IRAC Susceptibility Test Methods Series

Method No: 016

Version: 3 (June 2009)

Details:

Method:	No. 016 (Formally Method No 12c)	 *Photograph Courtesy of: Syngenta Crop Protection*
Status:	Approved	
Species:	Whiteflies, *Trialeurodes vaporariorum* and *Bemisia tabaci*	
Species Stage	Nymphs Eggs (If active has ovicidal activity).	
Product Class:	Neonicotinoids, pymetrozine, pyrethroids, organophosphates, buprofezin, pyriproxyfen and tetronic acid derivatives.	
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:
Ventilated insect holding cage (Approx. 50 x 50 x 50cm), potted host plants (plants must fit into holding cage and be of same age in all tests) , aspirator for transferring whiteflies, 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, Scissors

Method:
a) Host plant leaves are removed using scissors to until three fully expanded leaves remain (selected leaves should be of approximate same age). Each of these leaves is trimmed into a small rectangle 4 x 6cm approximately) and the plants placed within the holding cage.

b) Using aspirator adult whiteflies are collected and added at a rate of approximately 50-70 insects per leaf. The adult whiteflies are left for 24 hours to allow time for egg lay after which all the adults are removed.

The timing of the insecticide application depends on whether the target for the study is the nymph stage or the egg stage. (See table 1 for details)

TABLE 1: Time periods from adult removal until dipping

- *B. tabaci* eggs - 1 day
- *T. vaporariorum* eggs - 1 day
- *B. tabaci* larvae - 9 days
- *T. vaporariorum* larvae - 11 days

c) The required serial dilutions of insecticide solution are prepared in water using formulated insecticides (use of additional wetter is not recommended unless necessary). Approximately 100ml of insecticide solution is required for each solution. Water is used as a control solution

d) A single host plant is used for each concentration, with each of the three infested leaves counting as three replicates. Each infested leaf is independently fully emerged in the insecticide solution for a period of 20 seconds.

e) The potted host plants are returned to the holding cages and left for a further period until assessment (See table 2), assessments are based on specific criteria dependant on whether assessing larvicidal or ovicidal activity (see table 3 below). Holding cages are stored at 20^{o}C, 60% relative humidity and 16:8 hour lighting regime.

TABLE 2: Minimum time periods from adult removal until assessment*

- *B. tabaci* eggs - 10 days
- *T. vaporariorum* eggs - 12 days
- *B. tabaci* larvae - 16 days
- *T. vaporariorum* larvae - 20 days

*Timing may vary depending on whitefly biotype and development rate in different environmental conditions.

TABLE 3: Assessment criteria

- For larvicides, number of 2nd stage larvae (dead) and number of developing late-stage larvae (alive) are evaluated on the underside of the leaf to provide percentage mortality, which should be corrected for control mortality using Abbot's formula.

- For ovicides, total number of eggs and hatched larvae (alive) are evaluated on the underside of the leaf to provide percentage mortality, which should be corrected for control mortality using Abbot's formula.

IRAC Susceptibility Test Methods Series

Method No: 016

Version: 3 (June 2009)

Fig 1. Potted cotton plant which has been trimmed until only three leaf squares remain.

Fig 2. Cotton leaf which has been trimmed to an approximate 4x6 cm square.

Precautions & Notes:

1. If large numbers of eggs or nymphs (> 200) are present on the 4x6cm leaf square due to over infestation, randomly choose a smaller section of the leaf square for evaluation.
2. Where glass equipment is used it must be adequately cleaned with an appropriate organic solvent before re-use to prevent cross-contamination.
3. When handling chemicals and solvents, it is recommended appropriate protective equipment such as protective gloves; clothes and protective eye wear are worn.

Example Data:

Results from susceptible collected *Bemisia tabaci* nymphs
Mean of three replicates

Insecticide	Concentration (ppm)	No. Alive	No. Dead	% mortality	Corrected % mortality*
Control	0	125	6	4.6	
Imidacloprid	10	0	50	98.1	98.0
	3.33	8	35	77.4	76.3
	1.11	26	47	67.0	65.4
	0.37	39	27	36.0	32.9
	0.12	114	31	24.1	20.4
	0.04	97	7	6.1	1.6
Bifenthrin	5	19	60	81.2	80.3
	1.66	36	67	69.9	68.5
	0.55	35	24	37.8	34.8
	0.18	59	34	35.2	32.1
	0.06	67	8	10.3	6.0
	0.02	132	5	4.6	0.0

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

References & Acknowledgements:

Thanks to Ian Denholm and Kevin Gorman of Rothamsted Research for providing the method and for allowing publication by IRAC.

Figures 1 - 2 are courtesy of Syngenta Crop Protection.