Fungal Metabolism

Fungal secondary metabolites

1- Mycotoxins

a- Samples

Five samples of poultry feeds were used to isolate fungi.

b- Culture media

Use a general culture media purpose [potato dextrose agar (PDA)] and an antibacterial solution (antibiotic, e.g, chloramphenicol) to inhibit the bacterial growth associated with poultry feeds.

c- Antibacterial solution preparation

- Autoclaved 2.5 ml of distilled water (DW) in test tube.
- Add 250 mg of antibiotic, e.g, chloramphenicol to the autoclaved DW and shake the antibacterial solution.
- Add 1ml of the antibacterial solution for each 1000 ml (1L) autoclaved culture media (PDA) (100000 Mg/ml=100000 ppm*) to get 100 ppm concentration.

*Ppm=part per million.

d- Fungal isolation (Dilution method)

- Weighted 10 gm of poultry feeds and placed it in flask contain 100 ml DW (stoke), mixed using vortex.
- Prepare 6 test tube each contain 5 ml of DW.
- Take 1ml of stoke to the first tube that will be first dilution 1/ 10 (10^{-1}).
- Take 1ml of the first dilution to the second tube that will be the second dilution 1/ 100 (10^{-2}).
- Take 1ml of second dilution to the third tube that will be the third dilution 1/ 1000 (10^{-3}).
- Take 1ml of third dilution to the fourth tube that will be the fourth dilution 1/ 10000 (10^{-4}).

- Take 1ml of fourth dilution to the fifth tube that will be the fifth dilution 1/ 100000 (10^{-5}).
- Placed 1 ml of each filtrate dilution in a petri dish and then poured PDA contained the antibacterial solution (antibiotic), moved the dish for mixing the sample with the culture media.
- Leave it until solidify, then incubate for 5-7 days at 25-28°C under 24 hr. light.

Not: The dilution (1ml) can be distributed on surface of solid culture media.

e- Fungal diagnosis

Morphological characteristics, including colony colour, conidial shape, length, width and the number of septa and conidiophore length, should record and comparison should made with descriptions by classification key books. Specifically, classifying *Aspergillus flavus.*

f- Mycotoxin production (Aflatoxin)

1- Detecting Aflatoxin

- Cut 7 mm disc of one week-old of growing *Aspergillus flavus* and placed it on coconut extract agar (CEA) [20% (w/v) Coconut extract, 2.2% Bacto-agar, pH 6.9–7.0]
- and incubate for 5-7 days at 25-28°C.
- After incubation, check a blue fluorescent halo around the colony under 365 nm Uv light.