I2I Landscaping report: Lab efficacy testing of larvicides-Insect growth regulators.

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Acronym List

I2I	Innovation to Impact
IE	Emergence inhibition
IGR	Insect growth regulator
LITE	Liverpool Insect Testing Establishment
LSM	Larval source management
mL	Mililiters
SOP	Standard Operating Procedure
WHO	World Health Organization

Summary

Aim and key questions addressed	- To evaluate the biological activity of Insect growth regulators used in larviciding
Context	- Laboratory
Test item	- Mosquito larvae
Mosquito population	- Field mosquitoes or laboratory reared
Number of mosquitoes per replicate	- 25
Endpoints measured	- Mortality and emergence inhibition IE%
Exposure time	- Until adult emergence
Holding time	- Until adult emergence
Indicative of personal protection	- N/A
Suitable chemistries	- Insect growth regulators
Appropriate controls	- Negative control is solvent used for preparation of test insecticides
Relevant stage of production pipeline	- Mosquito characterisation

Characterisation of output	- Endpoints well defined
Accessibility	- Materials and set up need to be sourced and training is required
Cost	- Low cost, non specialised equipment required and straightforward method to perform
Level of validation and characterisation of outputs	- Not identified any formal validation
Outstanding questions, gaps and priorities	- N/A
Key references, related SOPs, guidelines and publications	- Report of the WHO informal consultation on the evaluation and testing of insecticides (World Health Organization - WHO, 1996) - Guidelines for 'Laboratory and Field testing of Mosquito Larvicides' developed by WHO in 2005 - I2I-SOP-042: Performing larval insecticide bioassays and larval susceptibility testing - Larval source management: a supplementary measure for malaria vector control (World Health Organization, 2013) - I2I-SOP-028: Insect growth regulators

Overview

Larval source management (LSM) is globally used to control malaria by targeting the immature stages of the mosquito vectors in their aquatic habitats preventing development and therefore reducing the abundance of adult vectors (World Health Organization, 2013). Integrating larval source reduction with adult mosquito control interventions e.g. insecticide treated bednets has been a highly effective strategy to control malaria.

There are four types of LSM: habitat modification, habitat manipulation, larviciding and biological control. Mosquitoes lay their eggs in various types of water bodies, depending on species, which can be man-made or natural, temporary, or permanent (World Health Organization, 2013). Once laid, eggs develop through to larval and then pupal stages, before eventually emerging as adults and leaving the aquatic habitat. Larvicides have various modes of action, such as suffocating larvae, interfering with their nervous systems, causing starvation, or inhibiting metamorphosis (Tusting *et al.*, 2013). There are five main groups of larvicides: oils and surface agents; synthetic organic chemicals; bacterial larvicides; spinosyns and insect growth regulators (IGRs)(World Health Organization, 2013). IGRs used for mosquito control fall into two groups: 1) chitin synthesis inhibitors which prevent the formation of a complete exoskeleton resulting in larval death and 2) juvenile hormone mimics that prevent the development of larvae and pupae into adults (World Health Organization, 2013).

Evaluation of mosquito larvicides, described by the WHO guidelines is in 3 phases: Phase 1: Laboratory studies, Phase 2: Small-scale field trials and Phase 3: Large-scale field trials. The scope of this report will focus on Phase 1: Laboratory studies, specifically bioassay methods for IGRs.

Define Accepted Methodologies

Are there existing standard SOPs/Guidelines detailing methodologies?

- Guidelines for 'Laboratory and Field testing of Mosquito Larvicides' (WHO, 2005)
- Report of the WHO informal consultation on the evaluation and testing of insecticides (WHO, 1996)
- Larval source management: a supplementary measure for malaria vector control (WHO, 2013)
- I2I-SOP-028: Insect Growth Regulator Larvicides

Are these sufficiently detailed?

The document 'Larval source management: a supplementary measure for malaria vector control (WHO, 2013) provides information on Insect growth regulators including modes of action and types of IGRs, but it does not describe how to carry out laboratory bioassays.

The 2005 WHO guidelines are an expanded and an updated version of the 1996 WHO guidelines. The 2005 WHO guidelines state it produces standardised procedures for testing larvicides. Information on materials, preparation of stock solutions, a larval feeding regime and data analysis is included in the guidelines. There are, however, parameters in these methods open to interpretation, and testing methods may vary between studies. The 2005 WHO guidelines also include a set of standardised guidelines for rearing methods of *Aedes* and *Culex* to ensure testing of homogeneous mosquitoes. There are no standardised methods for *Anopheles* mosquitoes however.

I2I-SOP-028: Insect growth regulator larvicides: provides instructions on performing the assay including details on materials required, a suitable feeding regime and data analysis.

Do these methods require specialised/non-standardised equipment and/or training?

Specialised training is required for the preparation of making up stock solutions, the identification of 3rd instar larvae to be tested. Mosquito handling skills, practice in conducting the assay, and knowledge of the methods of data analysis (use of log-probit software) are required. The identification of 'affected' larvae (ones that have not been completely separated from the pupal case) is also required alongside scoring moribund and dead larvae. The 2005 WHO guidelines also detail information on making a customised strainer to transfer test larvae.

Are there issues with the methods or their interpretation?

There are specific parameters that are not specified in detail in the 2005 WHO guidelines, as detailed below:

- The methods provide standardised instructions for rearing *Aedes* and *Culex* spp. mosquitoes to ensure that homogenous batches of mosquito larvae are tested. For other species it is stated to use these procedures subject to any necessary modifications for the biological requisites. Consequently, there are no guidelines for other species leaving the rearing methods open to interpretation and variations may occur between test sites.
- The 2005 WHO instructions state to add 0.1 or 1mL of the appropriate dilution to 100/200mL of water. The procedures state that this will not cause any noticeable variability in the final concentration. It is inconclusive if this has been tested, however. In comparison the I2I methods have standardised instructions and state to use a final volume of 200mL by adding 1mL of the appropriately diluted insecticide to 199mL of water.
- Distilled/non-chlorinated water is recommended for testing insecticides and tap water for controls. This difference in water type could be a confounding variable in any

mortality results obtained in the control test. In comparison the I2I SOP states to use deionised water for testing insecticides and controls.

What IGRs have the tests been used for?

The list of Prequalified Vector Control Products (WHO, 2023) includes the IGR larvicidal products as follows:

- Diflubenzuron
- Pyripoxyfen
- Novaluron

Are they validated, for which AIs/entomological effects, and to what extent?

This method is reported as being used frequently in published literature, however we have not identified any formal validation data.

What inputs need to be characterised? e.g., samples, mosquitoes, equipment

For laboratory testing the 2005 WHO guidelines and I2I SOP sufficiently detail the bioassay requirements, including the equipment required, recommended larval instar for testing and suggested feeding regime. Additional inputs that need to be characterised are detailed in 'Are there any issues with the method and their interpretation?'

Are endpoints clearly defined and appropriate? Who were they defined by?

The endpoints for IGRs as detailed in the I2I SOP and 2005 WHO guidelines are mortality and emergence inhibition (IE%). Emergence inhibition looks at the number of larvae that have not developed into successful adults. It is stated to record mortality every other day or every three days until adult emergence, recording any deformities. The experiment ends when all the

pupa/adults have either emerged or died. These endpoints are suitable due to their mode of action. IGRs are slow acting and causes larval death or prevent the development of larvae and pupae into adults.

Are their supporting SOPs? e.g., cleaning SOPs, mosquito rearing SOPs required.

- I2I-SOP-042: The Performance of Larval Insecticide Bioassays and Selections.
- LITSOP123: Test preparation, detailing the set-up of equipment required.
- LITSOP142: Equipment Cleaning in the LITE Laboratory Area, detailing general cleaning after testing.
- I2I-SOP-001: Bacterial Larvicide Bioassay (i.e. *Bacillus thuringiensis subsp. israelensis and Bacillus sphaericus*). This SOP focuses on bacterial larvicides only.

Define Current Use Practices

Does everybody use the same SOP?

The 2005 WHO guidelines are commonly used as 'guidance' when performing laboratory testing. This is reflected in the literature where the WHO, 2005 guidelines are commonly used and adapted for individual studies.

Are there differences of interpretation of the method?

The methodologies reported in published literature suggest that the guidelines have been adapted dependent on the study protocol, revealing inconsistencies in specific methodological details used for laboratory testing. Key parameters which may impact results of testing have listed in the section 'Are there issues with the methods or their interpretation?'

Are the results obtained largely consistent between studies?

Laboratory larvicide studies often test with different mosquito strains and different concentrations of active ingredients making comparability between studies difficult.

Is further development, refinement or validation of the method required? Based on priority, significance, and relevance of method.

The WHO guidelines have not been updated since 2005. Updated WHO guidelines providing standardised guidelines would be beneficial to ensure robust data from testing at the Phase 1 laboratory stage.

Identify Potential Sources of Variation

What are the sources of variability in the method and are there means to minimise or characterise these.

There are various methodological factors that would benefit from being standardised to prevent variability between studies. These are detailed below:

- Testing homogenous batches of mosquitoes: the 2005 WHO guidelines provide standardised methods for mosquito rearing but do not include *Anopheles*. Variations in rearing methods can cause variations in the fitness of the mosquito, affecting mortality.
- Using the same water type for insecticide testing and controls would be beneficial to ensure that conditions are standardised. Distilled/non-chlorinated water is recommended for testing insecticides and tap water for controls in the 2005 WHO guidelines.
- The stock solutions are made up for testing by the user. The WHO, 2005 methods state to add 0.1 or 1mL of the appropriate dilution to 100 or 200mL of water. This could potentially cause variation in results e.g. 1mL of solution added to 100mL of water in comparison to 0.1mL in 200mL of water could produce differences in the final concentration and volume of water.

- Scoring every second day or every third day an issue? The methods also state to score mortality every second or third day until adult emergence, recording daily may be more beneficial to reduce variability in mortality results between studies.

Do current method/s need to be adapted for new active ingredients/MoA/types of tool?

There are separate landscaping reports available for the different methods for lab efficacy testing of larval insecticides specific to insect growth regulators and insecticide bioassay methods. These are:

- I2I Landscaping report: Lab efficacy testing of larvicides-Insecticide bioassay methods
- I2I Landscaping report: Lab efficacy testing of larvicides-Bacterial bioassay methods

Are new methods required? Identify areas where current method/s are not suitable or sufficient.

N/A

Gaps in biological or other understanding that hinder method development or validation

N/A

Prioritisation – is there an issue that needs to be addressed, what specifics, how urgent is the need?

N/A

References

Tusting, L.S. *et al.* (2013) 'Mosquito larval source management for controlling malaria', *Cochrane Database of Systematic Reviews* [Preprint]. Edited by Cochrane Infectious Diseases Group. Available at: https://doi.org/10.1002/14651858.CD008923.pub2.

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