

6. Biolarvicides

6.1. Phase I

6.1.1. Duration

Evaluation should be carried out for 2 months.

6.1.2. Objectives

- To evaluate toxicity of the biolarvicide to mosquito larvae and determination of doses for treatment in Phase II
- To evaluate toxicity to larvivorous fish *Gambusia affinis* (Gambusia) and *Poecilia reticulata* (Guppy)

6.1.3. Preparation of biolarvicide suspensions and treatment

Fifty milligrams of candidate biolarvicide powder is weighed and placed in a 20 ml flask with 10 ml distilled water and 15 glass beads (6 mm diameter). The contents are thoroughly homogenised on a magnetic stirrer for 10 minutes. This homogenate can be placed in air tight glass vials and stored at 4°C for several months. From this homogenate, a stock solution is made in a test tube by adding 0.1 ml of the homogenate to 9.9 ml distilled water. This is mixed thoroughly on a Vortex agitator for few seconds. For the control, similar suspension of standard powder of *Bacillus thuringiensis* (*Bti*) or *Bacillus sphaericus* (*Bsp*) provided by WHO should be prepared.

From the stock solution, dilutions are directly prepared in 250 ml glass beakers or 300 ml plastic bowls previously filled with 150 ml distilled water. Suspension from stock solution is added to 3–4 replicates for each concentration. Stock solution is added to the replicates using micropipettes. The concentrations for testing will be 0.04 mg/l for which 120 µl, of stock solution respectively are added to the water in the containers. Further lower concentrations are made by serial dilution of this. Two controls are recommended for these experiments. For control-1, suspension of the standard *Bti* or *Bsp* should be used as per the need. For control-2, 150 ml distilled water should be used (to exclude mortality in water). For bioassays 20–25 late III or early IV instar larvae of *Ae. aegypti* or *An. stephensi* are used for *Bti*, while for *Bsp* evaluation *An. stephensi* or *Cx. quinquefasciatus* are used. Larvae are added to the cups prior to the addition of bacterial suspensions. Three to four replicates are used for each concentration and control.

Percent mortality should be calculated after 24 and 48 h of treatment by scoring dead and moribund larvae. Pupae emerged during the test should be excluded from the calculations. Mortality in control-2 in the range of 5 to 20% should be corrected using Abbott's formula. If mortality in control is more than 20%, tests should be repeated. In case of control-2 tests with more than 10% pupation should be repeated. $LC_{99.9}$ of the candidate and standard biolarvicide preparations should be calculated from mortality regression lines by probit analysis. Doses for application in Phase II are determined by multiplying the observed $LC_{99.9}$ value with a factor of 2 or 3.

6.2. Phase II

6.2.1. Duration

Total duration of this phase is 3 months.

6.2.2. Objectives

- To evaluate the candidate biolarvicide in different natural habitats
- To determine the effective dose and frequency of application in the field
- To assess the persistence of the biolarvicide formulation in different breeding habitats of the target vector species

Phase II trial should follow the procedures given under chemical larvicides (5.2.) Biolarvicides simulated trials are not recommended due to the absence of biotic and abiotic factors that would affect the potency of the formulation.

6.3. Phase III

6.3.1. Duration

Duration of this phase is 6 months.

6.3.2. Objectives

- To evaluate the efficacy against larvae of vectors in a locality
- To assess the persistence of biolarvicide
- To assess the operational dose and its frequency of use

Trial should be carried out as per the protocol given under Phase III for chemical larvicides (section 5.3.).

