# PATHOLOGY

# Standard operation procedure for collection of diseased plant specimens

**1. Title:** Standard operation procedure on collection of diseased plant specimens for processing and identification.

**2. Scope:** The method is applicable to all plant species.

**3.Purpose:**The purpose of this procedure is tocollect diseased plant species for identification and confirm the pest problem identified and reported is of any economic importance.

**4. Terms, Acronyms and Definitions:**

1. Disease score: the degree of infection during physical inspection of the tree species. Range:
2. 0% :no infection (healthy)
3. 1-15% : slight infection
4. 15-40%: moderate infection
5. 40-65%: severe infection(65-99.9)
6. Greater than 65% : death
7. 99.9-100% - Dead
8. Isolates: These are all microorganisms that grow out of the sample on media.
9. Causative agent (Pathogen): Organism responsible for the disease.
10. Saprophyte : Opportunistic organism

**5. Apparatus and Materials:**

1. Panga
2. Jembe
3. Secateur
4. Pruning saw
5. Polyethylene bags /Paper bags
6. Cooler box
7. Magnifying lens
8. Binocular
9. A tape measure
10. Paint brush
11. Labels
12. Marking pens
13. Plant press
14. Sisal twine

# 6. Reagent and chemicals:

1. Yellow paint /Orange
2. Ethanol
3. Painting brush
4. Lighter/match box

# 7.Method:

If the area for sampling is big i.e. 5 acres and above, a smaller sample plot of 100 by 100 meters is marked. However if the area is less than 5 acres , all the trees can be sampled, or random samples marked with yellow paint and taken using a line transect or quadrant. Random sampling should be done without bias.

The following steps are then taken;

7.1The sampling area is marked for tree sampling and numbered using yellow paint and brush.

7.2 Disease score for every marked tree is done based on percentage infection of the plant specimenwhether it is slight, moderate, severe, death or no infection.

7.3 Infected parts of the tree are sampled by picking the area between the dead and healthy sections, sterilized and packed in polyethylene (Polythene) bags and stored in a cooler box. The equipment used for cutting is sterilized between samples to prevent cross contamination.

7.4 The samples are transported to the laboratory for registration, isolation and identification of pathogens.

7.5 Isolation and identification of pathogen is done as per procedure on isolation and identification of pathogens of diseased plants.

**8.References:**

1. The Genera of fungi sporulating in pure culture, By J.A. Von ArX.
2. Illustrated Genera of imperfect fungi By H.L. Barnett and Barry B. Hunter.
3. Culture media laboratory preparations By OXO Limited London Exporters
4. Plant Pathology By Agrios

**Standard operation procedure on isolation and identification of pathogens from diseased plants**

**1. Title:** Standard operation procedure on isolation and identification of pathogens from diseased plants.

**2. Scope:** The method is applied to all infected plant species.

**3. Purpose:** The purpose is to isolate and identify disease causing agent and confirmation of the causal agent by means of pathogenicity testing.

**4. Terms, Acronyms and Definitions**

1. Pathogenicity test: It is the introduction of a causal agent into a healthy plant of the same species aimed at selecting the most effective strain of the pathogen with the capacity to produce the disease
2. Causal agent: microorganism responsible for the disease
3. Malt extract, Potato Dextrose agar: (Are)It is a growth media
4. Damp-chambering – subjecting a specimen to cold conditions to enhance disease symptoms.

**5.Apparatus and Materials:**

1. Spirit lamp
2. Desert knife
3. Scalpel blade
4. Culture room
5. Clean bench
6. Autoclave
7. Measuring cylinder
8. Conical flask
9. Non-absorbent cotton wool
10. Sterile petridishes
11. Oven.
12. Incubator
13. Desiccator
14. Forceps
15. Lighter
16. Wash bottle
17. Dropping bottles

# Reagents and chemicals

1. Ethanol
2. Culture media
3. Hydrogen peroxide
4. Thymol solution
5. Stain
6. Mercuric chloride
7. Sodium hypochlorite

# 7. Methodology

7.1 Malt – extract agar of 2% or 1.5% percent is prepared and sterilized in an autoclave, then poured into sterile petri-dishes and left to cool to semi-solid state.

* 1. Three replicates of small pieces of about 0.5 to 1.0cm2 from diseased samples are surface sterilized using 30% Hydrogen Peroxide, Mercuric chloride or Sodium hypochlorite for one minute. (specified time)
	2. Blot the small pieces or segments by blotting dry between two sterile filter papers.
	3. Plate three small pieces on sterile malt extract agar media on each petri-dish and incubate at room temperature (18oC to 22oC) for fungal growth on media.(for microorganism growth)
	4. Obtain pure cultures by sub-culturing (i.e. separation of individual microorganisms) from initial culture.
	5. Pick a bit of the sporulated cultures, mount them on a slide in a drop of stain and observe under a microscope for identification, based on morphological characteristics using reference cultures and books
	6. Identification, scoring and analysis of the data are done as per the table below.

Table 1: Pathogen scoring table

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample number**  | **Type of micro organism identified** | **Scores** | **Total** |
| **Replicate 1** |  **Replicate 2** | **Replicate 3** |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
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|  |  |  |  |  |  |
|  |  |  |  |  |  |

7.8 The causative agent of the disease is determined by calculating the mode of the isolated pathogen in the replicates.

7.9 Further confirmation of the causative agent of the disease is done by carrying out pathogenicity test.

7.10 The economic importance of the pest is determined after data analysis and interpretation.

7.11 An advisory report is compiled as per the format outlined below and sent to the client using the below format, highlighting the causative agents and the control measures either chemically, biologically or culturally

**Report format**

Client Name:……………………………………………………………………………

Client Contact:…………………………………………………………………………..

……………………………………………………………………………………………

……………………………………………………………………………………………

Sample location: …………………………………………………………………………

Sampled by : Client……………………… Staff…….…………………

Date sampled:………………………………………………………

Date received:……………………………………

Sample type: Roots:…… Leaves/ twigs……… Soil…… Fruits……Other……….

Disease symptoms:………………………………………………………………………

……………………………………………………………………………………………

……………………………………………………………………………………………

Isolates:…………………………………………………………………………………

……………………………………………………………………………………………

Preliminary examination:………..………………………………………………………

……………………………………………………………………………………………

Final examination:……………..………………………………………………………

……………………………………………………………………………………………

Causative agent:……………………………………………………………………………

………………………………………………………………………………………………

Control measures and recommendations:…………….……………………………………

……………………………………………………………………………………………

……………………………………………………………………………………………

Reporting officer:………………..……………………….…Sign…………………………

Approved by:…………………………………………………Sign…………………………..

Date:…………………….…………………

CC: Deputy Director Forest Productivity and Improvement

 Regional Director

*Disclaimer: Results are based on submitted samples.*

**8.References:**

1. The Genera of fungi sporulating in pure culture, By J.A. Von ArX.
2. Illustrated Genera of imperfect fungi By H.L. Barnett and Barry B. Hunter.
3. Culture media laboratory preparations By OXO Limited London Exporters
4. Plant Pathology By Agrios
5. A Note Book on Pathology in Kenya Forest Plantations by I.A.S. Gibson