SOP: Insect Growth Regulator Larvicides

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Timeline

Version	Date	Reviewed by	Institution
1	12/07/23	Katherine Gleave	LSTM, I2I
2			

Version Control[1]

Version	Date	Updated by	Description of update(s)
Annabel Murphy	04/07/23	Annabel Murphy	Updated: Format and structure under sub-headings and footnotes. Added

[1] Historical versions of SOPs can be found on the I2I website (https://innovationtoimpact.org/)

			glossary of terms and references.

Related documents

- I2I Best Practice SOP Library, 30 October 2020 (https://innovationtoimpact.org/)
- Bacterial Larvicide Bioassay, I2I-SOP-026
- The Performance of Larval Insecticide Bioassays LITSOP001.03
- Field Evaluation of Microbial Mosquito Larvicide Efficacy, I2I-SOP-027

1. Purpose

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

2. Background

Testing methods for the juvenile hormone (JH) analogues (juvenoids) and the chitin synthesis inhibitors differ. The JH is 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. Chitin synthesis inhibitors work by preventing the formation of chitin, a carbohydrate needed to from 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.

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. The delayed action of Insect Growth Regulators (IGRs) on treated larvae means that mortality is assessed every other day or every three days until the completion of adult emergence. 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.

3. Materials and equipment

- Finely ground yeast extract/rabbit pellets/ground fish food
- Fine mesh netting (mesh size small enough to prevent larvae from escaping)
- Small plastic disposable pipettes for transferring larvae
- Third instar lab reared larvae
- Milligram balance for weighing
- Flasks and transfer pipettes for serial dilutions
- Personal Protective Equipment, including nitrile gloves
- Small, sealable containers for transport of test species
- Cups

4. Procedure

4.1. Preparation of stock solution/suspensions and test concentrations

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.

4.2. Bioassays

- 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.

- 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.

- 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 ºC and for a photoperiod of 12 hours light/12 hours dark.

- 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."

- End the experiment when all the larvae or pupae in the controls have died or emerged as adults.

4.3. Data analysis

- Combine the data from all replicates of each concentration.

- 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.[2]

[2] IE% is calculated using the following formula (where T=percentage survival or emergence in treated batches and C=percentage survival or emergence in the control): IE (%)= 100 * (T x100/ 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.[3]

- IE values obtained from multiple concentrations should be subjected to probit regression analysis to determine IE_{50} and IE_{90} values (using computer software programs 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[4]:

    - 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 LC_{50} values are calculated and recorded.

    - A test series is valid if the relative standard deviation (or coefficient of variation) is less than 25 % or if confidence limits of LC_{50} 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 LC_{50} or LC_{90} values of other insecticides.

5. Additional data collection

[3] Abbot's formula: Adjusted mortality (%)= 100 x (X-Y) / (100-Y), where X is the percentage mortality and Y is the percentage mortality with the untreated control sample.

[4] LC_{50} and LC_{90} values are calculated from a log dosage-probit mortality regression line using computer software programs, or estimated using log-probit paper.

Record any deformities or morphogenetic effects that occur in either the molting immature mosquitoes or the emerging adults.

6. Deviations from standard protocol

7. Glossary of terms

IE	Adult emergence inhibition
IGR	Insect Growth Regulator
JH	Juvenile Hormone
LC	Lethal Concentration
LC_{50}	Lethal Concentration 50%
LC_{90}	Lethal Concentration 90%
mg	Milligram
mg/mL	Milligram per millilitre
ml	Milliltre
SOP	Standard Operating Procedure

8. References

Abbott, W.S. (1987). A method of computing the effectiveness of an insecticide. *Journalof the American Mosquito Control Association*, *3* (2), 302-303.