**Practical 6**

# BIOASSAY OF FUNGICIDES - INHIBITION ZONE TECHNIQUE AND SLIDE GERMINATION TECHNIQUE

## a) Inhibition zone technique:

This technique was introduced by Thornberry (1950) and called as paper disc plate method.

### Procedure

- Prepare PDA and sterilize it.
- Pour 15 ml of sterilised hot (80 to 90°C) agar medium into the bottom of 90 mm flat-bottomed Petri plates.
- After solidification, add 5 ml of lukewarm seeded agar containing (0.5-1 x 106 spores/ml and spread uniformly the seeded agar.
- Transfer the Petri plates to refrigerator and stack upside down to prevent
- Condensation of moisture.
- Prepare required concentration of the fungicide using sterile distilled water.
- Dip the sterile filter paper discs of 5mm diameter in the test chemical and transfer aseptically to seeded-agar plates after drying.
- Place three discs per plate (Maintain at least three such plates for each chemical)
- Incubate the inoculated Petri plates at 28 ±2°C for four days.
- Observe for clear zones of inhibition around each disc and measure from the centre of the filter paper disc.

**Note:** From these observations, the minimum inhibition concentration (MIC) is recorded. The MIC is the dosage at which no growth occurs on the impregnated seeded disc over a period of 7-10 days. It serves as a basis for comparing the efficacy of different test chemicals.

## b) SLIDE GERMINATION TECHNIQUE

**Aim**: To test the effect of fungicides on spore germination

Slide germination technique was introduced by Reddick and Wallace (1910) to determine fungicidal value of spray mixture or solution.

**Materials Required:** Clean glass slides (3 x 1" size), spore suspension of test fungus, Petri plates with three layers of blotting papers, Haemocytometer, microscope, pipettes, test chemicals (fungicides), sterile distilled water, beakers etc.

### Procedure

- Prepare spore suspension of test fungus grown on PDA (7 day old) by using sterile distilled water and adjust the final concentration to 50000 spores/ml using Haemocytometer.
- Prepare required concentrations of test fungicide using sterile distilled water.
- Place one drop of test fungicide at the centre of clean and sterilized glass slide and allow it to dry at 20-25$^{o}$C(Room temperature).

- Place one drop of spore suspension on the same spot where fungicidal suspension was placed with the help of pipettes.
- Incubate the fungus by placing the slides Petri plates containing moist blotters for 24 hours at 20-25$^{o}$C.
- Observe the germination of spores at six and 24 hours after setting of the experiment in low power/high power of the microscopes.
- Note the number of spores germinated in each concentration replication wise and calculate the percentage of spores inhibited from germination by using the following formula.

$$I = 100(C-T)/C$$

Where,

I = Inhibition percentage

C = No. of spores germinated in control

T = No. of spores germinated in treatment

Note:

i) The spore is said to be germinated if the length of the germ tube exceeds half the smaller diameter of the spore.

ii) Count at least one hundred spores for each concentration of test chemical.

Data obtained on the effect of different concentrations of the test chemicals and the germination of spores of test fungus is plotted on a graph paper to obtain 'Dose Response Curves'.