Methods for detection of fungal pathogens

1. Seed washing technique

This is useful in testing surface-borne, contaminating fungi like smuts, bunts, downy mildews, powdery mildews, rusts, etc.

Procedure
1. Place two grams of seed sample in a test tube and mix it well by adding 2 ml of sterile water for 5-10 minutes.
2. Centrifuge the supernatant solution at 200 rpm for 10 minutes and observe the sediments under a microscope for fungal structures.

2. Blotter test

Procedure
1. Line the lower lid of sterilized petri dishes with three layers of absorbent paper moistened with sterile water.
2. Drain off excess water and place 20–25 seeds manually with a forceps.
3. Evenly space the seeds to avoid contact with each other.
4. Incubate the seeds under near ultraviolet light in alternating cycles of 12-h light/darkness for 7 d at 20 $\pm$ 2 ^{0}C.
5. Examine the petri dishes under a stereo-binocular microscope for fungi developing on the seeds.

Profuse seedling growth may make interpretations difficult. This may be overcome by adding 2,4-D sodium salt to provide a 0.2% moistening solution.

3. Potato dextrose agar (PDA) method

Procedure
1. Prepare the medium by mixing 1 gram of Potato Dextrose Agar (PDA) powder in 100 ml of distilled water.
2. Sterilize the mixture in an autoclave for 15–20 min and cool to about 50°C.
3. Carefully pour the mixture into sterile petri dishes by lifting the lid enough only to pour in the agar to avoid contamination.
4. Allow it to cool and solidify for 20 min.
5. Surface-disinfect the seed by pretreating for 1 min in a 1% sodium hypochlorite (NaOCl) solution prepared by diluting 20 parts of laundry bleach (5.25% NaOCl) with 85 parts of water.
6. Place about 10 seeds (depending on size) on the agar surface with a forceps.
7. Incubate the petri dishes at 20–25°C for about 5–8 days.
8. Identify the seedborne pathogens on the basis of colony and spore characteristics.

Near ultraviolet light with a wavelength 300-380 nm (also called black light) may be required to promote sporulation.

Some times bacterial colonies develop on the agar and inhibit fungal growth making identification difficult. This can be overcome by adding an antibiotic such as streptomycin (500 ppm) to the autoclaved agar medium after it cools to 50–55°C.

4. Oatmeal agar method

Procedure

1. Prepare the medium by mixing 10 gram of ground oatmeal powder with 10g of pure agar in 1l of distilled water.
9. Warm on a stirrer hotplate mixing continuously until the agar has melted.
10. Sterilize the mixture in an autoclave at 220°C for 20 min and cool to about 50°C.
11. Mix in 4ml of tetracycline antibiotic solution (0.25g in 100ml) in one litre of media.
12. Carefully pour the mixture into sterile petri dishes by lifting the lid enough only to pour in the agar to avoid contamination.
13. Allow it to cool and solidify for 20 min.
14. Surface-disinfect the seed by pretreating for 1 min in a 1% sodium hypochlorite (NaOCl) solution prepared by diluting 20 parts of laundry bleach (5.25% NaOCl) with 85 parts of water.
15. Place about 10 seeds (depending on size) on the agar surface with a forceps.
16. Incubate the petri dishes at 20–25°C for about 10 days.
17. Identify the seedborne pathogens on the basis of colony and spore characteristics.

Near ultraviolet light with a wavelength 300-380 nm (also called black light) may be required to promote sporulation.