IRAC Susceptibility Test Methods Series

Method No: 020

Version: 3.3, May 2025

Details:

Method:	IRAC No. 020
Status:	Approved
Species:	Old world bollworm, *Helicoverpa armigera* Corn earworm, *Helicoverpa zea* Tobacco budworm, *Heliothis virescens* Southern armyworm, *Spodoptera eridania* Beet armyworm, *Spodopera exigua* Fall armyworm, *Spodoptera frugiperda* Cotton leafworm, *Spodoptera littoralis* Taro caterpillar, *Spodoptera litura*
Species Stage	Larvae (L2/L3)
Product Class:	This method is specifically recommended by the IRAC Diamide Working Group for evaluating the susceptibility status of **diamide insecticides (IRAC MoA 28).**

Spodoptera exigua larvae
Photograph Courtesy of: Syngenta Crop Protection

Comments:
For the purposes of this methodology the density of water is assumed to be 1.00g/ml

Description:

Distilled water, mixing bowl, weighing scales (0.001g accuracy),syringes/pipettes and beakers/test tubes for making dilutions, artificial diet (Stonefly *Heliothis* Premix Diet Formula)*, 10-20mm diameter well plates with sealable lid, protective gloves, artists paintbrush, fine forceps or seeker, binocular microscope or hand lens (optional), maximum/minimum thermometer. Large syringe or Icing bag for diet dispensing.

*Available from Ward's Natural Science Establishment, LLC (www.wardsci.com)

Method:

This method is an insecticide incorporated artificial diet assay. The following instructions are written as a guide to providing 20g of artificial diet per insecticide concentration utilised, which provides enough material to test 40 individual insect larvae per concentration.

(a) Collect a representative sample of insects from a field. It is recommended that egg masses (minimum of 10 masses), individual eggs (100 eggs) or larvae (minimum of 100 larvae) are collected to produce a F0 population of at least 50 adult moths from which an F1 population for testing can be reared. The insects should be reared on artificial diet and not be subjected to temperature, humidity or starvation stress after collection.

(b) Prepare accurate aqueous dilutions of the test compound from the identified commercial product. For initial studies, six widely spaced rates are recommended. Once a suitable rate range has been identified a narrower range of rates may be utilized, the modified rate range should include at least four doses which provide between 5% and 95% mortality of the target

insect (e.g. for diamide insecticides 5,1,0.2, 0.04 and 0.008 ppm)
4ml of insecticide solution is required per 1g of dry diet being prepared. The insecticide solution should be prepared at 1.25x the final dilution rate required. e.g. (400ml of insecticide solution at 1.25ppm is required to add to 100g of dry diet, resulting in diet containing 1 ppm concentration of insecticide).

NOTE: When preparing insecticide solutions always take into consideration the concentration of active ingredient in the formulation, e.g. INSECTICIDE-PRODUCT 5%EC contains 50g a.i/ l solution. (To prepare a stock solution containing 1.25ppm a.i.. a 40,000-fold dilution of the original 5% EC formulation needs to be prepared).

(c) Add 80ml of the insecticide solution (or distilled water for control) to a clean mixing bowl. Ensure that the insecticide solution is thoroughly mixed to suspend the insecticide solution.

(d) Weigh and add 20g of Stonefly Heliothis Premix Diet to the glass bowl and thoroughly mix all the ingredients together until a soft, smooth dough is obtained. Ensure protective gloves are worn during this procedure to avoid exposure to the insecticide.

Optional: A preservative mixture of formalin 0.1% + acetic acid 0.2% w/w can be added to the mixture for diet storage. However, where possible it is recommended to use a freshly prepared diet for the bioassay.

(e) Using a large syringe or icing bag, add 0.5-0.75ml of the dough to individual wells of a 10-20mm diameter well plate. A minimum of 24 wells per insecticide concentration should be prepared. Ensure each well is labeled with the insecticide and concentration utilized.

(f) Repeat steps (c) (d) & (e) for each of the insecticide concentrations utilized in the study and a control treatment. Ensure that equipment is cleaned and protective gloves are replaced between treatments to prevent contamination.

NOTE: treated diet may be used immediately or stored for a maximum of 7 days in a refrigerator (5ºC).

(g) Add a single late L2/ early L3 larvae (size will vary dependant on species tested) to each individual test well, using a fine artist's paint brush or fine tweezers. Once infestation of each plate is completed, seal the plate with a tight plastic lid/cover (ensure a tight seal between the top of the plate and the lid, preventing larvae from escaping).

(h) Store the containers in an area where they are not exposed to direct sunlight or extremes of temperature. Record maximum and minimum temperatures. A temperature of 24 (+/- 3°C), 30-60% RH and 16:8 light/dark regime is recommended.

NOTE: Do not stack trays on top of each other, as this can create variation in storage conditions for each tray.

(i) In the case of diamide insecticides, a final assessment of larval mortality (dead and live) is made after 7 days (168 hours). Larvae which are unable to make coordinated movement and cannot return to an upright position when turned upon their backs with a seeking pin or fine pointed forceps are to be considered as dead (combination of dead and seriously affected).

(j) Express results as percentage mortality, correcting for "untreated" (control) mortalities using Abbott's formula[1]. Untreated mortality should be recorded. It is recommended that the mortality data is utilised to perform a probit or logit-mortality dose response analysis to provide LC_{50} and LC_{90} estimates for each insecticide or insect population tested.

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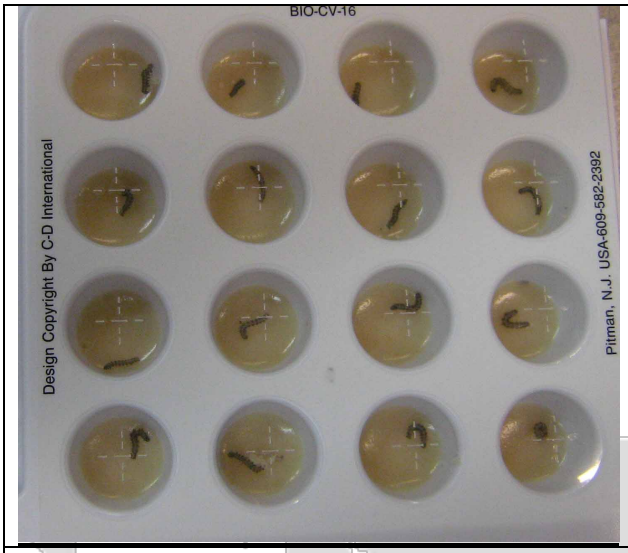

Fig 1: Diet trays after infestation
(Photograph courtesy of DuPont Crop Protection)

Percautions & Notes:

1. Disposable plastic equipment is preferred provided that it is not affected by the formulation constituents; glass equipment may be used but must be adequately cleaned with an appropriate organic solvent before re-use.

2. Insecticide products contain varied concentrations active ingredient(s). Ensure insecticide dilutions are based on active ingredient content (g a.i.). Some diamide insecticides are sold as pre-mixtures with other insecticides, these products should not be used to determine the susceptibility of insect populations to the single insecticide, as the mixture partner may have a significant impact on the mortality data generated.

3. Where possible, bioassays to measure the variation in insecticide susceptibility should run in parallel with a bioassay to measure the susceptibility of a known susceptible standard population of the target insect.

References & Acknowledgements:

This IRAC method is based on a methods developed by the University of Arizona (USA), DuPont Crop Protection , Syngenta Crop Protection, Bayer Crop Science and Nihon Nohyaku.

[1] Abbott's formula: corrected % mortaliy = 100x (1-(nT/nCo))

nT = survivors in treated diet,.
nCo = survivors in control.