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Identification & Management of Seed Borne Diseases




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Disease	Causal agent
Bean anthracnose	Colletotrichum lindemuthianum
Halo blight (bean)	Pseudomonas savastanoi phaseolicola
Common bacterial blight (bean)	Xanthomonas axonopodis phaseoli
Bean common mosaic	Bean common mosaic virus
Head smut (maize)	Sphacelotheca reiliana, Ustilago maydis
Gray leaf spot (Maize)	Cercospora zea-maydis
Maize leaf blight	Drechslera turcicum
Stalk rot / ear rot (maize)	Fusarium graminearum, F. verticillioides, F. proliferatum , F. subglutinans , Stenocarpella maydis
Bacterial blight (cow pea)	Xanthomonas campestris vignicola
Sclerotinia wilt & head rot (sun flower)	Sclerotinia sclerotiorum
Botrytis head rot (sunflower)	Botrytis cinerea

Reduced seedling vigour

Seed discolouration, Shrivelling, rotting & reduced size

Reduced seedling vigour

How does seed contamination occur?

Seed contamination or infestation

Pathogen itself or parts of it stick or mix with seeds during:

- Harvesting
- Extraction
- Threshing
- Selection
- Packing

Accompanying contamination

Physical mixing of the seed with pathogen's propagation organs

- Spores
- Sclerotium
- Nematode's galls
- Contaminated plant parts or soil particles containing pathogens

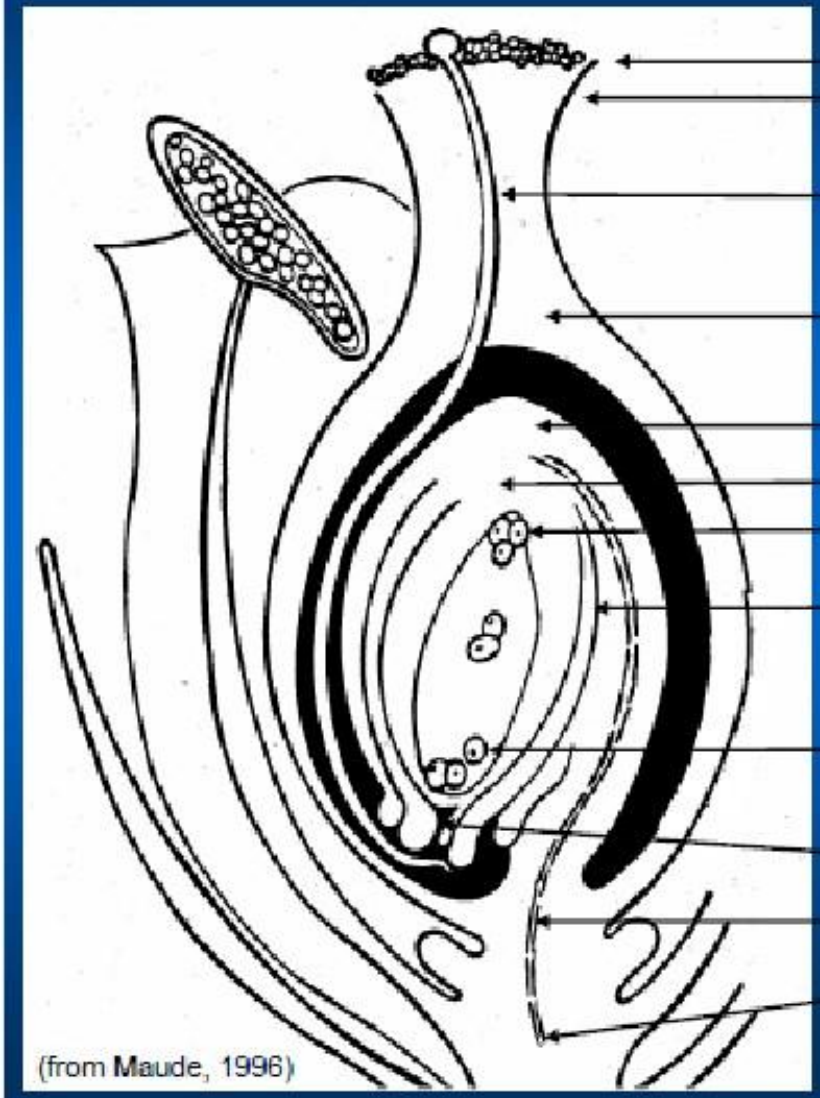
Location of pathogen in seed

- Infection of the embryo
- Under the seed coat
- In the endosperm or cotyledon
- On the surface of seed

How pathogens infect seed

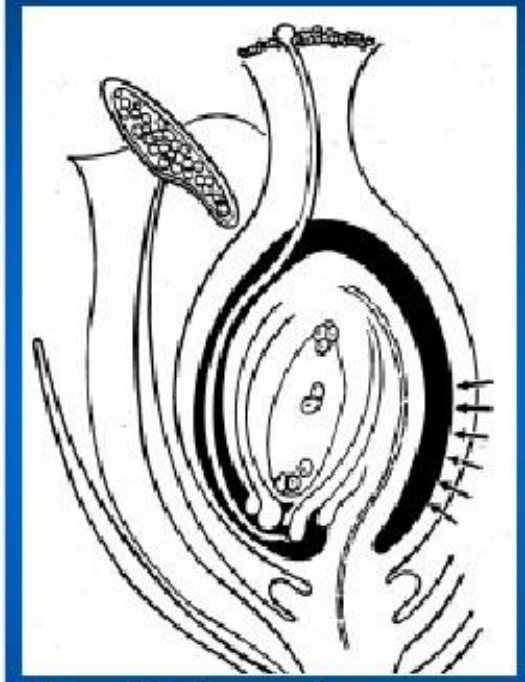
- Systemic Infection of the Seed
- Through flowers, fruits or funiculus
- Through the stigma
- Through the wall of the ovary or immature seed covers
- Through wounds & natural openings

Routes of active seed infection



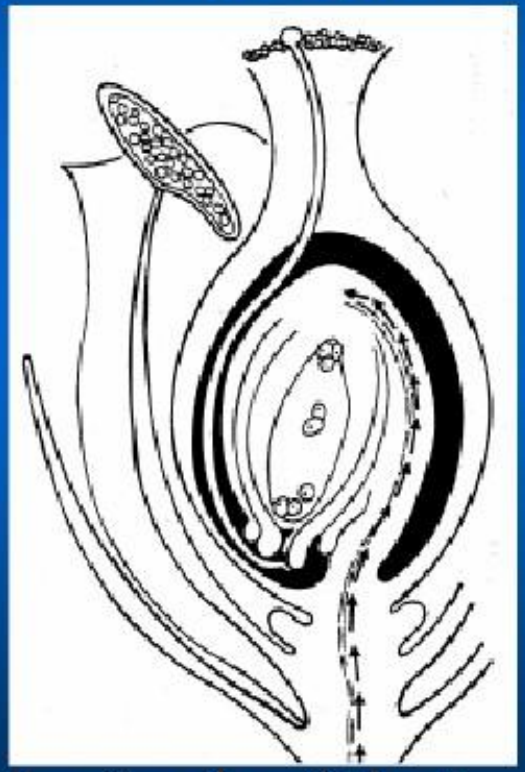
Routes of active seed infection

A. Penetration through ovary wall



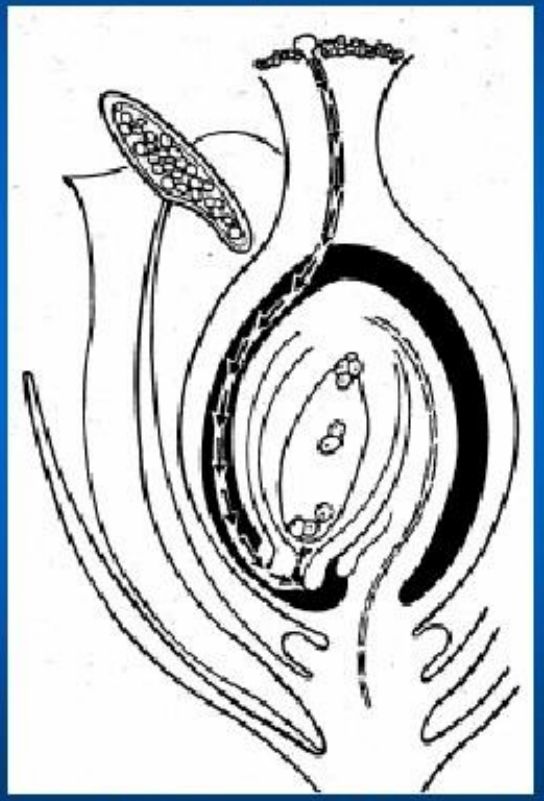
E.g.: *Cladosporium variabile* (spinach), *Botrytis* spp. (onion)

B. Systemic infection via vascular system



E.g.: Vascular wilt fungi, endophytes

C. Penetration through floral parts



E.g.: *Ustilago nuda* (grains) *Cucumber mosaic virus*

From Maude (1996)

Maize Lethal Necrosis Disease

Maize

Loose smut

Head Smut

Maize

Maize leaf blight

Gray leaf spot

Maize rust

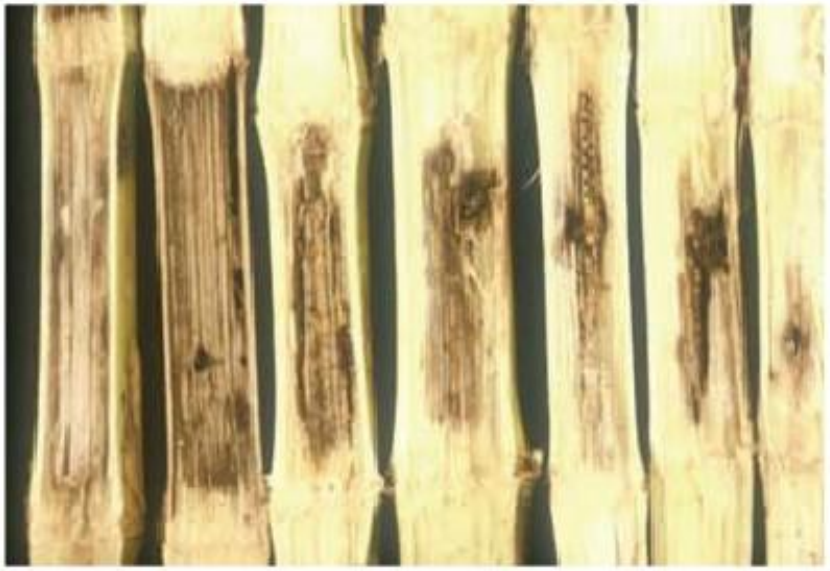
Maize

Fusarium stalk rot of maize

Charcoal rot

Diplodia stalk and ear rot of maize

Maize



Ear rot of maize

Maize

Fusarium ear rot

Diplodia

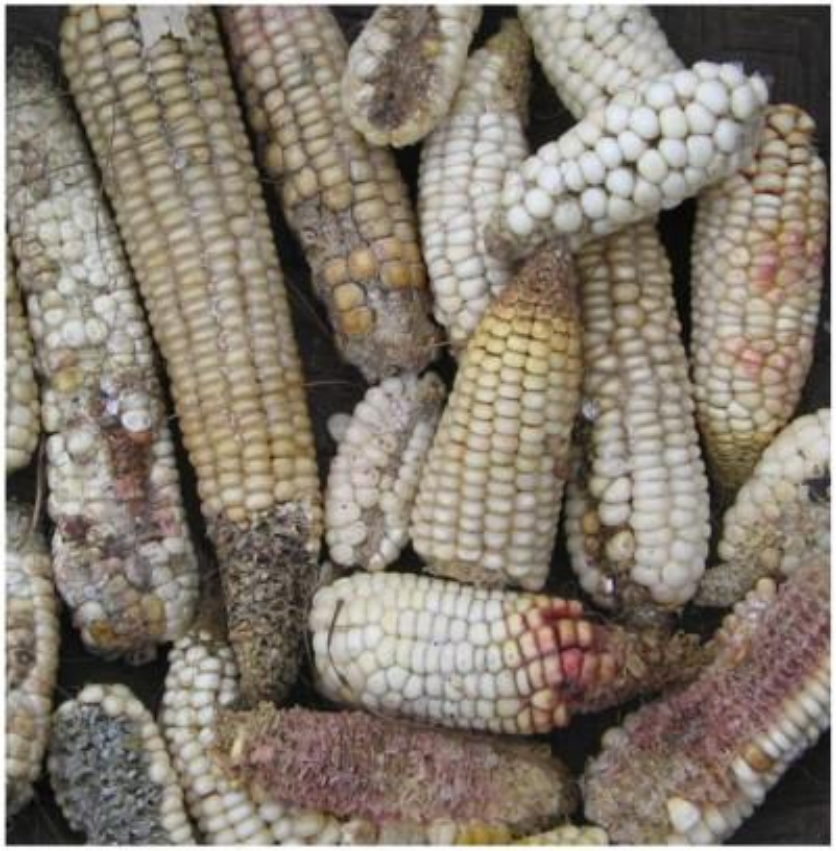
Fusarium ear rot

Trichoderma

Maize

Aspergillus ear rot

Gibberella ear rot

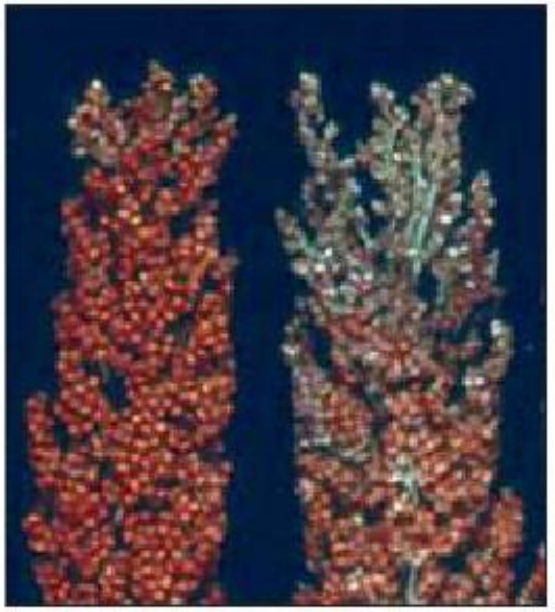


Sorghum

Anthracnose

Helminthosporium leaf blight

Target spot



Head blight

Wheat

Smut on wheat ears

Wheat kernels with smut symptoms

Wheat

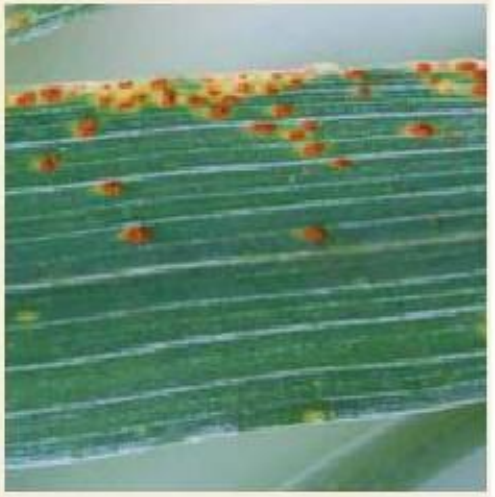
Wheat scab on ears

Wheat scab symptoms on kernels

Wheat

Loose smut

Stem rust



Leaf rust

Wheat

Powdery mildew

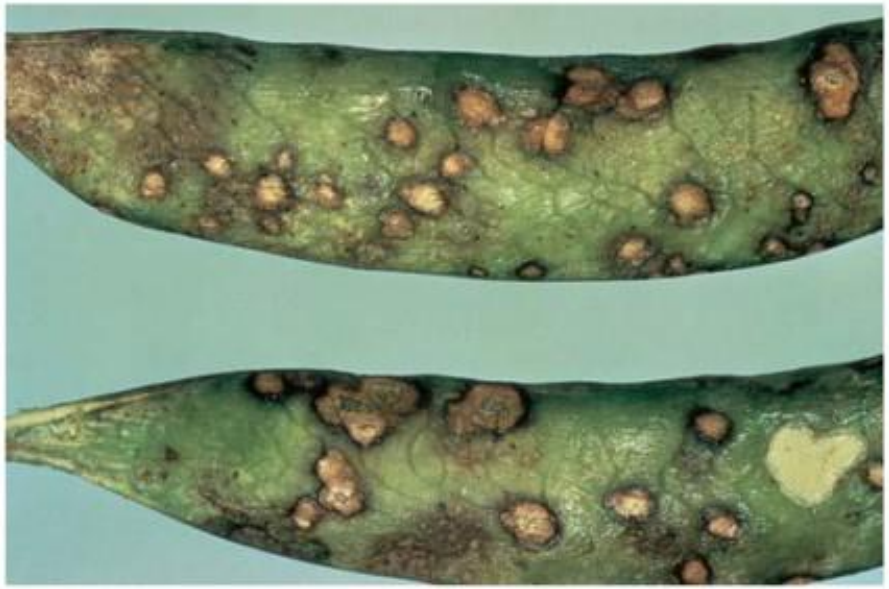
Barley yellow dwarf

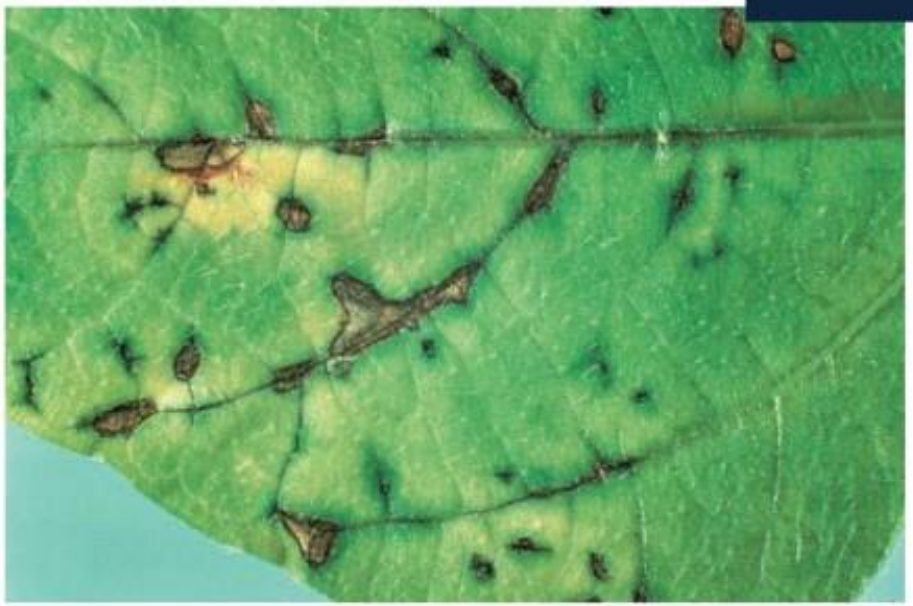
Rice blast

Rice

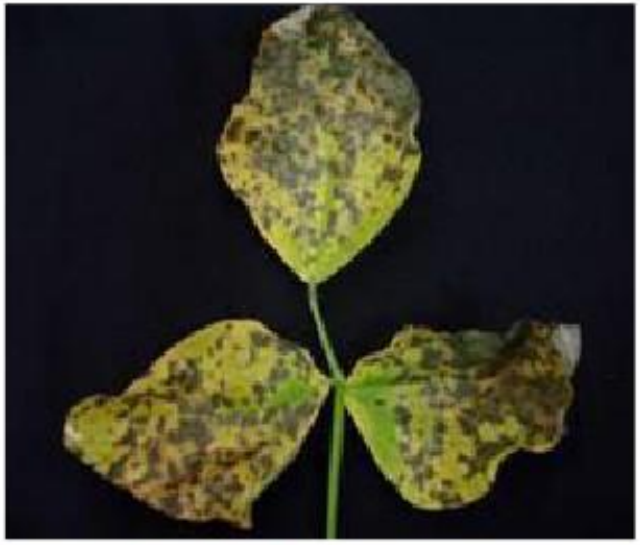
Bean anthracnose on pods and leaves

Bean





Angular leaf spot on bean



Sclerotinia on bean stems and pods

Bean

Aschochyta leaf spot

Web blight

Bean rust

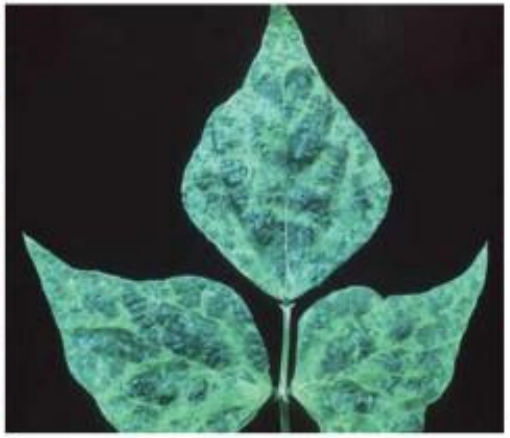
Beans

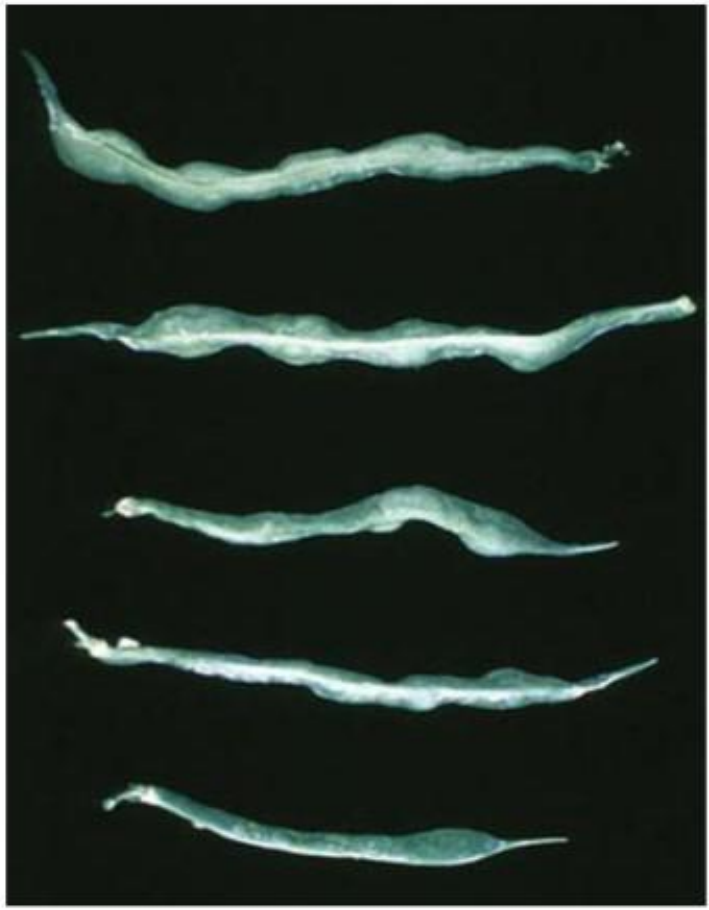
Root rots

Halo blight on bean

Bean

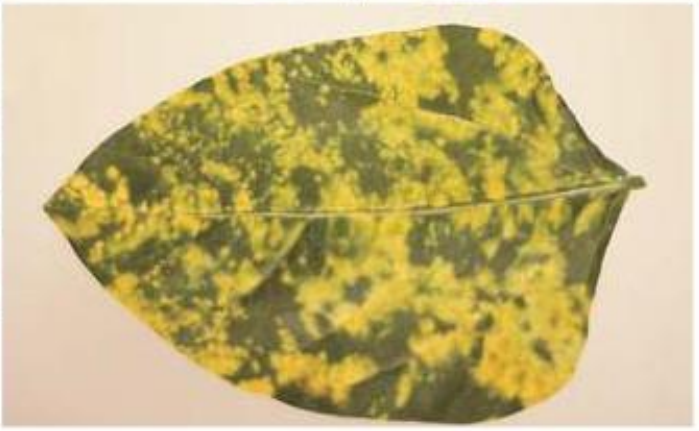
Bean virus diseases





Cowpeas

Virus diseases



Cowpeas

Bacterial blight

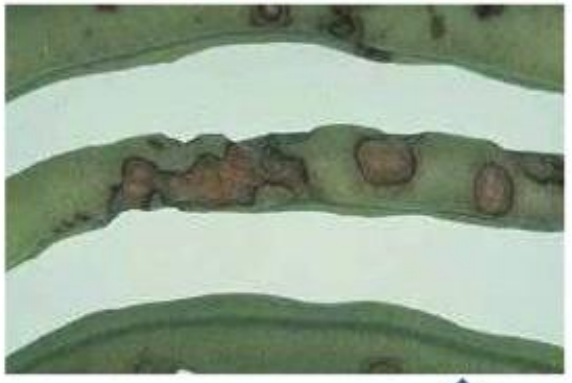
Aschochyta

Cercospora

Cowpea

Root rot

Rust



Anthracnose

Ground nut

Early leaf spot

late leaf spot

Alternaria leaf spot

Groundnut

Rust

Aspergillus crown rot

Wheat

Ground nut rosette

Virus diseases

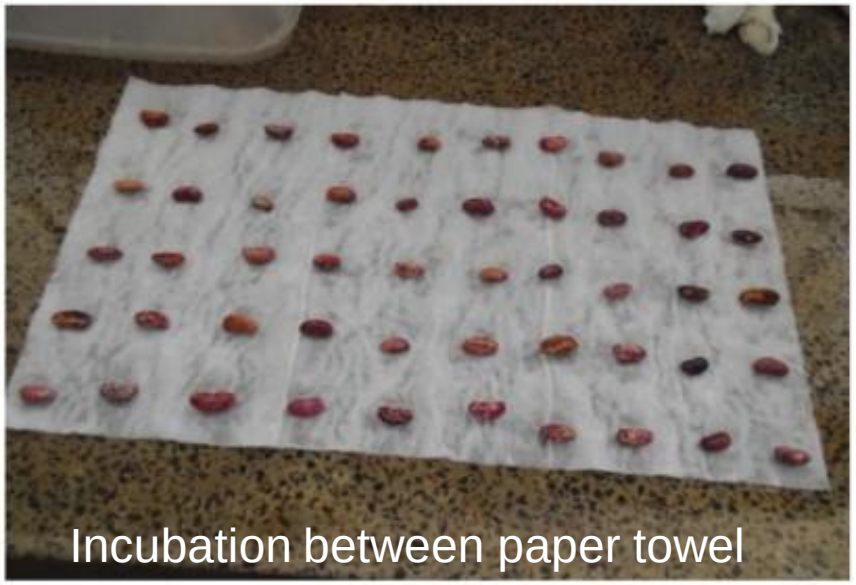
Green gram

Sunflower

Sclerotinia Head Rot of sunflower

Identification & Management of Seed borne Diseases

Shriveled and discoloured seed

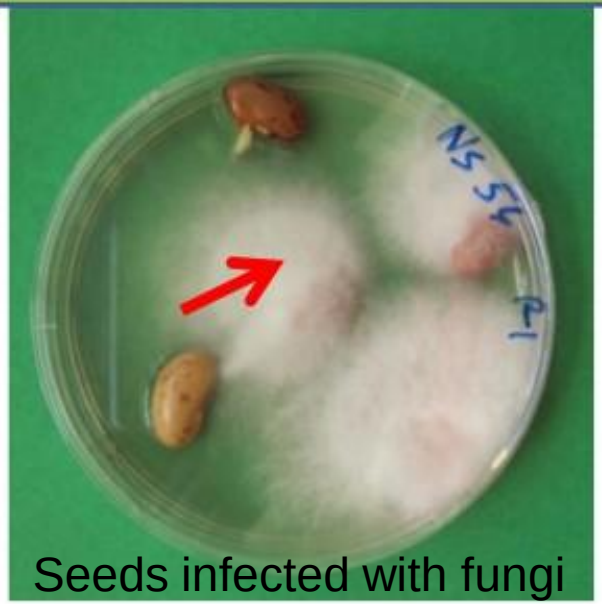


Incubation between paper towel

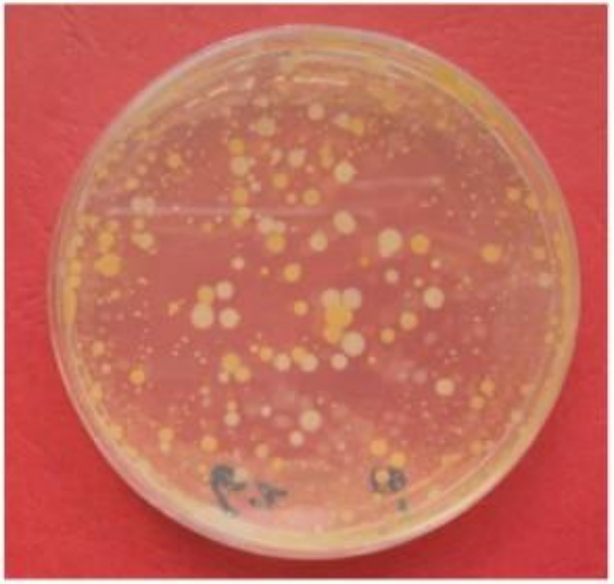
Incubation rolled paper towel

Incubation between paper towel

Infected seeds

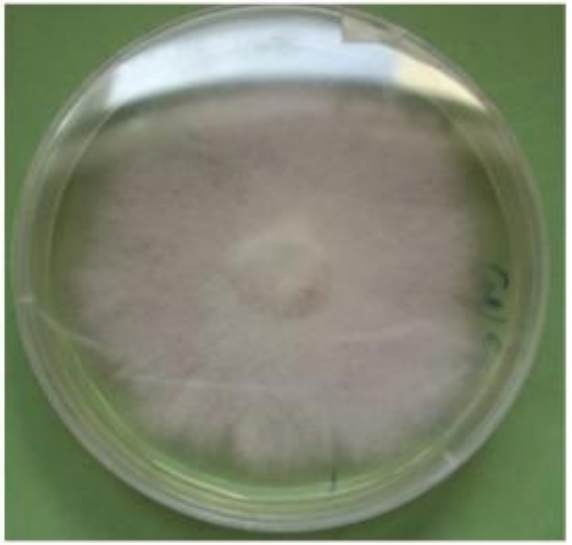


Seeds infected with fungi



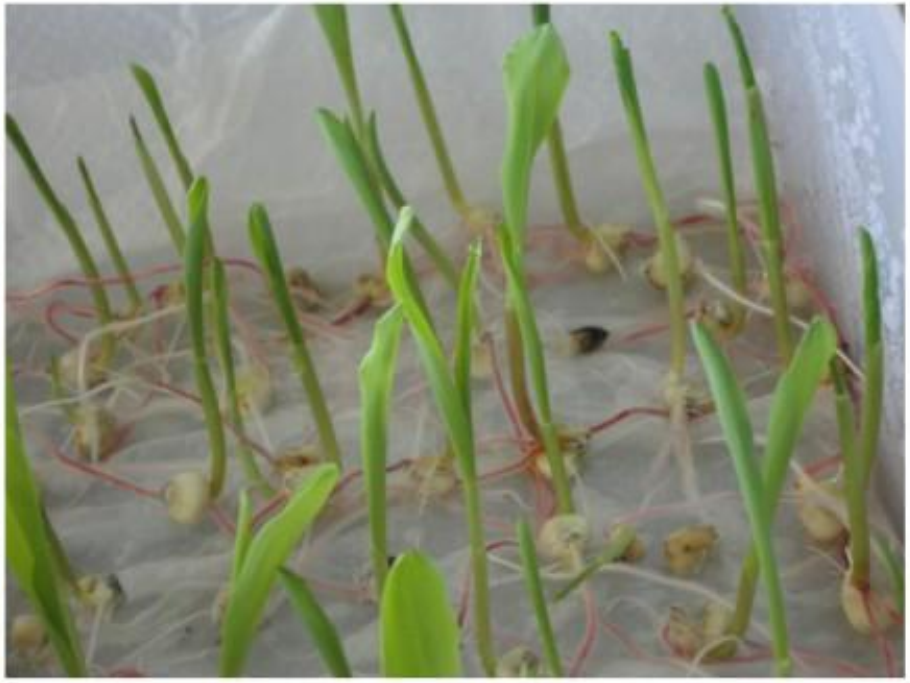
Bacterial isolated from infected seed

Seeds with symptoms of infection



Pure cultures of isolated pathogens

MANAGEMENT OF SEED-BORNE DISEASES



Previous cropping

- Seed production fields should be free from volunteer plants to avoid contamination of the crop seed by:
  - Any seed which is difficult to remove from the crop seed
  - Cross-pollination;
  - Seed-borne diseases transmitted from volunteer plants
  - The previous cropping shall be such that there is the least possible risk of any soil borne diseases being present which could subsequently be transmitted in the harvested seed.

Production in disease-free areas

- Dry areas with low humidity (use irrigation)
- Bean anthracnose and Bacterial blights of bean
- Altering time of planting
- Crop isolation from other fields containing possibly diseased plants

Good production practises

- Use of certified seed
- Minimize plant stress - fertilization & watering
- Weed management
- Well-drained soils
- Seed rate - proper plant density to promote rapid drying of foliage
- Destroy/ plough under crop residues
- Proper crop handling (wash hands & implements)
- Removal of infected plants (roguing)
- Avoid working in field when wet

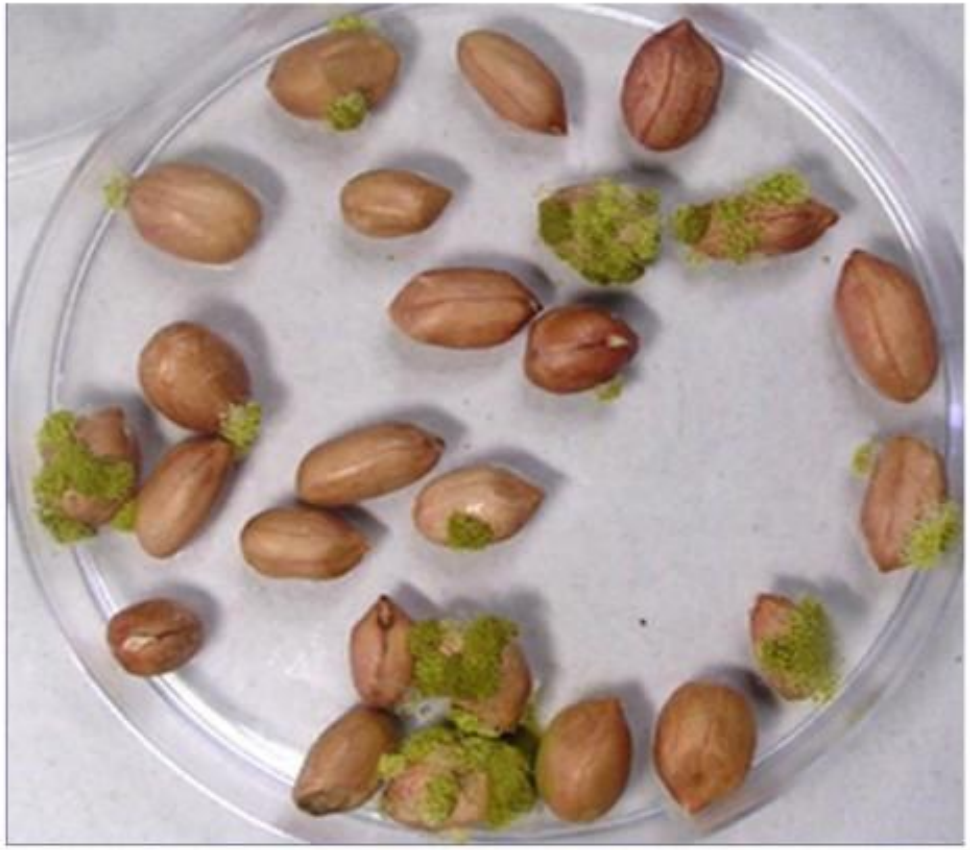
Eradicate disease-causing pathogen from production area

- Remove alternate hosts and volunteer host plants
- Crop rotation
- Sanitation - residue management
- Creating conditions unfavourable to pathogens
- Seed treatment
- Use resistant/ tolerant crop varieties
- Use of disease-free planting materials
- Spray protective fungicides
- Control of Insect Vectors

Isolation and Field Inspection

- Seed crops should be isolated from all sources of pollen
- contamination and seed-borne diseases
- Crop should be inspected at least once at appropriate stage of
- growth
- At least 20% of the crop of Certified Seed should be inspected
- Presence of any seed-borne disease should be at the lowest
- possible level

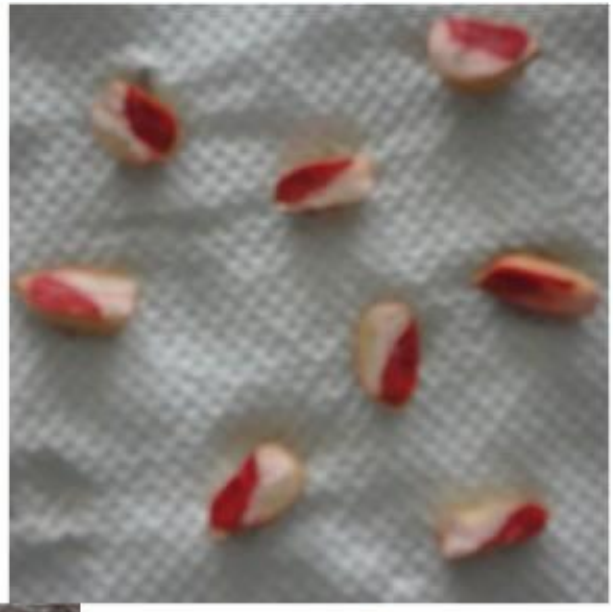
Seed health testing



Germination test

Diseases in seed crop production

Seed health test for seedborne pathogens



Fast green test for physical damage

Tetrazolium test for seed viability

Tolerated levels for seed borne diseases

Disease	Tolerance level
Head smut (maize)	2 plants per hectare
Loose smut (maize)	2 plants per hectare
Bunt (wheat)	1 head per 100 sq. m
Bunt (sorghum)	1 plant per 1,000 plants
Halo bight (bean)	None at inspection
Anthracnose (bean)	None at inspection
Common bacterial blight (bean)	None at inspection
Bean common mosaic	None at inspection
Bacterial blight (cow pea)	None at inspection
Botrytis head rot (sun flower)	5 plants per 1,000 plants
Sclerotinia wilt & head rot (sun flower)	5 plants per 1,000 plants

THANK YOU

SEEDBORNE FUNGAL PATHOGENS THAT CAUSE IMPORTANT DISEASES OF MAJOR CROPS:

PATHOGEN	CROP	DISEASE
Alternaria brassicicola	***Brassica* spp (Crucifers)**	**Black spot**
Mycosphaerella brassicicola	***Brassica* spp) (Crucifers)**	**Black ringspot**
Peronospora parasitica	***Brassica* spp (Crucifers)**	**Downy mildew**
Phoma lingam (Plenodomus lingam)	***Brassica* spp (Crucifers)**	**Black leg**

PATHOGEN	CROP	DISEASE
Alternaria porri	***Allium cepa* (onion)**	**Purple blotch**
Botrytis allii	***Allium cepa* (onion)**	**Damping off, gray mold, neck rot**
Cercospora arachidicola	***Arachis hypogaea* (Groundnut)**	**Leaf spot (Tikka disease)**
Cercospora personata	***Arachis hypogaea* (Groundnut)**	**Leaf spot (Tikka disease)**
Macrophomina phaseolina	***Arachis hypogaea* (Groundnut)**	**Root rot, stem rot**
Alternaria alternata	***Helianthus annus* (Sunflower)**	**Seed rot**

PATHOGEN	CROP	DISEASE
Alternaria zinniae	*Helianthus annus* (Sunflower)	Blight
Botrytis cinerea	*Helianthus annus* (Sunflower)	Gray mold
Macrophomina phaseolina	*Helianthus annus* (Sunflower)	Charcoal rot
Sclerotinia sclerotiorum	*Helianthus annus* (Sunflower)	White rot, wilt, stem rot
Alternaria ricini	*Ricinus communis* (Castor bean)	Capsule mold, seedling blight

PATHOGEN	CROP	DISEASE
Ascochyta phaseolorum	***Phaseolus vulgaris*** **(Bean)**	**Ascochyta leaf spot**
Colletotrichum lindemuthianum	***P. vulgaris*** **(Bean)**	**Anthracnose**
Elsinoe phaseoli	***P. vulgaris*** **(Bean)**	**Scab**
Fusarium oxysporum **f. sp. *phaseoli***	***P. vulgaris*** **(Bean)**	**Wilt**
Macrophomina phaseolina	***P. vulgaris*** **(Bean)**	**Ashy stem blight, charcoal rot**

PATHOGEN	CROP	DISEASE
Phaeoisariopsis griseola	***P. vulgaris* (Bean)**	**Angular leaf spot**
Sclerotinia sclerotiorum	***P. vulgaris* (Bean)**	**Sclerotial wilt**
Drechslera tritici-repentis	***Triticum aestivum* (Wheat)**	**Leaf spot, yellow spot**
Fusarium graminearum	***Triticum aestivum* (Wheat)**	**Head blight, scab**
Septoria nodorum	***Triticum aestivum* (Wheat)**	**Glume blotch**

PATHOGEN	CROP	DISEASE
Septoria tritici	***Triticum aestivum*** **(Wheat)**	**Speckled leaf spot**
Ustilago tritici	***Triticum aestivum*** **(Wheat)**	**Loose smut**
Drechslera teres	***Hordeum vulgare*** **(Barley)**	**Net blotch**
Rynthosporium secalis	***Hordeum vulgare*** **(Barley)**	**Scald**
Claviceps fusiformis	***Pennisetum typhoides*** **(Pearl millet)**	**Ergot**

PATHOGEN	**CROP**	**DISEASE**
Claviceps microcephala	***Sorghum vulgar* (Sorghum)**	**Ergot**
Colletotrichum graminicola	***S. vulgare* (Sorghum)**	**Anthracnose, red leaf, stalk rot**
Fusarium moniliforme	***S. vulgare* (Sorghum)**	**Seed rot**
Sclerospora sorghi	***S. vulgare* (Sorghum)**	**Downy mildew**
Sphacelotheca cruenta	***S. vulgare* (Sorghum)**	**Loose smut**

PATHOGEN	CROP	DISEASE
Sphacelotheca sorghi	***S. vulgare* (Sorghum)**	**Covered smut, grain smut**
Diplodia maydis	***Zea mays* (Maize)**	**Ear rot, seedling blight, stalk rot**
Drechslera maydis	***Zea mays* (Maize)**	**Blight, southern leaf spot**
***Exserohilum turcicum* (Syn. *Drechslera turcicum*, *Helminthosporium turcicum*)**	***Zea mays* (Maize)**	**Blight-Northern leaf blight**

PATHOGEN	CROP	DISEASE
Fusarium roseum **(Syn. *Gibberella zeae*)**	***Zea mays*** **(Maize)**	**Pink ear rot**
Fusarium moniliforme **(Syn. *Gibberella fujikuroi*)**	***Zea mays*** **(Maize)**	**Fusarium kernel rot**

SEED BORNE DISEASES AND THEIR IMPORTANCE

Prof. A. W. Mwang'ombe / Dr. R. D. Narla

What are seed borne diseases?

Figure 11. Seed from TSWV infected plants.
Courtsey of Texas Agricultural Extension Service - 1995.

- Seed-borne diseases are caused by pathogens such as fungi, bacteria, viruses and nematodes that live on the surface or interior of seed and have the potential to spread disease
- All true/vegetative seed are infected by the above pathogens
- Common seed borne pathogens
  - *Colletotrichum lindemuthianum and macrophomina phaseolina* (bean)
  - *Aspergilus flavus* (maize) *& A. parasiticus* (peanuts)
  - *Fusarium graminearum (*maize)
  - *Alternaria porri* (onion)
  - *Pyricularia oryzae* (rice)
  - *Bacteria - Xanthomonas campestris* (cabbage) *Ralstonia* in solanaceae
  - Nematode - *Aphelenchoides* besseyi (rice) - *Anguina* tritici (wheat)
  - Potato virus x
  - Bean common mosaic virus
  - Bean yellow mosaic virus
  - Maize dwarf mosaic virus

Some of the most important damages that pathogens can cause to seed are

1. Disease Transmission

Seed born pathogens transmit diseases between fields, regions and countries through seed and other planting material. For example, Diseases like Bacterial blight of paddy, Sclerotinia diseases of broad beans, common beans and recent cauliflower are transmitted through movement of improved seed.

- Importance of transmission can be realized when we know the seeding rate/ha (kg/ha), percentage seed infection and number of infected seeds/kg of seed.

E.g. Incase of Loose smut of wheat and Barley, with 0.1% seed infection bring 5000 infected seeds in a hectare of field. These 5000 give rise to equal number of infected plants (systemic) and in such cases yield losses are expected in the same ratio of 1:1

2. **Complete Loss or Reduction in Seed Germination**

Seed borne diseases/pathogens can be spread from the seed and infect the new plant in several ways.

- Upon sowing, moisture activates pathogens causing pre and post emergence damping off, eg: bean seeds infected with *Macrophomina phaseolina* cause 59% loss of germination. Soybean infected with C*ercospora kikuchii* – 12% loss of germination. Some of the pathogens like different species of *Fusarium*, *Pythium*, *Rhizoctonia*, *Sclerotinia*, *Altenaria* when also cause similar diseases in several other crops.

3. Seed abortion

Some of the seed borne pathogens like smut fungi in number of cereals and viruses like pigeon pea sterility mosaic virus cause heavy seed abortion resulting in 80-100% yield losses.

4. Reduction in seed quality

- Pathogen infections of seed often substantially reduce seed size resulting in weight reduction. Eg, leaf blight of sunflower – *Alternaria helianthi*, *A. zinnniae* infect the crop – severe leaf blight and yield loss of 80%

- Some other fungi – species of *Aspergillus*, *Fusarium*, infect standing maize causing seed rot.

- Sclerotisation, stromatisation and gall formation– *Claviceps fusiformis* – stromatisation of seed 60-70% yield losses in milllets.

Anguina tritici in wheat causes seed galls.

- Seed discolouration – a very important and wide spread symptom produced on seed indicating presence of pathogen – *Cercospora kikuchii* – soy bean, *Fusarium moniliformae* – sorghum, *Aschochyta pisi* – Sweet pea all result in reduction in market value

- Infected seeds are at risk of being contaminated with mycotoxins and nutritional changes

- Biochemical changes in seed products – Groundnuts infected with *A. flavus* gives inferior quality of oil through reduction of the refractive index

5. Reduction in yield

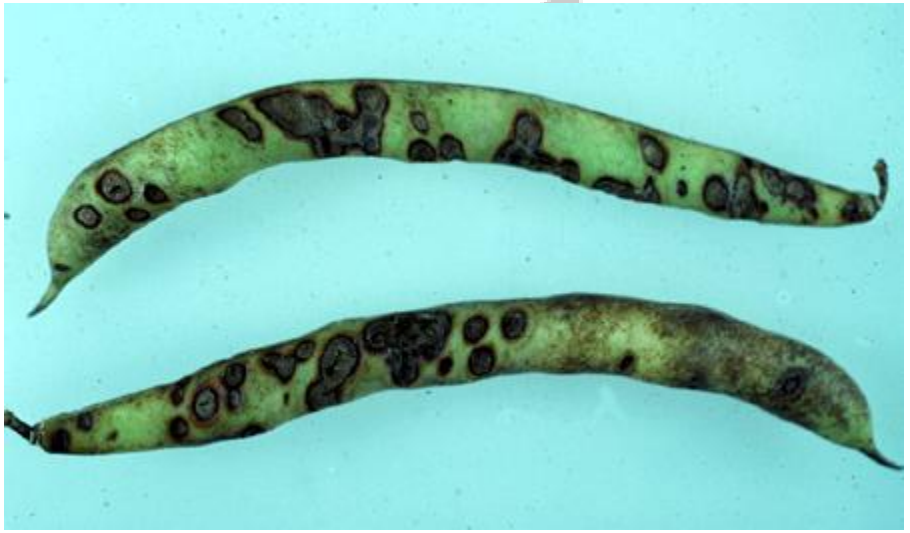
Great yield losses are experienced worldwide through seed born pathogens

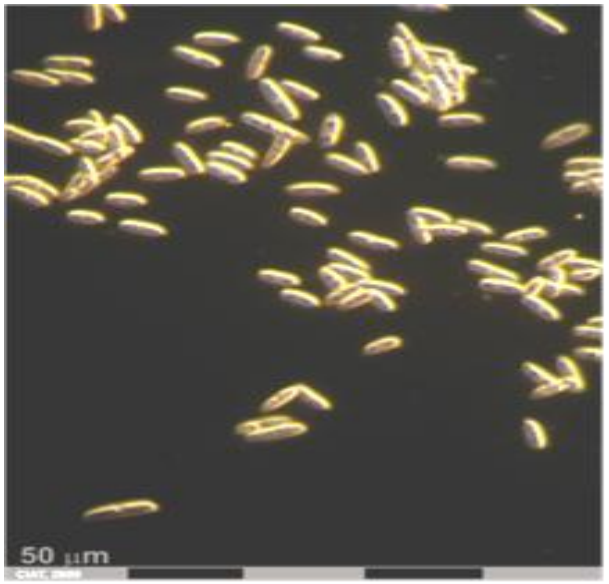
- **Fungal Seed borne Diseases**

Anthracnose

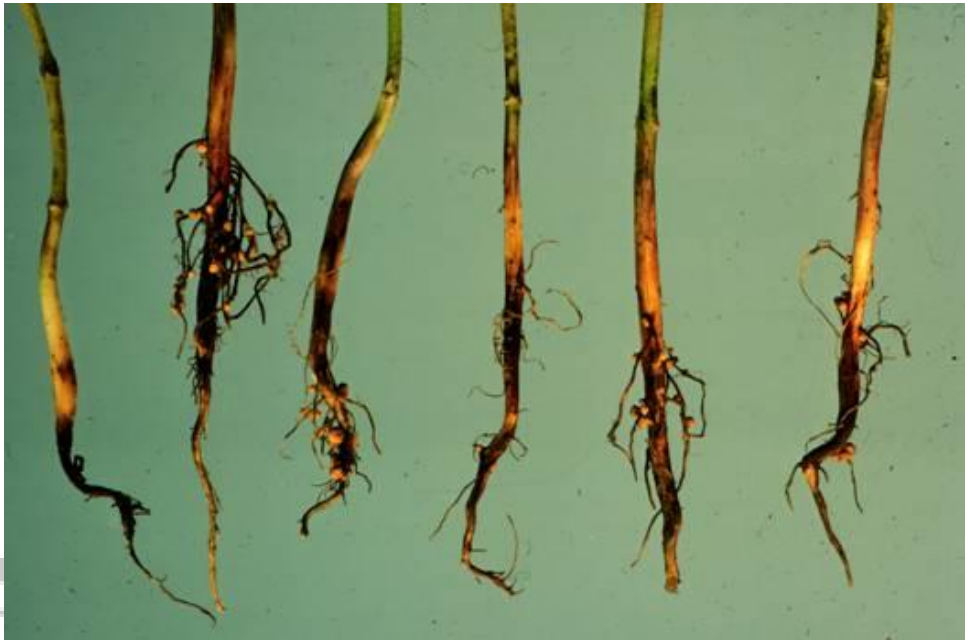
- Caused by *Colletotrichum lindemuthianum*

- The fungus is pathogenic to common bean, scarlet runner bean, mung bean, cowpea, and faba bean.

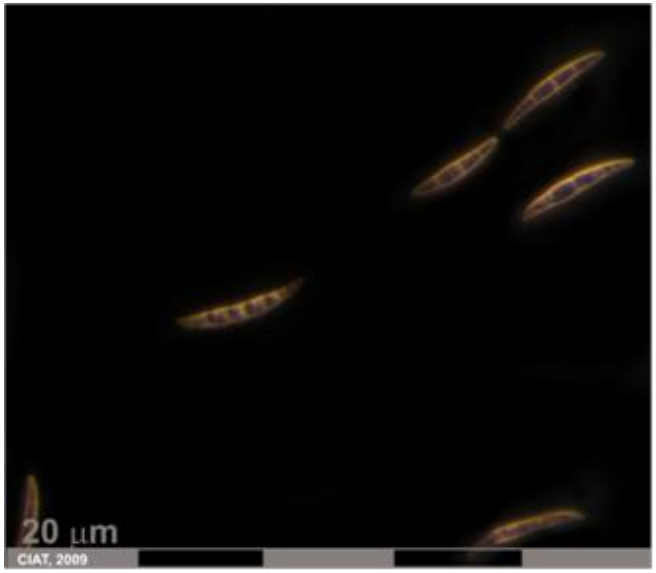




Fusarium root rot

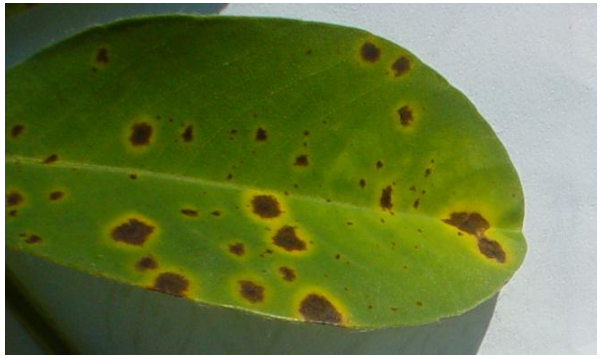


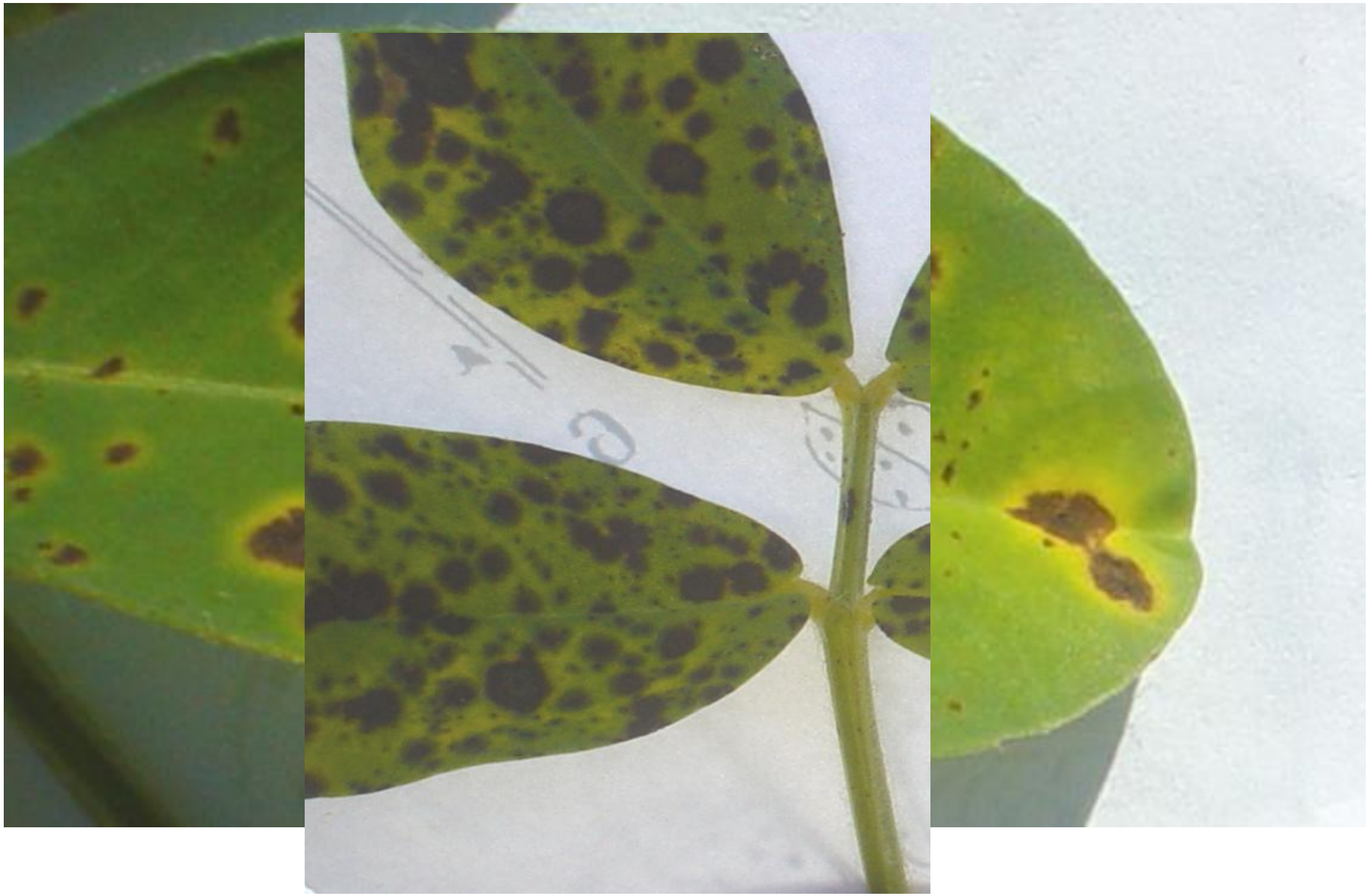
- Caused by *Fusarium oxysporium*
- may complex with *Rhizoctonia solani* and *Pythium* spp.



Disease: Groundnut leaf spot
Causal agent: *Cercospora arachidicola*

- Disease symptoms - small chlorotic spots appear on leaflets 10 days after infection
- In five days, spots develop into mature, sporulating lesions
- Identification by morphology of conidiophores

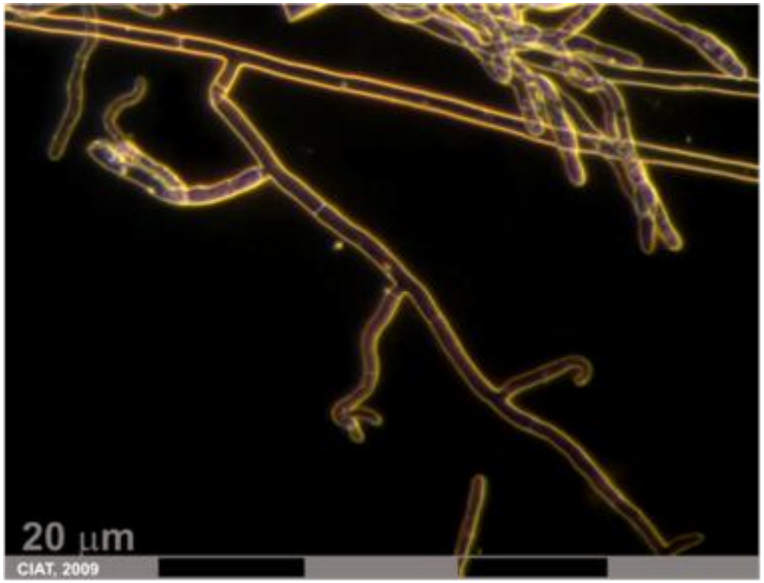




Late leaf spot damage on grondnut

Rhizoctonia root rot

- Caused by the soilborne fungus *Rhizoctonia solani*.



Southern blight

Caused by *Sclerotium rolfsii*

- Attacks a wide range of crops in africa

Southern Blight on tomato stem

Angular leafspot caused by *Phaeoisariopsis griseola*

Fusarium headblight

- Causal agent: *Fusarium graminearum*

White mold

- Causal fungus *Sclerotinia sclerotiorum*.

- also known as Sclerotinia stem rot

Finger millet blast

Pyricularia grisea

- **Bacterial Seed borne Diseases**

Halo blight of beans caused by *P*. (*syringae*) *savastanoi pv phaseolicola* –

Bacterial blight of beans

Causal agent: *Xanthomonas axonopodis* pv. *phaseoli*

Fuscous Blight-

Xanthomonas axonopodis pv. phaseoli (*syn. Xanthomonas campestris pv. Phaseoli)* *var. fuscans*

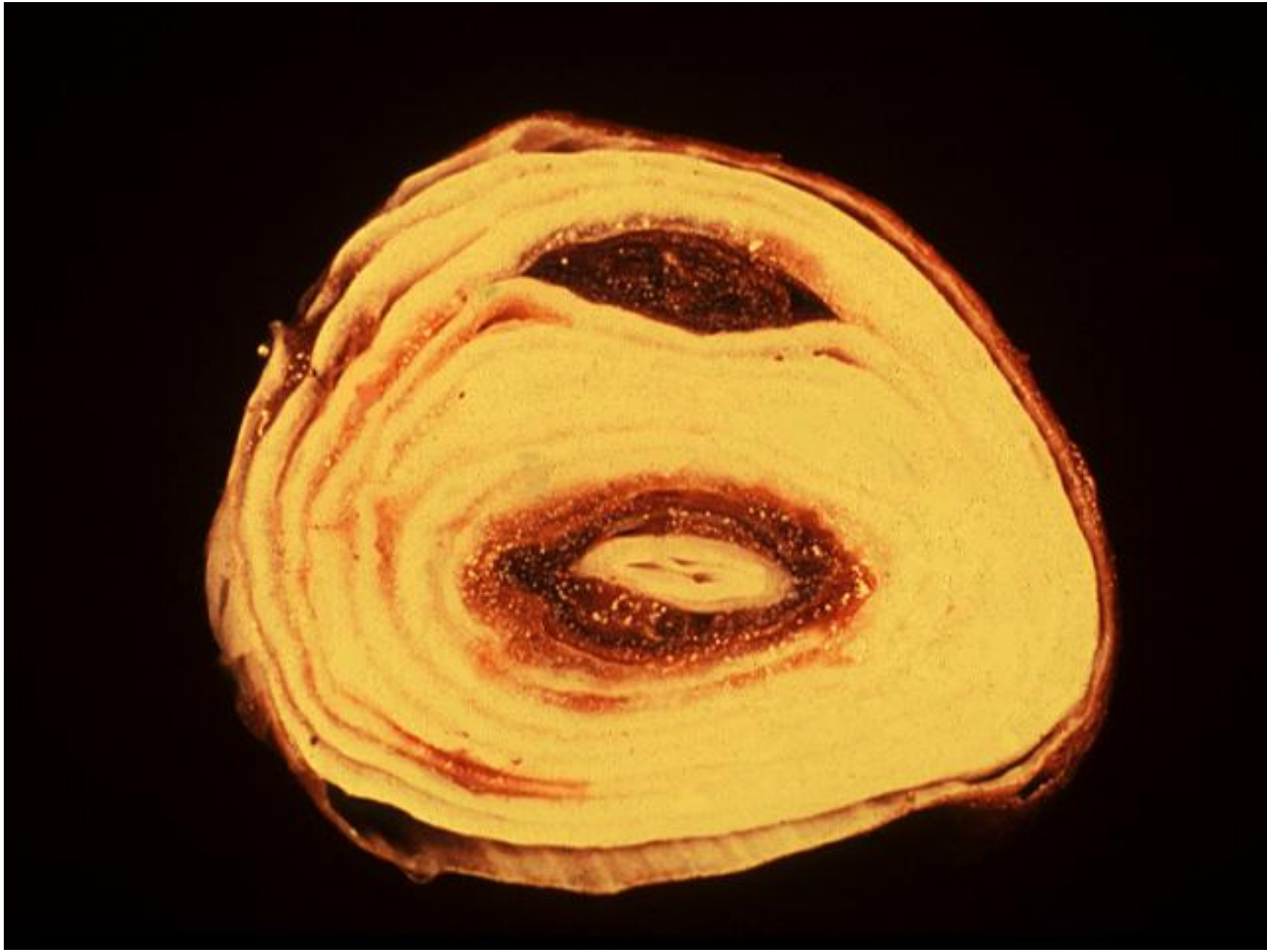
Seed borne Nematodes

Wheat gall nematode *Anguina tritici*

White tip disease of rice by *Aphelenchoides besseyi*

Aphelenchoides besseyi

Ditylenchus dipsaci on faba beans causing stunting



Ditylenchus dipsaci on onions causing bulb rot

Ditylenchus dipsaci

Ditylenchus damage on maize

Seeds *infected with Ditylenchus*

Seed borne viruses

Bean Common Mosaic Virus

Pea seed borne mosaic virus

Tomato spotted wilt virus

- " Global losses in food production due to seed born diseases are important negative factors in world agriculture
- Total amount of annual global loss is equivalent to total amount of food need for the entire population of Latin America (with exception of storage fungi)
- Such waste cannot be accepted as a natural law
- Seed born diseases must be controlled"

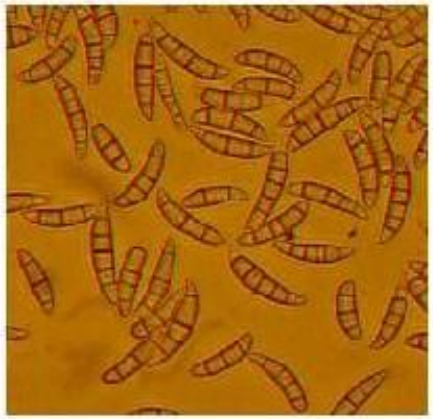
Paul Neerguard

I am sure all of us as seed scientists and technologists appreciate these statements.

Seed Quality Assurance, Management and Control Processes

Diagnostic Methods For Seedborne Diseases



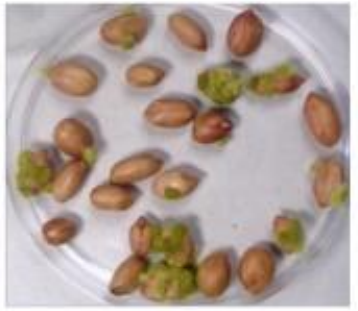



Prof. Agnes W. Mwang ómbe/ Prof. James W. Muthomi

Department of Plant Science and Crop Protection
University of Nairobi

Effects of Pathogen infection of seed

- A decrease in germinability
- Discoloration
- Biochemical changes
- Heating
- Mustiness and total decay
- Mycotoxin production



Location of Seedborne Pathogen

- Embryo
- Endosperm
- Seedcoat
- Surface borne

Objective of Seed Health

- 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

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

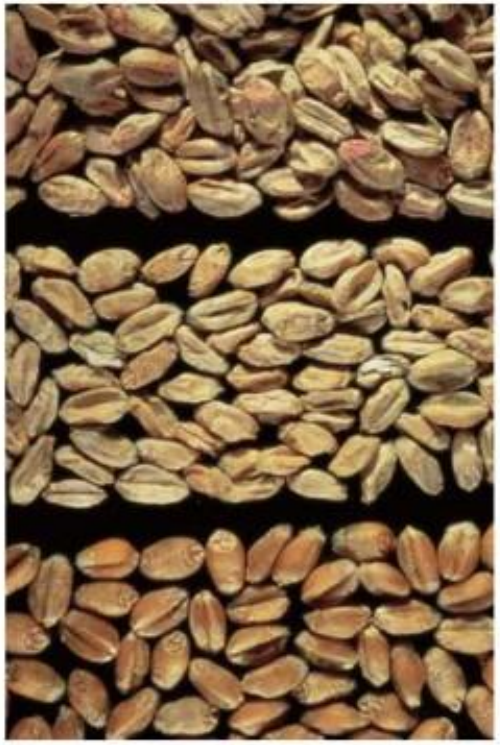
- Provides quick information on insect, disease and mechanical damage to the seeds

The fruiting structures of fungi

- 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

Physical abnormalities include:

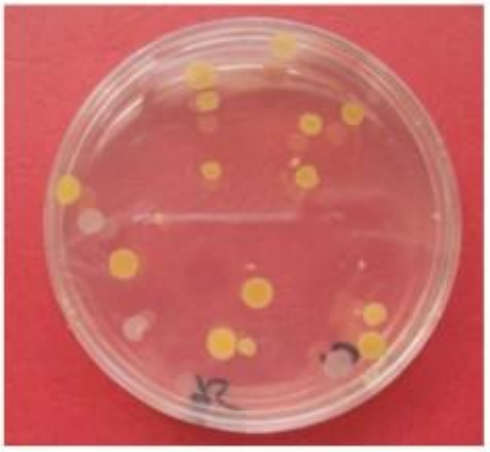
- Shriveling of the seed coat
- Reduction or increase in seed size
- Discoloration or spots in the seed coat

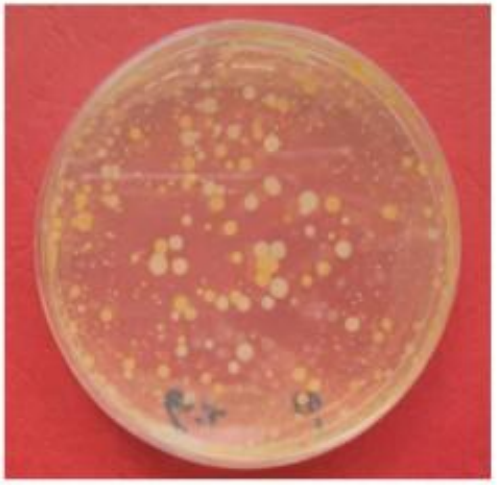


Seed washing test

- 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

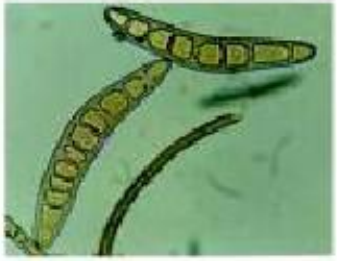
Washing test seed assay





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



Blotter Method

Simple and inexpensive way to detect seedborne fungi

Procedure:

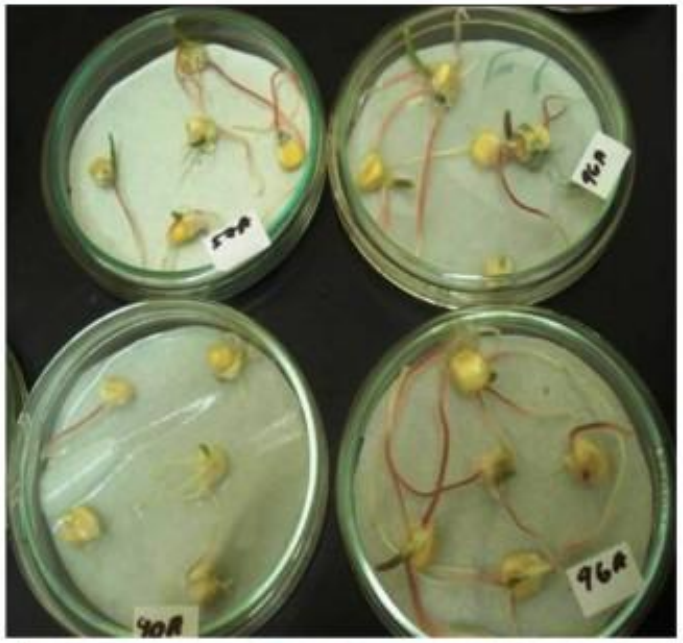
1.9.5-cm Pyrex glass or clear plastic petri plates containing 2-3 layers of blotter papers moistened with distilled water.

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.

Diagnostic Methods for Seed borne Diseases



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.

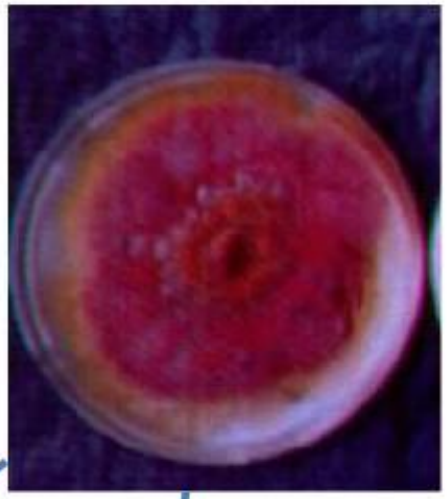
Diagnostic Methods for Seed borne Diseases

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.

Plating

Purify

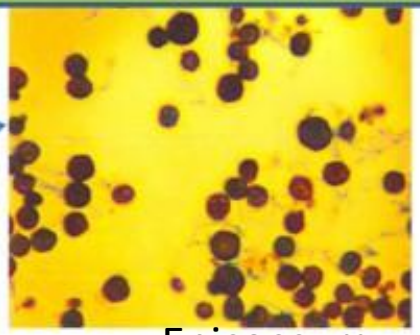


Microscopy

Chlamydospores

Spores

ISOLATION AND MICROSCOPY

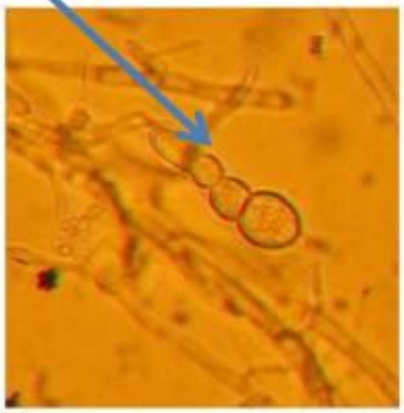


Epicoccum

Penicillium

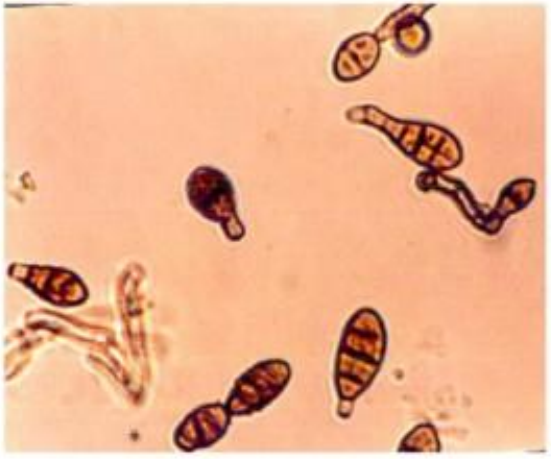
Fusarium

Spores



Chlamydospores

Alternaria

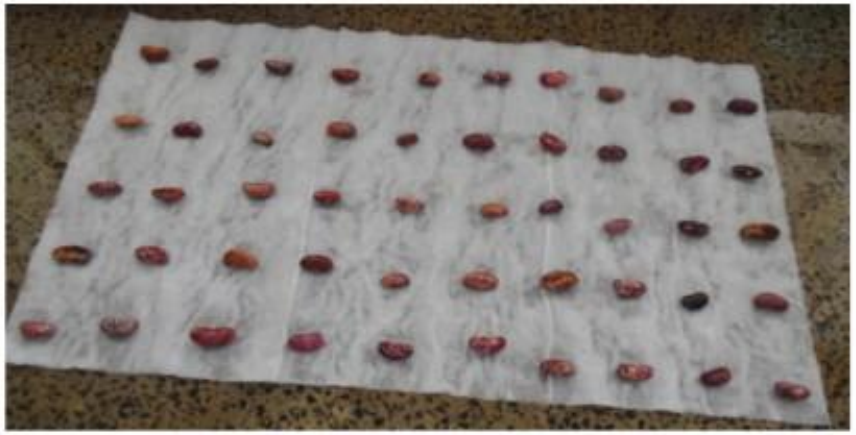


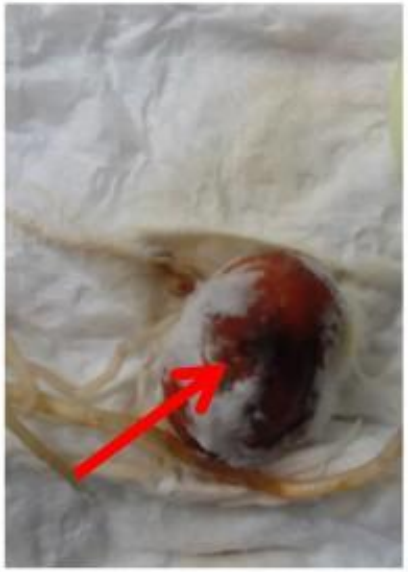
Paper towel method

- 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.

- 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)

Diagnostic Methods for Seed borne Diseases



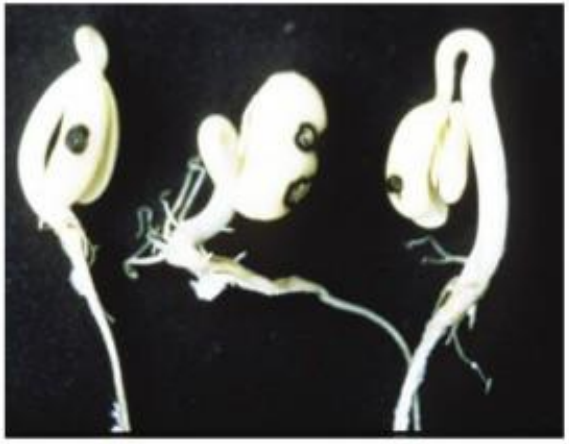


Seedling symptom test

- 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.

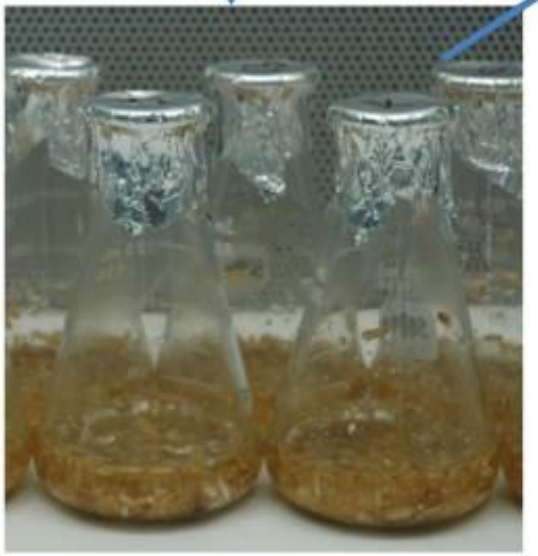
Diagnostic Methods for Seed borne Diseases

Detection 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

Infected seeds



Soak overnight in sterile saline

Serial dilution in sterile distilled water

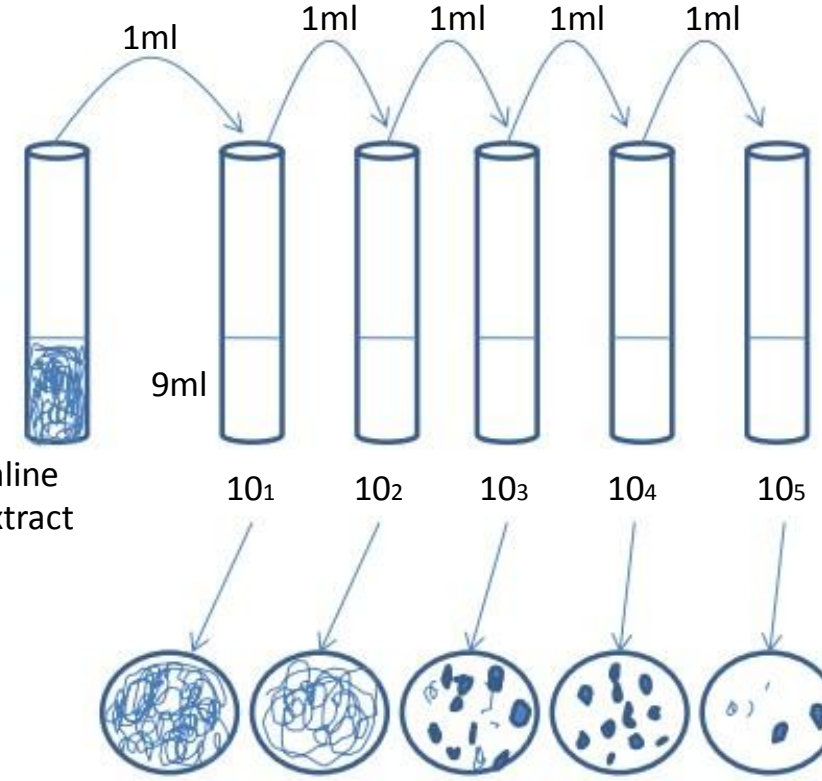
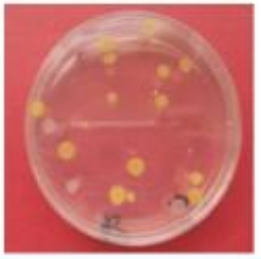
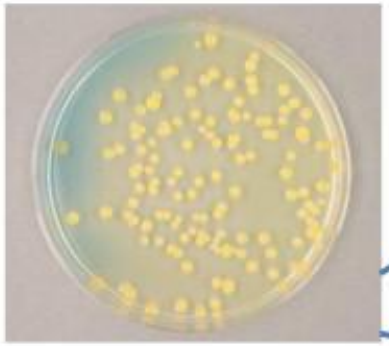


Plate 1ml of each dilution in molten agar medium. Incubate and count the number of colonies for each dilution. Determine bacterial population by multiplying the number of colonies by the dilution factor



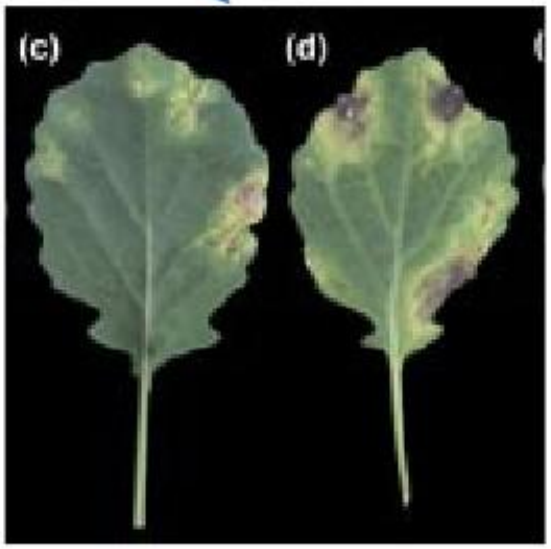
Pathogenicity test



Isolated bacteria

Inoculate on germinated bean cotyledons

Water soaking sympom



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

Detection of seed born Pathogens

Routine Testing Methods for Seed Health

Prof. A. W. Mwang'ombe/Narla, R.D. /Michael Starr

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?

- Seed 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

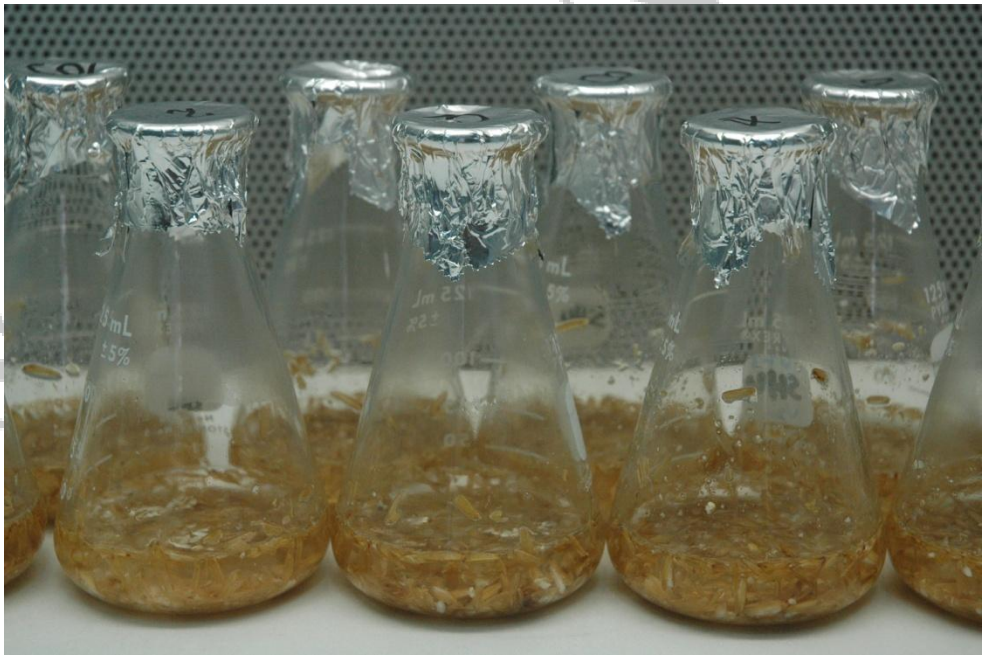
- Detects insect/mechanical damage
- Useful for purity analysis (weed and any other seed contamination, stones, etc)

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)

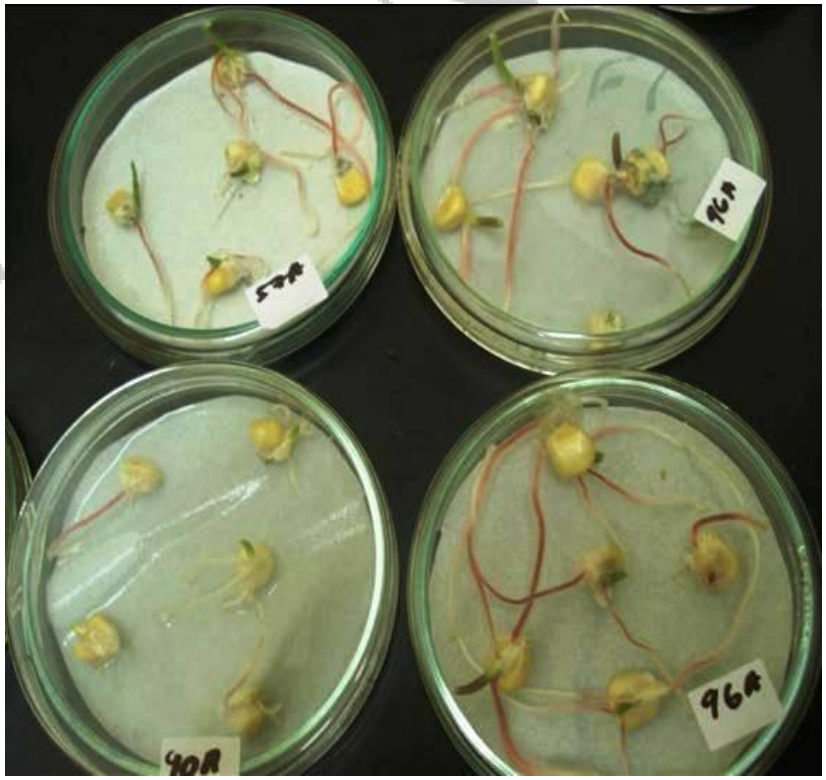
3. Blotter test

- 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.