IRAC Susceptibility Test Methods Series

Version: 1.0 (7th February 2023) *Method No: 037*

Details:

Method:	No: 037	 *Blatella germanica* Photograph courtesy of BASF
Status:	Approved	
Species:	*Blatella germanica*	
Species Stage	Adults & Nymphs	
Product Class:	Carbamates (1A), Organophosphates (1B), GABA-gated chloride channel antagonists (2A, 2B) Pyrethroids (3A), nAChR agonists (4), Sodium Channel Blockers (22A, 22B)	
Comments: This method has only been approved and validated for species and active ingredients indicated.		

Objectives:

Susceptibility Baseline: ☒ Resistance Monitoring: ☒

Description:

Materials:

- Aerated insect-proof containers
- Forceps for transferring insects
- CO_2 for anesthetizing insects
- Glass beakers/vials for test liquids
- Test insecticides
- Pipette for liquid
- Weighing balance for solid products
- Microsyringe or micropipette for topical application
- Acetone or comparable solvent for topical application
- Dry dog food
- Dental wick
- Microcentrifuge tubes
- Petri dishes (60 x 15 mm)
- Filter paper discs (42.5 mm)
- Water bath/heat block
- Sonicator for dissolving insecticides in solvent

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Methods:

- Collect enough insects from an infested location or laboratory colony (for baseline studies) and keep them in an aerated insect-proof container. Ensure that insects are not subjected to excessive stress after collection (temperature, humidity, starvation, etc.). Transfer insects to the laboratory as soon as possible. Record sample details such as collection date and location and other information that may be useful for tracking samples and interpreting susceptibility results later on.

- After arriving in the lab, allow the insects to recover overnight prior to testing. Dry dog food can be used as a food source, and a water source can be constructed by placing a dental wick in a microcentrifuge tube filled with water. Select uniform individuals/life stages for testing (e.g. 4th instar nymphs or adults).

- Prepare appropriate test dilutions of technical grade active ingredients (AI) in 100% acetone or comparable solvent (test solvents beforehand to ensure that they are not toxic or harmful to the insects). 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 (5-100%) for a clear concentration response for the insecticide(s) being evaluated. Rates can be formulated as µg AI/µl to deliver the desired concentration per insect (µg/insect).

- Anesthetize the insects with CO_2 and apply a 1 µl drop on the dorsal pronotum of each insect with a microsyringe or micropipette.

- Place 5-10 treated individuals in each Petri dish. Each dish is considered one observation. Include at least four observations for each concentration of the insecticide. Prepare at least four additional observations for solvent controls.

- All dishes with treated insects should be labeled and arranged randomly and kept at 27±2ºC in an incubator with 60% relative humidity under dark conditions.

- Assess mortality between 3-7 days after infestation (test length may vary depending on the AIs being evaluated). Mortality can also be recorded daily to determine changes in mortality over time. For longer experiments, provide a food (dry dog food) and water source in each dish. Insects unable to right themselves when probed should be considered dead.

- Express results as a percentage mortality. Correct for 'untreated' (control) mortalities using Abbott's formula[1] (Abbott 1925). The corrected 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.
nCo = survivors in control.

- If mortality is greater than 20% for the solvent control treatment, the study should be considered as invalid. Mortality at the highest rate must be 100%, and at least three datapoints should have mortality >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 reuse 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 Corteva and Syngenta.