SOP: Performing Larval Insecticide Bioassays and Larval Susceptibility Testing

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Prepared by

Name	Role	Institution
Annabel Murphy	Contributor	LSTM, I2I
James Court	Contributor	LITE, LSTM

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Related documents

- I2I Best Practice SOP Library, February 2024 (https://innovationtoimpact.org/resources/)
- The Performance of Larval Insecticide Bioassays and Selections LITSOP011
- Methods for Performing Calculations and Dilutions I2I-SOP-043

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1. Purpose

To provide guidelines for the performance of larval insecticide bioassays. This standard operating procedure (SOP) provides instructions on the performance of susceptibility testing.

2. Background

Larviciding is an effective tool, either alone or alongside other control interventions (such as indoor residual spraying and long-lasting insecticide nets) as part of an insecticide resistance management strategy. It works by targeting the immature (larval and pupal) stages of the mosquito vector, reducing the quantity that survive to adulthood thus reducing the transmission of vector borne diseases.

Larval insecticide bioassays expose 1^{st}-4^{th} instar laboratory-reared mosquito larvae to larvicide treated water at concentrations within the larvicide's activity range. Mortality is recorded at 24 hours (or longer depending on the study protocol) post exposure. The data collected can in turn be used for, (but is not limited to) the following: exploring insecticide resistance mechanisms in the mosquito, determining diagnostic concentrations, and identifying the lethal concentrations that induce 50% and 90% mortality (LC_{50} and LC_{90}) or 50% and 90% inhibition of adult emergence (IE_{50} and IE_{90}), and calculating resistance ratios between strains.

3. Materials and equipment

- Disposable deli pots
- Disposable plastic trays with lid
- Pasteur pipettes
- 10mL pipette and tips
- 1000µl pipette and tips
- 200µl pipette and tips
- 10µl pipette and tips
- 1^{st}-4^{th} instar larvae from mosquito colony to be tested (100 for each test and 25 for each control)
- Insecticide to be tested
- Deionised water
- Elastic bands
- Cotton wool
- Metal sieve
- Glass bottles with lids for chemical waste disposal
- Measuring spoons (for example Drop, Smidgen, Pinch, Dash and Tad set by Norpro, for feeding
- Funnel (200mL)
- Gloves, lab coat, protective glasses
- Permanent markers
- Fine mesh strainer

4. Procedure

Dilutions

4.1. Put on a lab coat, gloves and protective glasses. Work in a fume cupboard if necessary, dependent upon the chemical (refer to material safety data sheet (MSDS).
4.2. Refer to I2I-SOP-043 'Methods for Performing Calculations and Dilutions' to prepare the solutions and place each dose in a labeled amber glass container (some compounds can be sensitive to light, refer to (MSDS) with a chemically resistant lid to reduce evaporative loss.

Addition of Insecticide and Control Material

Run one replicate of a negative and positive control alongside each test. The negative control is the solvent used in preparation of the insecticide dilutions. The appropriate positive control is dependent on the study protocol.

Using permanent marker label disposable deli pots to be used for the bioassay with the the following: date of testing, mosquito colony, insecticide, concentration and replicate number.

4.3. To each deli pot, add 199mL deionised water. Do this by adding 200mL of water using a 200mL funnel and remove 1mL of water using a 1000µl pipette.
4.4. To the negative control, add 1mL using a 1000µl pipette acetone only to give a total volume of 200mL.
4.5. Refer to the dilutions in Table 1. In the exposure pots, using a 1000µl pipette add 1mL of the pre-diluted insecticide. To avoid contamination start with the lowest concentration working towards the highest using a different pipette tip for each insecticide and control. Once control/exposure material has been added, gently stir the water to ensure an even distribution.
4.6. If using a stock concentration to make multiple concentrations of the same insecticide, top-up each deli pot with acetone if required so that the final volume is 200mL (see Table 1).
4.7. To the positive control, using a 1000µl pipette add 1mL of the appropriate pre-diluted insecticide to give a total of 200mL.
4.8. Record the time insecticide or control material is added to the last test and wait a minimum of 1 hour to ensure all acetone/solvent material has evaporated.

Table 1: Examples of different insecticide concentrations, diluted with the appropriate amounts of pre-diluted insecticide and acetone.

Amount of deionised water in each deli pot	Dilution	Acetone to add	Total end volume per deli pot
199mL	1,000µl (Highest concentration) (1,000µl pipette)	0µl	200mL
199mL	100µl (100µl pipette)	900µl	200mL
199mL	10µl (10µl pipette)	990µl	200mL
199mL	1µl (1µl pipette)	999µl	200mL
199mL	0.1µl (Lowest) (1µl pipette)	999.9µl	200mL

Addition of Mosquito Larvae

4.9. Using a clean Pasteur pipette, count 25 larvae (both male and female are acceptable) into a clean petri dish. Use larvae of 1st-4th instar dependent on study protocol.[2] This is one replicate; 4 replicates are recommended per insecticide/strain combination.

4.10. Remove excess water by holding the tip of the pipette flat againt the bottom of the petri dish, leaving just enough water to be able to transfer the larvae into the deli pots.

4.11. Pipette the larvae into each of the exposure or control pots-this is the exposure start time.

4.12. Secure the top of the pots with a piece of netting held in place with an elastic band (see Figure 1).

[2] Larvae in the 4th stage of development are used by taking larvae 1 day before the first pupae are expected from the colony being tested.

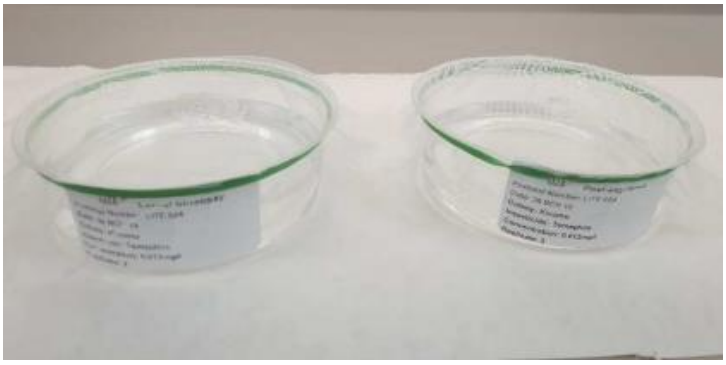

Figure 1: Example of disposable deli pots with a piece of netting secured with an elastic band. Figure taken from LITSOP011.

Feeding mosquito larvae and larval scoring

4.13. Feed each test a standard amount of diet every 24 hours. For example, a level measure from the 'drop' measuring spoon equates to 20mg of ground Tetramin fish flakes.

4.14. Count larval mortality in the exposure/control pots (or transfer to a petri dish using a Pasteur pipette to make scoring easier). Remove dead larvae and place in a deli pot with 5% sodium hypochlorite and disposed of.To make up 5% sodium hypochlitie use a measuring cylinder to add 25mL of sodium hypochlorite to 475mL of water

4.15. Using a fine mesh strainer, separate any dead larvae from the sodium hypochlorite solution. Dead larvae should be disposed of as biological waste. Dispose of the sodium hypochlorite solution as chemical waste.

4.16. Record larval mortality. Dead larvae are those that cannot be induced to move when probed with a pipette. Moribund larvae are incapable of rising to the surface or diving when the water is disturbed. Moribund larvae should be recorded and included in percentage mortality calculations. Larvae which have pupated during the test should be discarded and recorded as survivors.

End of the Exposure Period

4.17. Set up a second set of deli pots labelled with the following: date of testing, mosquito colony, insecticide, concentration and replicate.

4.18. Add 200mL clean deionised water to each deli pot.

4.19. After the exposure period, transfer the larvae to the corresponding post-exposure pots using a metal sieve or aquarium fish net. To avoid contamination of insecticides, start with the lowest concentration working towards the highest, and use a different sieve/net for each insecticide and control.

4.20. Use a glass bottle and funnel to collect the exposure water and dispose of waste liquid as chemical/biological waste.Leave larvae to recover for a minimum of 1

hour and score final mortality. Score mortality within 2 hours of a scoring time point, including the recovery period.

4.21. Pour larvae from 'post exposure' pots into a deli pot with 5% sodium hypochlorite to kill them. Seperate the dead larvae from the sodium hypochlorite using a fine mesh sieve and dispose of the dead larvae as biological waste and the sodium hypochlorite as chemical waste.

Results and Data Interpretation

4.22. Present results for each 'test group' as percentage mortality at specified time points as per the study protocol.

4.23. If control mortality is greater than 20%, or more than 10% have pupated in any control 'test', reject and repeat the entire 'test group' to which is belongs.

4.24. Reject a 'test' where more than 10% of the larvae have pupated, and there are <20 or >30 larvae.

4.25. If control mortality for a 'test group' is between 5-20% apply Abbot's foumula.[3]

5. Additional data collection

Record the exposure start time of testing, the humidity and temperature at the start of testing and subsequent recording time points.

6. Deviations from standard protocol

All deviations from the standard protocol should be noted in data collection sheets. See Appendix 1 for data collection sheet.

7. Glossary of terms

- IE_{50} Inhibition of adult emergence 50%
- IE_{90} Inhibition of adult emergence 90%
- IRS Indoor Residual Spraying
- LC_{50} Lethal concentration of larvicide for 50% mortality
- LC_{90} Lethal concentration of larvicide for 90% mortality
- LITE Liverpool Insect Testing Establishment
- LLIN Long Lasting Insecticidal Net
- MSDS Material Safety Data Sheet
- Mg Milligram
- mL Millilitre
- PPE Personal protective equipment

[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.

8. References

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

Record sheet templates

Larval Bioassay Record Sheet

Test Date:				
Colony:				
Age of mosquito larvae:				
Time finished adding insecticide/control material:				
Environmental conditions:	**Exposure start** **Temperature (°C):** **Humidity (RH%):**	**24 hours** **Temperature (°C):** **Humidity (RH%):**	**48 hours** **Temperature (°C):** **Humidity (RH%):**	**72 hours** **Temperature (°C):** **Humidity (RH%):**

Row	Insecticide	Concentration	Exposure start time	Exposure end/recovery start time	Recovery end time	24 hour time point (Scoring)	48 hour time point (Scoring)	72 hour time point (Scoring)	Total larvae number

1									
2									
3									
4									
5									
6									
7									
8									
9									
10									