IRAC Susceptibility Test Methods Series

Method No: 005

Version: 4 (December 2012)

Details:

Method:	No 005
Status:	Approved
Species:	*Nilaparvata lugens* *Nephotettix cincticeps*
Species Stage	Stage 5 nymphs and adults Stage 3-4 nymphs for insect growth regulator MoAs
Product Class:	Suitable for all insecticides

Nilaparvata lugens

Comments:
Method evaluation varies with mode of action, refer to methods point f) for guidelines.

Field populations of hoppers may be collected by hand or by suction device and kept in holding cages containing potted rice plants. Insects should be collected at random from several points in a field and from a few fields in an area, then pooled together as parent stock.

Objectives:

Susceptibility Baseline: ☒ Resistance Monitoring: ☒

Description:

Materials:

- Transparent plastic or glass tubes, or suitable glass jars for holding treated plants
- Bacteriological agar (e.g. DIFCO no. 1, though other brands will be suitable)
- Containers for preparation of insecticide dilutions
- 30-50ml plastic syringes
- 100-1000µl micro-pipettes for liquids or microbalance for solids
- Extravon (Invadin) or a similar non-ionic wetting agent.
- Paper towels.
- Maximum/minimum thermometer.
- Containers and suction device (mouth aspirator) for collecting insects.
- Untreated rice seedlings (BPH susceptible cultivar) 10-12 days old in pots

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a) Prepare agar according to manufacturer's instructions and allow it to cool but not solidify. As soon as it is lukewarm (approx. 37°C) pour enough agar into each pot of rice to cover the soil surface. Note that if the agar is too hot it will run straight through the pot. The cool, but still liquid agar, will stay on the soil surface and quickly solidify. The agar helps to prevent soil from falling out of the pots when the plants are dipped into the insecticide solution and also makes it easier to find dead or affected hoppers during assessment.

b) Make test solutions in water containing 0.03% w/v Extravon (or similar non-ionic wetter) using formulated insecticide. At least five to six concentrations are required. A series of concentrations should be chosen to give a range of mortality for a clear dose response. The volume of each test concentration needs to be large enough to allow the whole plants to be dipped; 300-400ml per solution is recommended.

c) Invert the pots and dip seedlings completely into the test solutions for 10 seconds. It is very important that the seedlings are dipped all the way into the solution to ensure that all exposed plant parts are in contact with the insecticide. After dipping, revert the pots and allow the seedlings to dry, ensuring that the replicates do not touch during this time. Drying normally takes 10-15 minutes depending on the ambient relative humidity. When the plants are dry, place them into individual plastic or glass tubes.

d) Collect suitable hoppers from the holding cage using a suction device. Ensure that only one target life stage is used per test, do not mix life stages or short winged and long winged forms in one test. For insect growth regulators use 3rd to 4th instar larvae; for other mode of actions (MoAs) use 5th instar or adult insects. Note which life stage is used for the test.

e) Infest 10-15 insects per tube. There should be at least three replicate tubes per insecticide concentration.

f) Different MoAs require assessment at different time points. Chose the appropriate assessment timing from the list below:

Carbamates (1A)	24 and 48h
Organophosphates (1B)	24 and 48h
Neonicotinoids (4A)	3 days (72h)
Tetramic/tetronic acids (23)	5 days (120h)
Chitin biosynthesis inhibitor (16)	5 days and 10 days
Selective homopteran feeding blockers (9B)	7 days and 18 days

g) Count and record numbers of live and dead insects. Insects that fall onto their backs and cannot recover a normal posture should be counted as dead.

h) Untreated mortality should be recorded. Express results as percentage mortality and correct against untreated mortality using Abbott's formula.

i) Discard data if control mortality is more than 20%.
j) Record test temperature, which should be between 25 and 30°C.
k) The Group 9B insecticides, selective homopteran feeding blockers are slow acting and in the lab have little to no significant direct effect on adults. Plants should be treated and infested as outlined in points a) to e). Assess the adult mortality seven days after treatment and infestation and then remove the adults from the test plants. Keep the treated plants in the test holding conditions for another eleven days, then count and record the number of newly emerged nymphs. Calculate the average nymph emergence for each treatment. LC50 and LC90 values cannot be calculated in this case because the assessment criterion is not mortality. However the response of different populations to the range of Group 9B insecticide doses applied can be compared.

References & Acknowledgements:

A video of the full method is available on the IRAC website and can be viewed via the IRAC Method Team page (http://www.irac-online.org/teams/methods/) or directly on YouTube via the link http://www.youtube.com/watch?v=Pazc28TzHhM

Figures:

Fig. 1: Plant pots with agar poured over the soil surface

Fig. 2: Plants are dipped fully in test solutions for 10 seconds. It is important that all of the exposed plant material is dipped. There should be at least three replicate per insecticide concentration.

Fig. 3: Treated plants are infested with 10-15 insects per tube ensuring that only one target life stage is used per test.