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Seed health testing

Second Lecture /3rd. stage

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2023-2022

Infected part of the seed

1- Surface contamination:

Eg. Many smut fungi

such as grain smut, smut of barley.

2- seed coat infection:

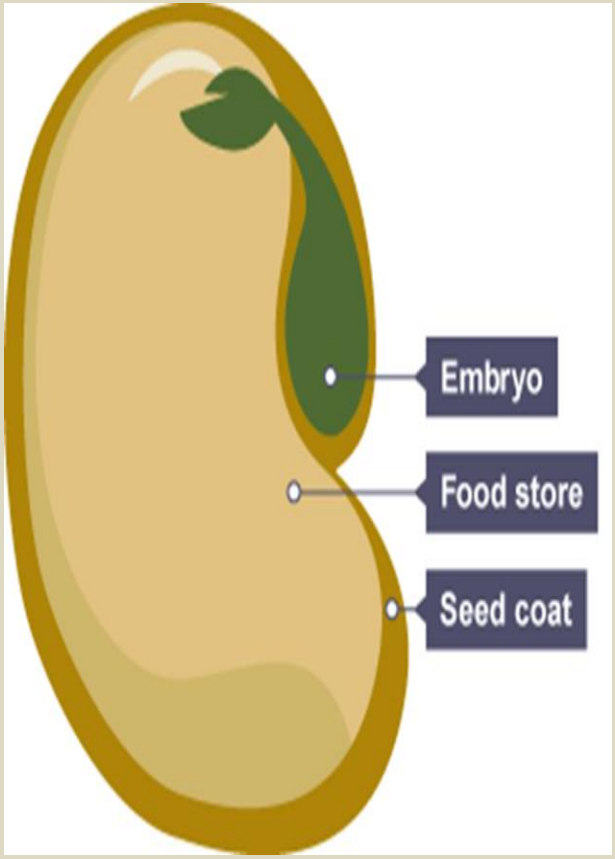
many of seedborne bacteria.

3- endosperm infection:

Fusarium moniliforme the causal agent of seedling blight of sorghum

4- embryo infection:

Loose smut of wheat and barley



Seed Health Testing

Are techniques to determine the presence or absent of (fungi, bacteria, viruses and nematode) in the seed.

Seed Health Testing methods

1- Visual examination of dry seeds.
2- Washing test.
3- blotter method.
4- Agar plate method.
5- seedling test.
6- bioassay test
7- serological test.
8- PCR test.
9- staining test

Objective of seed health testing

1- evaluation of the planting value of seed samples.

2- evaluating the loss of viability of seeds during storage.

3- seed certification.

4- Quarantine

5- seed treatment.

CHOOSING SEED HEALTH TESTING METHODS

More than one method may be available for detection of a particular seed borne pathogen.

The selection of a method depends upon the purpose of the test, i.e., whether the seeds are to be tested for seed certification, seed treatment, quarantine, etc.

If for quarantine purposes, then highly sensitive methods are preferred because it is important to detect even traces of inoculum.

Seed health testing for detection of seed borne fungi

1- visual examination of dry seed

2- seed washing test

3- blotter test

4- agar plate test

5- seedling test

6- staining test

Visual examination:

The dry seeds are examined either by naked eye or with the microscope.

infected seeds exhibit different symptoms produced by fungi on seed surface (seed rot, shrunken seed, discolouration of seed coat, shrivelling, abnormality of seed size and small size of seeds).

Also dry seeds are tested for the presence of admixtures such as sclerotia or resting structures of fungi.

Visual examination

Examination by naked eye

Examination by microscope

Example of dry seed infection

Brown discoloration and small size of seeds caused by *Ascochyta rabiei*

Gary mold disease cause shrivilling seeds

Example of dry seed infection

Ergot disease shows strucuture on rice seeds

Corn smut symptoms

Washing test:

This technique is used for detecting seedborne fungi carried as spores on seed surfaces.

The method is quick and can be adapted for detecting chlamydospores, oospores, smut spores, and conidia of *Alternaria, Cephalosporium, Curvularia*, *Drechslera, Fusarium, Peronospora*, and *Pyricularia*.

Washing seed test

Procedure:

1- 50 grams of seeds are taken in a flask with 100 ml of water and shaken for 10 min on a mechanical shaker or by hand.

2- The suspension is examined as such or the suspended spores are concentrated by centrifuging at 3000 rpm for 15–20 min.

3- The supernatant is discarded, and the spores are again suspended in 2 ml of lactophenol.

4- This suspension is then examined under the microscope for the presence of spores and conidia.

Blotter test

The blotter test is a simple and inexpensive means of detecting pathogens associated with seeds.

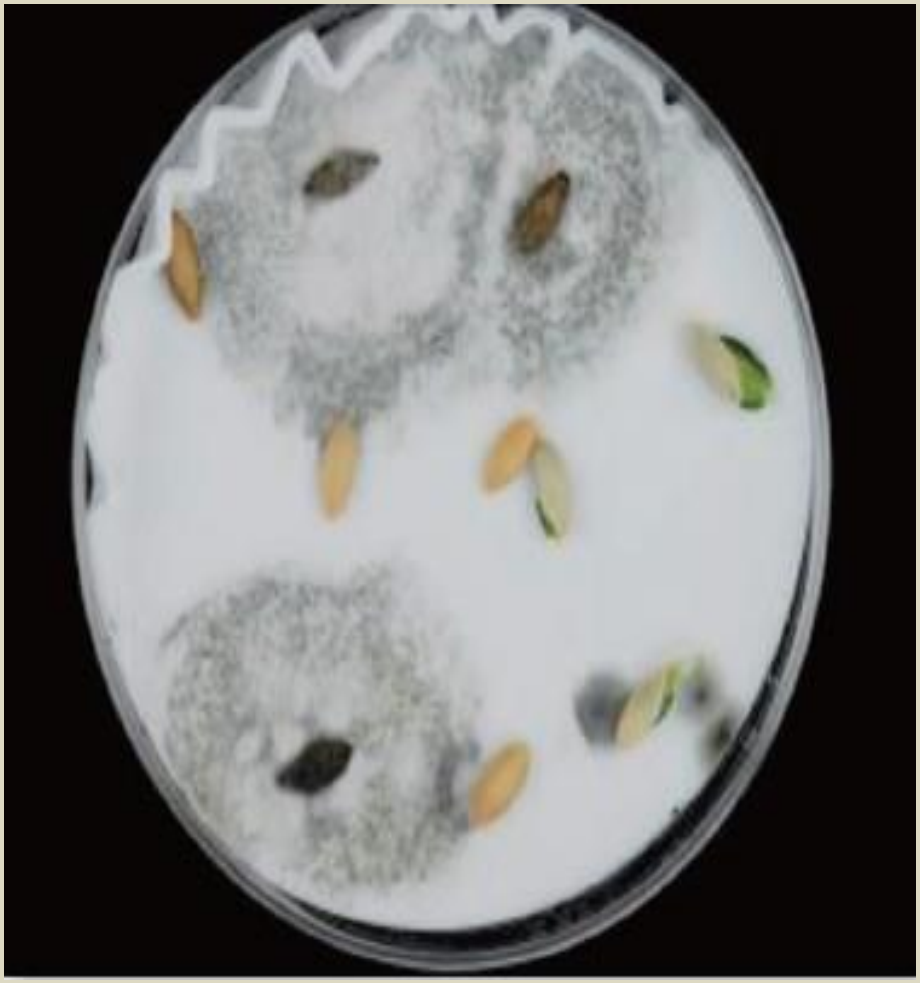
The basic principle in this method is to provide a high level of relative humidity, and optimum temperature conducive for fungal development.

Blotter test

Seeds are typically surface sterilized and planted on moist blotter paper.

They are incubated for 7-10 days. Blotters are observed for the development of fungal pathogens such as *Fusarium spp.* and *Alternaria spp.*

The pathogens are confirmed by microscopic examination to identify typical characteristics



Modifying Blotter test

freezing blotter test

2,4-dichlorophenoxy acetic acid test

Freezing blotter test

This method uses as modification of blotter test.

the dead seeds act as a natural substrate for the growth of fungi.

Fungi like (*Fusarium* and *Septoria* in cereals, *Phoma* in sugar beet and *Pyrenophora graminea* and *P. teres* in barley) can be detected by this method.

Freezing blotter test

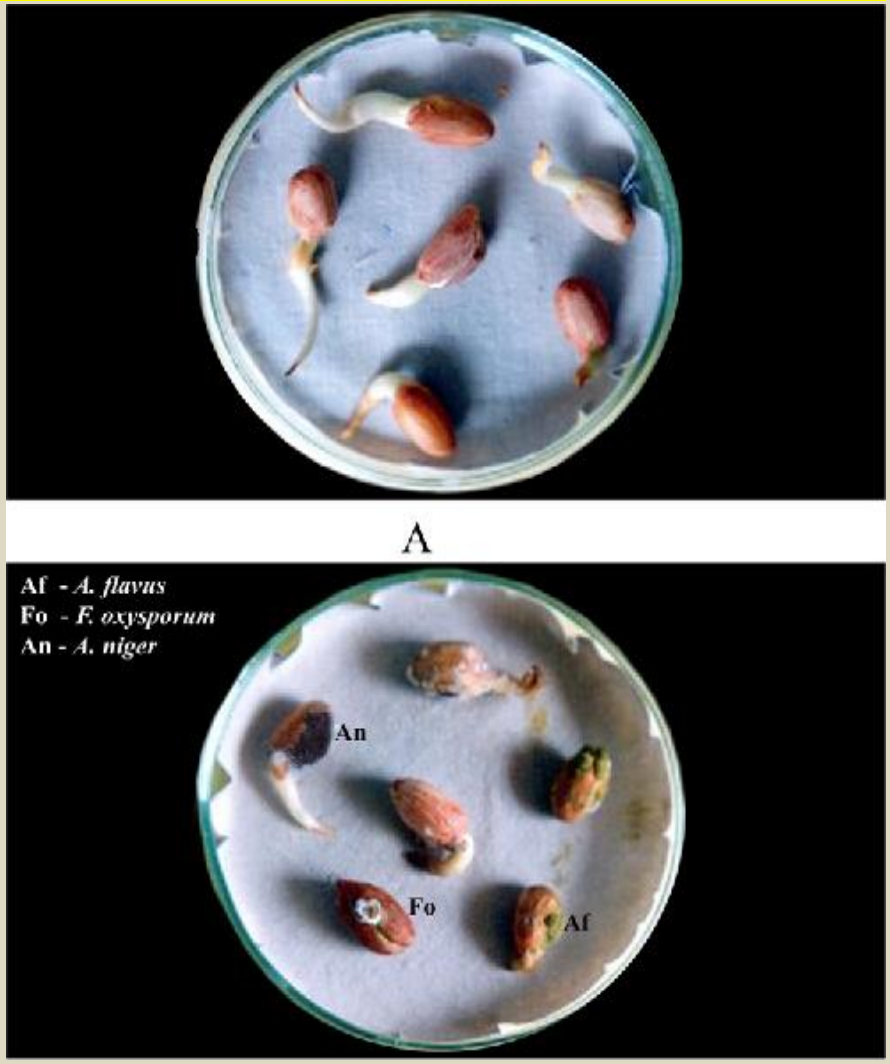
1- Soak seed in 2% bleach for 3 minutes and rinse with distilled water.

2- Place seeds evenly spaced on 2 -4 layers of moist blotter in a transparent plastic box and covered.

3- Incubate box at 20 C for 2 days (12 h light and 12 h dark per day).

4- Transfer box to freezer at -20C for 1 day.

5- Replace box in incubator at 25 C (12 h light and 12 h dark per day) for 4 days.



2,4-dichlorophenoxy acetic acid test

This is a modification of the blotter method. During incubation, seeds germinate and obstruct observation.

Most fungal structures are found associated with the seed coat and this seed coat displacement results in difficulties in evaluation and identification of microorganisms.

To overcome this problem, 2,4-D (2,4-dichlorophenoxy acetic acid) in agar media is used.

2,4-D (2,4-dichlorophenoxy acetic acid) is a herbicide used to suppress germination of tested seeds before incubation.

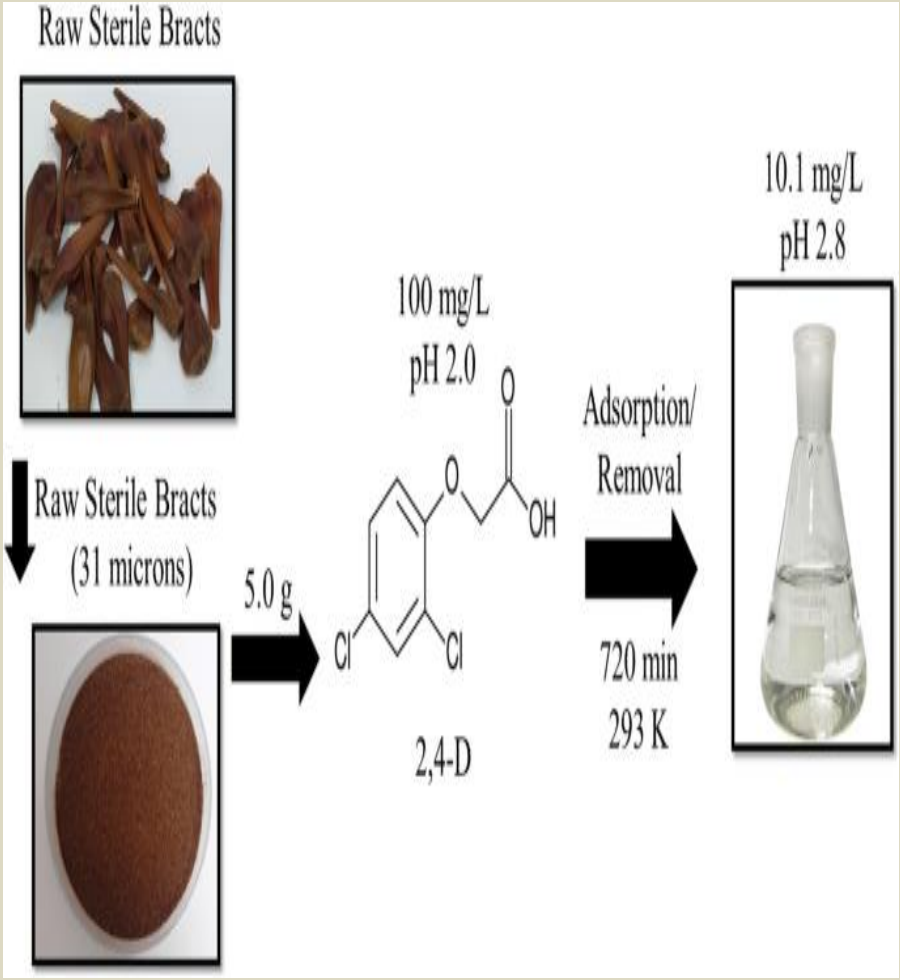
2,4-dichlorophenoxy acetic acid test

the blotter is soaked in 0.2 per cent solution of 2,4-dichlorophenoxy acetic acid instead of water.

Incubation and other conditions remain the same as in the blotter method.

This method used for the presence of *Colletotrichum lindemuthianum in soybean seeds.*

Also to detect *Pyricularia oryzae* on rice seeds.



Agar plate method

Seeds are typically surface sterilized and placed on selective or semi-selective culture media (Malt extract and PDA).

They are incubated for 7-10 days. Phomopsis spp., Fusarium spp. *Alternaria, phoma and pyricularia* and Verticillium spp. are some of the pathogens that can be identified by this method. Suspect organisms are confirmed by microscopic examination and the number of infected seeds are recorded.

Seedling test

This was used for the detection of seed-borne pathogens that could not be identified by routine seed testing in the laboratory. It was based on symptoms that develop on seedlings in a controlled environment favorable to disease development. There are number of grow out test for seedling symptoms as follow:

1- test tube agar method

2- soil methods

3-sand or brick stone method

Test tube agar method

15 ml water agar is taken in test tube, sterilized and solidified with a slight slant.

2. One seed is sown in each test tube and incubated at 28 ± 1 °C with 12 hours alternating cycles of light and darkness.

3. Seedlings are examined after 14 days for the typical symptoms of disease in the coleoptiles.

4. The symptoms can be easily studied being visible on roots as well as on green parts.

Seedling Growing test (soil)

Procedure:

seeds were grown in pots containing sterilized soil. The seeds were watered adequately. Temperature and humidity are in optimum rate

symptoms are observed after incubation for 2–4 weeks depending on the kind of seed and temperature