IRAC Susceptibility Test Methods Series

Method No: 022

Version: 3

Details:

Method:	No: IRAC No. 022
Status:	Approved
Species:	*Tuta absoluta*
Species Stage	Larvae L2 (size: 4-5 mm)
Product Class:	Oxadiazins (IRAC MoA 22), anthranilic diamides (IRAC MoA 28), spinosyns (IRAC MoA 5)

Tuta absoluta larvae
Photograph Courtesy of: DuPont Crop Protection

Comments:
In order to obtain homogeneous *Tuta absoluta* larvae (same age, nutritional and general health condition), it is highly recommended that insects collected from the field (F_0 generation) are brought to a laboratory and reared to the F1 generation for evaluation of insecticide susceptibility.

Objectives:

Susceptibility Baseline: ☒ Resistance Monitoring: ☒

Description:

Materials:
Insect-proof containers, scissors, fine forceps, fine pointed brush, seeking pin, beakers and syringes / micropipettes for test liquids (solutions and EC formulations), accurate balance for solids and SC liquid formulations, syringes/pipettes/micropipettes for making dilutions, binocular microscope or hand lens, wire net or paper towels, 10-15 cm^2 cell plates with sealable lid*, filter papers, protective gloves, maximum/minimum thermometer, untreated tender/ young tomato leaflets.
Optional: a light box (glass surface table with a fluorescent light source underneath).

* Suggested model: Bio-Serv, Rearing tray white ref: RT32W and Bio-assay Tray Lid-4 cells ref: RTCV4

Methods:
This method is a leaf-dip bioassay to be performed preferably with F1 L2 larvae (4-5mm in size):

a) Collect a representative sample of insects from a field. These may be larvae suitable for immediate testing (least preferred as these larvae may be contaminated from unknown previous field treatments or otherwise parasitized, etc.) or individuals (larvae/ pupae/ eggs) to be reared to second instar larvae F_1 generation (preferred, homogeneous cohort). The insects should not be subjected to temperature, humidity or starvation stress after collection. In order to obtain a representative sample of insects from any given field, ideally a minimum of 100 larvae or pupae should be collected from each field to be tested, in order to establish a colony of at least 50 adults. The collection of late stage larvae (e.g. 4^{th} instar) is recommended because they will require less plant material to develop, and will have shorter rearing time in the lab, and moth emergence will be synchronous. These moths are then reared to obtain enough L2 larvae for the bioassays.

b) Collect sufficient non-infested, untreated tomato leaves. Although the test will be done using single leaflets, it is preferable to collect entire leaves uniform in size. Tender young whole leaves are preferred. Do not allow leaves to wilt by keeping them in a moist environment (sealed plastic bag).

c) Prepare accurate dilutions of the test compound from the identified commercial product. For initial studies, six widely spaced rates are recommended. The use of a wetter/spreader (non ionic adjuvant) is highly recommended in order to obtain optimal leaf coverage. The adjuvant solution should be used for the "untreated" control solution in place of water alone. As the addition of a wetting agent can significantly affect the performance of an insecticide product in a bioassay, it is essential that details of the wetting agent and concentration used are recorded with any summary data and that only data generated with the same type of wetting agent and concentration are compared for susceptibility measurements.

d) Dip leaflets individually in the test liquid for 3 seconds with gentle agitation, ensuring the entire surface is emerged equally. Then dry the treated leaflets on a wire net with upper leaf surface (abaxial surface) facing skywards, or on paper towels (least preferred). Do not allow the leaflets to wilt. Dip the same number of leaflets per treatment (dose) and treat sufficient leaf material to avoid starvation stress in the "untreated" during the test. Do the same procedure for all the doses, starting with the "untreated" control (wetter solution), then followed by the more diluted dose and advancing progressively to the higher concentrations.

e) Prior to placing the leaflets in the bioassay cell units, place a slightly moistened filter paper covering the bottom of each cell. Around 0.2 ml distilled water should be sufficient to moist the filter paper and keep the leaf material turgid throughout the bioassay period. Excess water or water drops need to be removed.

f) When the surface of the leaflets is completely dry, place the leaflets in the labeled containers (one leaflet per cell unit), which must be suitable for holding enough leaf material in good condition for 3 days.
Note. Tomato leaves are quite fragile and sensitive. Maintaining the tomato leaflets intact – avoiding cutting them into measured pieces - helps keep the leaflets in good conditions for the period of the bioassay.

g) Begin the transfer of L2 larvae to the bioassay cell units, using a fine soft brush and taking extreme care not to damage the very fragile larvae. The following method is recommended in order to minimize larvae mortality due to handling: place leaves infested with L2 larvae on a light box (glass surface table with a fluorescent light source underneath), so that larvae can be clearly viewed through the leaf epidermis. The larvae can be easily located by forcing them to move with softly touching the leaf surface using a fine paintbrush. Once an L2 larva is detected, a small leaf square is cut around it with a sharp scalpel. The leaf square (with the larva) is lifted with a brush or fine forceps and is placed on the tomato leaf in the bioassay tray. In a few minutes the larva will start looking for food on the fresh

tomato leaf provided in the bioassay tray.
Start the infestation process with the untreated control cell units (1 larva per cell) and then continue by ascending order of concentration of insecticide. Avoid cross contaminations, e.g. brush touching treated leaflets (in case it happens, immediately wash the brush well, before continuing the infestation). Each dose should have at least 32 larvae (32 cell units or one tray if using suggested model from Bio-Serv*).
Note. As developmental time can vary between populations and slight differences in rearing / environmental conditions, the following length measurement can be used to classify L2 larvae: 4-5mm.

h) When the infestation is finished, close the trays carefully, sealing the cells with their lids (each lid closes 4 cells, if using suggested model from Bio-Serv*).

i) Store the bioassay trays in an area where they are not exposed to direct sunlight or extreme temperatures. Record maximum and minimum temperatures. If possible, maintain a temperature of 25 ± 2°C, 60-70% RH, and 16:8 light:dark photoperiod regime.

j) Perform evaluations 72 hours after placing the larvae in the trays:
Evaluation of the effects on the larvae: Larvae which are unable to make coordinated movement from gentle stimulus with a seeking pin or fine pointed forceps to the posterior body segment are to be considered as dead (combination of dead and seriously affected).
Anti-feeding effects (percentage damage to the leaf or larval growth) may also be recorded for additional information.
Evaluation of leaf damage: Since uniform leaves were chosen at the beginning of the assay registering leaf damage as % of total leaf area mined is the preferred method.

Express effects on larvae as percentage "affected", correcting for untreated control (wetter solution) mortality using Abbott's formula. It is recommended that the mortality data is utilized to perform a probit or logit dose-response analysis to provide LC_{50} and LC_{90} estimates for each insecticide and insect population tested. If the % of "affected" larvae in the untreated control is above 20%, the bioassay is considered to be of inferior quality and should be repeated. Ideally, control mortality should not exceed 10-15%.

Precautions & 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. Insecticide products contain varied concentrations of active ingredient(s). Ensure insecticide dilutions are based on active ingredient content (g a.i.). Some diamide insecticides are sold as pre-mixtures with other insecticides, these products should not be used to determine the susceptibility of insect populations to the single insecticide, as the mixture partner may have a significant impact on the mortality data generated.

References & Acknowledgements:

This IRAC method is based on a method developed by DuPont Crop Protection in Brazil. The method has been validated by several researchers in Europe: Dr. T. Cabello (University of Almeria, Spain), Dr. P. Bielza (University of Cartagena, Spain), Dr. E. Roditakis (NAGREF, Greece) and Pr. C. Rapisarda (University of Catania, Italy).