_g$
- Weight of total Ash = $(G-E)g$
- Total ash in sample = $\frac{(G-E)}{(F-E)} \times 100 = H\%$
- Total ash $\%$ on DM basis = $\frac{H \times 100}{DM\% \text{ in sample}}$

(v) **Total soluble sugars**: Total soluble sugars were determined by anthrone sulphuric acid method of loewus (1952) given by Peach and Tracey (1955) as stated earlier.

**Determination of Minerals**:

(a) **Calcium**: Calcium was estimated by the method described by Pathak, et al., 1996 p. 36. Calcium is a mineral precipitated with the help of ammonium oxalate in the form of calcium oxalate, which is
treated with conc. H$_2$SO$_4$ to release oxalic acid to be titrated against standard potassium permangmate (N/10 KMnO$_4$) solution. The volume of 0.1N, KMnO$_4$ utilized in reaction is multiplied by a factor 0.002 g to obtain the calcium content in the sample taken for precipitation.

The ash content of fruit body was extracted in Volumetric flask and mixed thoroughly after making the volume up to the mark. The aliquot (25 to 50 ml) was transferred with a pipette into a 250 ml beaker and then diluted with equal volume of distilled water. About 2-3 drops of methyl red indicator and 10 ml of saturated ammonium oxalate solution were added with stirring the contents in beaker with a glass rod. The glass rod was not removed from the beaker during the complete process. After that the dilute NH$_4$OH was added drop wise and mixed till the colour become light pink (pH 2.5 to 4). An excess of ammonium hydroxide turned the colour brownish yellow. In such case a few drops of dilute HCl was added and mixed to revive light pink colour for complete precipitation of calcium in the form of calcium oxalate. The precipitate in beaker was heated to boil. It was then cooled and kept covered beneath a bell Jar over night for the formation of coarse granules. Light pink colour of the content in beaker was maintained by adding more drop of HCl, if necessary.

Next morning the content in beaker was filtered through a what man filter paper No. 40. First super nant was decanted and then precipitate on filter paper was given several washings with hot distilled water to make oxalate free. After complete filtration, the filter paper with precipitate was removed from the funnel, brought to the original beaker carefully without any loss of precipitate, unfolded and then hanged tilted
in the beaker with the support of obliquely placed glass rod. Below the filter paper, about 10ml of dilute $\text{H}_2\text{SO}_4$ (20%) was drawn in a graduated pipette and dropped over the filter paper. About 10ml of dilute $\text{H}_2\text{SO}_4$ (20%) was drawn in a graduated pipette and dropped and washed the precipitate into the beaker. The washed filter paper was again folded and kept back on its funnel. The dissolved precipitate in beaker was heated (60-70°C) and titrated against $\text{N/10 KMnO}_4$ solution while hot. The end point was indicated by a faint pink colour. At this point filter paper was placed in the beaker and stirred with the glass rod. A few more drops $\text{N/10 KMnO}_4$ was added with stirring to revive a faint pink colour at the end point. Pink colour persisted for at least 30 seconds. The volume of $\text{N/10, KMnO}_4$ used for titration was recorded.

A factor for determining calcium (Ca) has been derived as follows

$$1000 \text{ ml of } \text{N. KMnO}_4 = 20 \text{ g Ca}$$

$$50 \text{ ml of } \text{N KMnO}_4 = \frac{20}{1000} \text{ g} = 0.02 \text{ g}$$

Or

$$1 \text{ ml of } \text{N/10 KMnO}_4 = 0.002 \text{ g}$$

$$\text{Ca (\% on DM basis) = } \frac{T \times 0.002 \times V \times 100 \times 100}{A \times S \times D} \times 100$$

Where $T =$ volume of $\text{N/10 KMnO}_4$ used

$V =$ Volume of mineral extract

$A =$ aliquot of mineral extract used for calcium precipitation

$S =$ Weight of air dry sample ashed

$D =$ Dry matter (%) in sample
The following conversion factors are used for presenting the content of calcium in the material
(i) $\text{CaO} = \text{Ca} \times 1.4 \text{ g}$
(ii) $\text{CaO} \times 0.7143 \text{ g}$

(b) **Copper (Cu)**:

The copper was estimated by the method described by Pathak et al., 1996 p. 48. The copper present in mushroom was digested with an acid mixture and treated with sodium diethyl dithiocarbomate to form a coloured complex compound. The intensity of colour in known volume was measured at 440 nm in a photoelectric colorimeter.

**EDTA**: Sodium salt solution was prepared by dissolving 25g sodium salt of EDTA in 500 ml distilled water. To it 0.5 g of sodium diethyl dithiocarbomate and 100 ml of chloroform were added and shaken (5-6 times) till the colour. After that 8ml of 0.1% solution of cresol red was mixed thoroughly and the solution was preserved for use in a glass stoppered glass bottle and labelled.

**Copper Standard**: The copper standard was prepared by dissolving 982 mg of copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in some distilled water in a volumetric flask (250ml) and 5ml of conc. $\text{H}_2\text{SO}_4$ was then added to it. The contents in volumetric flask were mixed and volume was made upto the mark by adding more distilled water. This standard solution contained 1 mg copper per ml. A reagent blank was prepared and used for zero setting in the colorimeter.

A calibration curve was drawn by processing graded volume of standard copper solution in a range of 0 to $4\mu \text{Cu per ml}$.
(c) **Iron (Fe)**: Iron in fruitings of *A. bisporus* was estimated by the method used by Pathak et al., 1996 p. 48. The development of blood red colour of ferric ion on reaction with potassium thiocyanate was used for the determination of Iron in the biological materials.

An aliquot (6.5ml) of mineral extract was taken in a conical flask. To it 1 ml. of dilute HSO$_4$ (30%), 1 ml of potassium persulphate solution and 1.5 ml of potassium thiocyanate solution were added and mixed thoroughly. A red colour of ferric ion developed which was measured within short time (15.20 minutes) at 540nm in a colorimeter. A calibration curve was drawn by processing different dilutions of standard ferrous ammonium sulphate solution and used for the determination of Iron content in test material:

(d) **Zinc (Zn)**: Zn was determined by the method given by Jackson (1958) p., 402. The acid form of diphenylthiocarbozone (dithizone) is soluble in CCl$_4$ whereas the ammonium salt is soluble in water containing a slight excess of NH$_4$OH. Dithizone forms complexes with Zn, Cu, Co and Ni which can be extracted from a water solution into CCl$_4$ at pH values between 8 and 10. In the present procedure, Cu, Ni, Co and Pb are held in carbonate complex form in the aqueous layer where as the Zn is separated into the CCl$_4$ layer at pH 8.8 The needed reagents included Zinc free distilled water prepared by redistillation in a pyrex still or passage through an ion exchange column; 1N HCl prepared by distillation of approximately 6N HCl in a Pyrex still and dilution of the condensate to 1 N : 1 N NH$_4$OH prepared by distillation of concentrated NH$_4$OH into zinc free water in a pyrex container packed in ice (or by collection) of anhydrous NH$_3$ in a pyrex container of zinc free water.
ACS purity CCl₄ or (redistilled sulphur free CCl₄, stored in the dark); thymol blue indicator; and the following special reagents:

(i) **Corbamate solution, 0.2 per cent**: A 0.2 per cent solution was prepared by dissolving 0.2 gm of sodium diethylthiocarbamate in 100 ml of Zinc free water. This solution keeps satisfactorily in a brown bottle, if kept in a cool, dark place.

(ii) **Dithizone solution in CCl₄** (0.01 per cent): Into a 4-liter separatory funnel, 0.2 gm of diphenylthiocarbazone and 1 liter of CCl₄ were placed and the solid was brought into solution by frequent agitation for about 15 minutes. To this solution 2 litres of Zinc-free 0.02 N NH₄OH was added and the mixture was shaken to transfer the dithiozone to the aqueous phase. The CCl₄ (light green colour) was discarded and the aqueous phase was rinsed with several 100 ml portions of CCl₄. Then 500 ml of CCl₄ and 50 ml of zinc free 1 N HCl were added. The mixture was shaken to transfer the dithizone to the CCl₄ layer, then CCl₄ dithizone phase was diluted to 2 litres. The solution was stored in a glass-stoppered pyrex bottle, in a refrigerator.

(iii) **Ammonium citrate 0.4 M**: To 90 gm of ammonium citrate, enough water was added to make 1 litre. To this solution, enough zinc-free concentrated NH₄OH was added to bring the pH to 8.5. The zinc impurities were removed by extraction with portions of dithizone in CCl₄ in a large separatory funnel until the latter reagent no longer changed the colour and then with portions of CCl₄ until the citrate solution was free from dithizone colour.

(iv) **Standard Zinc solution**: Exactly 0.1 gm of pure zinc was dissolved in 50 ml of 0.02 N H₂SO₄ and diluted to 1 litre to give a 100
μgm/ml concentration of Zn. A secondary dilution of 10ml to 500ml gave a 2 μgm/ml working standard.

A solution containing Zinc in 30 to 40 ml of 0.02 N HCl was placed in a 125 separatory funnel, 25ml of 0.01 N NH OH was added to the CCl₄ phase and the mixture was shaken again for 3 minutes to extract the excess dithizone into the aqueous phase.

A 5 ml aliquot of the organic phase was taken with a pippett and diluted with CCl₄ to 25ml. The solution thus obtained was mixed and transferred to a colorimeter tube and the light transmission was measured at a 535 (or 540) μm light maxima. The amount of zinc present was determined by reference to a standard curve prepared in an identical manner with known amount of Zinc.

(e) Manganese (Mn): Manganese was estimated by Method given by Jackson (1958) p. 318. The needed reagents consisted of distilled water, filter paper to fit Buchner funnel, 1N NH OAC of pH-7, 30 per cent H₂O₂, concentrated HNO₃ and 1 N NH OAC of pH-7 to which 0.2g of hydroquinone had been added to each 100ml.

To 25g sample 250ml of N NH OAC of pH-7 was added. The flask was stoppered and shaken in the machine for 30 minutes and then allowed to stand at least for 6 hours with frequent shaking. The sample mixture was then filtered through a Buchner funnel and the filtrate was evaporated to dryness on the steam hot plate. The excess ammonium acetate was destroyed by moistening the residue with water and re-evaporating it to dryness. The residue was then treated with 30 per cent H₂O₂ and HNO₃ and the beaker was covered and digested on the steam plate for 30 minutes. The sides of the beaker were washed down with a fine steam.
of water from a wash bottle. The solution was transferred to a 150ml beaker if that size was not used for the original evaporation. It was important that this transfer was not to be made before the \( \text{HNO}_3 \) and \( \text{H}_2\text{O}_2 \) treatment. The cover glass on the beaker was next supported on glass hooks and the solution was evaporated to dryness to complete the oxidation of the organic matter and the removal of the \( \text{H}_2\text{O}_2 \).

**Development of HMnO\(_4\) Colour**: Ten ml of 85 per cent \( \text{H}_3\text{PO}_4 \) was added to the beaker, a cover glass was placed on it and the solution was warmed over a burner to boiling and then cooled to about 50°C to avoid spattering when diluted with water. The cooled acid was diluted with 10 ml of water and the solution was mixed by rotating the beaker. Approximately 0.2 g of sodium paraperiodate was added to the beaker and was covered and the solution was heated on the steam plate until a purple colour appeared. Then 75 ml of the purified water diluent was added and heating was continued for 40 minutes or until the purple colour no longer increased. Approximately 0.1 g additional periodate was added near the end of the period of digestion.

The hot solution was transferred to a 100 ml volumetric flask with the purified water diluent. The flask was closed and the solution was mixed, allowed to come to room temperature and diluted to exactly 100 ml with the purified water diluent. A portion of this solution was compared with the standard HMnO\(_4\) solution, by means of a photoelectric colorimeter with 540 mu light (525 to 545mu, as explained for the standard).

The HMnO\(_4\) colour obeys Beer's law with 540 mu light. The normality of Mn in the test solution was calculated from the percentage
transmission. The manganese was calculated to mg Mn⁺⁺ per 100 g sample.

\[ \text{mg Mn}^{++} \text{ per 100 gm sample} = N \times 100 \text{ml} \times \frac{100}{5} \]

Where, \( N = \) normality in the 100 ml of test sample.

\[ S = \text{gm sample weight represented in the aliquot} \]

\[ \text{ppm Mn}^{++} = \text{Meg Mn}^{++} \text{ per 100g sample} \times 549.3 \]

**Assay for different amino acids of *A. bisporus***

Edible fungi have been used as food by man since time immemorial. For many years, there has been a general belief that these edible fungi have high nutritive value. In an attempt to document the nutritional status of button mushroom, in general, investigations on amino acid composition of protein of *A. bisporus*, the most popular edible fungus of this group in India were undertaken.

The fruit bodies of *A. bisporus* were collected from the cultivated mushroom bed. Fresh well developed, clean and healthy sporophores were selected, dried at 70°C ± 5°C to constant weight, finely powdered and defatted in soxhlet apparatus using solvent ether. Qualitative amino acid determination was done in alcoholic extract using ascending paper chromatography with butanol, acetic acid and water in ratio of 4:1:5 by volume as solvent. The quantitative determination of amino acid was done with the help of amino acid autoanalyser.
Observations on the competitor moulds and disease of white button mushroom

Like other crops, mushroom are also affected by a large number of biotic and a biotic factors. Harmful fungi in large numbers were encountered in compost and casing soil during the cultivation of white button mushroom. Many of these act as competitor moulds there by adversely affecting spawn run and consequently the yield where as others attack the spawn and fruit bodies at various stages of crop growth producing distinct disease symptoms. Some times there is a complete crop failure depending upon the stage of infection, substandard compost preparation and various environmental conditions. Most of the growers cultivate button mushroom under natural climatic conditions. Poor hygienic conditions and lack of cook out facilities help in the perpetuation of various pathogens. Poorly treated or untreated casing materials also introduce large number of pests and pathogens.

The mushroom beds laid with spawned compost for different experimental purposes were intensively surveyed during the cropping season in 1998-99 and 1999-2000 from November to March to locate the beds affected by undesirable fungi and to ascertain the extent of damage at various temperature and humidity conditions.

Small pieces from substrate showing undesirable fungi were directly picked up with the help of sterilized forceps. These were then studied directly under the microscope for identification. The isolation of these fungi were also made from these contaminated pieces to collect them in polythene bags. These were then sterilized in 0.1 per cent mercuric chloride solution for 15 seconds and finally transferred onto culture plates under aseptic conditions and incubated at 28°C for 10 days. Pure culture of fungi were obtained by hyphal tip culture method and maintained on PDA slants.
Economics of Mushroom Cultivation

The study on the economics of mushroom production was made on the data obtained from the mushroom cultivation unit, K. S. S. P. G. College, Ayodhya (Faizabad) U.P. The primary data were collected from the cultivation farm during the period of October 1998 to March 2000 through a prestructured and pretested schedule.

To analyse the economics of mushroom production, it was essential to study the cost of production which consisted of fixed and variable cost including depreciation on the cost of cultivation room and investment on equipments and interest on fixed farm resources. The variable cost included value of compost, value of spawn, labour charges, electricity charges, casing soil, pesticides and insecticides etc. The financial ratios based on incom statement, which served as guideline for measuring the returns from an investment was also used. These ratios were divided into two categories viz. (a) Efficiency ratios which related the expenses to grass income and (b) Profitability ratios which related the income to capital investment.

(a) Efficiency ratio: The following efficiency ratios were used:

(i) Operating ratio = \[
\frac{\text{Total operating farm expenses (in Rs.)}}{\text{Grass farm income (in Rs.)}}
\]

(ii) Grass ratio = \[
\frac{\text{Total farm expenses (in Rs.)}}{\text{Grass farm income (in Rs.)}}
\]

(iii) Expense structure ratio = \[
\frac{\text{fixed cash expenses (in Rs.)}}{\text{Total cash expenses (in Rs.)}}
\]
Less than 1 efficiency ratio (i.e. operating, grass and expense structure ratio indicated the generation of additional return (income from an investment).

B. **Profitability ratio**: This related the income to capital investment and included:

(i) **Capital turnover ratio** = \( \frac{\text{Grass income (in Rs.)}}{\text{Total capital investment (in Rs.)}} \)

(ii) **Rate of return on capital** = \( \frac{\text{Net return to capital (in Rs.)}}{\text{Average capital investment (in Rs.)}} \)

The following measures of project worth were used to assess the economic feasibility of mushroom farming:

Pay back period was the length of time period from the beginning of the project that equated the net value of incremental production streams to the initial capital investment, i.e., the present value of total cash inflows from an investment equalized the total cash-out-flows.

Benefit-cost-ratio was obtained by dividing the present worth of benefit stream by the present worth of cost stream. Infact, this ratio measured the return or benefit per unit of cost.

For establishing the relationship between the grass returns from mushroom production and various independent variables, the production function analysis was performed.