



AGRI MAGAZINE

(International E-Magazine for Agricultural Articles)

Volume: 02, Issue: 04 (April, 2025)

Available online at <http://www.agrimagazine.in>

© Agri Magazine, ISSN: 3048-8656

Diseased Plant Sample Preservation: Importance and Techniques

*Trishita Bera

Dept. of Pl. Pathology, CPGS-AS, Central Agricultural University (I), Meghalaya, India

*Corresponding Author's email: trishitapathology@gmail.com

Preserving plant disease samples is crucial in plant pathology for accurate diagnosis, research and tracking the spread and evolution of pathogens, ultimately aiding in effective disease management and crop protection. Diseased plant sample preservation is a specialized field that navigates the complex and dynamic nature of plant tissues and their interactions with pathogens. Unlike animal samples, plant tissues possess rigid cell walls, diverse metabolic pathways, and varying degrees of susceptibility to environmental degradation. These factors necessitate tailored preservation strategies that consider the specific plant species, the nature of the disease and the intended downstream analyses. These preserved samples become invaluable resources for understanding disease mechanisms, identifying resistance genes and developing novel control strategies. Herbarium specimens, which often include diseased plant samples, serve as historical records of plant diseases and their impact on plant populations. These collections provide valuable insights into the evolution of plant pathogens and their adaptation to changing environments. So, here we will take a deeper dive into the importance and techniques used for diseased plant sample preservation.

Keywords: Disease control, Herbarium, Preservation, Wet preservation

Introduction

The preservation of diseased plant samples is a crucial practice with significant implications for agriculture, research, and environmental science. Diseased plant sample preservation involves employing various techniques to maintain the integrity of plant tissues affected by pathogens (fungi, bacteria, viruses) or other disease-causing agents. This process aims to halt the degradation of the sample, preserving the disease's characteristics for future analysis. The primary objective is to ensure that the disease-causing agent and the plant's response to it remain intact, allowing for accurate identification and study. This enables researchers and practitioners to understand the disease's etiology, epidemiology, and potential control measures.

The urgency in preserving diseased plant samples stems from the rapid progression of plant diseases and the degradation of plant tissues during post-harvest or after sampling. Fungal spores can germinate, bacterial populations can proliferate and viral RNA can degrade within hours, making timely preservation critical for accurate diagnosis and research. Moreover, the preservation of diseased plant samples extends beyond merely capturing the pathogen; it also aims to maintain the integrity of the plant's response to infection. This includes preserving the intricate cellular and molecular changes that occur during disease development, such as the activation of defence pathways, the accumulation of secondary metabolites and the alteration of tissue structures.

Importance

- **Accurate Diagnosis and Identification**

Identifying Pathogens- Preserved samples allow plant pathologists to accurately identify the specific pathogen causing a disease, whether it's a fungus, bacteria, virus or other agent.

Distinguishing Diseases- Preserved samples help differentiate between similar-looking diseases, ensuring that the correct control measures are implemented.

Understanding Disease Cycles- Samples can provide insights into the life cycle of pathogens, including how they spread and persist, which is essential for developing effective control strategies.

Molecular techniques applied to preserved samples allow for the identification of new pathogens and the characterization of disease resistance genes.

- **Research and Tracking**

Historical Records- Preserved samples serve as a valuable resource for studying historical epidemics and understanding the evolution of plant pathogens over time.

Tracking Pathogen Spread- Herbarium specimens and other preserved samples can be used to track the geographic distribution and spread of plant pathogens, helping to anticipate potential outbreaks or epidemics.

Understanding Pathogen Evolution- Studying changes in pathogen populations over time, using preserved samples, can help researchers understand how pathogens adapt and evolve and develop strategies to stay ahead.

- **Conservation of Plant Genetic Resources**

Plant diseases can threaten native plant populations and disrupt ecological balance. Preserved samples help document the impact of diseases on biodiversity and inform conservation efforts. Herbarium specimens, including diseased samples, serve as valuable records of plant-pathogen interactions and contribute to our understanding of plant evolution.

- **Agricultural Productivity**

Accurate diagnosis and research enabled by sample preservation contribute to improved crop health, leading to increased agricultural productivity and food security. Research using preserved samples contributes to the development of disease-resistant crop varieties, reducing reliance on chemical pesticides and ensuring stable food production.

- **Supporting Sustainable Agriculture**

Accurate disease diagnosis and research using preserved samples contribute to the development of sustainable agricultural practices. This includes promoting integrated pest management, reducing reliance on chemical pesticides, and conserving natural resources.

- **Voucher Specimens and Scientific Validation**

Verifying Scientific Data- Voucher specimens, which are preserved samples associated with a scientific publication, allow researchers to verify the identity of the organisms studied and ensure the reliability of the research findings.

Long-Term Reference- Preserved samples can be used as a reference for future research, even after the initial study is completed, as names and classifications can change over time.

Preservation types

Dry preservation – Herbarium Specimens: An herbarium is a collection of preserved plant specimens, including whole plants or parts of it, usually dried and mounted on sheets for scientific study and reference. Creating and maintaining of herbaria, serves for scientific research, education and documentation of plant-disease diversity.

Following methods are commonly followed in dry preservation

- **Pressing-** This involves placing plant material between sheets of absorbent paper (like newspaper) and applying pressure using weights or a plant press. It's effective for flattening leaves, flowers, and thin stems. It is a very common method for herbarium specimen creation.
- **Desiccation-** Using desiccants like silica gel, sand or borax mixtures to absorb moisture. Effective for preserving the three-dimensional shape and color of flowers.
- **Freeze-Drying (Lyophilization)-** A more advanced technique that involves freezing the plant material and then removing the water under vacuum. Preserves the natural and delicate shapes and color remarkably well. This is a more expensive method, requiring specialized equipment.

Wet Preservation- This is a method of preserving plant specimens in liquid solutions, which is particularly useful for maintaining the three-dimensional structure and, to some extent, the original color of delicate plant tissues. Wet preservation is ideal for fleshy plant parts, such as fruits, flowers, and some algae, that would be distorted or damaged by drying.

4% formalin solution, FA solution (Formaldehyde Alcohol solution) and FAA solution (Formaldehyde Aceto Alcohol solution) are mainly employed for wet preservation.

Color Preservation: While colours can fade over time, wet preservation can help retain more of the original coloration compared to dry preservation. For colour retention through wet preservation following methods are followed:

1. Saturated Copper acetate method (for green colour preservation)
2. Keefe's Solution (Green preservation)
3. Hesler's Reagent Preservation (Colour preservation)

Materials required: Small knife, Polythene bags, Razor, Blade, Scissors, Magnifying glass, Cotton, Knife, Newspaper, Envelops, Pencil, Markers, Notebook, Blotting paper/Tissue paper, Plant sample-pressing objects (Wood or iron or heavy books), blank album, butter paper, Specimen bottles, distilled water, tags.

Specimen- The specimen for herbarium may be a single sporocarp or a part of it or the host (i.e. stem, bark, fruit, inflorescence) with the disease in it. It should be about 25-40 cm long and up to 26 cm wide, which will fit on a standard herbarium mounting sheet of dimension 42*27 cm. Plant parts which are very large may be cut into sections. Long and narrow specimens such as grasses and sedges can be folded once or twice at the time of pressing. So, by this way plant height of 1.6 m can be pressed onto a single sheet. For small diseased plant samples, a number of individuals can be placed on each sheet.

Procedure to be followed for

Dry Preservation

1. Identification of the disease sample in the field.
2. Careful collection of the specimens and putting them in moist polythene bags to avoid withering.
3. Washing of diseased specimens like roots and stems to remove soil and other dirt particles that may stick on its surface.
4. Drying of specimens by pressing them gently between two sheets of blotting paper.
5. After that, in fresh blotting paper the specimens are kept under pressure.
6. Specimens have to be regularly checked for any kind of mold growth. If necessary, sun exposure can be provided for few minutes.
7. The specimen will be ready for further preservation purpose within few days.
8. Leaf specimen is to be mounted on herbarium sheets or to be kept in butter paper. Root and stem specimens are preserved in specimen bottles.
9. Labelling- The specimen has to be labelled with disease name, host name, causal organism, locality, date of collection, name of the collector/scientist.

Wet Preservation

The cleaned disease samples are just put into the required mixtures of solutions (may be subjected to boiling for colour preservation). Then the specimens have to be labelled with disease name, host name, causal organism, locality, date of collection, name of the collector/scientist, who identified the disease.

4% formalin solution preservation- The specimens are washed properly with clean water to remove dirt and dust and then they are put into 4-5% formalin solution in specimen bottles. Here, chlorophyll of the specimen is not retained at all.

Composition: 40 ml of formaldehyde (40%), 15 ml of ethyl alcohol, 1 lit. of distilled water.

Keefe's Solution (Green preservation, wet cum dry method)- Keefe's solution, a method for green preservation of plant specimens, involves immersing them in a solution of 50% ethyl alcohol, formalin, glycerine, cupric chloride, and uranium nitrate, then drying them for herbarium preparation.

Composition: 90 ml of 50% ethyl alcohol, 5 ml of formalin, 2.5 ml of glycerine, 20 g of cupric chloride, 2.5 g of uranium nitrate.

Saturated copper acetate method- Green plant parts can be preserved without deteriorating the normal green colour. It involves boiling green plant specimens in a mixture of glacial acetic acid (50%) saturated with copper acetate crystals and water, then rinsing with water and preserving in 5% formaldehyde.

Composition: Glacial acetic acid (50%), copper acetate/Cupric acetate, 4-5% formalin solution.

First, the stock solution has to be made with 50% glacial acetic acid saturated with copper acetate. One part of stock solution is added with four parts of water and heated up to boiling point. The diseased sample is immersed in the boiling solution for 10-15 mints (the natural green colour will be vanished and regained after sometime which will be replaced by Cu-acetate). Specimen is taken out and washed in cold running water thoroughly. Finally, it is preserved in 4-5% formalin solution and properly labelled.

Hesler's Solution Preservation (Colour preservation)- It retains the colour of fruits and stems. After washing properly, specimen is dipped into the Hesler's solution.

Composition: 50 g of Zinc chloride, 25 ml formaldehyde (40%), 25 ml of glycerol, 1 lit. of distilled water.

Discussions

The choice of preservation method depends primarily on the specific objectives of the study, the type of plant material and the available resources. With proper preservation protocol we can store or preserve samples for hundreds of years. Not only the specimen's structure or shape will remain intact but their colour can also be preserved. Herbariums are further stored in museums or herbaria. Museums use wet preservation to display and preserve delicate plant specimens.



Fig 1: Boiling of sample in saturated copper acetate method



Fig 2: 4% formalin solution preservation



Fig 3: Hesler's solution for color preservation

Conclusions

In the face of global challenges such as climate change, emerging plant diseases and the need for sustainable agriculture, the preservation of diseased plant samples becomes even more critical. Effective plant sample preservation is paramount for ensuring the integrity and longevity of specimens, thereby enabling accurate and reliable scientific research. No single preservation method is universally applicable; rather, the optimal technique must be carefully selected based on the research goals and the characteristics of the plant material. These preserved samples are vital for developing resilient crop varieties, implementing effective disease management strategies and ensuring food security for a growing population. It is a critical step in the fight to maintain healthy plant ecosystems.

References

1. Nelson S.C., Bushe B. C. (2006) Collecting Plant Disease and Insect Pest Samples for Problem Diagnosis, Soil and Crop Management, SCM-14: 1-3.
2. Hamim I. (2015) Study of Collection and Preservation of Diseased Plant.
3. Das A.P. (2021) Herbarium Technique, Instrumentation Manual: 78-91. University of Florida Herbarium (FLAS). Preparation of Plant Specimens for Deposit as Herbarium Vouchers [cited 2025]. Available from: <https://www.floridamuseum.ufl.edu/herbarium/methods/vouchers/>