6.

Bioassays to Detect Insecticidal Activity

Bioassays are widely employed to investigate the substances responsible for the bioactivity of natural products from biological sources. Insect bioassays are used to evaluate candidate compounds as to their effects on test animals.

Bioassay with *Plutella xylostella*

Plutella xylostella, diamond back moth (DBM) (Lepidoptera, Noctuidae) is a serious pest of cruciferous crops in Sri Lanka and throughout the world. The life cycle of the moth is about 17-21 days, and 13-14 generations per year have been observed in tropical climates like in Sri Lanka. The larvae of DBM feed on the foliage of cruciferous plants from seedling stage to harvest, reducing the yield and quality of the produce. DBM has a short life cycle, high fecundity and is easy to culture under laboratory conditions. The adverse effects of exposure to test chemicals are also easily detectable. Therefore DBM is extensively used as a test insect in insect bioassay experiments.

Exposure Techniques

Test insets may be exposed to test chemicals by several methods. The leaf dipping bioassay and topical application are the most commonly used methods. Topical application requires a very small amount of the test chemical and the cuticle should be permeable to the chemical to observe the biological effect. This technique is more suitable for determining LD50 of pure active compounds.

In the leaf dipping bio assay exposure technique, the active compounds may enter in to the insect body through all means such as contact, ingestion or inhalation and therefore will reflect most of the adverse effects of the test chemical(s). However the technique requires more numbers of the test insect and is a suitable bio assay technique for selecting active fractions from crude extracts. This bioassay technique can be carried out with minimum facilities. The active test chemicals can be further tested under green house conditions and field testing is possible after converting the test compound in to a usable form (a formulation).

Leaf Dipping Bioassay

The bioassay may be conducted under choice and no-choice conditions.

Preparation of test solutions:

i. Prepare stock solution (4000 ppm) of each extract - dissolve the dicholoromethane extract (20 mg) in acetone (1 ml) and dilute to 5 ml with water. Methanol extracts are dissolved directly in distilled water (5 ml) using ultrasonication where necessary, to homogenize the solution.

ii. Prepare a series of concentrations of each extract by diluting the stock solution with distilled water.

iii. Add a commercial detergent (Teepol® or Triton X-100®, 40 µl) to each test solution.

iv. Cut leaves from the outer layers of green house grown cabbage plants with same maturity into 2.7 cm diameter discs using a cork borer. Dip discs in to the test solution for 5 seconds, hold vertically to allow excess solution to drip off and place on rack to dry.
Allow to dry for 2 h and use the disks for DBM bioassay.

No-choice experiments

Place a treated disc in a plastic Petri dish (4.5 cm diameter) and introduce 5 second instar larvae into it. Place a moistened cotton wool block (5 x 5x 5 mm) inside each petri dish to retard drying of the discs.

Make the following observations

Count the number of larvae which were moribund/dead and measure the feeding area (mm^2) 24 and 48 HAI.

Also record % pupation, % adult emergence, mean developmental period and developmental abnormalities if any.

Choice experiments

Arrange alternatively treated and untreated cabbage leaf discs (3 from each) radially in the Petri dish and release 10 second instar larvae into the center of the petri dish. Provide uniform illumination using fluorescent tubes hanging over the petri dishes.

Observations to be made:

Record the number of larvae settled on treated and untreated discs, Measure their feeding area (mm^2) 24 and 48 HAI.

Data analysis: The data should be subjected to appropriate statistical analysis for conclusion.

Reference

Redfern, R.E. 1983, Insect Bioassays. In CRC Handbook of Natural Pesticides: Methods Vol.1 (eds. N.B. Mandawa), pp 479-488, CRC press, Florida.