IRAC Susceptibility Test Methods Series

Method No: 023

Version: 1

Details:

Method:	IRAC No. 023	 *Myzus persicae* *Photograph Courtesy of DuPont*
Status:	Approved	
Species:	Peach-Potato Aphid (*Myzus persicae*)	
Species Stage	Nymphs (2-3rd Instar)	
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. Small ventilated plastic boxes with lids (*Blackman boxes*, approx 5x8x2 cm), small pieces of synthetic sponge (5x2x2 cm), 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). *Brassica rapa* (Chinese cabbage) is the recommended choice of host plant for *Myzus persicae.* Choice of host plant should be recorded for future reference.

(c) Small primary leaves (cut from Chinese cabbage plants ~ 3 weeks old) are removed from the host plant at the petiole using sharp scissors, leaving as much petiole as possible. The lower leaf edges are trimmed with the razor blades to allow the leaf to fit inside the ventilated box (see figure 1). The leaves are then placed inside the plastic box with the abaxial side facing the box opening. A small piece of synthetic sponge is then placed around the petiole to provide support. Calculate the number of leaves that need to be prepared per insecticide treatment (concentrations tested x replicates + control replicates). A minimum of 3 replicates should be utilized.

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(d) 4 young apterous adult aphids (10-12 days old) from the collected population are transferred using an artist's paint brush on to the abaxial side of the leaf surface. The boxes are sealed with the lid and then placed upright in water-filled trays under 21°C, 70% RH 16/8 light/dark photoperiod conditions. The boxes are left for 48 hours until the leaf is infested with up to 50 aphid nymphs at which point the original adult aphids are removed.

(e) 5 days after infestation of the leaves with the adults, 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.

(f) Transfer 7 ml of test solution to 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.

(g) Gently remove the aphid infested leaves from the plastic boxes.

(h) With the aid of fine forceps, 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.

(i) 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.

(j) 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, 70% RH and 16:8 light/dark regime is preferred.

(k) After a 72 hour holding period, the leaves 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 fine paintbrush are to be considered as dead (combination of dead and seriously affected). Aphids that have fallen into the container are also counted and evaluated.

(l) 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 (e.g. POLO PC) to provide LD50 and LD90 estimates for each insecticide or insect population tested.

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Fig 1: Prepared Chinese Cabbage Leaf in plastic box

Fig 2: Vial with Chinese cabbage leaf inserted and placed in plastic container.

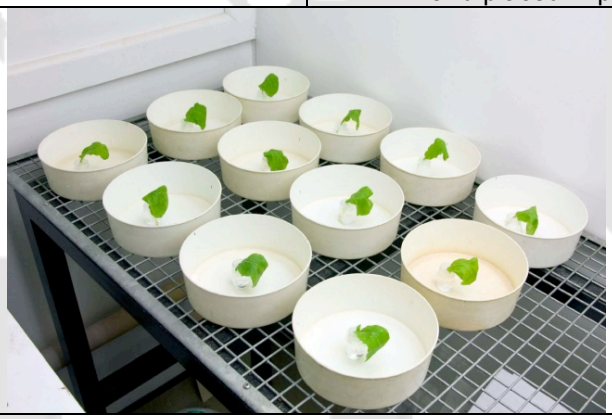

Fig 3: multiple replicates/concentrations

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.

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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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