IRAC Susceptibility Test Methods Series

Method No: 004

***Version: 3** (June 2009)*

Details:

Method:	No 004 (Previous Method No. 4a)	
Status:	Approved	
Species:	*Panonychus ulmi* *Tetranychus spp.* *Panonychus citri*	
Species Stage	Adult	
Product Class:	bromopropylate, cyhexatin, dicofol, formetanate and propargite	*Photograph Courtesy of:* *Whitney Cranshaw, Colorado State University* *Tetranychus spp*
Comments:		

Description:

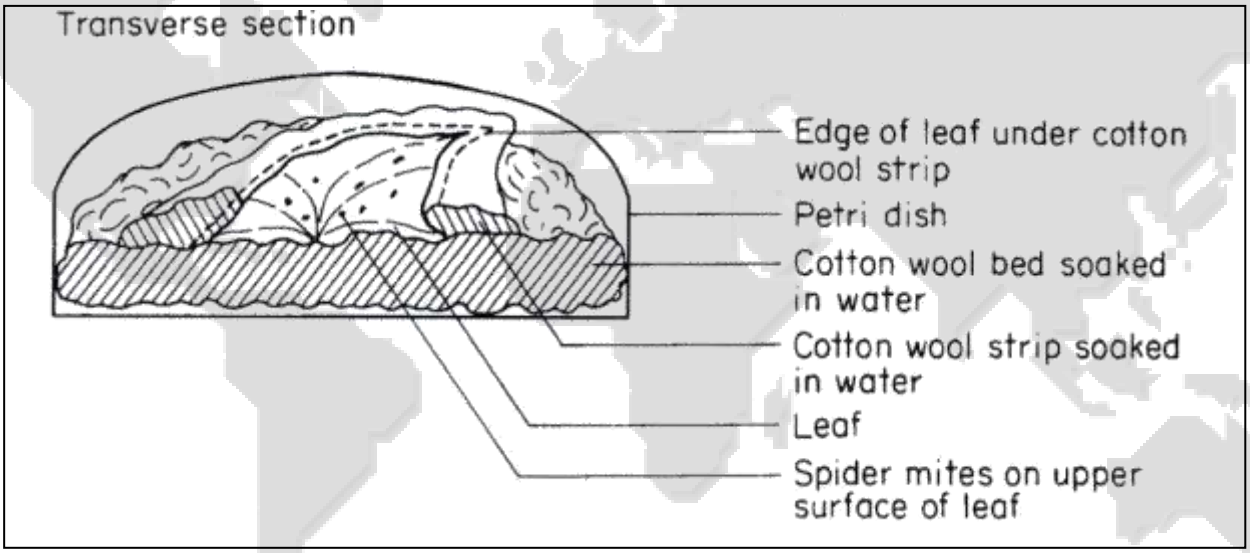
Materials:
Petri dishes (9-cm diameter), absorbent cotton wool, untreated leaves (for *Tetranychus* spp., leaves of kidney beans are particularly suitable; for *P. ulmi* use apple or plum leaves; for *P. citri* young leaves of sour orange, *Citrus aurantium*, or sweet orange, *Citrus sinensis*, may be used), scissors/guillotine for cutting cotton wool into strips for uniform dimensions, small forceps, fine sable brushes (to reduce risk of cross contamination, it is advisable to use separate brushes for manipulating mites on each set of treated leaves), beakers or glass jars (ca. 250-ml capacity) for test liquids, Agral or similar wetting agent, binocular microscope, equipment to prepare standard solutions, maximum/minimum thermometer.

Method:

(a) Collect adult mites from the field. A population of at least 10 adult mites per leaf is recommended.
(b) Collect a supply of untreated leaves. For *P. ulmi*, apple leaves may be used. However, use of leaves from related shrubs such as myrobalan (*Prunus cerasifera*) provide a more durable experimental substitute.
(c) Prepare test liquids. The use of a wetter is not recommended (see note).
(d) Agitate test liquids and dip individual leaves for 5 s. Allow surface liquid to dry from leaves before placing them in Petri dishes. Use a minimum of five replicates per treatment.
(e) Place a layer of cotton wool over the base of Petri dish. Add tap water to the point of saturation, but avoid build-up of standing water.
(f) Place treated leaves, top surface uppermost, on wet cotton wool base.
(g) Cut strips of cotton wool approximately 1 cm in width and soak in tap water. Lay cotton wool strips around the perimeter of each treated leaf, half over the leaf and half over the cotton wool bed. Place a small piece of damp cotton wool around the petiole of each leaf.

(h) Use a binocular microscope or hand lens to ensure that there are no gaps between the leaves and cotton wool strips. Remove any mites already on the leaves.
(i) Use a fine sable brush to place 10 adult female mites on the surface of each treated leaf.
(j) Maintain tests at 21 ± 3°C in a room where they are not exposed to direct sunlight and at 65-90% relative humidity. The Petri dishes must be left open.
(k) Using a hand lens or binocular microscope, assess mortality after 72 or 96 h depending on speed of action of the test compound. Use a fine sable brush to stimulate individual mites. If they are incapable of walking, record them as dead.
(l) Express results as percentage mortality and correct for untreated mortality using Abbott's formula. Untreated mortality should be recorded.

Diagram:

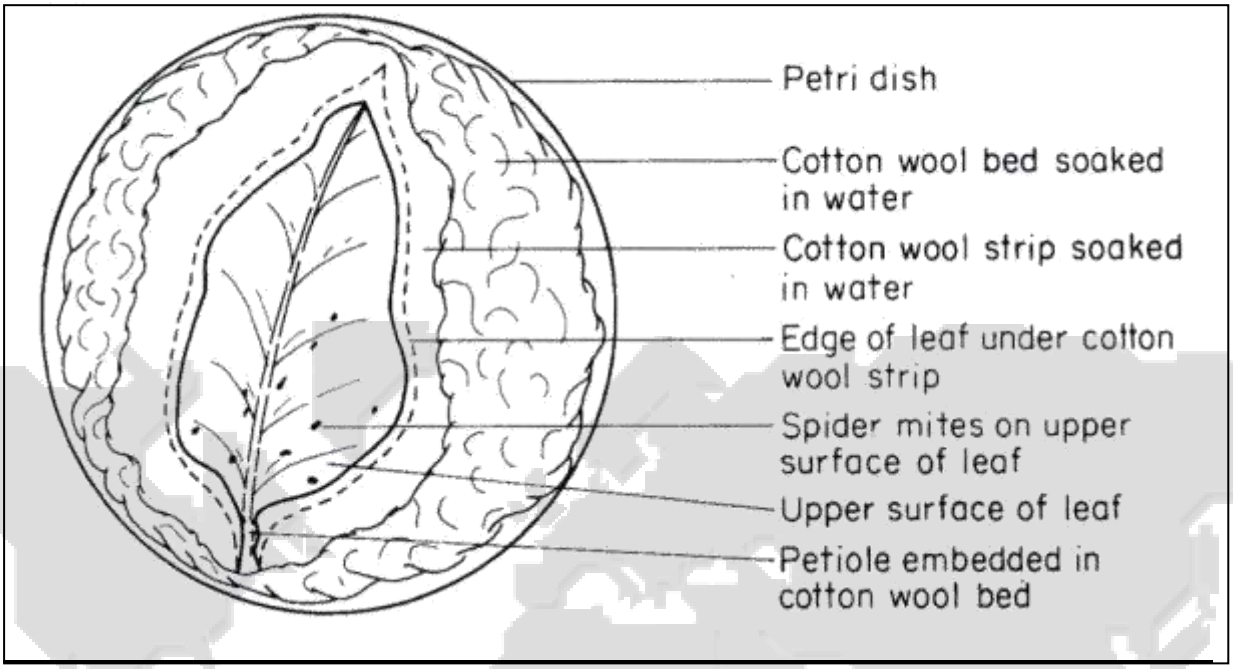


IRAC Susceptibility Test Methods Series

Method No: 004

Version: 3 *(June 2009)*

Plan



Percautions & Notes:

Notes

For *Tetranychus spp*. which lives mainly on the lower leaf surface, the leaves may need to be placed lower surface uppermost.

Since formulated compounds are used, addition of wetter is considered unnecessary, particularity in tests where a high discriminating dose is used for determining resistance. However, in special cases, e.g. where low doses are to be used for determinations of LD_{50} values and/or with citrus leaves, addition of a wetter may be necessary to achieve leaf wetting. In this case, the type and concentration of wetter should be noted and quoted with results. Escaped adults should be disregarded from calculation of percentage mortality.

References & Acknowledgements:

None