

## IRAC Susceptibility Test Methods Series

Method No: 003

Version: 3 (June 2009)

### Details:

Method:	No: 003 (Formally Method No 3)	 <p>Photograph Courtesy of: Whitney Cranshaw, Colorado State University <i>Tetranychus spp</i></p>
Status:	Approved	
Species:	<i>Panonychus ulmi</i> <i>Tetranychus spp.</i>	
Species Stage	<i>P. ulmi</i> (summer eggs) <i>Tetranychus</i> (eggs)	
Product Class:	clofentezine hexythiazox tetradifon	
Comments: None		

### Description:

#### Materials:

Petri dishes (9-cm diameter), filter paper to fit Petri dishes, cotton wool, *untreated* apple or plum leaves, small scissors, small forceps, fine pointed brush or cocktail stick, beakers or glass jars (ca. 100-ml capacity) for test liquids, 1-ml disposable plastic syringes for liquids or weighing balance for solids, hand lens (minimum 10 x) or binocular microscope, maximum/minimum thermometer.

#### Methods:

- (a) Cut square sections about 1.5 x 1.5 cm from chemically untreated apple or plum leaves. Use young leaves, but not before they are fully expanded. Leaves must be in good condition. Use a minimum of four replicates (leaf sections) per treatment.
- (b) Place these sections, upper surface uppermost, on a sheet of moist filter paper on moist cotton wool in open Petri dishes.
- (c) Collect apple leaves with adult mites, and with the fine pointed brush or cocktail stick transfer 10 – 15 females onto each leaf section. Maintain at a minimum temperature of 20°C, minimum photoperiod 16 h and a high light intensity, but not in direct sunlight.
- (d) After 24 h, check that the female mites have laid eggs. Aim for at least 20 eggs per leaf section. If there are not enough eggs, leave for a further 24 h. Do not leave longer than 48 h.
- (e) When sufficient egg numbers have been obtained, remove the mites with the fine pointed brush or cocktail stick. Record the time when this is done.
- (f) Prepare appropriate test dilutions of formulations in water. The use of a wetter is not recommended.
- (g) Agitate test liquids and then dip the leaf sections for 5 secs. Dip equal number of control leaf sections in water only.
- (h) Record the number of eggs per leaf section.
- (i) Return leaf sections to Petri dishes and maintain in conditions described above. Record maximum and minimum temperatures. Moisten cotton wool daily.

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- (j) Using a hand lens or binocular microscope observe leaf sections daily until there has been complete (or nearly complete) hatch on the untreated (water only) leaf sections. Record numbers of un-hatched eggs on treated and untreated leaf sections.
- (k) Express results as percentage mortality and correct for untreated mortality using Abbott's formula. Untreated mortality should be recorded.

### **Precautions & Notes:**

If the lids are left off, the leaf sections may dry out and, unless the cotton wool can be moistened at least daily, the test may be invalidated by excessive control mortality. In such circumstances, the method may have to be modified to suit the local conditions, e.g. use lids with holes cut in them to reduce water loss without creating a condensation problem.

For *Tetranychus* spp. which live mainly on the lower leaf surface, the leaf sections may need to be placed lower surface uppermost. Leaves of kidney beans are particularly suitable.

### **References & Acknowledgements:**

None