PRESERVATION OF PLANT PATHOGENS AND DISEASE SPECIMENS:

A pathogen is defined as an infectious, biological agent that often causes diseases or illness to its host. Pathogens are believed to be a vital part of the evolutionary process, as a result eliminating them would cause imbalance in ecosystem as well as genepool. As a result, their conservation becomes necessary.

Plant disease specimens can be preserved in several ways, the method varies according to the type of specimen in hand. Preservation techniques can be of two types –

- ✓ Dry preservation
- ✓ Wet preservation

The following type of techniques are carried out for preservation:

1. Flat materials such as leaves and thin stems may be dried and fastened to sheets of heavy paper. Cellulose tapes make good fasteners. The specimen should be dried before mounting by placing between blotters, several sheets of newspapers, or pieces of corrugated paper board alternated with several sheets of newspapers. Leaves are pressed (same for stems) between the layers and keep in a warm place to dry. Drying should be carried out for 1 or 2 days to prevent moulding of specimens.
2. Small, herbaceous plants or twigs of large trees are preserved using herbarium sheets.
3. Thick specimens can be dried by exposure to air or with some heat by keeping in small boxes. Sometimes the specimens are glued to the bottom of the box. Both flat and thick dry specimens can be mounted behind transparent film or glass. For thick specimens cotton or other soft materials may be used in a box with the transparent material for a cover.
4. For the purpose of retaining color in dry herbarium specimens, calcium chloride can be used. Fine, granulated, anhydrous calcium chloride was used in an amount about double the water content of the tissue to be dried. The specimen was preserved between sheets of paper under relatively heavy object for several hours followed by transfer to two new sheets of paper which was placed on a flat layer of calcium chloride in a plastic bag. Air was removed from the plastic bag containing specimen and desiccant, the bag was sealed and heavy object placed on it.
5. Soft, gelatinous or fleshy plant materials such as fruits, roots or cedar-apple rust galls with gelatinous tendrils must generally be preserved in a solution in sealed

jars. Formula for good preservative solution is – water, formaldehyde (40%), ethyl alcohol (95%).

Other such solutions include –

- ✓ 5% formalin solution
- ✓ FA solution (formaldehyde alcohol solution)
- ✓ FAA solution (formaldehyde aceto-alcohol solution)

6. For the purpose of retaining color during wet preservation some of the methods that can be followed are – saturated copper acetate method and Hesler's preservative for colored fruits.
7. Other alternatives include drying in desiccant powder such as desiccant silica gel. This technique is used where it is essential to preserve the shape of a delicate plant organ such as flower.
8. Cryopreservation is a new method for preservation of plant pathogens. It refers to the storage of biological/pathogenic samples (fungal/bacterial) at ultra low temperature, usually that of liquid nitrogen at -196°C and is considered as ideal means for long term preservation of germplasm of pathogens. It can also be carried out at cryogenic temperature of -80°C using dry ice. Cultures are stored in cryovials which are subsequently submerged in liquid nitrogen.
9. Lyophilization is another new method which is also called freeze-drying method and is one of the most suitable method for long term preservation of pathogens. The process works by freezing the material, then reducing the pressure and adding it to allow the frozen water in the material to sublimate. It gives better results for the preservation of several bacteria, yeast and sporulating fungi but not much effective for non-sporulating fungi. Cultures of pathogens are subjected to stress while carrying out desiccation using vacuum and are lyophilized better in this stage.
10. Cell lines and microorganisms cannot be held in culture indefinitely due to the gradual rise in toxic metabolites, use of nutrients and increase in cell number due to growth. Subculture is thus used to produce a new culture with a lower density of cells than the original culture, fresh nutrients and no toxic metabolites hence allowing continued growth of cells without risk of cell death. A subculture is new microbial/pathological by transferring some or all cells from a previous culture to fresh growth medium. This process is called subculturing the cells.

Cultivation and characterization of pathogens alone is not adequate without specific preservation techniques that do not alter the morphology, physiology or genetics of the pure strains. Thus careful preservation is indispensable in the fields of teaching, industrial applications as well as for future research perspectives.