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PP-420

Practical-05

BIOASSAY OF FUNGICIDES - POISONED FOOD TECHNIQUE

The principle involved in this technique is to 'poison' the nutrient medium with a fungi toxicant and then allow a test fungus to grow on such a medium. In this technique a solid or liquid medium can be used.

With solid medium

Procedure

1. Prepare potato dextrose agar (PDA) medium in flasks and sterilise.
2. Add requisite quantity of the fungicide to the medium (at Luke warm stage) so as to get a desired concentration.
3. Mix the fungicide thoroughly by stirring
4. Pour the medium into sterilised Petri plate and store them in refrigerator till required
5. Grow the culture of the test fungus on PDA medium.
6. Cut small discs (5 mm), of the test fungus culture (7 day old) with a sterile cork borer.
7. Transfer aseptically the culture discs in the centre of a Petri plate containing the medium with a certain amount of fungicide.
8. Maintain suitable checks where the culture discs are growth under the same conditions on PDA without fungicide.
9. Incubate the inoculated Petri plates at 28 ± 2^0C temperature in incubator
10. Measure the fungus colony diameter when the colony growth in control plates is full.
11. The colony diameter, compared with check, is taken as a measure of fungi toxicity.
12. Per cent inhibition over control (check) is calculated by the following formula.

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

Where, I = Inhibition percentage,

C = Growth in control (check),
T = Growth in treatment

Note: The growth of fungal colony in treated plates is determined by excluding 5 mm fungal inoculation discs from measured diameter of the colony.

The data obtained on the effect of different concentrations of the test chemical on growth of test fungus are plotted on a graph paper to obtain dosage response curves.

With liquid medium

Procedure

1. Prepare liquid medium such as Blakeslee's medium or Richard's medium in flasks and sterilise.
2. Add requisite quantity of the fungicide to the medium so .as to get a desired concentration.

2. Mix the fungicide thoroughly by stirring
3. Transfer aseptically the 5 mm culture discs (7 day old culture) of test fungus into conical flasks containing poisoned medium.
4. Maintain control-flasks where the culture discs are grown under the same conditions on medium without fungicide.
5. Incubate the inoculated flasks at 28 ± 2°C temperature in an incubator.
6. Take observations when growth of the test fungus is full in control flasks (visual rating on 1-10 scale)
7. Filter the contents (mycelium) through pre- weighed whatman filter paper.
8. Dry filter papers in an oven at 105°C for 48 hours to a constant weight.
9. Weigh the dried filter papers along with the mycelium.
10. Calculate the weight of mycelium by subtracting weight of filter paper from the weight of filter paper + weight of mycelium and find mean of each sample.
11. Prepare a graph illustrating the growth increments in dry mycelia weight of the test chemical.
12. The dry weight of the fungus, compared to check, is taken as a measure of fungi toxicity.