and engineers.

Infrastructure

A Mushroom Farm

A **mushroom farm** is the business of growing fungi. All mushroom growing techniques require the correct combination of humidity, temperature, substrate (growth medium) and inoculum (spawn or starter culture). A tentative layout for a mushroom farm of 20-30 ton per acre production capacity is given below (Fig. 7 and Fig. 8):

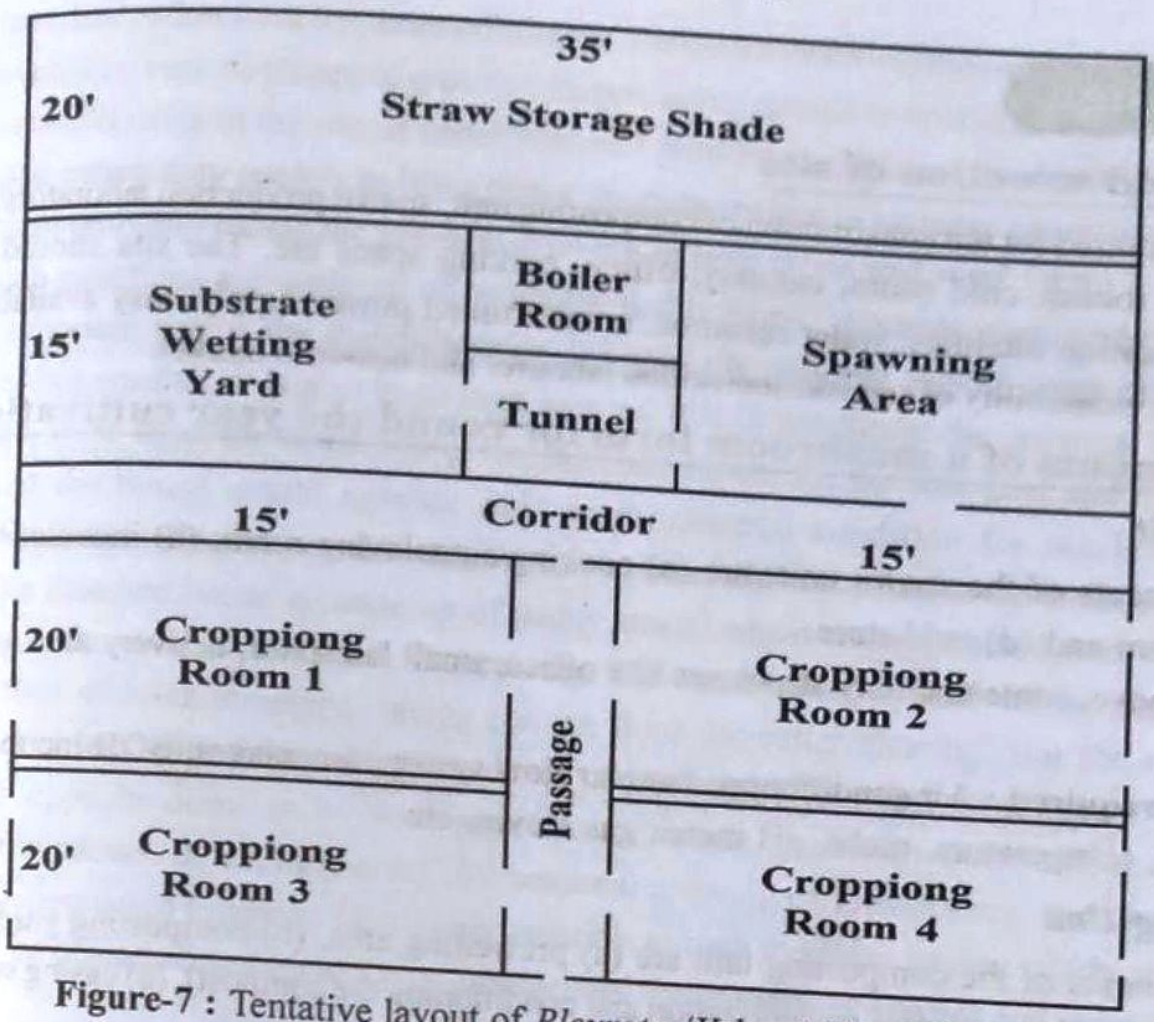

Figure-7 : Tentative layout of *Pleurotus/Volvariella* cultivation farm

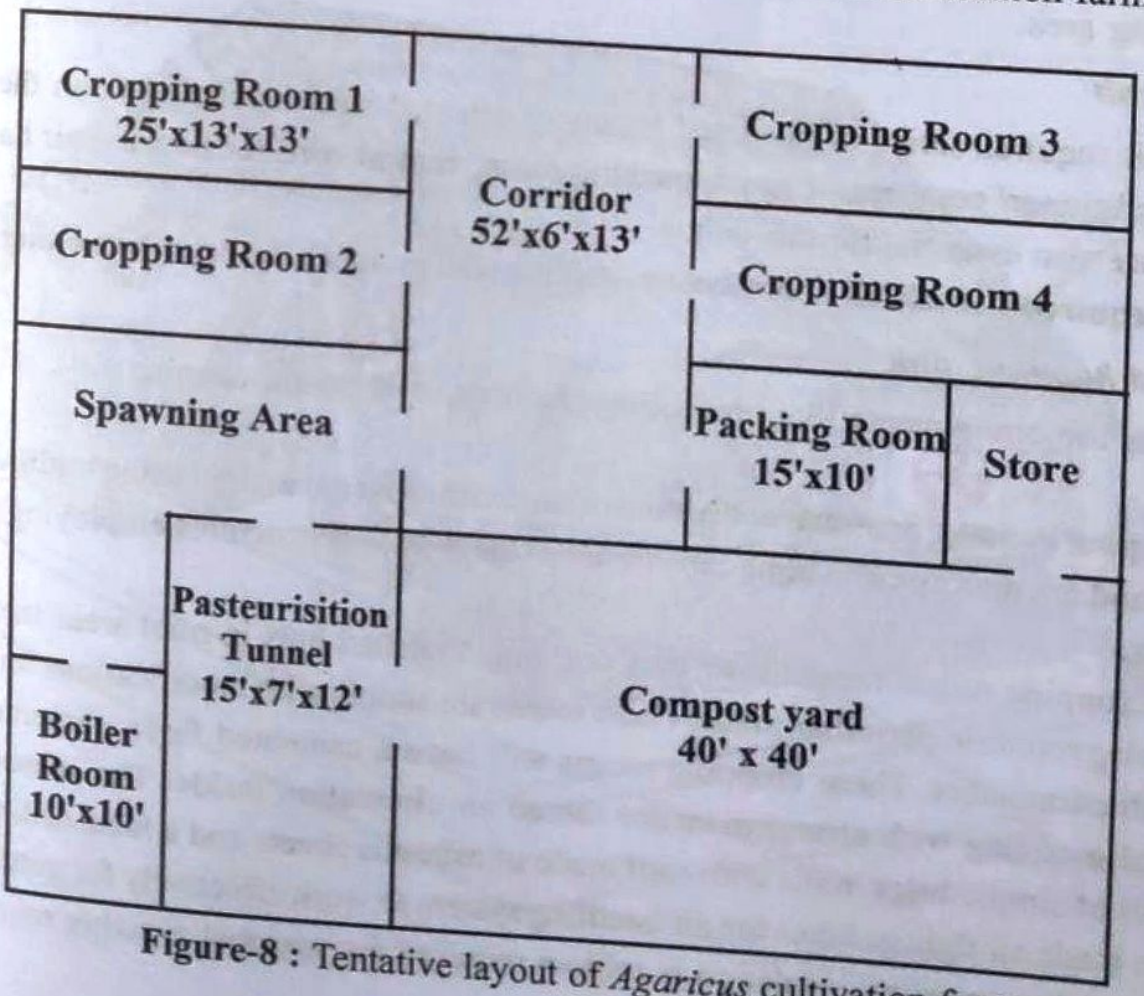

Figure-8 : Tentative layout of *Agaricus* cultivation farm.

Farm Design

I Land and selection of site

Land is required for the construction of composting unit, spawn production laboratory, mushroom cropping rooms, cold room, cannery, office, parking space etc. The site should have good communication facilities, water resource, uninterrupted power supply, easy availability of raw materials, availability of semiskilled/skilled labourer and nearer to market.

II Components of a mushroom farm for round the year cultivation

a. Spawn unit

Major components of the spawn units are (a) cooking/autoclaving room, (b) inoculation room, (c) incubation room and (d) cold store.

Besides the above, some ancillary structures like office, small lab space, delivery area, etc. may also be required.

Machineries required : Air conditioners, laminar flow system, autoclaves, BOD incubators, boiler, boling cattles, refrigerators, racks, pH meter, gas stoves, etc.

b. Composting Unit

Major components of the composting unit are (a) prewetting area, (b) composting yard, (c) Phase-I bunker, (d) phase -II tunnels [for pasteurization and conditioning the compost], (e) casing soil chambers, and (f) spawning area.

c. Cropping unit

A cropping unit requires series of insulated rooms of selected size depending upon the production targets. Air conditioner/ compressor room, packing room, central corridor housing air handling units and pipelines are also essential for this unit.

Machineries required : Insulated doors, central chilling station, racks, trolleys, harvesting trays, etc.

d. Post harvest handling unit

This unit needs the components like precooling chamber, cold room, canning hall, laboratory and store.

Seasonal cropping rooms : Seasonal cultivators of the mushroom are mainly using traditional methods of cultivation and are mainly cultivating mushroom in the thatched structures employing long method of composting.

The seasonal cropping rooms ranges from mud and pole thatched huts in rural areas to modern state of the art growing rooms in periurban areas. These rooms are simple with modifications for maintaining various growth parameters. These cropping rooms will have a cemented floor, cemented walls and ceiling or a false ceiling with arrangement for forced air circulation inside. The seasonal cropping rooms are built of simple brick walls with roof made of asbestos sheets and a false ceiling. The room is more or less made air tight to make the air handling system to work effectively for getting necessary air circulation during growing. Insulation is seldom required for seasonal growing rooms, as it will

not allow heat dissipation from the room efficiently. These simple rooms are used for seasonal mushroom growing, coinciding various phases of growth with prevailing outside temperatures. No energy is generally used for heating/cooling of the rooms under seasonal growing conditions. However, few units in plains have installed heavy duty coolers to bring down the temperature in summer conditions.

Most common cropping rooms for seasonal growing in both urban and rural set up is **thatched house** (Fig. 9). Such structures are made up of bamboos, paddy straw/ sarkanda grass and polythene sheets. The most important part is the thatch because it keeps the required indoor temperature maintaining cooler under hot weather. It is also to be kept in mind that to encourage the growers, locally available materials are to be used for constructing the thatched house for the low cost and at the same time ensuring that the house would provide right environmental condition for mushroom yield. The recommended thatched house was 8m×6m but it may be made according to the availability of land and resource. The thatched house is made up of paddy straw/ wheat straw, bamboo and wooden support. Sometimes plastic cover is also used, if necessary. The construction of such house may be performed through earthen or brick structure, having cement floor for better clearing. But the room should be always thatched roof with false ceilings made up of jute bags. The room should have windows on all four sides in opposite direction for well ventilation. Door(s), preferably double door, may be placed between the windows. In the hilly areas, for seasonal growing of mushrooms, such houses are made with bamboo frame and synthetic fiber cloth materials at both the sides having paddy straw insulation. Racks are to be placed according to the requirement of the growing of species and quantity of the same.

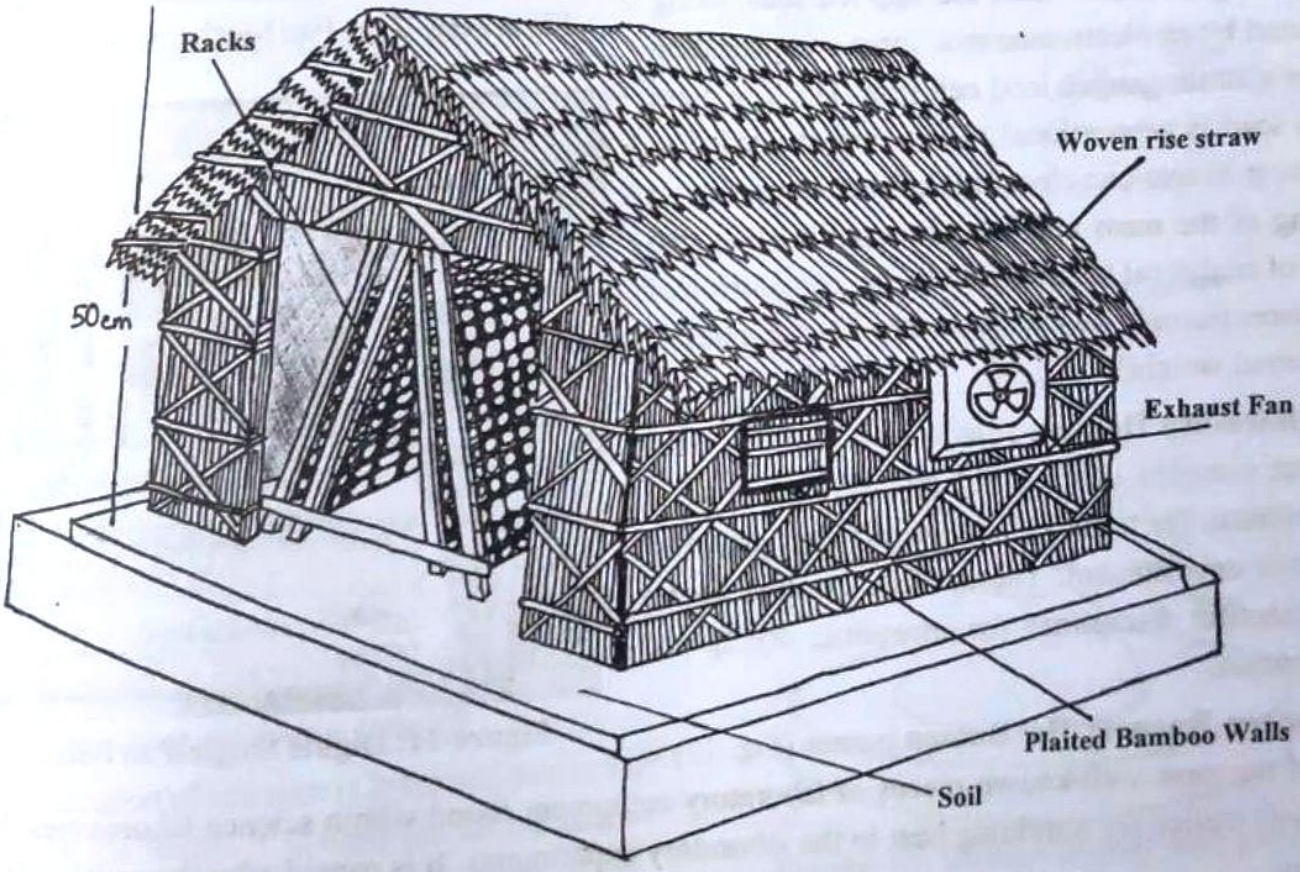

Figure-9 : Thatched house with racks for mushroom cultivation.

B Appliances and Tools

Instruments and appliances required for mushroom cultivations are weighing balance, heater, compound microscope, bunsen burner/ spirit lamp, laminar air flow apparatus, autoclave, water bath, hot air oven, pH meter, BOD incubator, humidifier, drum for boiling straw, hand chopper or chaff cutter etc.

1. Weighing Balance : A balance is a measuring device/instrument used to measure the mass of an object. Weighing is a common source of error that can be very difficult to detect in the final analyses of the results. For chemical or biological experiments, accurate amount of chemical should be weighed by using a balance. However, no experiment can be conducted without a balance. There are several types of balances used for weighing such as physical balance or single pan balance, chemical or analytical or digital electrical microbalance. All of these kinds of weighing balance are utilized in most of the laboratories. In mushroom cultivation, pan balance and electronic single pan balance are often used.

(i) Two-Pan Mechanical Balance : A Double-Pan balance is a scale having 2 pans which are balanced against each other (Fig. 10). The scale works like a see-saw, of which 2 pans attached to a beam over a centered pivot point.

Figure-10: Two-Pan Mechanical Balance

(ii) Digital Single-Pan Balance: These are usually top loading balances with the applied load being measured by an electromagnetic force compensation unit or a strain gauged load cell (Fig. 11). The mass of the load is proportional to the current needed to balance it. Single-pan electronic balances give a direct reading of the mass applied whereas the other two types of analytical balance rely on the comparison of two forces (an unknown weight with either an external or internal weight).

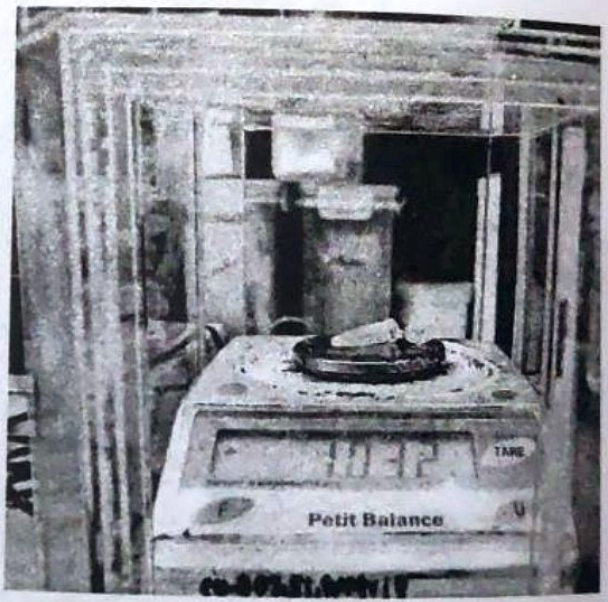

Figure-11: Digital Single-Pan Balance

2. Laboratory Heater: A laboratory heater is used to heat samples (both solid and liquid) to a set temperature, for a given amount of time, within an enclosed environment. The devices are used across the scientific disciplines for annealing, drying and sterilization.

3. Bunsen Burner: The Bunsen burner (Fig. 12) is one of the most well-known pieces of laboratory equipment found within science laboratories. It is the basic device for providing heat in the laboratory experiments. It is named after the name of R.W. Bunsen.

Parts of Bunsen Burner

(i) The Base: Heavy metallic base is connected to a side gas tube. Gas passes through a small hole called Nipple or Nozzle and enters into the burner tube.

(ii) The Burner Tube: It is a long metallic tube with two holes diametrically opposite to each other near the lower end which form the air vent. The tube can be screwed at the base and the gas from the nozzle mixes with the air coming through the air vent and burns at its upper end.

(iii) The Air Regulator: A short metallic cylindrical sleeve with two holes which is diametrically opposite to each other. For regulating the flow of air through the air vent, size of its hole is adjusted by rotating the sleeve.

The air flow needs to be correctly adjusted so that the temperature of the flame becomes quite high which is known as non-luminous flame. Three distinctly visible parts of the Bunsen flame are described below:

Principal Parts of Bunsen Flame (Fig. 12)

1. The Inner Dark Cone, A E C

It is innermost dark cone, just above the burner tube consisting of unburnt gases. This zone is the coldest one without any combustion.

2. The Middle Blue Cone, A D C E A

It is middle part of the flame where gas particles get heated up to incandescence and glow but do not burn. As the combustion is incomplete in this part, the temperature is not so high.

3. The Outer Non-luminous Mantle, ABCDA

It is purplish outer cone and the hottest part of the flame. As it is in direct contact with the atmosphere, combustion is quite complete in this zone.

Bunsen identified six different regions in these three principal parts of the flame (Fig. 12):

(i) The upper oxidising zone (f)

The location of this zone is in the non-luminous tip of the flame. It is used for all oxidation processes where highest temperature of the flame is not essential.

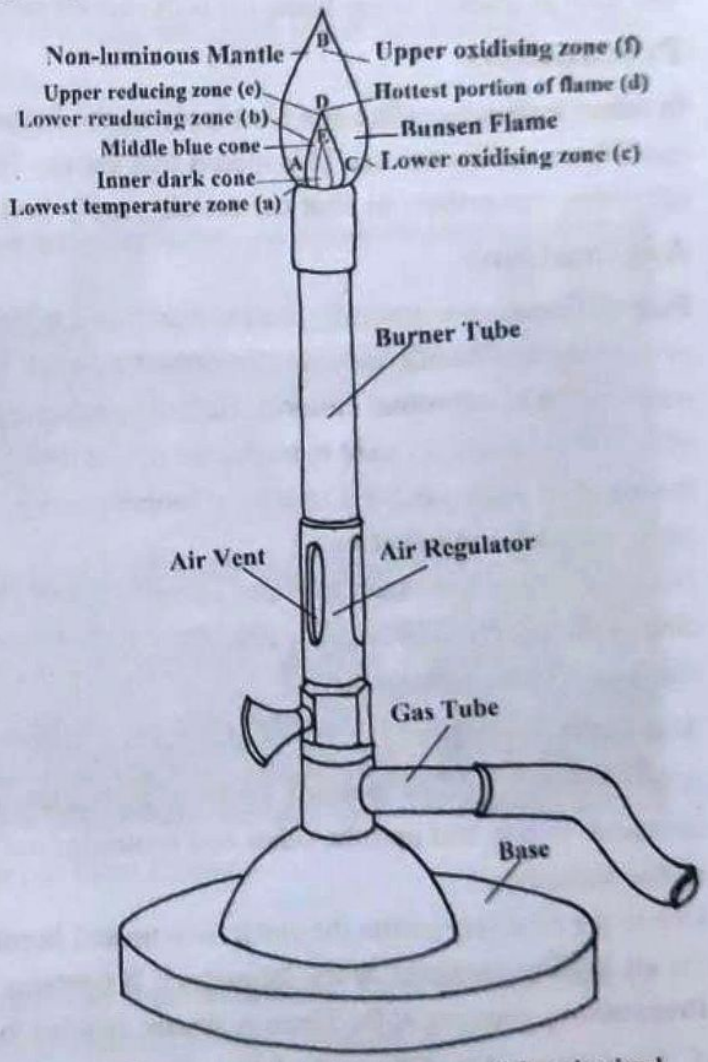

Figure-12: Bunsen Burner with principal parts of Bunsen Flame

(ii) Upper reducing zone (e)

The tip of the inner blue cone is upper reducing zone, rich in incandescent carbon.

(iii) Hottest portion of flame (d)

About one-third of the height of the flame and approximately equidistant from inside and outside of the mantle (i.e. the outermost cone of the flame) is the hottest portion of flame, known to be as fusion zone.

(iv) Lower oxidising zone (c)

Adjacent to the outer border of the mantle near the lower part of the flame is known as lower oxidising zone.

(v) Lower reducing zone (b)

The inner edge of the outer mantle near to the blue cone is the lower reducing zone where reducing gases mix with the oxygen of the air.

(vi) Lowest temperature zone (a)

The zone as marked in the flame (*a*) is the lowest temperature zone which is adjacent to the tube mouth.

Precautions

In some instances, when the air supply to the burner is increased, the flame blows out or detaches itself from the burner tip. This means that the gas flow is top great and needs to be cut down. While relighting remembers to shut off the air supply first.

Applications

Bunsen burners are generally used to heat liquid in order to induce chemical reactions. The sterilization of inoculation needle/loop is performed through this burner. It is also used during transfer and purification of microbial cultures. Before opening the cotton plug, mouth of culture tubes/flasks need to be flamed so that the contamination through the mouth is reduced. Sterilization of tools by using Bunsen burner/spirit lamp is called **incineration.**

Disadvantages : Bunsen burners cannot control the temperature like electronic heaters. The use of open flame is highly dangerous and risky, needs appropriate precautions.

4. Spirit Lamp

A spirit lamp (Fig. 13) contains a wick of cotton thread dipped in a spirit container in one end and the other end protrudes out through the nozzle of the container.

Figure-13: Sprit Lamp

Due to the capillary action the spirit rises up and burnt. As the flame is nonluminous, thus can be used for all heating purposes in the laboratory. It contains a cover which is used to put off the lamp.

Precaution : Blowing at the flame is strictly avoided to put off the lighted burner.

5. Laminar Air Flow Cabinet

A laminar flow cabinet (Fig. 14) is an enclosed bench, designed to prevent contamination. This

instrument consists of an air blower in the rear side of the chamber producing airflow with uniform velocity along parallel flow lines. There is a special filter system of high efficiency particulate air filter (HEPA), capable of removing all airborne particles (≥ 0.3 mm) to maintain sterile conditions.

Parts and working mechanism of laminar air flow cabinet

A laminar flow cabinet consists of a filter pad, a fan, a HEPA filter and one cabinet containing one fluorescent tube and one UV tube.

- The fan sucks the air through the filter pad where dust is trapped.
- Afterwards, the prefiltered air has to pass the HEPA filter to remove contaminating fungi, bacteria, dust etc.
- Sterile air flows into the working area to minimize the risk of contamination.
- Laminar air flow cabinets are normally made of stainless steel with no gaps or joints thereby blocking the entry of the contaminants in the working zone. Laminar air flow cabinet is also known as **clean bench** because the air for the working environment is thoroughly cleaned by the precision filtration process.
- Inside the cabinet a fluorescent tube and a UV tube are fitted which is regulated by two separate switches fitted outside the apparatus.
- Switch on the UV light for a period of 30 mins so as to kill the germs, if any present in the area of working space.

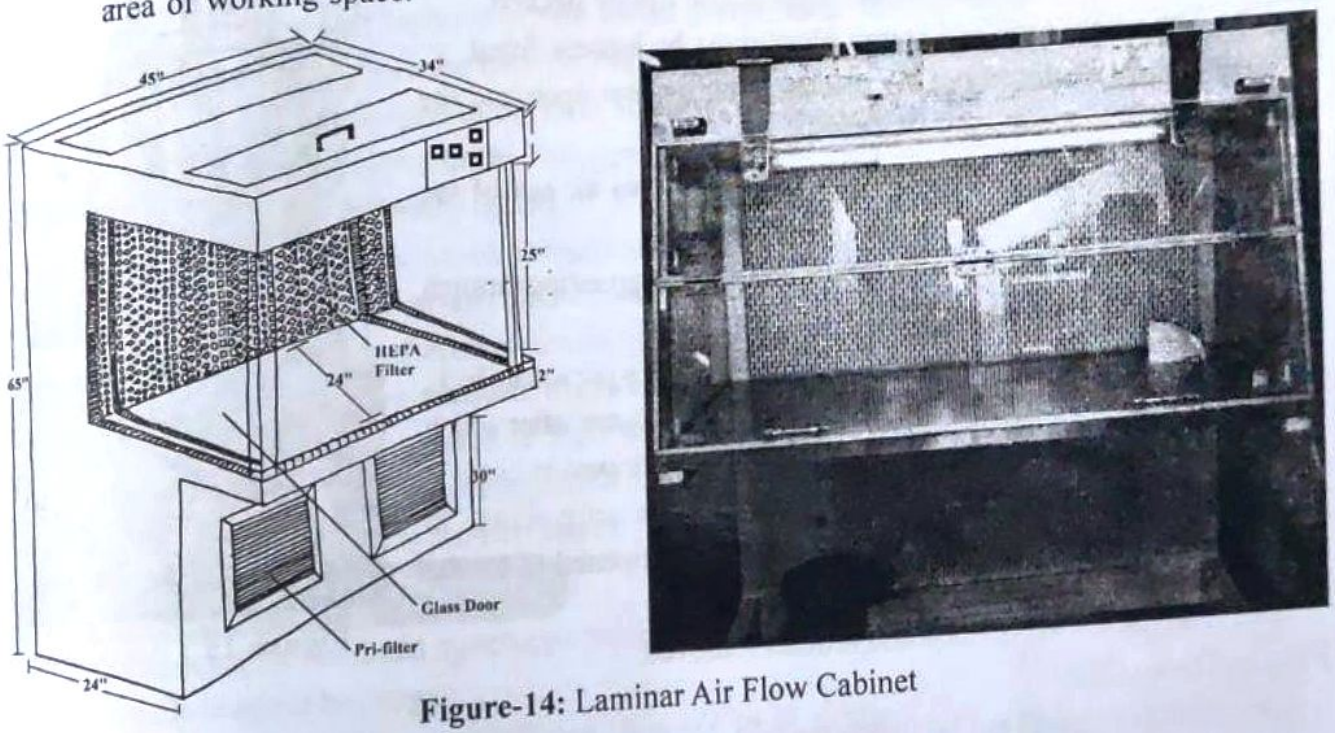

Figure-14: Laminar Air Flow Cabinet

Types of Laminar air flow cabinet

Laminar Flow Cabinets can be produced as both horizontal and vertical cabinets. There are many different types of cabinets with a variety of airflow patterns for different purposes. These are as follows:

a) Horizontal Laminar Flow Cabinet
b) Vertical Laminar Flow Cabinet

c) Laminar Flow Cabinets and Hood

d) Laminar Flow Benches and Booth

For mushroom cultivation, horizontal laminar air flow cabinet is used.

Horizontal Laminar Flow Cabinet

In this apparatus, the air flow comes through prefilter, changes its direction and is processed across the work in a horizontal direction. It creates a homogeneous air flow in the chamber. The constant flow of filtered air protects material and product from contamination.

6. Autoclave: A simple autoclave (Fig. 15) consists of vertical or horizontal cylindrical body with a heating element, a perforated tray to keep the articles, a lid that can be fastened by screw clamps, a pressure gauge, a safety valve and a discharge tap.

Different types of autoclave

- Simple "pressure cooker type" laboratory autoclave,
- Steam jacketed downward displacement laboratory autoclave, and
- High pressure prevacuum autoclave

Operation of autoclave:

The followings are to be kept in mind during the operation of autoclave.

- The articles to be sterilized must not be tightly packed.
- The screw caps and cotton plugs must be loosely fitted.
- The lid is closed but the discharge tap is kept open and the water is heated.
- As the water starts boiling, the steam drives air out of the discharge tap.
- When all the air is displaced and steam start appearing through the discharge tap, the tap is closed.
- The pressure inside is allowed to rise up to 15 lbs per square inch.
- At this pressure the articles are held for 15 minutes, after which the heating is stopped and the autoclave is allowed to cool.
- Once the pressure gauge shows the pressure equal to atmospheric pressure, the discharge tap is opened to let the air in.
- The lid is then opened and articles removed.

Figure-15 : Autoclave

Precautions

- Articles should not be tightly packed.
- The autoclave must not be overloaded.
- Air discharge must be complete and there should not be any residual air trapped inside.
- Caps of bottles and flasks should not be tight.
- Autoclave must not be opened until the pressure has fallen or else the contents will boil over.
- Articles must be wrapped in paper to prevent drenching; bottles must not be overfilled.

Advantage

- Very effective way of sterilization, quicker than hot air oven.

Disadvantages

- Drenching and wetting or articles may occur.
- Trapped air may reduce the efficacy.
- It takes long time to cool.

7. Water Bath : The water bath (Fig. 16) has container or vessel filled with heated water and the temperature of which is maintained at a constant level. It is used to incubate samples over a period of time at a constant temperature.

Figure-16 : Water Bath

Main parts of water bath

a) **Container or tank bath:** It is an insulating box made up of steel.
b) **Heater:** The insulating box is fitted with electrode heating coil.
c) **Thermometer:** An indicator to mark the correct temperature of the system.
d) **Thermostat or regulator:** The temperature is controlled through this thermostat.

Types of water bath

i. **Circulating water bath:** It is also called stirrer water bath in which water is thoroughly distributed throughout the bath resulting in a uniform temperature. An electric motor with rotary magnet is flanged to the bath bottom.
ii. **Non-circulating water bath:** This type of water bath depends mainly on convection instead of water being uniformly heated. It is less accurate in terms of temperature control.
iii. **Shaking water bath:** An additional control for shaking which moves liquid from one place to another is present in this type of water bath.

8. Hot Air Oven : It is a box shaped structure fitted with a thermostat control, temperature indicator, meshed shelves and must have adequate insulation (Fig. 17).

Articles to be sterilized are exposed to high temperature (160°C) for duration of one hour in an electrically heated oven. The heat is transferred to the article by radiation, conduction and convection. This method was introduced by Louis Pasteur.

Operation of hot air oven

a. Articles to be sterilized must be perfectly dry before placing them inside to avoid breakage.
b. Articles must be placed at sufficient distance so as to allow free circulation of air in between.
c. Mouths of flasks, test tubes and both ends of pipettes must be plugged with cotton wool.
d. Articles such as petri dishes and pipettes may be arranged inside metal canisters and then placed.

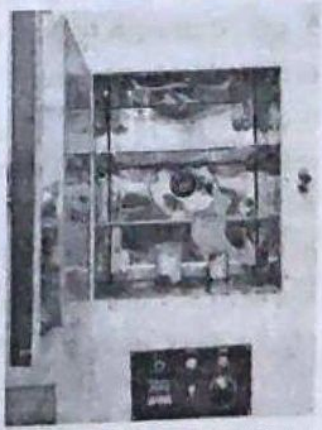

Figure-17: Hot Air Oven

e. Individual glass articles must be wrapped in aluminium foil.
f. Placed the objects on shelves of oven after which metallic doors were firmly closed.
g. The main switch was then put on and the thermometer (marking upto 200°C) was inserted and the temperature was allowed to rise.
h. The thermometer unit was adjusted at that temperature.
i. The objects to be sterilized are subjected to a temperature of around 170°C for around 90 mins.
j. The main switch should be then put off and the sterilized objects were only removed after the temperature in the oven sufficiently feels near to the room temperature.

Precautions

The hot air oven must not be opened until the temperature inside has fallen below 60°C to prevent breakage of glassware.

Advantages

- It is an effective method of sterilization of heat stable articles.
- The articles remain dry after sterilization.
- A dry heat cabinet is easy to install and has relatively low operating costs.
- It penetrates materials.
- It is non-toxic and does not harm the environment.
- It is non-corrosive for metal and sharp instruments and
- This is the only method of sterilizing oils and powders.

Disadvantages

- Time consuming method because of slow rate of heat penetration and microbial killing.
- High temperatures are not suitable for most materials e.g. plastic and rubber items.
- Since air is poor conductor of heat, hot air has poor penetration.
- The time and temperature required will vary for different substances and overexposure may ruin some substances.
- Cotton wool and paper may get slightly charred.
- Glasses may become smoky.

9. pH Meter : A typical pH meter (Fig. 18) is an electronic instrument consisting of special measuring probes (a glass electrode and a reference electrode) connected to an electronic meter that measures and displays the accurate pH reading of the solution. The glass electrode is a half-cell and the calomel electrode/ reference electrode is another half-cell.

It is used for measuring the pH (acidity or alkalinity) of a liquid (though special probes are

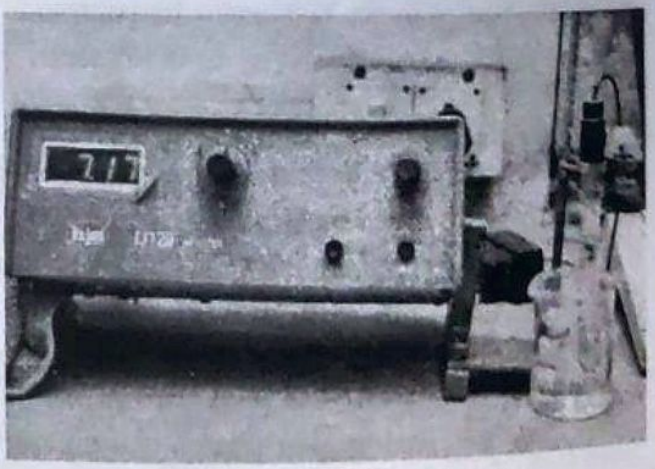

Figure-18 : pH Meter

sometimes used to measure the pH of semi-solid substances). Maintenance of pH values is an important parameter for the growth and process of any organism.

Principle: pH can be defined as a negative log of hydrogen ions (H^+) concentration:

$$pH = - \log 10 (H^+) = 7;$$

where, p= Power and H= Hydrogen

pH is the degree of acidity and alkalinity of a solution on a scale 1 to 14. pH values 1 to <7 show the acid values, pH 7 neutrality and pH >7 to 14 alkalinity. The pH of water is 7 at 25° C. The acid is proton donor and the base is the proton acceptor (acid = base + H^+) i.e. acid dissociates and produces hydrogen ion concentration (H^+).

Application

i. By using a pH meter, pH of an unknown solution is measured.
ii. It is widely used for the diagnosis of various disorders in human body.
iii. It is also used in the field of agriculture, pharmaceuticals, brewing, dyeing, printing, jam & jelly manufacturing and corrosion prevention.

Precautions

i. A pH meter should be carefully treated in order not to damage the electrode.
ii. The electrodes should always be held in distilled water to prevent the electrode from being non-functional.

10. Incubator : An incubator (Fig. 19) is an instrument that consists of copper/steel chamber, around which warm water or air is circulated by electric current or by means of small gas flame to get accurate temperature containing storage system.

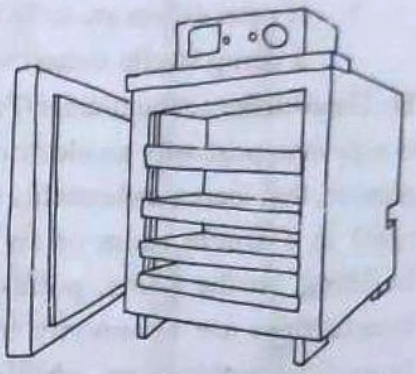

Figure-19 : Incubator

Working Principles

a. Temperature greatly influences the microbial growth. Therefore, instrument is generally designed that can allow the desired microorganism to grow at a particular temperature.
b. It is operated to allow the microbial growth on a suitable medium under proper temperature. In an incubator, the variation in temperature should not be more than one degree.
c. Some microbes are to be grown at lower temperatures for specific purposes. The BOD is a temperature control incubator which can maintain temperatures from 50°C to as low as 2-3°C is used for incubation in such cases.

Structure

(i) The incubator is made up of double walled chamber adjusted to a desired temperature. A box or container with insulated walls and a door fitted with a latch to close the door firmly.
(ii) It is done by using an external knob controlling the thermostat system. The gap between two walls is insulated to check heat conduction.
(iii) On the front of the base on one side is a knob which can switch-on and switch off the instrument. In the centre of the front or besides the knob, a bulb is fitted to indicate whether the instrument is off or on.

(iv) On the backside a thermostat is fitted to regulate the desired temperature. So, the temperature of the incubator is kept constant due to its control by using thermostat.

(v) A thermometer is inserted from the outside of the incubator through a hole in the centre of its roof to read the temperature of the inside chamber.

(vi) Its base contains a heating unit heated through electricity.

(vii) The internal chamber is provided with one or more shelves.

(viii) Now, sophisticated incubators are available with humidity and oxygen control systems.

Precautions

1. The door of the incubator should be opened only when necessary.
2. If tubes are to be incubated for a long time or at higher temperature, the medium may become too dry due to excessive evaporation. In such cases cotton plug should be pushed inside the neck of the tube. The tube should be covered by a rubber cap so as to cover the plug.
3. If petri dishes are to be incubated for a long time they may be placed in a moist chamber with a damp sterile cotton wool at the bottom.

11. Humidifier : A humidifier (Fig. 20) is a device, primarily an electrical appliance, that increases humidity (moisture) in a single room or an entire building. In the home, point-of-use humidifiers are commonly used to humidify a single room, while whole-house or furnace humidifiers, which connect to a home's HVAC system (Heating, Ventilating, and Air Conditioning system), provide humidity to the entire house.

■ Working Principles

All humidifiers operate in different ways but they all have the same purpose to produce healthier air. Humidifiers deliver great results by balancing out the dry air in the house with moisture.

This all begins with the water tap line that is connected to a current water source for the humidifier.

- While the water inlet valve permits the humidifier to

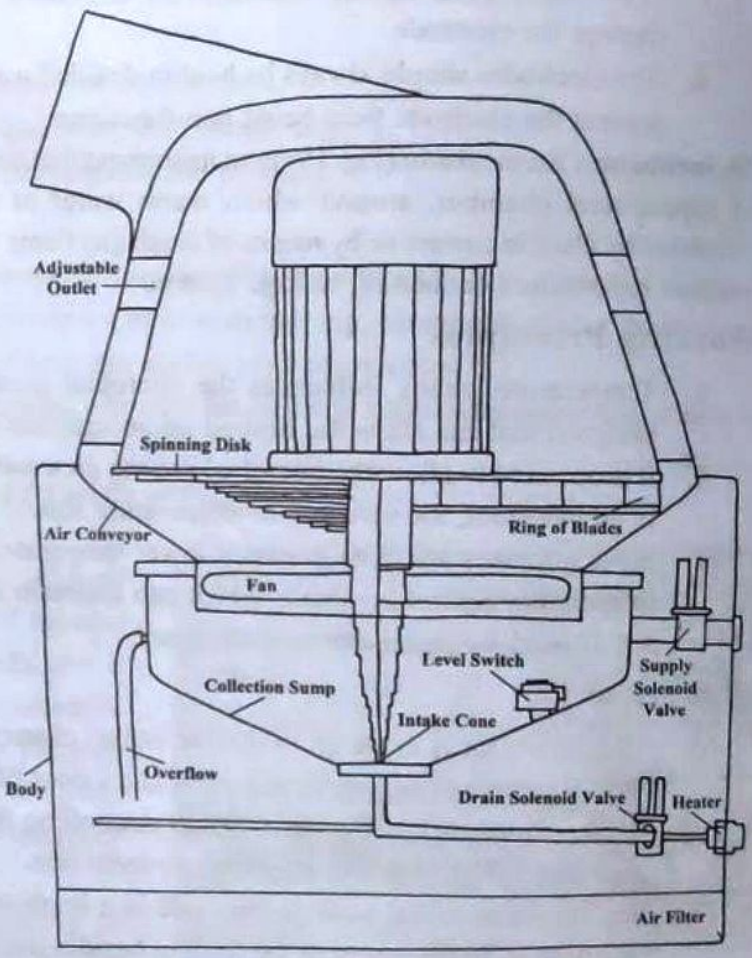

Figure-20 : Humidifier

receive water flow, the orifice controls the amount of water flow to the valve for economical purposes.

- The water is then distributed through the water feed tube and into the evaporator pad, which holds the water while it is being evaporated to produce humidified air.
- From the evaporator pad, the water migrates to the drain pan. This leads to an invisible mist that overpowers your indoor air.
- Once all of the dry air is bombarded with a moist, denser air, there will be a dynamic change in the atmosphere of the house.

Tools required for mushroom cultivation: Inoculating needle, inoculating loop, dissecting needle, scalpels, forceps, scissors, thermometer etc.

Inoculating Needle and Loop

These are most commonly used tools for mushroom cultivation. An **inoculation needle** (Fig. 21) is a very simple laboratory equipment used to transfer and inoculate living microorganisms. It is made of platinum, tungsten or nichrome which tip was bent to form a hook, fixed into a metallic rod with steel screw shaft and a handle. The needle straight wire is used for transferring culture from solid medium. Even smaller amount of liquid culture can be manipulated by using straight needle.

Inoculation loop, (Fig. 21) also called a smear loop or microstreaker, is a simple tool used for transferring inoculum for streaking and retrieve an inoculum from a culture of microorganisms. This is most commonly used tool where a wire made of platinum, tungsten or nichrome with a loop of about 5 mm was fixed into a metallic rod with steel screw shaft and a handle. The loop should be such so as to retain a small circular film in it by dipping it in solution.

Both inoculation hook and loop are sterilized either by using Bunsen burner or hot heating coil wire till the needle or loop become red hot. After wire is cooled, these are generally used in transferring culture from medium. The loop and wire also used for picking small quantities of solid materials from a microbial colony, and can be used to inoculate either a liquid or solid medium. Both loop and straight wire must be flamed immediately after use so that contamination is avoided.

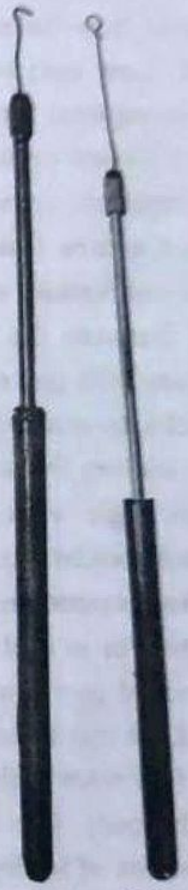

Figure-21: Inoculating Needle and Inoculating Loop

A Glass Goods required for mushroom cultivation

Glass goods like culture tubes, petridishes, beakers, conical flask, funnels, graduated measuring cylinder, graduated pipette, glass milk/saline bottle etc are required for mushroom cultivation units.

B Miscellaneous requirements for mushroom cultivation

Miscellaneous requirements viz. test tube rack, pipette stand, glass marker pen, absorbent and non-absorbent cotton, small knife, rubber bands, muslin/cheese cloth, jute rope, coconut rope or

plastic ropes, plastic Sheet, polythene bag, gunny bags, culture rack, stove, sieves, water sprayer etc.

(i) Plastic bags

Plastic bags (polypropylene plastic bags) of different sizes are required for mushroom cultivation. Breadth×length of mushroom bags are 6×8 inch for spawn production, 18×26 inch for making packets of straw substances, 7×10 inch for short term storage and marketing of mushrooms and variable size for long term preservation of dry mushrooms. 1-2 mils (1 mil = 25.4 μm) thick bag is well suited to most situations.

(ii) Sieves

Sieves are generally made of bamboo cane, plastic or iron net, which is required to drain out excess water from boiled grain.

(iii) Low cost stoves

It is required for preparation of medium boiling of spawn grain, substrate etc.

(iv) Vessel or boiling kettle

Aluminium or steel vessels working on electrically, kerosene or gas are used for boiling of grains.

(v) Culture Rack

For cultivation, steel or wooden rack of 32×82 (breadth×height)×height as convenient to keep gap 22 between the rack and wall, and 32 between two racks is normally used. Each rack contains 4 selves with gap of 22 . and the lower most shelf is 1ft. above the ground.

Racks are made up of the angle iron for horizontal and vertical support with iron mesh strips used for the shelves for housing compost. Length (vertical axis) of the racks is generally made up of 4-5 cm thick angle while horizontal support is made up of 3.5-4 cm thick. Width of each shelf on the racks should not be more than 135 cm in any case as width more than that creates hindrance in performing various operations during cropping and most important of that is harvesting. Cultivation can be done in bags or in shelved beds. Five to seven rows of shelves (depending on height of the room) can be provided one above the other in the racks keeping a minimum distance of 60 cm in between. This distance can slightly be narrowed down if cultivation is employed in shelved beds. In such a case all the four sides of the shelf should be provided with 15-20 cm high iron sheets for housing the compost in the beds. If more than 5 shelves on each rack are kept in the room than there should be the provision of trolley running in between two rows of racks just above the fourth shelf for carrying out the various operations. Depth of the compost in shelves is generally kept at 15-20 cm while bags can be filled up to the maximum height of 30 cm. A room of standard size (60×17×12 ft) can accommodate 2 rows of racks each 135 cm wide. This will absorb 9 ft. of the room and the rest 8 ft can be used to have one central path of 90 cm and 2 side paths of 75 cm. Length of each rack would be 52-55 ft.

C Chemicals

1. Disinfectant
2. Chemicals for culture media
3. Chemicals for spawn production
4. For long term storage

Sterilization

Sterilization is the most important step in successful mushroom cultivation along with maintaining of hygiene in every step. It generally refers to denote the process that eliminates, removes, kills, or deactivates all forms of biological agents (in particular referring to microorganisms such as fungi, bacteria, viruses, spores, unicellular eukaryotic organisms such as Plasmodium, etc.) present in a particular substrate, area, surface, equipment, foods, medications, or biological culture medium. A mushroom grower is always to be cautious in disinfection through sterilization and maintaining hygiene in mushroom cultivation, otherwise the desired yield would not be achieved.

Principal ways of sterilization : Sterilization can be done in the following ways :

A Physical Method

1. Thermal (Heat) methods
   - (i) Dry Heat Sterilization
     - a. Sunlight
     - b. Red heat
     - c. Flaming
     - d. Incineration
     - e. Hot air oven
   - (ii) Moist Heat Sterilization–Autoclaving
   - (iii) Pasteurization
   - (iv) Tyndallization
2. Radiation method
3. Sterile Filtration method
4. Sonic and Ultrasonic vibrations method

B Chemical Method

1. Surface sterilization by chemicals
2. Sterilization by fumigation

Methods of sterilization

A Physical Method

1. **Thermal Heat Method :** Heat sterilization is the most widely used and reliable method of sterilization of articles that can withstand heat, involving destruction of enzymes and other essential cell constituents.
   - This method of sterilization can be applied only to the thermo stable products and moisture sensitive materials.
   - Heat acts by oxidative effects as well as denaturation and coagulation of proteins.
   - Those articles that cannot withstand high temperatures can still be sterilized at lower temperature by prolonging the duration of exposure.

Factors affecting the sterilization by heat

- **Nature of heat:** Moist heat is more effective than dry heat.
- **Temperature and time:** Temperature and time are inversely proportional. As temperature increases the time taken decreases.
- **Number of microorganisms:** More the number of microorganisms, higher the temperature or longer the duration required.
- **Nature of microorganism:** Depends on species and strain of microorganism, sensitivity to heat may vary. Spores are highly resistant to heat.
- **Type of material:** Articles that are heavily contaminated require higher temperature or prolonged exposure. Certain heat sensitive articles must be sterilized at lower temperature.
- **Presence of organic material:** Organic materials such as protein, sugars, oils and fats increase the time required.

Action of heat

- Dry heat acts by protein denaturation, oxidative damage and toxic effects of elevated levels of electrolytes.
- The moist heat acts by coagulation and denaturation of proteins.
- Moist heat is superior to dry heat in action.
- Temperature required to kill microbe by dry heat is more than the moist heat.
- Thermal death time is the minimum time required to kill a suspension of organisms at a predetermined temperature in a specified environment.

■ Fundamentally, heat can be classified into two types, viz.,

(i) Dry heat (160-180°C) : Sterilization for thermo stable products.

(ii) Moist heat (121-134°C) : Sterilization is used for moisture resistant materials.

(i) Dry heat sterilization: This method is used for destruction of microorganisms through the use of dry heat. In dry heat sterilization technique, killing of microorganisms increases with longer exposure (1.5-3 hours) of lethal temperature (160-180°C) than moist heat sterilization. The standard way is at least two hours at 160°C dry heat. A rapid method, heats to 190°C for 6 minutes for unwrapped objects and 12 minutes for wrapped objects. This method is used for sterilizing glass and steel goods.

The benefit of dry heat includes good penetrability and non-corrosive nature which makes it applicable for sterilizing glass wares and metal surgical instruments. It is also used for sterilizing non-aqueous thermos-stable liquids and thermos-stable powders.

■ Principle of Dry heat sterilization

Sterilizing by dry heat is accomplished by conduction. The heat is absorbed by the outside surface of the item, then passes towards the centre of the item, layer by layer. The entire item will eventually reach the temperature required for sterilization to take place.

Dry heat does most of the damage by oxidizing molecules. The essential cell constituents are destroyed and the organism dies. The temperature is maintained for almost an hour to kill the most difficult of the resistant spores.

Mechanism of action

- Destroys bacterial endotoxins.

Advantages

- Most widely used and reliable method of sterilization, involving destruction of enzymes and other essential cell constituents.

Disadvantages

- It can be applied only to the thermo stable products.

Various available methods of dry heat sterilization are as follows-

(a) Sunlight: It is responsible for spontaneous sterilization in natural conditions. By killing bacteria suspended in water, sunlight provides natural method of disinfection of water bodies such as tanks and lakes.

The microbicidal activity of sunlight is mainly due to the presence of ultra violet rays in it. In tropical countries, the sunlight is more effective in killing germs due to combination of ultraviolet rays and heat.

Advantages

- This is a natural method for sterilization.
- This is cost effective.
- No man power is required.

Disadvantages

- Time dependent.
- Not possible to sterilize glassware, chemicals, culture media, etc.

(b) Red heat: Articles such as bacteriological loops, straight wires, tips of forceps and searing spatulas are sterilized by holding them in Bunsen flame till they become red hot.

Advantages

- This is a simple method for effective sterilization.
- This is cost effective.

Disadvantages

- This process is restricted to those articles that can be heated to redness in flame.

(c) Flaming : This is a method of passing the article over a Bunsen flame, but not heating it to redness. Small objects like needle, forceps, scalpel, etc. are usually sterilized by dipping in ethanol and subsequently passing by several times through oxidizing flame of Bunsen burner or spirit lamp until it glows red ensuring that any infectious agent is killed. On the other hand, the articles such as mouth of test tubes, flasks, glass slides and cover slips are passed through the flame a few times for sterilization.

Advantages

- This is a simple method for effective sterilization.
- This is cost effective.

Disadvantages

- Even though most vegetative cells are killed, there is no guarantee that spores of different microorganisms too would die on such short exposure.
- This method too is limited to those articles that can be exposed to flame.
- Cracking of the glassware may occur.

(d) Incineration : This is a method of destroying contaminated material by burning them in incinerator. Articles such as soiled dressings; animal carcasses, pathological material and bedding etc. should be subjected to incineration.

Advantages

- It is suitable only for those articles that have to be disposed.

Disadvantages

- This technique results in the loss of the article.
- Burning of polystyrene materials emits dense smoke, and hence they should not be incinerated.

(e) Dry heat sterilization by hot air oven : Articles to be sterilized are exposed to high temperature (160°C) for duration of one hour in an electrically heated oven. The heat is transferred to the article by radiation, conduction and convection.

Metallic instruments (like forceps, scalpels, scissors), and glass wares (such as petri-dishes, pipettes, flasks, all glass syringes) are generally sterilized by this method.

The most common time-temperature relationships for sterilization with hot air sterilizers are

- 120 minutes at 150°C (300°F),
- 60 minutes at 160°C (320°F),
- 30 minutes at 170°C (340°F) and
- 15 minutes at 180°C (360°F).

(ii) Moist heat sterilization: Moist heat sterilization involves the use of steam in the range of 121-134°C. Steam under pressure is used to generate high temperature needed for sterilization. Saturated steam acts as an effective sterilizing agent. This method is used for sterilization of culture media and all other implements necessary for cultural work. Sterilization is done at 15lb/sq inch pressure or 121°C temperature for 15 minutes. Soil or cereal grains are sterilized for 1 hour. In some cases, soil sterilization is done consecutively for 2-3 days. Autoclave is used to serve the purpose. In an autoclave the water is boiled in a closed chamber. As the pressure rises, the boiling point of water also raises. In an autoclave the relationship between pressure and boiling temperature of water is given below (Table 9).

Table-9: The relationship between pressure and boiling temperature of water

PRESSURE (lb/sq inch)	Temperature (°C)
0	100
1	102.3
3	105.7
5	107.8
7	111.7
9	114.3
10	115.6
12	118.0
15	121.0
18	124.3
20	126.0
25	130.0
30	135.0

Advantages

It has more penetrative power than dry air, it moistens the spores (moisture is essential for coagulation of proteins), condensation of steam on cooler surface releases latent heat, condensation of steam draws in fresh steam.

(iii) Pasteurization: Pasteurization is a heat treatment process, named after scientist Louis Pasteur, who demonstrated this technique in the year 1860. It is a general term for the process of heating sufficiently to destroy almost all non-sporulating microorganisms. In this process the materials are exposed to temperature 63°C for 30 min or 72°C for 15 sec or 89°C for 1 sec.

(iv) Tyndallization: Tyndallization is named after John Tyndall. The process involves boiling of materials for 20 minutes in free flowing steam (100°C), cooling, incubating for a day, and then repeating the same for three to four times. The basis of this process is to allow heat-resistant surviving perennating bodies and spores of the previous boiling steps to germinate or grow to form the heat-sensitive vegetative stage which will be killed in the next exposure. By this way through repeated exposure finally all the microorganisms will be killed.

1. **Radiation:** Sterilization of different heat liable components can be achieved using electromagnetic or particulate radiation. Many types of radiation are used for sterilization like electromagnetic radiation (e.g. gamma rays and UV light), particulate radiation (e.g. accelerated electrons). Ultra violet ray (from a germicidal lamp) and X-ray are commonly used for killing microorganisms. The major target for these radiation is microbial DNA.

Radiation sterilization with high energy gamma rays or accelerated electrons has proven to be a useful method for the industrial sterilization of heat sensitive products.

Mechanism of action

- Ionization of nucleic acids.

Application

- Radiation sterilization is generally applied to articles in the dry state; including surgical instruments, sutures, prostheses, unit dose ointments, plastics.

Advantages

- It is a useful method for the industrial sterilization of heat sensitive products.

Disadvantages

- Undesirable changes occur in irradiated products; an example is aqueous solution where radiolysis of water occurs.

3. Sterile filtration method: Thermo labile liquids can be sterilized by microfiltration by using membrane filters with pore size of 0.2-0.45 μm or sintered glass filters with the help of vacuum pump. These filters are able to remove smallest organisms like bacteria. The major mechanisms of filtration are sieving, adsorption and trapping within the matrix of the filter material.

Application

- Various applications of filtration include removing bacteria from ingredients of culture media, preparing suspensions of viruses and phages free of bacteria, measuring sizes of viruses, separating toxins from culture filtrates, counting bacteria, clarifying fluids and purifying hydrated fluid.
- It is used to remove microbes from heat labile liquids such as serum, antibiotic solutions, sugar solutions, urea solution.
- Sterilizing grade filters are used in the treatment of heat sensitive injections and ophthalmic solutions, biological products and air and other gases for supply to aseptic areas.
- They are also used in industry as part of the venting systems on fermenters, centrifuges, autoclaves and freeze driers. Membrane filters are used for sterility testing.

Advantages

- Membrane filters are commonly used to remove particles from solutions that can't be autoclaved.
- Filtration is aided by using either positive or negative pressure using vacuum pumps.
- It is used for both the clarification and sterilization of liquids and gases as it is capable of preventing the passage of both viable and non-viable particles.

Disadvantages

- Filtration does not kill microbes.
- Does not differentiate between viable and non-viable particles.

4. Sonic and Ultrasonic vibrations method: Sound waves of frequency >20,000 cycle/second kills bacteria and some viruses on exposing for one hour. Microwaves are not particularly antimicrobial in themselves, rather the killing effect of microwaves are largely due to the heat that they generate.

Application

They are used to clean and disinfect instruments as well as to reduce microbial load.

Advantages

High frequency sound waves disrupt cells.

Disadvantages

This method is not reliable since many viruses and phages are not affected by these waves.

B Chemical Method

1. Surface sterilization by chemicals: Outer surface of Petri dish is sometimes sterilized by dipping in 10 ml of propylene oxide in an open dish placed inside an air tight bell jar. There are other surface sterilizing agents as mentioned below (Table 10): -

Table-10 : Some surface sterilizing agents

SURFACE STERILIZING AGENT	CONC. (%)	TREATMENT TIME (minutes)	RINSING AGENT
Formaldehyde solution	5	1-5	70% ethyl alcohol and sterile distilled water.
Hydrogen peroxide (H_2O_2)	3	1-5	Sterile distilled water.
Potassium permanganate	2	1-5	Sterile distilled water.
Sodium or calcium hypochloride	0.35	1-5	Sterile distilled water.
Ethanol	70	1-5	Sterile distilled water.
Mercuric chloride ($HgCl_2$)	0.01	1-5	70% ethyl alcohol followed by sterile distilled water
Silver nitrate ($AgNO_3$)	1	1-5	2% sterile NaCl solution followed by sterile distilled water.

1. Sterilization by fumigation: The chemically reactive gases such as formaldehyde and ethylene oxide possess biocidal activity. Ethylene oxide is a colourless, odorless, and flammable gas.

Mechanism of action: The mechanism of antimicrobial action of the two gases is assumed to be through alkylation of sulpohydryl, amino, hydroxyl and carboxyl groups on proteins and amino groups of nucleic acids.

Ideal Condition: The concentration ranges (weight of gas per unit chamber volume) are usually in range of 800-1200 mg/L for ethylene oxide and 15-100 mg/L for formaldehyde with operating temperatures of 45-63°C and 70-75°C respectively.

Advantages

- Penetrating ability of gases.