

Standard Operating Procedure (SOP)

Insect Growth Regulator Larvicides

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1. Purpose

This procedure is to ensure that the biological activity of larvicides other than bacterial products and conventional insecticides is conducted systematically.

2. Scope

Testing methods for the juvenile hormone (JH) analogues (juvenoids) and the chitin synthesis inhibitors differ.

- a) JH analogues interfere with the transformation of late instar larvae to pupae and then to adult, whereas chitin synthesis inhibitors inhibit cuticle formation and affect all instars and immature stages of the mosquito.
- b) The delayed action of Insect Growth Regulators (IGR_s) on treated larvae means that mortality is assessed every other day or every three days until the completion of adult emergence.
- c) The effect of both types of IGR on mosquito larvae is expressed in terms of the percentage of larvae that do not develop into successfully emerging adults, or adult emergence inhibition (IE %).

3. Definitions

- a) Juvenile hormone: A central regulator of insect post-embryonic development and life history traits. JH are secreted by a pair of endocrine glands behind the brain called the *corpora allata*. JHs are important for the production of eggs in female insects.
- b) Chitin synthesis inhibitors: Chitin synthesis inhibitors work by preventing the formation of chitin, a carbohydrate needed to form the insect's exoskeleton. The inhibitors prevent the new exoskeleton from forming properly, causing the insect to die. Chitin synthesis inhibitors can kill eggs by disrupting normal embryonic development.
- c) Moribund: Pertaining to test larvae that show signs of life but are incapable of locomotion.

4. Abbreviations

- a. IGR – Insect Growth Regulator
- b. IE – Inhibition Emergence
- c. ml – millilitre
- d. mg – milligram
- e. LC – lethal concentration

5. Procedure

a. Preparation of stock solutions or suspensions and test concentrations

- i. The preparation of test solutions or suspensions and bioassay set-ups are the same as for fast acting compounds. Technical materials are generally soluble in organic solvents and stock solution (1%) should be made by dissolving 200 mg in 20 ml. Formulated materials should be diluted with water and serial dilutions made in the same manner.

b. Bioassays

- i. Use third instar larvae for testing JH analogues and chitin synthesis inhibitors. The accurate initial count of larvae is essential because of the cannibalistic or scavenging behaviour of larvae during the long exposure period.

- ii. Provide a small amount of food (finely ground yeast extract, rabbit pellets, or ground fish or mouse food) at a concentration of 10mg/l at two-day intervals until mortality counts are made. Suspend the food particles in water and one or two drops added per cup. The larvae in the control are fed in the same manner as those in the treated batches. Cover the test and control cups with netting to prevent successfully emerged adults from escaping into the environment.
- iii. Count mortality or survival every other day or every three days (depending on protocol) until the complete emergence of adults. Hold the test containers at 25-28 °C and for a photoperiod of 12L: 12D.
- iv. At the end of observation period, the impact is expressed as IE% based on the number of larvae that do not develop successfully into viable adults. In recording IE% for each concentration, moribund, and dead larvae and pupae, as well as adult mosquitoes not completely separated from the pupal cases, are considered as “affected”.
- v. End the experiment when all the larvae or pupae in the controls have died or emerged as adults. Record any deformities or morphogenetic effects that occur in either the molting immature mosquitoes or the emerging adults.

6. Data Analysis

- a. Combine the data from all replicates of each concentration.
- b. Total or mean emergence inhibition can be calculated on the basis of the number of third stage larvae exposed. The overall emergence of adults reflects activity. IE% is calculated using the following formula :

$$IE (\%) = 100 \left[\frac{T \times 100}{C} \right],$$

Where T = percentage survival or emergence in treated batches and C = percentage survival or emergence in the control.

- c. If adult emergence in the control is less than 80 %, the test should be discarded and repeated. Where the percentage is between 80 % and 95 %, the data are corrected using Abbott’s formula :

$$Mortality (\%) = \frac{X - Y}{X} \times 100$$

Where X = percentage survival in the untreated control and Y = percentage survival in the treated sample.

- d. IE values obtained from multiple concentrations should be subjected to probit regression analysis to determine IE_{50} and IE_{90} values (using computer software programmes or estimated from log-probit paper). Preferably, 6 concentrations within the 10-90 % mortality are included the bioassay to allow for probit analysis. The data analysis procedures below should be as follows:
 - a) LC_{50} and LC_{90} values are calculated from a log dosage-probit mortality regression line using computer software programmes, or estimated using log-probit paper.

- b) Bioassays should be repeated at least three times at each selected concentration, using new solutions or suspensions and different batches of larvae each time. Standard deviation or confidence intervals of the means of LC₅₀ values are calculated and recorded.

- c) A test series is valid if the relative standard deviation (or coefficient of variation) is less than 25 % or if confidence limits of LC₅₀ overlap (significant level at $P < 0.05$). The potency of the chemical against the larvae of a particular vector and strain can then be compared with the LC₅₀ or LC₉₀ values of other insecticides.