Detection of seed born Pathogens

Routine Testing Methods for Seed Health

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Seed health

- Seed is usually tested to establish seed health status

- Seed health refers primarily to presence or absence of disease causing microorganisms such as fungi, bacteria, viruses, and animal pests such as nematodes and insects, but physiological conditions such as trace element deficiency may also be involved (International Rules of Seed Testing(ISTA, 1985),

Why Seed testing?

- *S*eed testing is required to establish whether seed is infected.
- To detect the most important seed-borne pathogens
- Testing seed before sowing identifies potential disease problems and allow steps to be taken to reduce the disease risk.
- Laboratory testing is usually required, as infected seed may have no visible disease symptoms.

Why Seed testing?

- Many crop diseases can be seed-borne and significant crop losses can result from the use of infected seed
- Uncontrolled movement of infected seed between regions can result in the rapid expansion of the area affected by these diseases.
- Therefore, laboratory testing is usually required, as infected seed may often have no visible disease symptoms.

Detection of seed born pathogens is done by the following Methods

Non-incubation methods

1. Dry seed inspection (visual examination)
2. Seed washing test

Incubation methods

3. Blotter test
4. Agar test
5. Seedling symptom test

1. Dry seed inspection (Visual examination)

- A qualitative test that detects fungal/bacterial seed infection by discoloration in seed coat, abnormal size or shape
- Best for fungi producing visible structures like sclerotia, stromata etc

- Detects insect/mechanical damage
- Useful for purity analysis (weed and any other seed contamination, stones, etc)

Dry seed inspection procedure

1. Acquire a sample
2. Inspect all seed parts carefully with naked eye and remove, identify non-seed matters
3. Carefully examine for seed galls, sclerotia and smut balls
4. Using hand held lens, examine for presence of discoloration and fungal structures, spores or spore deposits adhering on seed coat.

2. Seed washing test

- Applicable solely for seed born fungal pathogens
- A known amount of seed in suspended in known amount of sterile distilled water

Washing test seed assay

Washing test procedure

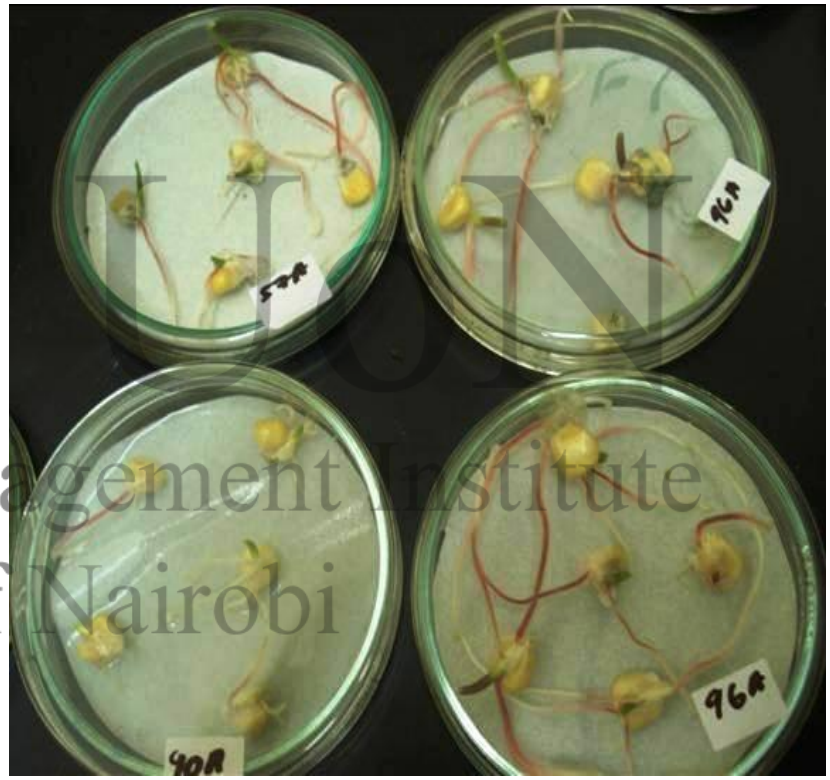
1. Transfer 50g seed taken from 1kg to Erlenmeyer flask and add 100 ml water and 1 drop tween 20
2. Shake for 5 min, and sieve through cheese cloth
3. Transfer filtrate in to centrifuge tubes (1500-2000 rpm, 3min)
4. Pour off liquid and invert tubes
5. Add 1 drop of Shears solution and mount on a slide and observe under microscope (X100 -X400)

3. Blotter test

- Seeds are incubated for 7 days at 20-22 ^{0}C
- Fungi associated with the seeds are then examined and identified under microscope

Blotter test procedure

- Line petri dishes with 3 filter papers (blotters) sterile, soaked in distilled water
- Spread seeds in Petri dishes at regular intervals (10 or 25/dish)
- Incubate at 20-22 ^{0}C for 7 days in alternating cycles of 12hrs light/darkness using near ultraviolet (NUV)
- Examine seeds after 7 days under microscope and identify the pathogens



4. Agar test

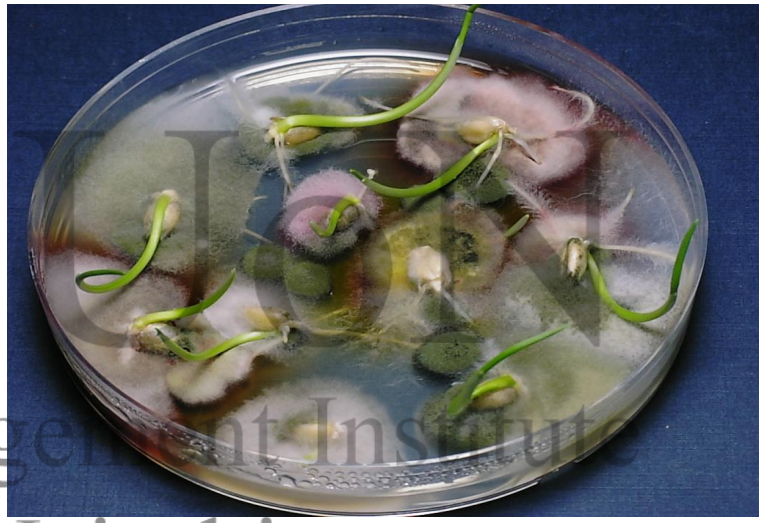
- Seed borne fungi are also detected and identified based on characters of colonies on agar directly developing from seed.

Preparation of the agar media

- Calculate the amount of agar medium for testing e.g. 400 seeds of a sample. The amount of agar will depend on the number of seeds to be plated in each petri dish (10 small sizes sees per dish, e.g. rice and 5 large sized seeds per dish, e.g. beans, soybeans)
- Sterilize agar medium in conical flasks or in Pyrex bottles if required add 0.3 g streptomycin sulphate in 1000 ml agar.
- Before pouring, let the agar medium cool down to around 50oC. Add antibiotic in the agar medium, if required e.g. 0.3 g streptomycin sulphate in 1000 ml agar.
- Since streptomycin sulphate is toxic, wear gloves while weighing and pouring it into the molten agar medium.
- Pour the medium in sterile petri dishes, approximately 15 ml per dish. Pouring should be done on a clean table room which has been decontaminated e.g. in a LaminarAir flow bench. Let the dishes solidify completely before plating seeds.

Agar test procedure

1. Surface sterilize the seeds
2. Plate seeds on agar on petri plate using sterile forceps
3. Incubate for 7-10 days
4. Observe the plates for fungal colonies from day 2 onwards
5. Observe colonies under microscope
6. Fungi are identified based on colony characteristics
7. Percentage of infections is calculated



8. For bacterial isolations, nutrient agar is used.

5. Seedling symptom test

Some of the seed-borne pathogens/obligates or deep seated infections cannot be grown on blotters or agar media. Therefore, seed has to be planted either in sterile soil or paper towels

When these are provided normal conditions for seed germination, after days of incubation, seeds germinate and if infected, produce characteristic symptoms of the pathogen

These effects can be seen if seeds are sown on suitable substrate and seedling grown under environmental conditions which support expression of such effects.

Seedling symptom test procedures

1. Paper towel test

Sterilised seeds are sown on paper towels, 1-2cm apart depending on seed size. These seeds are rolled so that each seed is in an individual roll, then incubated for 2-3 weeks under sterile conditions providing appropriate relative humidity for seed germination and symptom development.

Observe the symptoms and identify the pathogen

Seedling symptom test procedure

2. Growing on test

Seeds are in sterile soil either in individual pots or seed trays and appropriate conditions for seed germination are provided.

Pots are incubated under controlled conditions for seedlings to grow and develop symptoms.

Symptoms are observed, pathogens isolated and identified.

Apart from the above routine procedures, sophisticated techniques like ELISA, PCR also can be used to detect seed born pathogens.

Freeze blotter test

- To detect specific fungi, seed is incubated in ideal fungal growing conditions.
- The seed is briefly frozen before incubation to prevent germination.
- Observation for distinctive growths of fungal species of quarantine concern is made.

Serological test

- ELISA (enzyme linked imunosorbent assay), which employs antibodies conjugated to an enzyme, to greatly amplify and signal the presence of amounts of viral antigens.
- Dot Immunobinding Assay (DIBA)
- Tissue blot immunoassay