IRAC Susceptibility Test Methods Series

Version: 1.0 (7th February 2023) *Method No: 035*

Details:

Method:	No: 035	 Photograph courtesy of BASF
Status:	Approved	
Species:	*Amrasca sp*	
Species Stage	Nymphs	
Product Class:	Organophosphates (1B) (Dimethoate, Acephate), Flonicamid (29), Pyropenes (9D)	
Comments: This method has only been approved and validated for species and active ingredients indicated.		

Objectives:

Susceptibility Baseline: ☒ Resistance Monitoring: ☒

Description:

Materials:

- Plastic container (6.5 cm diameter) with ventilated lid (~40 holes with a diameter small enough to avoid escape of jassid nymphs)
- Sterilized agar
- Glass beaker for dilution of chemicals
- Pipettes for making dilutions
- Pipettes for transferring warm agar
- Paper towel
- Autoclave for sterilized water
- Plastic screen for leaf drying
- Forceps
- Petri-plates
- Fine brush
- Healthy insecticide-free cotton leaves
- Jassid culture
- Plastic trays for holding experimental containers.

Methods:

- Prepare 1% agar by mixing agar powder in distilled water and autoclave (or microwave) at ~120ºC for 20 minutes.

- With the help of a pipette, pour warm agar (~14 ml) into the base of each plastic container to form a 4 mm deep agar layer and allow it to solidify (Fig. 1A).

- Dissolve technical grade active ingredients (AI) in a 1:1 acteone:water stock solution, where the acetone or comparable solvent (test solvents beforehand to ensure that they are not toxic or harmful to the insects) can range from 98 – 100%. Mild heat and sonication can be used to aid dissolving AIs in solvent. Prepare appropriate test dilutions with a water diluent of 0.01% (v/v) of a nonionic surfactant (e.g. Triton X-100). Formulated insecticides may be able to be dissolved in water, check manufacturer recommendations. Ensure that the product is completely dissolved. Select a series of concentrations (4-6 rates) to give a range of mortality for a clear concentration response for the insecticide(s) being evaluated.

- Prepare appropriate test dilutions of technical grade active ingredients (AI) in 1:1 acetone:water diluent with 0.01% (v/v) of a nonionic surfactant (e.g. Kinetic or Triton X-100). Formulated insecticides may be able to be dissolved in water, check manufacturer recommendations. Ensure that the product is completely dissolved. Mild heat and sonication can be used to aid dissolving AIs in solvent. Select a series of concentrations (4-6 rates) to give a range of mortality for a clear concentration response for the insecticide(s) being evaluated.

- Select fresh cotton leaves from untreated healthy cotton plants and prepare at least 4 leaf discs (6.5 cm diameter) per treatment. Each leaf disc is considered as an replication. To remove potential contaminants, clean the leaf discs by dipping in sterilized water for 4 to 5 sec. and allow them to dry on paper towel (15 to 20 min) (Fig. 1 B & C).

- Treat these leaf discs with different concentration of chemicals by dipping in solution for 5 sec. and place the dipped leaf discs on plastic screen for drying (15 to 20 min) as shown in Fig. 1 D & E.

- Once the agar has cooled and the treated leaf disc is dried, place one leaf disc adaxial (upper) surface down in each container as shown in Fig. 1F.

- Introduce 5 jassid nymphs (3rd instar) with a fine brush onto each leaf disc (more than 5 individuals may cause high natural mortality) and close the container. Use at least 4 observations (4 leaf-disc) with a minimum of 20 insects/treatment.

- Keep the containers in a growth chamber under 16L/8D photoperiod at 25 ± 2ºC, RH- 65%.

- Assess mortality at various timings up to 120 hours (5 days) after treatment by counting the live nymphs/adults for each observation.

- Express results as percent mortality. Correct for 'untreated' (control) mortalities using Abbott's formula[1] (Abbott 1925). The mortality data can be subjected to a probit or logit dose response analysis to calculate an LC_{50} or LC_{90}.

[2] Corrected % mortality = (% alive control - % alive treated) x 100% / (% alive control)

[1] Abbott's formula: $Corrected\% = \left(1 - \frac{nT}{nC}\right) * 100$

nT = survivors in treatment.
nC = survivors in control.

- If mortality is greater than 20% for the control treatment, the study should be considered as invalid. Mortality at the highest rate must be 100% for baseline/susceptible populations, and at least three datapoints should have mortality of >0% and <100%.

For more information on validation, refer to "IRAC Susceptibility Test Methods Series." https://irac-online.org/test-methods/

Precautions & Notes:

a) Where glass equipment is used, it must be adequately cleaned with an appropriate organic solvent and/or lab detergents before re-use to prevent cross-contamination.

b) Different batches of technical grade insecticide may vary in concentration of active ingredient (usually between 85-99% AI). It is recommended to use high purity AI where possible. Purity needs to be taken into account when preparing the test solutions.

References & Acknowledgements:

1. Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18: 265-267.
2. Püntener W., 1981 Manual for field trials in plant protection second edition. Agricultural Division, Ciba-Geigy Limited.
3. IRAC Susceptibility Test Methods Series. Insecticide Resistance Action Committee, Test Methods. URL: https://irac-online.org/teams/methods/documents (accessed 1/12/23).
4. Methodology was provided by BASF and Syngenta.

Fig 1. A) Pouring agar in vial. B) Cleaning leaf disc in sterilzed water. C) Drying leaf discs on a paper towel. D) Dipping the leaf disc in the chemical. E) Drying the treated leaf discs on a screen. F) Treated leaf disc inside the vial.