IRAC Susceptibility Test Methods Series

Method No: 12a

Version: 1

Details:

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Status: Approved

Species: Whiteflies, *Trialeurodes vaporariorum* and *Bemisia tabaci*

Species Stage: Adults

Product Class: Neonicotinoids, pymetrozine, pyrethroids, organophosphates and other whitefly adulticides

Photograph Courtesy of:
Central Science Laboratory, Harpenden
Archives, British Crown

Comments: This method is suitable for use with commercial formulations of insecticides. This method was developed by Rothamsted Research, UK and validated for endorsement as an IRAC approved method by BASF and Syngenta.

Description:

Materials:
Small (3-5cm diameter) Petri-dishes with gauze-covered ventilation holes in lids, sharpened metal tube for cutting leaf discs (diameter 2mm less than Petri-dish diameter), agar powder, aspirator for transferring whiteflies, carbon dioxide (cylinder), Glass flasks or disposable plastic cups (150-200ml) for serial dilutions of insecticide, syringes/pipettes for making dilutions, binocular microscope or hand lens, paper towels, maximum/minimum thermometer.

Method:

(a) Ventilate the Petri-dish lids with small holes with a diameter too small for the whiteflies to escape or glue gauze mesh over larger holes.

(b) Prepare agar by mixing 1% w/w agar powder with distilled water, heat until boiling and then allow to cool while constantly mixing. After cooling for approximately 10 minutes, pour the warm agar into the bases of the Petri dishes to a depth of 3-4 mm. NOTE: Different brands of agar powder may necessitate using a different concentration than 1%, so experiment first to determine the required agar to water ratio for the required level of gelling.

(c) Use the metal tube to cut leaf-discs from clean, untreated host plant leaves.
(d) Immerse the leaf discs into serial dilutions of formulated insecticide for 20 seconds (use of additional wetter is not recommended) and air dry on paper towels. Control discs are dipped in distilled water only.
(e) Prepare three replicates per concentration, including the control.
(f) Once the agar has cooled and set, lay a leaf disc adaxial (upper) surface down, into each Petri-dish.
(g) Place 15-20 healthy whiteflies using a small mouth or venturi operated aspirator onto each leaf disc. If necessary, lightly anaesthetize the whiteflies using carbon dioxide to facilitate transfer. Place the ventilated lid onto the Petri-dish base and ensure a close-fitting seal to prevent the whiteflies escaping.
(h) When the whiteflies have recovered from narcosis (if carbon dioxide is used), invert the dishes so that the leaf disc is abaxial (lower) surface down and the adults oriented normally.
(i) Hold the dishes under natural light or in a light cycling incubator at 20 ± 2ºC.
(j) Assess mortality at 48 hours after treatment using a binocular microscope or hand lens.
(k) Express results as percentage mortalities, correcting for "untreated" (control) mortalities using Abbott's formula. Untreated mortality should be recorded.

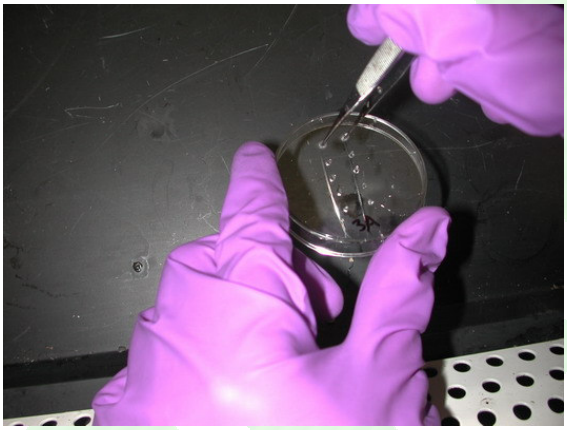

Figure 1. Preparing Petri dishes with ventilated lids

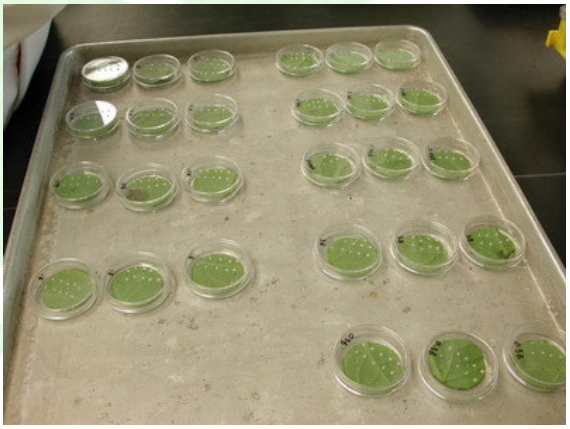

Figure 2. Bioassay in progress showing Petri dishes containing treated leaf disks and whiteflies

Precautions & Notes:

1. Where glass equipment is used it must be adequately cleaned with an appropriate organic solvent before re-use to prevent cross-contamination.

Example Data:

Results from field collected *Bemisia tabaci*
Totals of three replicates

Insecticide	Concentration (ppm)	No. Alive	No. Dead	% mortality	Corrected % mortality*
Control	0	62	2	3.1	
Imidacloprid	0.06	53	3	5.4	2.4
	0.1	57	8	12.3	9.5
	0.6	40	19	32.2	30.0
	3	7	60	89.6	89.3
Pymetrozine	0.3	45	6	11.8	9.0
	1	40	16	28.6	26.3
	3	31	20	39.2	37.3
	10	13	33	71.7	70.8

*Abbott's formula: Corrected % mortality = (% alive control - % alive treated) x 100% / (% alive control)

References & Acknowledgements:

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Figures 1 - 2 are courtesy of BASF.