IRAC Susceptibility Test Methods Series

Method No: 018

Version: 3.4

Details:

Method:	IRAC No. 018
Status:	Approved
Species:	Diamondback Moth *(Plutella xylostella)*
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)**** <u>This method is also suitable for the following insecticide classes (IRAC MoA class):</u> Carbamate (1A)* Organophosphate (1B)* Organochlorine (2A)* Fiprole (2B)* Pyrethroid (3A)* Spinosyn (5)* Avermectin (6)* Benzyl urea (15)** Diacylhydrazine (18)*** Indoxacarb (22A)* Metaflumizone (22B)* Pyridalyl (un)*

Plutella xylostella larvae
Courtesy of BASF

Comments:

Mortality assessment period may vary depending on insecticide mode of action

The following guidelines may be used:

*72 hours assessment period
**96 hour assessment period
***120 hour assessment period (addition of fresh plant material may be necessary to avoid starvation). Larvae should go through full molt before mortality assessment.

Description:

Materials:
Insect-proof containers, scissors, forceps, fine pointed brush, beakers for test liquids, syringes/pipettes for liquids or weighing balance for solids, syringes/pipettes for making dilutions, binocular microscope or hand lens (optional), untreated leaves of a host plant, paper towels, maximum/minimum thermometer, filter papers, seeking pin or fine forceps.

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(a) Collect a representative sample of insects from a field. These may be larvae suitable for immediate testing, or eggs/L1 larvae for rearing to the appropriate stage or material from which an F1 population for testing can be reared. The insects should not be subjected to temperature, humidity or starvation stress after collection.

(b) Collect sufficient non-infested, untreated host plant leaves. Whole leaves are preferred or, for some crops, the distal portions. Do not allow leaves to wilt by keeping them in a humid environment (plastic bag). *Brassica oleracea* (cabbages, cauliflowers & collards) are the recommended choice of host plant; however *Brassica rapa* (chinese cabbage, turnip) is also suitable. Choice of host plant should be recorded for future reference.

(c) Prepare accurate dilutions of the test compound from the identified commercial product. For initial studies, six widely spaced rates are recommended. The use of additional wetter is only recommended for highly waxed leaf material, in which case this wetter solution is used for the "untreated" (control) solution in place of water alone. As the addition of a wetting agent can significantly affect the performance of an insecticide product in a bioassay, it is essential that details of the wetting agent are recorded with any summary data and that only data generated with the same agent and concentration are compared for susceptibility measurements.

(d) Dip leaves individually in the test liquid for 10 seconds with gentle agitation and place to surface-dry on paper toweling (abaxial surface facing skywards). Ensure the entire leaf surface is emerged equally and do not allow the leaves to wilt. Dip the same number of leaves per treatment (a minimum of four replicate leaves per concentration is recommended), and treat sufficient leaf material to avoid starvation stress in the "untreated" during the test. Commence dipping the "untreated" first and work up through the test dilutions (lowest to highest).

(e) Place the treated surface-dry leaves in the labeled test containers, which must be suitable for holding enough leaf material in good condition for up to 96 hours.

Optional: A filter paper can be placed inside the base of the container to absorb any excess condensation.

(f) Add equal numbers of L2 larvae to each container, so that a minimum total of 40 larvae are used per treatment, divided between at least four replicate containers. Seal the containers with the container lid.

As development time can vary between populations of *Plutella xylostella*. The following length measurement can be used to classify L2/L3 larvae: 3-5mm.

(g) Store the containers in an area where they are not exposed to direct sunlight or extremes of temperature. Record maximum and minimum temperatures. If possible a mean temperature of 25°C, 60% RH and 16:8 light/dark regime is preferred.

(h) In the case of diamide insecticides, a final assessment of larval mortalities (dead and live) is made after 96 hours. For other insecticides please see guidelines provided at the top of this document. Larvae which are unable to make coordinated movement away from gentle stimulus with a seeking pin or fine pointed forceps to the posterior body segment are to be considered as dead (combination of dead and seriously affected). Anti-feeding effects (percentage damage to the leaf or larval growth) may also be recorded for additional information.

(i) Express results as percentage mortalities, correcting for "untreated" (control) mortalities using Abbott's formula. Untreated mortality should be recorded. It is recommended that the mortality data is utilised to perform a probit or logit dose response analysis to provide LD50 and LD90 estimates for each insecticide or insect population tested.

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 know susceptible standard population of the target insect.

References & Acknowledgements:

This IRAC method is based on the IRAC Method No. 007 for leaf feeding Lepidopteran and Coleoptera. It has been modified specifically for diamide insecticides and *Plutella xylostella* with significant contributions from Anthony Shelton (Cornell University, Geneva, USA).