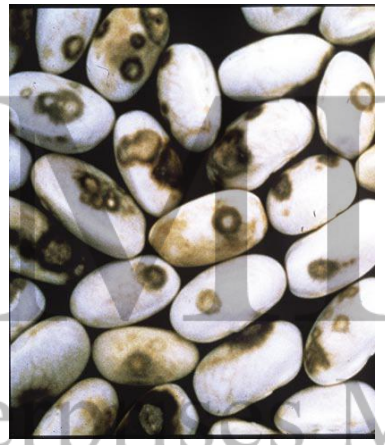
SEED ENTERPRISE MANAGEMENT INSTITUTE (SEMIs)

Seed Quality Assurance, Management and Control Processes

24th April – 6th May 2017

Seed Health Testing Procedures

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Objective of Seed Health Testing

- Testing for Quarantine
- Testing for evaluation of planting value
- Testing for certification scheme
- Testing for advisability of seed treatment
- Testing seeds for storage quality of for feeding
- Testing for resistance of cultivars

Methods in Seed Health Testing

- Visual examination of dry seed
- Seed washing test
- Blotter method
- Washing test
- Agar plate method
- Growing-on test
- Pathogenicity test

Inspection of dry seeds

- Provides quick information on insect, disease and mechanical damage to the seeds

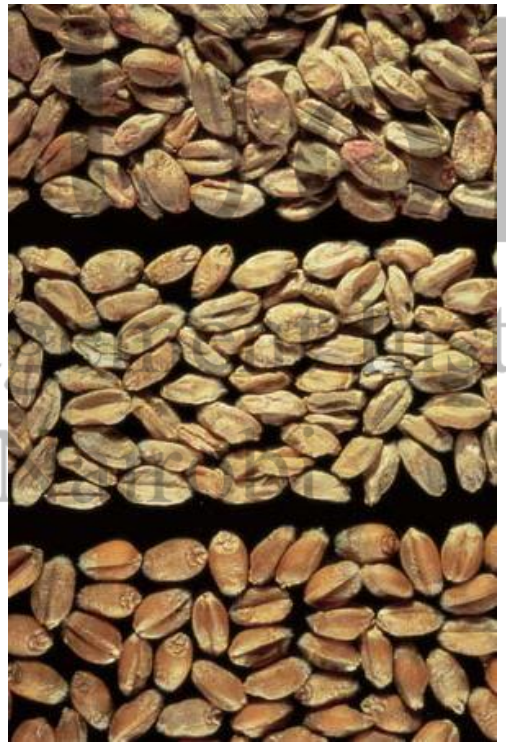
<u>The fruiting structures of fungi</u>

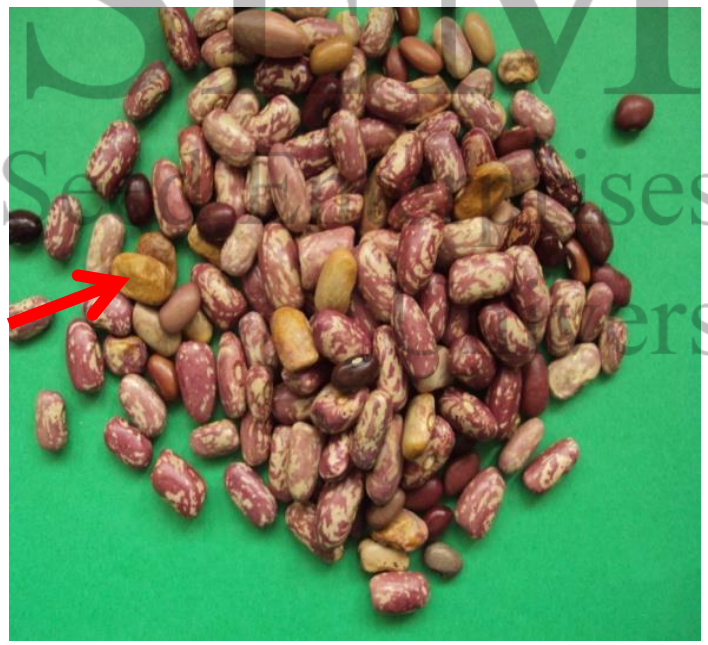
- Acervuli, pycnidia, pericthecia, sclerotia on the seed surface or submerged in the seedcoat
- Sclerotia loosely mixed with seeds
- Individual spores or spore masses on the seed surface

Seed Health Testing Procedures

Physical abnormalities include:

- ❑Shriveling of the seed coat
- ❑Reduction or increase in seed size
- ❑Discoloration or spots in the seed coat



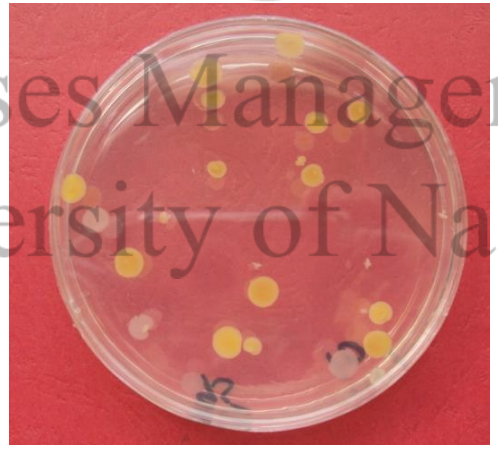


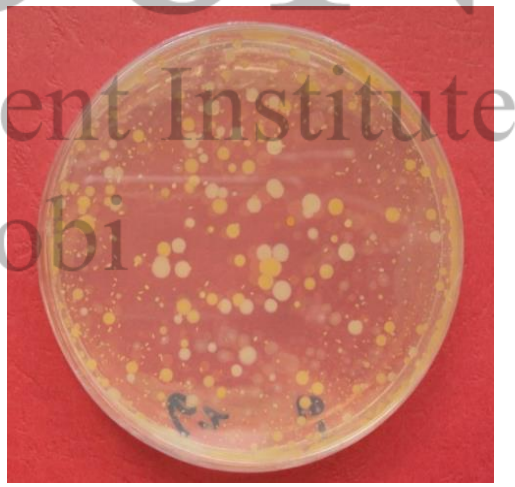
Seed Health Testing Procedures

Seed washing test

- Applicable solely for seed born fungal and bacterial pathogens
- A known amount of seed in suspended in known amount of sterile saline (8.5% NaCl) overnight
- Extract is plated on agar medium and incubated
- Count number of colonies to determine CFU/seed for bacteria

Washing test seed assay





Seed Health Testing Procedures

Procedure

1. Transfer 50g seed taken from 1kg to Erlenmeyer flask and add 100 ml sterile water and 1 drop tween 20
2. Shake for 5 min, and sieve through cheese cloth for fungi
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)
6. For bacteria, soak seeds in saline overnight; plate extract on agar medium

Seed Health Testing Procedures

Seed Washing test to detect of seedborne bacteria

e.g. Halo blight and common bacterial blight of bean

- Suspend seeds in sterile saline plus Tween 20 (0.02% v/v)
- Soak subsamples overnight (16–18 h) at 5 ± 4 °C).
- Shake on to obtain a homogenous extract.
- Prepare a tenfold dilution series from the seed extract.
- Plate each dilution & undiluted seed extract selective media.
- Incubate inverted plates and examine after 4-5 days
- Subculture suspect colonies to sectored plates of KB.
- Pathogenicity test of isolated bacteria by inoculation on cotyledons of bean seedlings of known susceptibility

Seed Health Testing Procedures

Infected seeds

Soak overnight in sterile saline

Serial dilution in sterile distilled water

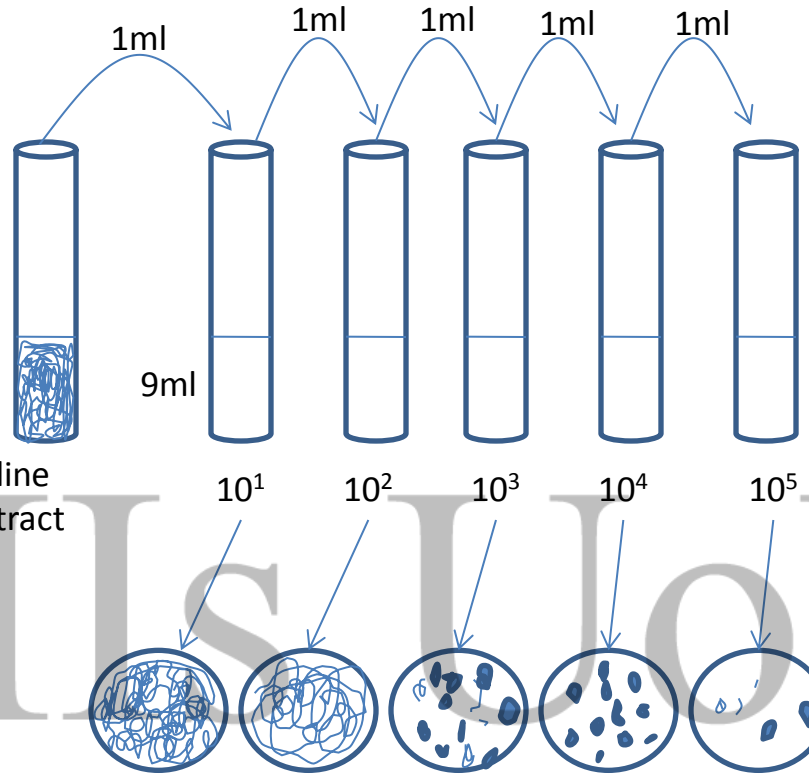
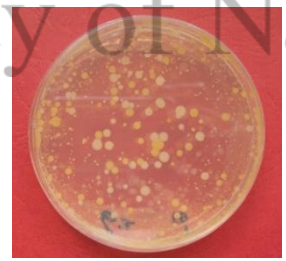
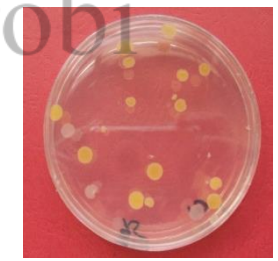


Plate 1ml of each dilution in molten agar medium. Incubate and count the number of colonies for each dilution. Determine bacterial population by multiplying the number of colonies by the dilution factor





Blotter Method

Simple and inexpensive way to detect seedborne fungi

Procedure:

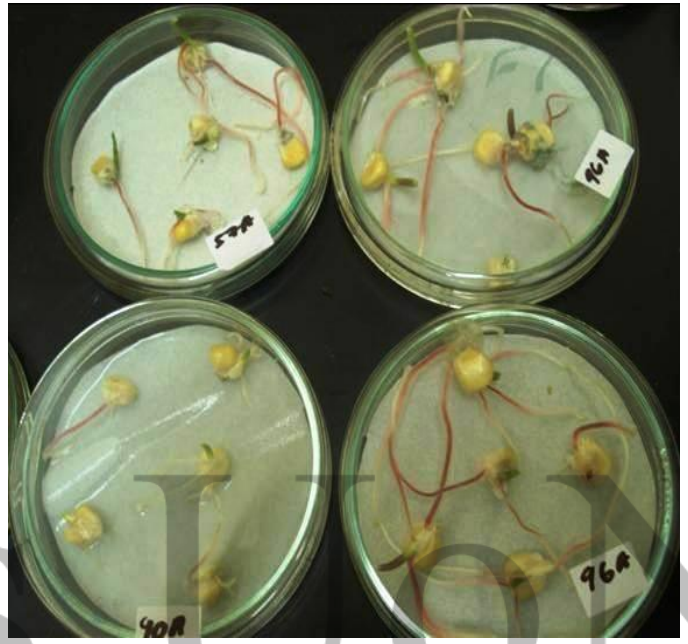
1.9.5-cm Pyrex glass or clear plastic petri plates containing 2-3 layers of blotter papers moistened with distilled water.

2.Place seeds working sample equidistant on the petri plates

3.Incubate seeds at 22 °C under a 12-h light and 12-h dark cycle.

Results: Express results as a percentage infected seeds of the number of total seeds.

Seed Health Testing Procedures



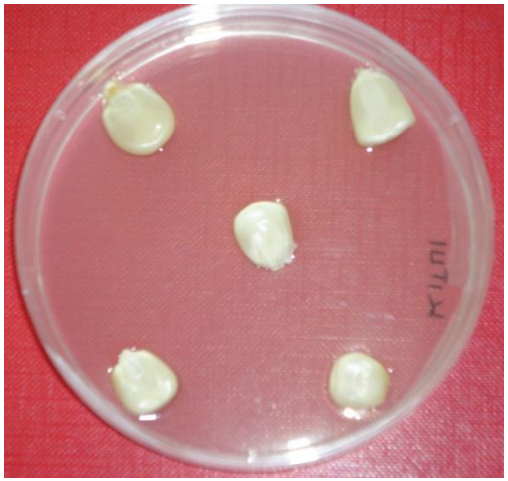
Agar plate method

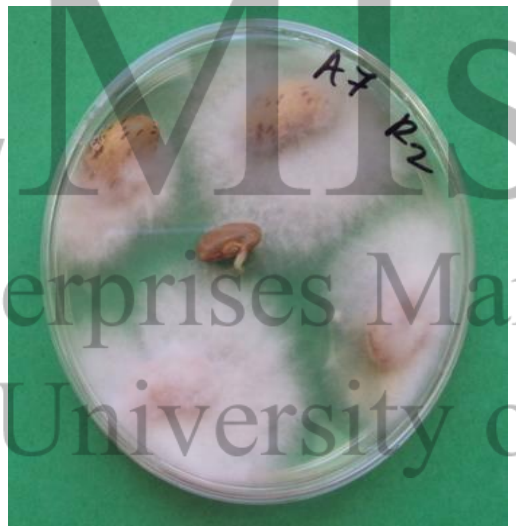
- Detects and identifies seedborne fungi through colony characteristics which they exhibit when grown on nutrient agar.
- Media - water agar, potato dextrose agar, potato sucrose agar, Czapek-Dox agar, malt extract agar.
- Germination inhibitors – herbicide or sodium chloride

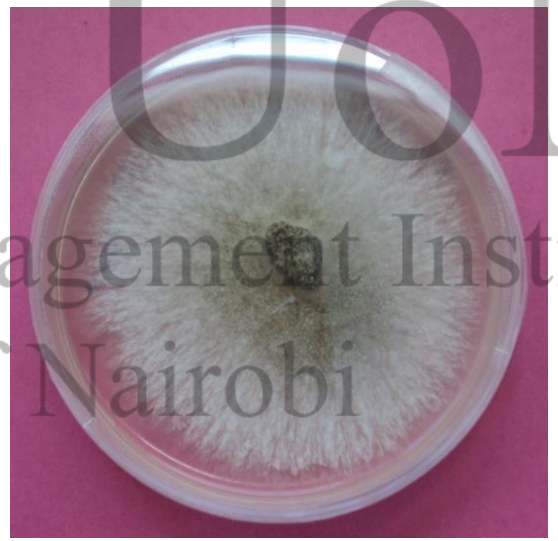
Procedure:

1. 400 seeds pretreated with 1% sodium hypochlorite for 10 min.
2. Place seeds agar media in 9.5-cm petri dishes.
3. Incubate at 22 °C for 5-8 days, either under alternate cycles of NUV light and darkness, or in darkness.

Seed Health Testing Procedures







Seed Health Testing Procedures

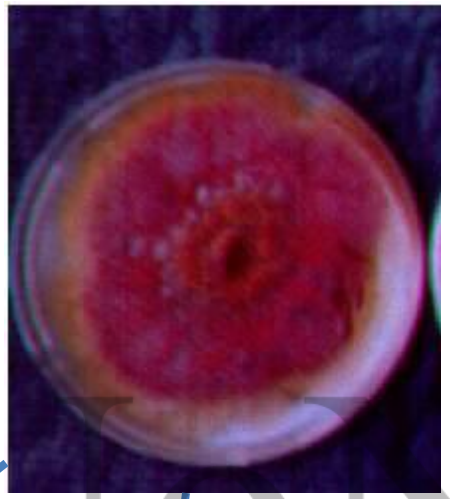
Results:

- Examine characteristic pathogen colonies, beginning on the third day and continuing through the eighth day of incubation.
- Also examine seeds under a stereo binocular microscope.
- View spores and other fungal structures under a compound microscope to distinguish the fungal forms.
- Express results as a percentage of seeds infected.

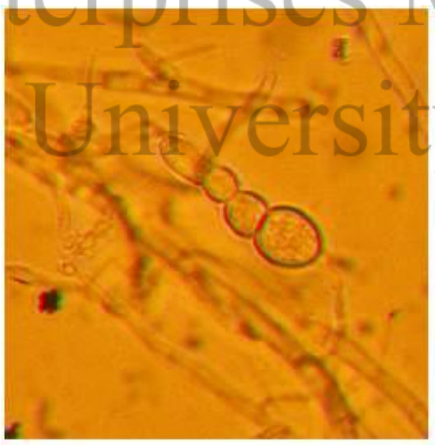
Seed Health Testing Procedures

Plating

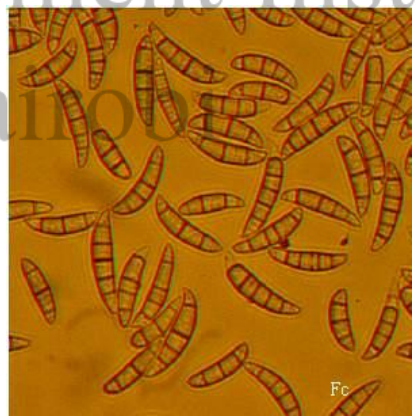
Purify



Microscopy



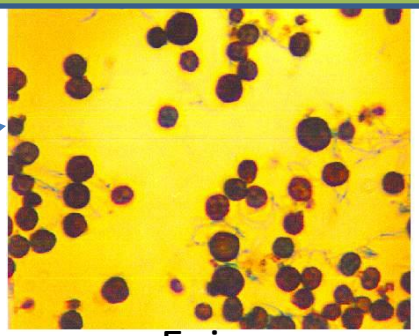
Chlamydospores



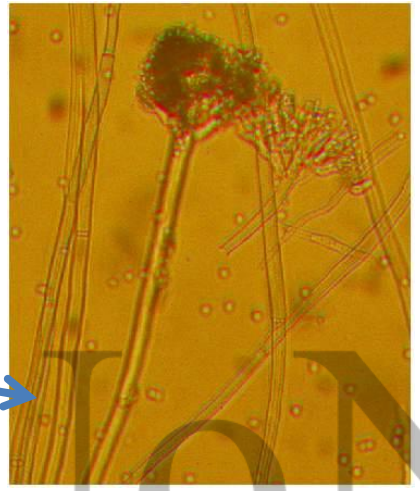
Spores

Seed Health Testing Procedures

ISOLATION AND MICROSCOPY



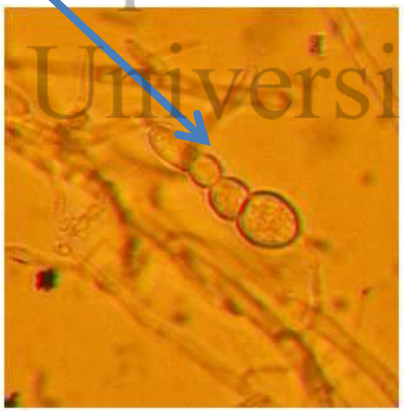
Epicoccum



Penicillium

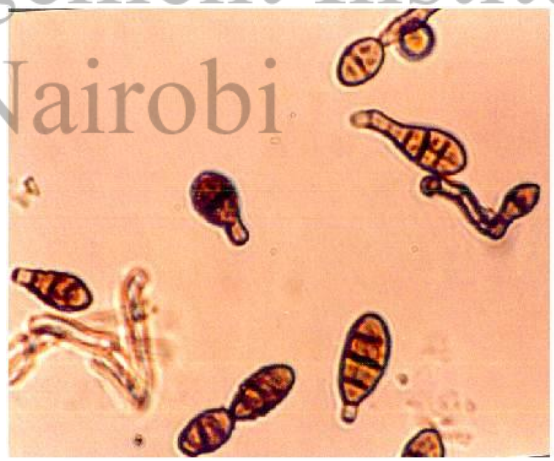
Fusarium

Spores



Chlamydospores

Alternaria



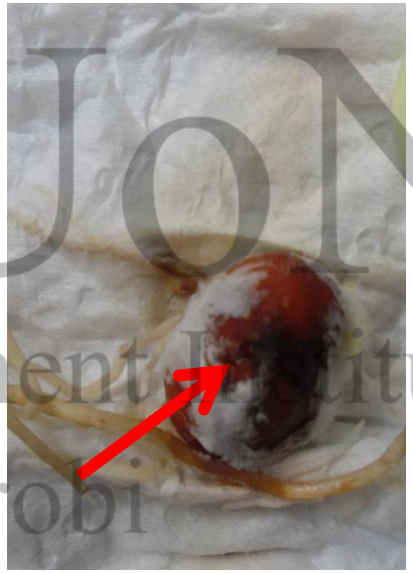
Paper towel method

- Seeds are submerged in a solution of 2.5% sodium hypochlorite for 5 min, rinsed in sterile distilled water and blotted dry.
- Spread the seeds in replicates of 50 on double sheets of wet paper towelling 350 x 450 mm.
- Cover seeds with one sheet of wet paper towelling.
- Fold the paper twice lengthways and cover it with a sheet of polythene to maintain the moisture during incubation.

Seed Health Testing Procedures

- Incubate for 7 days at 20 ºC in darkness.
- Examine seeds by naked eye for growth of fungi.
- Observe seeds under dissecting microscope for fungal structures.
- Mount fungal growth on micrscope slides & observe under high-power microscopes (mag ×200)

Seed Health Testing Procedures

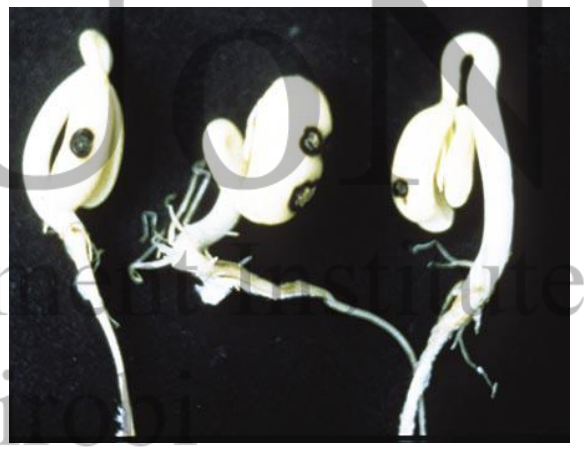


Seedling symptom test

- Some of the seed-borne pathogens/obligates or deep seated infections cannot be grown on blotters or agar media.
- Detects seedborne fungal, viral, and bacterial pathogens which are readily transmittable
- seed are planted either in sterile soil, sand or paper towels

a) Paper towel test

- Sterilized seeds are sown on paper towels, 1-2cm apart depending on seed size. seeds are rolled so that each seed is in an individual roll,
- incubate for 2-3 weeks under sterile conditions providing appropriate relative humidity for seed germination & symptom development.
- Observe the symptoms and identify the pathogen



b) Growing-on test

Detects seedborne fungal, viral, and bacterial pathogens which are readily transmittable.

Procedure:

- Sow seeds on a suitable medium (sterilized soil, sand, on paper towel or water agar) under optimal conditions for germination.
- Incubate under controlled conditions for seedlings to grow & develop symptoms.
- Observed characteristic symptoms, pathogens isolated & identified.

Seed Health Testing Procedures

Seed Health Testing Procedures

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


THANK YOU FOR THE AUDIENCE