Notes

34

PROVIDING ORGANISMS FOR LABORATORY WORK

Laboratory exercises are an integral part of learning science. A lot of equipment is required for a laboratory course in physical sciences. For biological sciences, on the other hand, living or preserved organisms have to be provided for the study of anatomy, physiology, histology and animal behaviour. In this lesson, you will learn about methods of culturing organisms in the lab, maintaining an animal house for live animals used in the laboratory and using equipment such as nets and press for collection and preservation of plants and animals required for lab exercises.

OBJECTIVES

After reading this lesson you shall be able to :

- *identify and list the organisms which are usually cultured in the laboratory;*
- *list various animals generally needed in a biological laboratory;*
- *list the materials required and describe methods of culturing some common protozoans such as Amoeba and Paramecium, Hydra, Rhizopus, Drosophila*
- *describe the method of growing root tips of onion in the laboratory;*
- *mention the facilities required in an animal house for rearing animals;*
- *explain the measures to keep the surroundings of animal clean;*
- *explain measures for personal hygiene of these handling the animals;*
- *state the measures to feed animals in the animal house;*
- *list various steps to be taken for care of sick animals;*
- *list various equipment required for collection of flora, fauna such as nets vasculum, plant press and mention their uses;*
- *outline the organisation of a typical biology laboratory;*
- *state need for proper ventilation in the lab especially as an outlet for fumes;*
- *list measures to prevent fire hazards.*

34.1 CULTURING ORGANISMS IN THE LABORATORY

Certain organisms can be collected from nature and then multiplied inthe laboratory. Growing large population of organisms in the laboratory by providing space and nutrition is termed **Culturing**. For research work, few organisms are collected from nature or bought from dealers and then maintained and grown and multiplied on a large scale. In the school and college laboratories organisms are cultured on a small scale specifically for laboratory use by individual students.

34.1.1 Preparation for Culturing Organisms

Four points have to be kept in mind while culturing organisms or rearing them for laboratory work. These are :

(i) knowledge of location or habitat where a particular organism may be found;

(ii) methods of collection;

(iii) methods of culture that is kind of vessel to be used to grow them; kind of food to be given to them and ways of protecting them from enemies;

(iv) methods of preservation for future use.

34.2 COMMON ORGANISMS CULTURED IN THE LAB

Organisms are cultured in the lab for morphological, taxonomic cytological, genetic and behavioral studies. Following are some organisms commonly cultured in the Biology laboratory.

(i) Paramecium and Amoeba belong to the phylum Protozoa. They are obtained from fresh water ponds and easily cultured. Being microscopic in size, stained slides of these protozoans are prepared for observing their structure. Living specimens are studied under the microscope for ciliary and pseudopodial movement.

Fig. 34.1 Paramecium

Fig. 34.2 Amoeba

(ii) *Rhizopus*, the bread mould is a fungus. Its structure and its stages of life cycle can be studied from a lab culture of the bread mould.

(iii) *Hydra* is a cnidarian. It is difficult to rear it but can be obtained from the ponds where it sticks to leaves of aquatic plants.

(iv) *Drosophila* is the fruitfly with which breeding experiments were done by early geneticists and many genetic principles were discovered. In the laboratories, all over the world it is cultured for experiments on Behaviour, Genetics. Cytology and Evolution because of its short life history, easy culture and prolific reproduction rate.

(v) Onion root tips are grown especially for the study of mitosis. Onion or *Allium cepa* has sixteen large chromosomes and slides made from onion root tips clearly show the four phases of mitotic cell division.

Fig. 34.3 Hydra

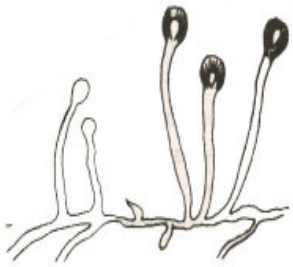

Fig. 34.4 Rhizopus-bread mould

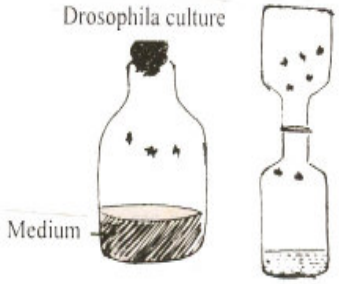

Fig. 34.5 Drosophila-the fruitfly

34.2.1 Culturing Paramecium

Material required : Vegetable remains from ditches, grass, leaves, jam bottles or any other jars, cotton, glass tube which can be made into a micropipette.

Procedure : Half fill jars with grass, leaves and vegetable ramains. Add water to almost fill the jars. Leave for a week. If kept at 70° to 80° F, results are better. This is the stock culture.

Pure Culture : Boil grass blades and seeds in water for 20 minutes. Divide the vegetable matter in different bottles and allow them to stand. Bacteria will grow and appear as a scum on the surface. Take a drop on the slide and locate ***Paramecia*** (Fig. 34.1) Use micropipette to draw in *Paramecia* by placing it near *Paramecia* which will be drawn up the micropipette by capillary action. Add them to other jars in which *Paramecia* will grow and divide and a pure culture will be obtained.

34.2.2 Culturing Amoeba

Amoebae occur at bottom of container or on surfaces of leaves and stems when pond water is collected.

Into a shallow dish, pour 100 ml of distilled water. Add three grams of rice and contaminate with water mould such as *Saprolegnia.* Leave in a warm place for a week. Inoculate with *Amoebae* (Fig. 34.2) from a good culture, taking material from bottom of container. After culture is well established that is Amoebae feed and divide, add occasionally few grains rice and add fresh distilled water.

34.2.3 Culturing *Hydra*

Hydra (Fig. 34.3) is difficult to rear but can be obtained from fresh water ponds sticking to blades of leaves of aquatic plants. Pick aquatic vegetation and place it in jars of pond water. Care has to be taken not to let the specimens become too warm. Generally, when hydra float upon surface of water, the amount of oxygen is insufficient. So to dislodge them, leave in the dark overnight. They will float on the surface. Quickly pick up and transfer to new culture dishes equally fast or they will stick to the pipette.

34.2.4 Culturing bread mould - *Rhizopus*

Rhizopus or ***Mucor*** often occur on stale bread. It grows rapidly and can be easily cultured.

Material required : Slice of bread, moist chamber made from tin can.

Method : Take piece of bread. Slightly moisten and keep in a closed container for two or three days. The best place would be some warm dark corner. White cottony growth appears with black dots scattered on it. The black dots are sporangia (Fig. 34.4) with lots of spores. If a bit of the cottony growth is mounted in a drop of water, the general structure of ***Rhizopus***, its sporangia and spores are visible.

34.2.5 Culturing *Drosophila*, the fruit fly

If an empty jam bottle containing an overripe banana is kept at a fruit shop, very soon tiny red eyed fruit flies will fly into it. These can then be transferred into culture bottles.

Jam bottles or milk bottles can be cleaned and boiled for use as culture bottles. The culture medium is prepared by heating water and dissolving one gram of agar in it. One gram of yeast, 5 grams of brown sugar and 7.5 grams of cornflour are then added. Heating is continued till the mixture is semi solid and can be poured into the culture bottle. A drop of Propionic acid is added to the medium to inhibit fungal growth.

Flies can be transferred into the culture bottles easily as fruitfly is negatively geotactic (moves upwards against gravity). Thus when an empty bottle is inverted on a jar containing ***Drosophila*** (Fig. 34.5) the flies move into the inverted bottle. A paper strip is inserted between the mouths of the two bottles and the upper bottle with the flies is removed. These flies can then be transferred to a fresh culture bottle.

The optimal culture temperature for ***Drosophila*** is 25°C and ***Drosophila*** culture is kept in a BOD maintained at this temperature. In case the culture has to be maintained at room temperature, September to March is the best time.

34.2.6 Growing onion root tips

Material required : Coplin jars, or wide mouth bottles/100 m1 beakers onions, scalpel, water.

Procedure : Take an onion and scrape off the dry roots from the bulb to expose the disc. Fill a coplin jar with tap water and place the onion bulb on it such that the disc touches the water. Place this near the window to get enough light for three to four days. Roots will start growing and tips can be clearly seen. (Fig. 34.6).

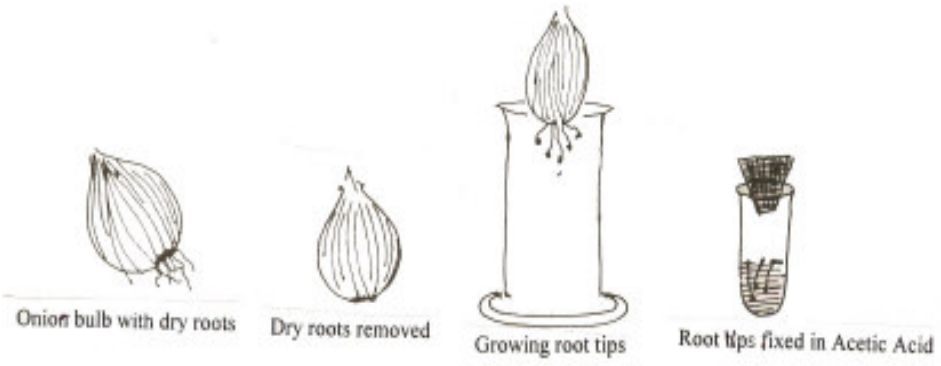

Fig. 34.6 Growing onion root tips

Onion root tips are used for preparing slides to observe various phases of mitosis as cells forming the root tip are rapidly dividing cells.

Preparation of slides showing stages of mitosis

To prepare cell division slides a squash preparation of onion root tips is essential.

Material required : 1 : 3 Aceto Alcohol, 1 N Hydrochloric acid, 1% Acetocarmine stain, slides and cover slips.

Procedure

Cut off root tips and fix in 1 : 3 Aceto Alcohol (1 part of glacial acetic acid and 3 parts of Absolute Alcohol) in a watch glass bottle. After five minutes, put the root tips in 1 N HCl in a watch glass and warm them. Remove HCl by washing in water and leave in the stain, 1% Acetocarmine for five to ten minutes. Carmine is a dye obtained from the cochineal bug and the stain is prepared in Acetic Acid. Remove the stained root tip on a clean slide and tease with a needle. Place a cover slip. Put the slide on a filter paper. Fold the filter paper to cover the slide and gently soak the extra stain. Apply pressure with thumb on the cover slip where the teased root tip is. This is called squashing and root tip cells then spread out on the slide and when viewed under the microscope, stages of mitosis can be seen. Care has to be taken not to shake the coverslip.

INTEXT QUESTIONS 34.1

1. Name three organisms cultured in the laboratory.

   .....

2. Why are root tips grown in the laboratory.

   .....

3. How are *Drosophila* transferred from one cultrue bottle to another?

   .....

4. Where is *Hydra* collected from?

   .....

5. On what is *Rhizopus* grown?

   .....

34.3 TIPS ON THE BIOLOGY LABORATORY

In the Biology laboratory plants and animals are handled all the time. It becomes absolutely necessary to clean the working tables and wipe with an antiseptic before and after lab work starts. There should be arrangement for proper disposal of used up animals and plants. This would prevent attack by microorganisms and smell of rotting plants and animals. The fear of spreading infection would also not be there.

An exhaust fan is absolutely essential in a biology lab. It not only removes (i) odour of animals but also (ii) fumes of formalin used to preserve certain animals. Also (iii) chemicals are used for certain experiments - their fumes are removed when the air inside the lab is made to circulate with the use of an exhaust fan.

A fire fighting equipment and antiburn ointment such as Burnol should also be kept in the laboratory. Since explosive chemicals and spirit lamps or bunsen burners are required for experiments, it is better to take safety measures.

The biology laboratory needs to be well lit and working table should receive enough natural light. The chemicals should be kept on a shelf in one corner of the room.

34.4 REARING ANIMALS IN ANIMAL HOUSE AND CAGE MANAGEMENT

Animals such as frogs, rats, cockroaches, leeches etc. are used for the study of organ systems and other anatomical details. These animals have to be procured before hand and kept in the animal house. An animal house is a room earmarked for keeping animals required for dissection. Provision for proper ventilation, exhaust and water taps for washing animals are absolutely essential in the animal house. Insect cages can be made with cake tins or cardboard boxes (Fig. 34.7).

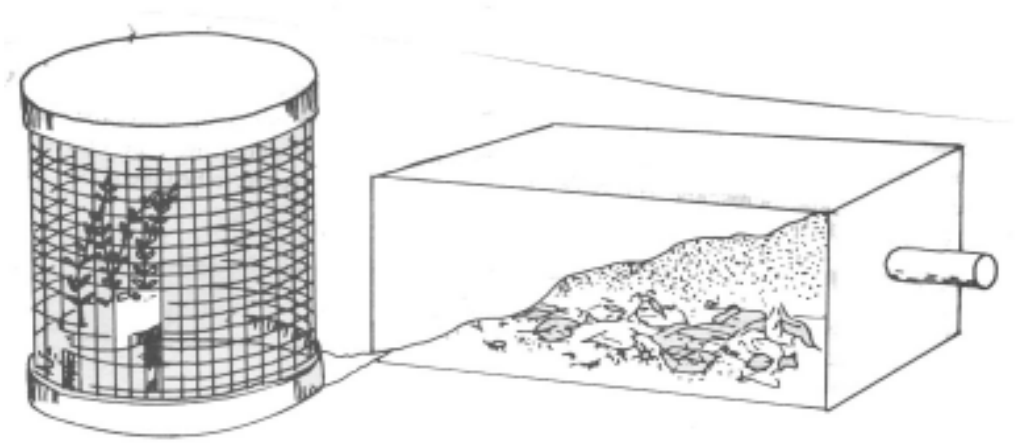

Fig. 34.7 Insect cages that can be made at home

Animals are reared in the animal house in cages. Cockroaches are kept in cages with wire mesh forming the floor and walls of the cage and a lid is on the top to take out the cockroaches with the help of tongs. (Fig. 34.8). Cages for rats are made of crowbars. A door on the side is for introducing rats on taking them out. A sliding tray is kept at the bottom to remove rat droppings. (Fig. 34.9).

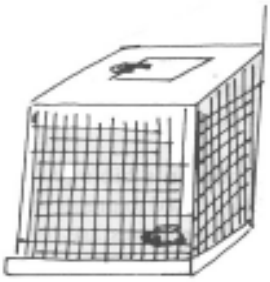

Fig. 34.8 Cockroach cage

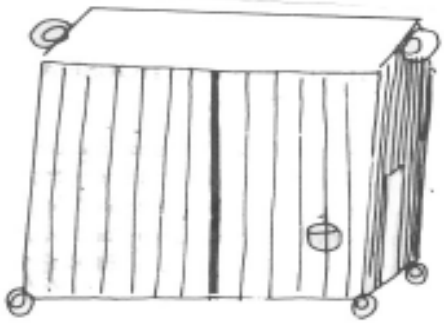

Fig. 34.9 Rat cage

Frogs are kept in the froggery. Froggery is a cement tank usually built in the botanical garden so that insects coming to the garden become available as food. A mud substratum is made in the cement tank which is kept wet. The tank is covered with a wire mesh and there is provision for taking out and introducing frogs.

Food, temperature, humidity and sanitation are important factors in the rearing of animals that is cage management. Proper feeding is of prime importance. The amount of food given should be such that it is consumed completely. Overfeeding should never be done. Also if too much food is given, it may rot and the animals may catch infection. Cockroaches are given wet filter paper bits or bread crumbs to eat. Rats are fed with gram, potatoes, carrots etc. They are voracious eaters and have to be fed two or three times, otherwise hungry caged rats maul and injure each other. Frogs trap insects that hover around.

On weekends when the institution is closed somebody is put incharge of feeding the reared animals. Providing water is as important as giving food and bowl full of water is kept in the cage. In case an animal is sick, it should be immediately isolated.

Workers incharge of handling animals for laboratory work have to keep in mind certain precautions. They should wear gloves. Frogs or rats should be held from the waist region to avoid injury by teeth or claws in case of rats and slipping away of frogs.

34.5 EQUIPMENT REQUIRED FOR COLLECTION OF FLORA AND FAUNA

A few items of equipment have to be carried on collection trips.

A. For carrying the collected material following vessels would be required

(i) **Plastic buckets**.

(ii) Small **vials** with stoppers.

(iii) **Plastic bags** with rubber bands.

(iv) **Vasculum** and **plant press** which are described later.

B. For picking out flora sticking on rocks or ground and aquatic animals sticking to rocks.

(i) Pocket knife for prying sessile plants and animals from rocks or hard substratum. The knife should be oiled before use and also used with care so that it does not injure any part of the collector.

(ii) Geology pick for turning rocks and chipping off specimens.

(iii) Hammer and chisel for collecting lichens and deep rooted plants on rocks.

(iv) Nails

C. Night collection

Nocturnal animals (active during night) and intertidal specimens have to be collected at night. For this it is essential to carry

(i) Flash light

D. For culturing bacteria, the following is necessary

(i) Petriplates for keeping the medium

(ii) Culture tubes

(iii) Wire needle and wire loop

E. For trapping insects or other animals

(i) Insect trap or

(ii) Berlese funnel about which you will learn in lesson 35.

(iii) Nets are required not only for trapping insects but also various other animals. Various kinds of nets are available about which you shall learn later in the chapter.

F. For plant collection, sheets, gummed tape, cables, water proof pen are also required.

34.6 VASCULUM AND PLANT PRESS

34.6.1 Vasculum

Vasculum is made up of a metal cylinder with a sliding door usually worn on a strap over the collectors shoulder into which plant specimens are placed. (Fig. 34.10).

Polythene bags and paper bags are also used for putting fruits, seeds and small specimens after collection.

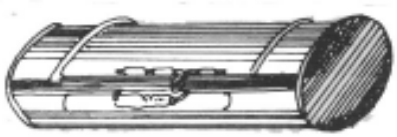

Fig. 34.10 Vasculum

Fig. 34.10 Vasculum

34.6.2 Plant Press

Plant press is an indispensable tool for pressing fresh plant specimens to subsequently dry them and mount permanently.

Plant press is made of wood and is of two types.

(i) **Lab press :** Is shown in Fig. 34.11. It is heavier than field press.

(ii) **Field press :** Is lighter in weight

A student can make his own plant press from plywood at low cost.

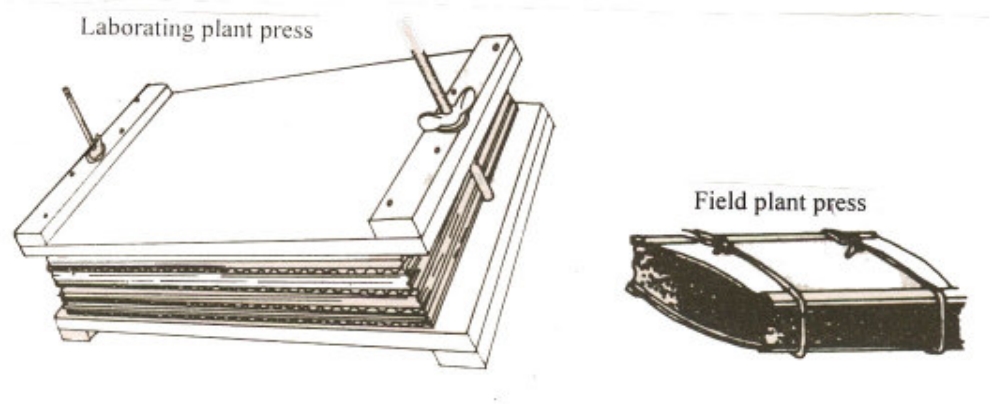

Fig. 34.11 (a) Laboratory Plant Press and (b) Field Plant Press

34.7 NETS

Several kinds of nets are used for collecting fauna. Following are some of them

(i) **Biological dredge :** A dredge consists of a strong net attached to a heavy frame which is pulled along the substrate in order to obtain plants and animals. It can be used in fresh water and the sea. Collector can use it from a boat also (Fig. 34.12).

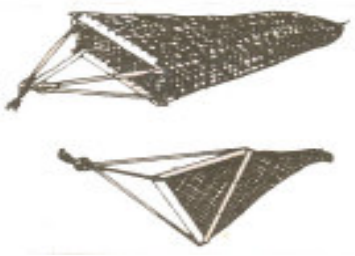

Fig. 34.12 Biological dredge

(ii) Plankton nets have fine or coarse mesh with a tapered tip.

(iii) Insect nets are of (a) dredge type (b) aquatic dip type or sweep net. (Fig. 34.13)

(iv) Fish nets are of various kinds (Fig. 34.14).

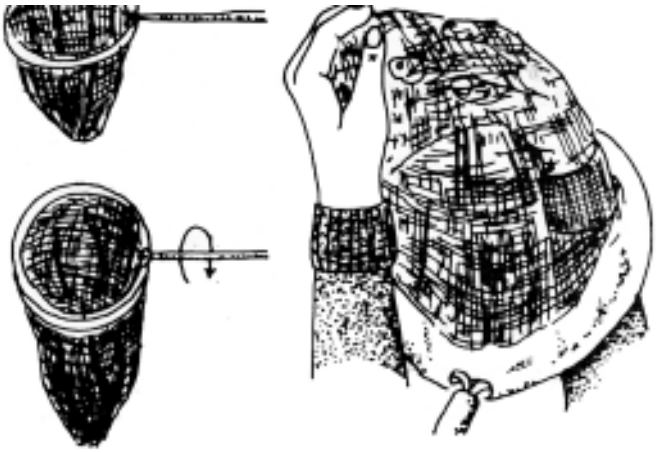

Fig. 34.13 Dip and Sweep net

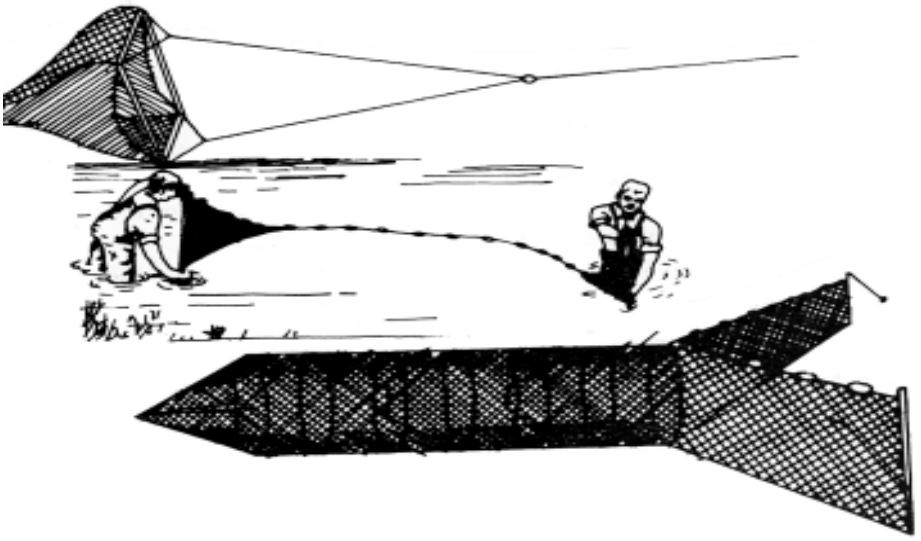

Fig. 34.14 Fish net

INTEXT QUESTION 34.2

1. How are formalin fumes prevented from collecting in the lab?

   .....

2. Mention any two factors which are important for cage management.

   .....

3. Name the equipment required to release flora or fauna from rocks.

   .....

4. What is a vasculum?

   .....

5. What is a biological dredge used for?

   .....

WHAT YOU HAVE LEARNT

- Certain organisms can be collected from nature and multiplied in the lab. This is called 'culturing'.
- While preparing cultures, one should know
  - (i) the location or habitat from which a particular organism can be collected.
  - (ii) the method of collection
  - (iii) the method of culture and
  - (iv) how to preserve organisms for future use.
- *Paramecium* and *Amoeba* can be cultured after collecting them from pond. Parameciam are grown in bottles containing grass, leaves etc. on which bacteria is growing. *Amoeba* is cultured in shallow dish with rice and water mould in it.
- *Hydra* can be cultured in a jar with aquatic vegetation.
- *Rhizopus* or bread mould is cultured on stale bread.
- *Drosophila* or fruit fly are cultured in empty milk bottles containing medium having yeast, brown sugar, and cornflour. They are transferred by placing empty bottle on the medium and the fruit flies fly up.
- Onion root tips are grown in coplin jars with water and used for the study of cell division by making squashes.
- Biology lab should have an exhaust fan to remove odour and fumes of formalin and other chemicals.
- A firefighting equipment should also be kept in the lab.
- Animals required for dissection are reared in cages. Cages for different organisms are built differently.
- Food, temperature, humidity and sanitation are important factors for cage culture. Food has to be given in adequate quantity and arrangements made for feeding during weekends.
- Plants can also be kept in cages.

- Items of equipment required for collection of fauna and flora are (a) for keeping collected specimens or (b) for picking specimens attached to rocks. (c) for culturing microorganisms and (d) for trapping insects or other animals.
- For keeping collected specimens, plastic buckets, vials, plastic bags and vasculum are required.
- For picking specimens free of substratum, a knife or pick are required.
- Flash light is required for night collection.
- Insect trap or Berlese funnel is to trap insects.
- Nets of different kinds are for collecting insects, fish, plankton etc.
- A vasculum is a metal cylinder with a sliding door carried by the collector on the shoulder.
- A plant press is used for pressing fresh plant specimens. There are two kinds of plant press - lab press and field press.
- A dredge is a net attached to a metal frame which can scrape the ground to collect specimens.

TERMINAL QUESTIONS

1. Describe the method of culturing any one protozoan.
2. How can you culture bread mould on a piece of bread?
3. How can you prepare onion root tip squash to study mitosis?
4. Why should there be an exhaust fan in a biology lab?
5. Write a note on animal house.
6. What all should you carry if you go on an excursion to collect animals?

ANSWERS TO INTEXT QUESTIONS

34.1
1. Amoeba/Paramecium/Hydra/Rhizopus/Drosophila (any three)
2. To study mitosis/cell division.
3. By placing an empty bottle above culture bottle.
4. Aquatic fresh water plants.
5. Bread

34.2
1. By installing an exhaust fan.
2. Food/temperature/humidity/ventilation
3. Pocket knife/Geology prick
4. A metal container for collecting plants
5. Collecting aquatic flora and fauna