IRAC Susceptibility Test Methods Series

Method No: 007

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

Details:

Method:	No: 007 (Formally Method No. 7)	 *Heliothis larvae Photograph Courtesy of: Syngenta*
Status:	Approved	
Species:	Leaf-eating lepidoptera (including *Heliothis*, *Helicoverpa*) and Coleoptera on cotton, vegetable and field crops	
Species Stage	Larvae	
Product Class:	organophosphates, carbamates, pyrethroids, organochlorines and insect growth regulators	
Comments: None		

Description:

Materials:
Insect-proof containers, scissors, forceps, fine pointed brush, beakers for test liquids, syringes/pipettes for liquids or weighing balance for solids, syringes/pipettes for making dilutions, binocular microscope or hand lens, untreated leaves, paper towels, maximum/minimum thermometer.

Method:

(a) Collecta representative sample of insects from a field. These may be larvae suitable for immediate testing, or eggs/L1 larvae for rearing to the appropriate stage or material from which an F1 population for testing can be reared. The insects should not be subjected to temperature, humidity or starvation stress after collection.

(b) Collect sufficient non-infested, untreated leaves. Whole leaves are preferred or, for some crops, the distal portions. Do not allow leaves to wilt.

(c) Prepare accurate dilutions of the test compound from identified commercial product. For initial studies, five widely spaced rates are recommended. The use of additional wetter is only recommended for highly waxed leaf material, in which case this wetter solution is used for the "untreated" (control) solution in place of water alone.

(d) Dip leaves individually in the test liquid for 5 s with gentle agitation and place to surface-dry on paper towelling. Do not allow to wilt. Dip the same number of leaves per treatment, and treat sufficient leaf material to avoid starvation stress in the "untreated" during the test. Commence dipping the "untreated" first and work up through the test dilutions.

(e) Place the treated surface-dry leaves in the labelled test containers, which must be suitable for holding enough leaf material in good condition for up to 3 days.

(f) Add equal numbers of neonate larvae (*Heliothis/Helicoverpa*) or recently molted L2 larvae to each container, so that a minimum total of 40 larvae are used per treatment, divided between at least four replicate containers. It only one leaf surface is accessible to the larvae, ensure that this is the correct one for the species involved. If cannibalism is a problem (e.g. *Heliothis* and *Helicoverpa* spp.), reduce the number or larvae per container, but increase the replication.

(g) Store the containers in an area where they are not exposed to direct sunlight or extremes of temperature. Record maximum and minimum temperatures. If possible a mean temperature of 25°C is preferred.

(h) In the case of rapidly acting compounds, a final assessment of larval mortalities[2] is made after 48 h. For slowly acting compounds (e.g. benzoylureas, insect growth regulators etc.) a first assessment is made at 72 h, when the leaves are changed for fresh leaves treated, as before, with the appropriate insecticide dilution. The containers are held for a further period before the final assessment, either for 72 h or until larvae in the "untreated" (control) have moulted again.

(i) Express results as percentage mortalities, correcting for "untreated" (control) mortalities using Abbott's formula. Untreated mortality should be recorded.

Percautions & Notes:

1. Disposable plastic equipment is preferred provided that it is not affected by the formulation constituents; glass equipment may be used but must be adequately cleaned with an appropriate organic solvent before re-use.

2. At each assessment, larvae are classed as either: (a) unaffected, giving a normal response (such as taking a coordinated step) when gently stimulated by touch, or (b) dead or affected, the latter giving an abnormal response to stimulation or showing abnormal growth which should be described. For some benzoylureas, colour changes are reliable indicators of effectiveness and should be recorded. Thus, % response (‚mortality') will include both dead and affected.

References & Acknowledgements:

None