IRAC Susceptibility Test Methods Series

Method No: 024

Version: 1

Details:

Method:	IRAC No. 024	 *Aphis gossypii* *Photograph Courtesy of Syngenta*
Status:	Approved	
Species:	Cotton Aphid (*Aphis gossypii*)	
Species Stage	Apterous adult & 4th instar nymphs	
Product Class:	This method is specifically recommended by the IRAC Diamide Working Group for evaluating the susceptibility status of **cyantraniliprole,** which belongs to the diamide group of insecticides (IRAC MoA 28).	
Comments: Cyantraniliprole SC formulations are recommended for this bioassay.		

Description:

Materials:
Beakers for test liquids, syringes/pipettes for liquids or weighing balance for solids, syringes/pipettes for making dilutions. razor blade, scissors, fine pointed brush, glass vials (1.5cm diameter x 7cm height), parafilm, large plastic container (17cm diameter x 6cm height), liquid Fluon®, cotton wool, binocular microscope or hand lens (optional), untreated leaves of a host plant, maximum/minimum thermometer,seeking pin or fine forceps.

Method:

(a) Collect a representative sample of insects from a field for rearing to the appropriate stage 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. Do not allow leaves to wilt by keeping them in a humid environment (plastic bag). *Gossypium hirsutum* (Cotton) is the recommended choice of host plant for *Aphis gossypii.* Choice of host plant should be recorded for future reference.

(c) Two week old cotton seedlings are cut at the petiole (leaving the petiole long enough to reach the base of the vial) with sharp scissors and remove the larger side leaves. Calculate the number of seedlings that need to be prepared per insecticide treatment (concentrations tested x replicates + control replicates). A minimum of 3 replicates should be utilized.

(d) Prepare accurate dilutions of the identified commercial product. For initial studies, six evenly spaced rates are recommended. Distilled water should be used as a control treatment.

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(e) Transfer 7 ml of test solution to the 7 ml glass vials, each labeled with the appropriate insecticide concentration. A minimum of three test vials per insecticide concentration should be utilized. The vials are then sealed with parafilm, before a small (5mm) cut is placed in the parafilm covering the opening of the vial.

(f) Insert the leaf petiole into the insecticide filled glass vials through the small cut in the parafilm. The petiole should reach the base of the glass vial.

(g) 40 apterous aphids from the collected population are transferred using an artist's paint brush on to the top side (adaxial) of the leaf surfaces.

(h) After infestation, place the glass vial/infested leaf into the inner center of a large plastic container (17cm diameter x 6cm height), where the inside rim of the container has been coated with a thin layer of liquid Fluon®. Fluon® can be applied using cotton wool or cotton bud.

(i) 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 22°C and a 16/8 light/dark regime and 70% RH is preferred.

(j) After a 72 hour holding period, the seedlings are removed from the glass vials and an assessment of mortalities (dead and live) is made utilizing a binocular microscope. Aphids which are unable to make coordinated movement away from gentle stimulus with a seeking pin, fine pointed forceps or paint brush are to be considered as dead (combination of dead and seriously affected). Aphids that have fallen/moved into the container are also counted and evaluated.

(k) 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.

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Fig 1: Vial with cotton leaf inserted and placed in container

Fig 2: multiple replicates

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 methods developed by DuPont USA and Rothamsted Research in the UK. We would particularly like to acknowledge the contribution of Steve Foster from Rothamsted Research.

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