Laboratory and field evaluation of bio-larvicide *Bacillus thuringiensis* isolate of Nepal against *Aedes aegypti*

Ganga Gharty Chhetri (G.C)

Lecturer

Trichandra Multiple campus

Central Department of Microbiology

Tribhuvan University

Authors and Affiliations

Ganga GC*[a, b], Megha Raj Banjara[a], Ishan Gautam[c], Prakash Ghimire[a], Komal Raj Rijal[a]

[a]*Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal.*
[b]*Trichandra Multiple Campus, Ghanta Ghar, Kathmandu, Nepal.*
[c]Natural History Museum, Tribhuvan University, Swayambhu, Kathmandu, Nepal.

Background

- Dengue disease rate is increasing in all urban areas of Nepal.
- All 77 districts of Nepal (EDCD 2023).

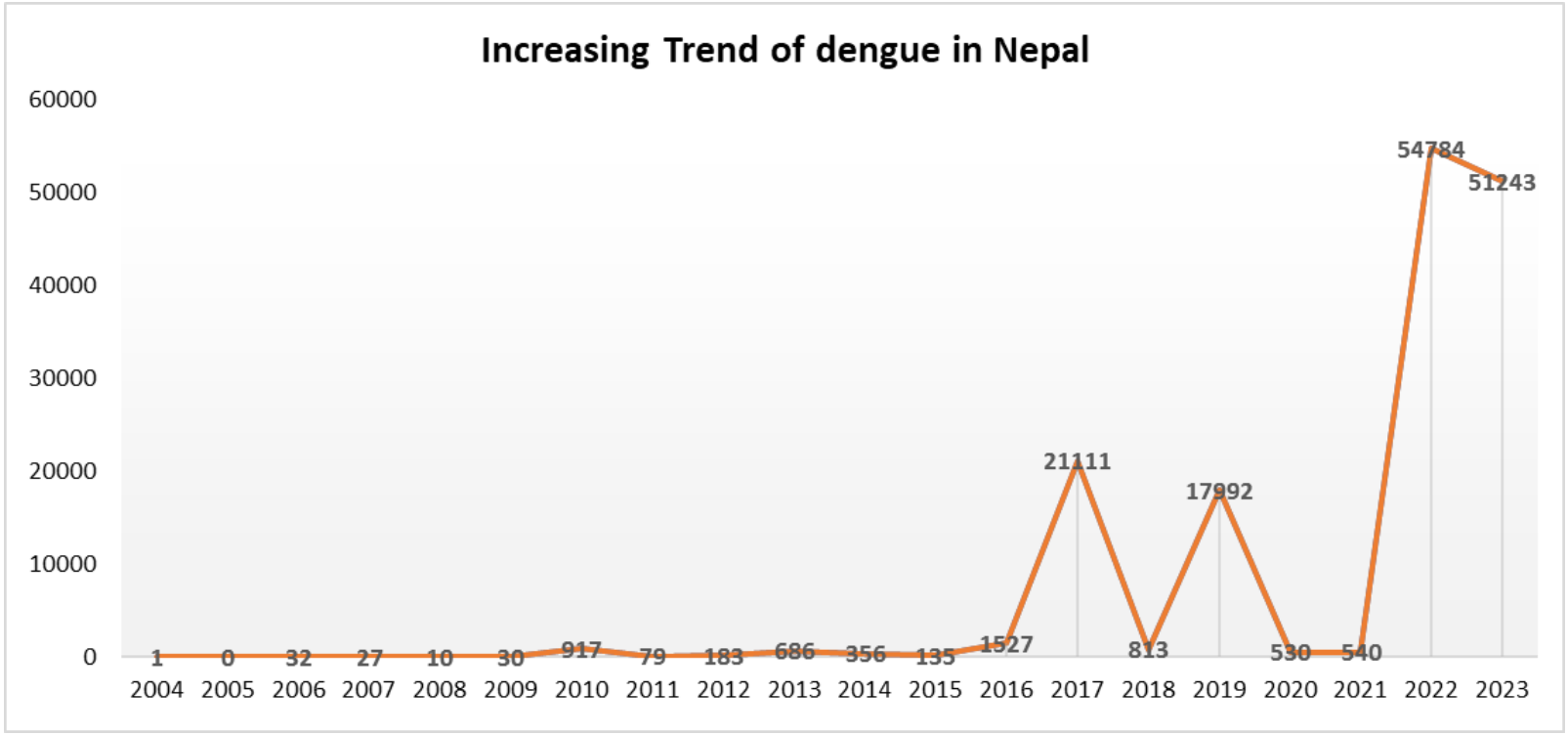
Aedes aegypti

Aedes albopictus

- ❖ Transmitting the dengue viruses.
- ❖ In Nepal *Aedes aegypti* is the main vector for transmitting dengue virus (Poudel, 2023).

Dengue Control Measures

- ❖ Search and destroy campaign,
- ❖ Is by Environmental management and manipulation in all urban areas.
- ❖ But the disease rate is increasing in order.



(EDCD 2023 situation update)

Urgent need for new tools

- To decrease the burden of dengue diseases, in Nepal.
- Multiple and safe tools should be implemented to control dengue vectors.
- Tools like- Biological control, Sterile insect technique (SIT), *Wolbachia,* etc., should be included in the vector control program.

Rationale

- *Aedes aegypti* has a different behavior than the other mosquito vectors.
- It breeds indoors in domestic containers, day biter.
- Larval source management (LSM) without emptying water containers by using a biological control agent like *Bacillus thuringiensis* (Bt) is a novel work.
- A new intervention in Nepal.
- The research aims to isolate a mosquito larvicidal *Bacillus thuringiensis* (Bt), a future tool to control mosquitoes.

Objective 1

Isolation of *Bacillus thuringiensis* from soil

Identification Conventional method 16srRNA

Objective 2

Screening for mosquitocidal activity

Formulation of bio-larvicide

Objective 3

Evaluation against *Aedes aegypti* Laboratory

Field trial

METHODOLOGY

Isolation and Identification

Soil Samples

Acetate Selection

Travers *et al* 1987

Bacillus spp

Identification
Conventional

Bacillus thuringiensis
Coomassie Brilliant Blue staining
(Rampersad *et al* 2002)

METHODOLOGY

Pure culture in NA agar Plate

Primary Screening

Bacillus thuringiensis (Bt)

Spherical crystal protein

Larval Source/ Field Collected Larvae

Mortality of larvae Observed after 24 hours incubation

WHO (2005) Guidelines for laboratory and field testing of mosquito larvicides

METHODOLOGY

Quantitative bioassay Against Laboratory reared *Aedes aegypti*

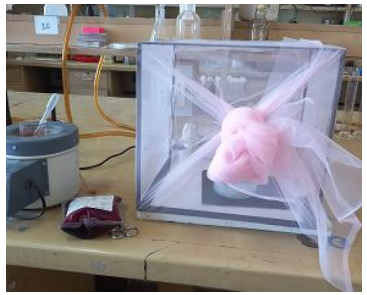
Liquid Formulated
Bio larvicide Bt

- **6** Different concentrations of Bt
- Four replicates
- 3 consecutive days

WHO (2005) Guidelines for laboratory and field testing of mosquito larvicides

METHODOLOGY

Rearing of *Aedes aegypti*



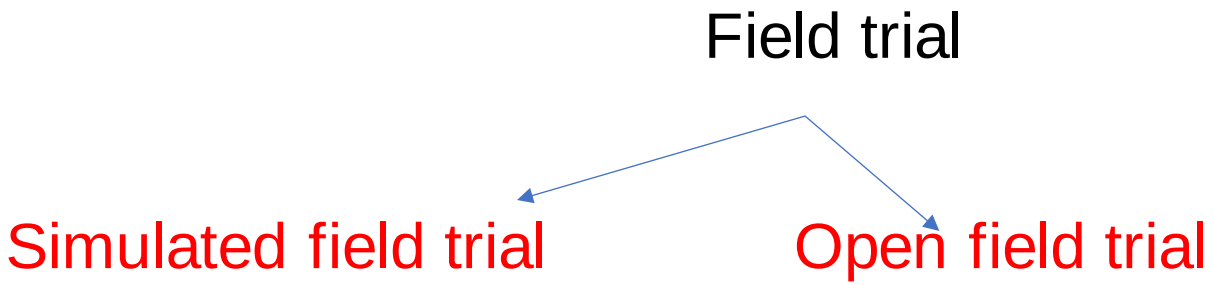
Insectary (Cage)

Eggs in a White Paper

Hatching of Eggs in an enamel tray

Hatched larvae

METHODOLOGY



Simulated field trial

- Small containers (buckets) of capacity 1500ml filled with water were placed under a shaded condition by using six different concentrations of formulated Bt and adding 25 reared *Aedes aegypti* larvae once a week to observe the residual effect of the formulation.

WHO (2005) Guidelines for laboratory and field testing of mosquito larvicides

Open Field trial

- At 20 different sites.
- Containers like –tires, buckets, tubs, jars, drums, etc. placed in open field and under shaded places.
- In open spaces where rainwater gets collected in the containers naturally.
- Different concentration of bio-larvicide Bt was added to the water containers containing the larvae of mosquitoes.

Data Analysis

- The data was analyzed in Microsoft Excel and manually.
- Probit analysis, mean lethal concentration, LC_{50} and LC_{90} was determined.
- The mortality percentage was calculated by using the formula
- Mortality (%) = (x-y/x) x100
- Where x = percentage survival in the untreated control and y = percentage survival in the treated sample (World Health Organization, 2005).
- Graphs and tables were used to present the residual effect of bio larvicide.

Results and Discussion

Table: 1 Number of native Bt obtained from soil

Soil	Site	Bt isolates
454	Geographical region of Nepal	1395

- Every soil sample contains ≥ 3 different types of Bt isolates.
- Soil sample is a rich source of Bt.

Results and Discussion

Preliminary screening for larvicidal activity of Bt

Table: 2 Larvicidal activity of Bt

Spherical Crystal Protein	Larvicidal Bt	Non Larvicidal Bt
808	4	804

Mosquitocidal Bt

- 14P2A
- 3P1B
- 8P2A
- 7P2A

- Not all Spherical crystal proteins or insecticidal crystal proteins (ICPs) producing Bt were larvicidal.
- Indicate the specificity of the ICPs toxin. The composition matters not the shape of ICPs

Results and Discussion

Morphology of insecticidal crystal proteins (ICPs)

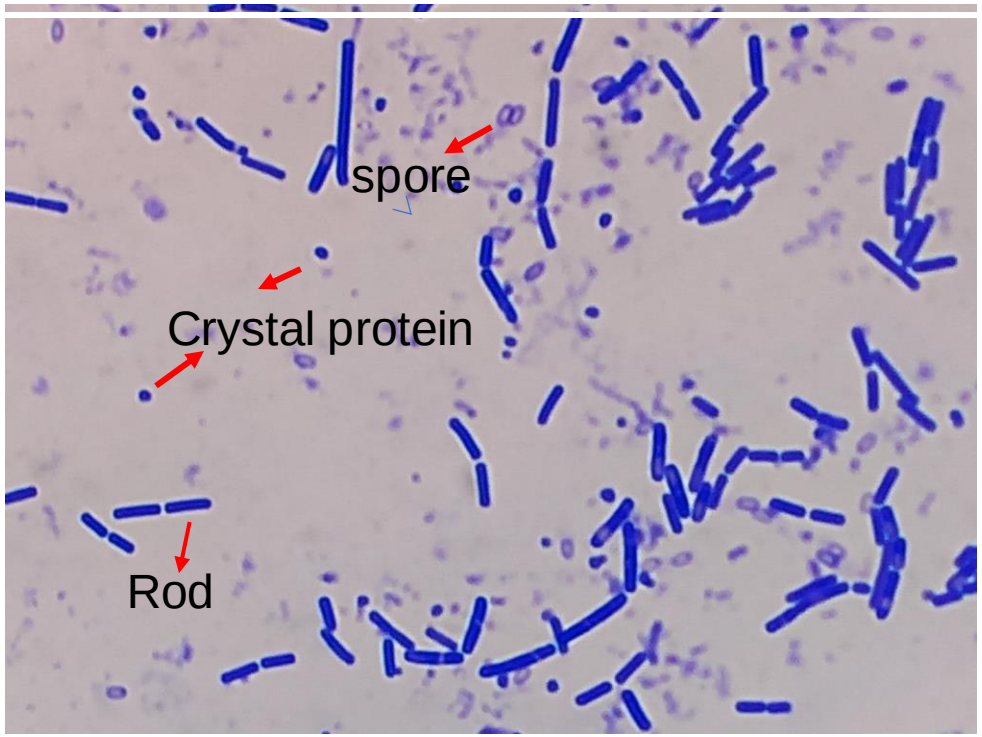


Figure 1. Light microscopic observation of Bt ICPS in Coomassie Brilliant blue stain.

Results and Discussion

16srRNA sequence

- 99% identity towards the *Bacillus thuringiensis* var *israelensis* strain AM 65-52.
- WHO-recommended strain.
- Isolates confirm as Bti

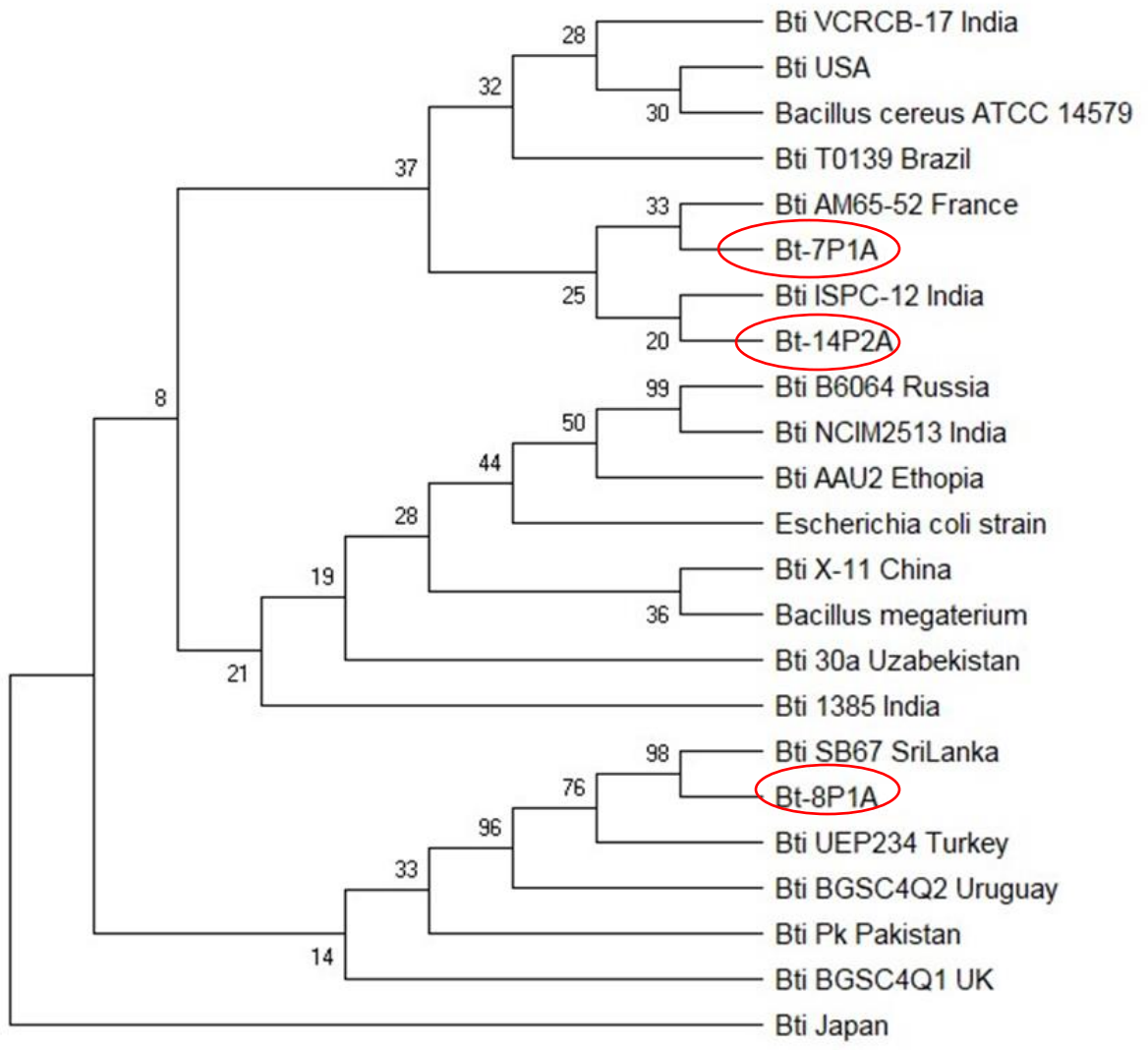


Figure 2. Neighbor-joining Phylogenetic tree based on the 16srRNA gene sequences using MEGA software.

Results and Discussion

Quantitative bioassay against *Aedes aegypti* larvae

❖ >1PPM concentration of Bt was effective in causing 100% mortality of *Aedes aegypti* larvae Fig 3.

- LC_{50} 6.45 PPM
- LC_{90} 37.15 PPM

The potency of Bt was 16611.5 ITU/mg

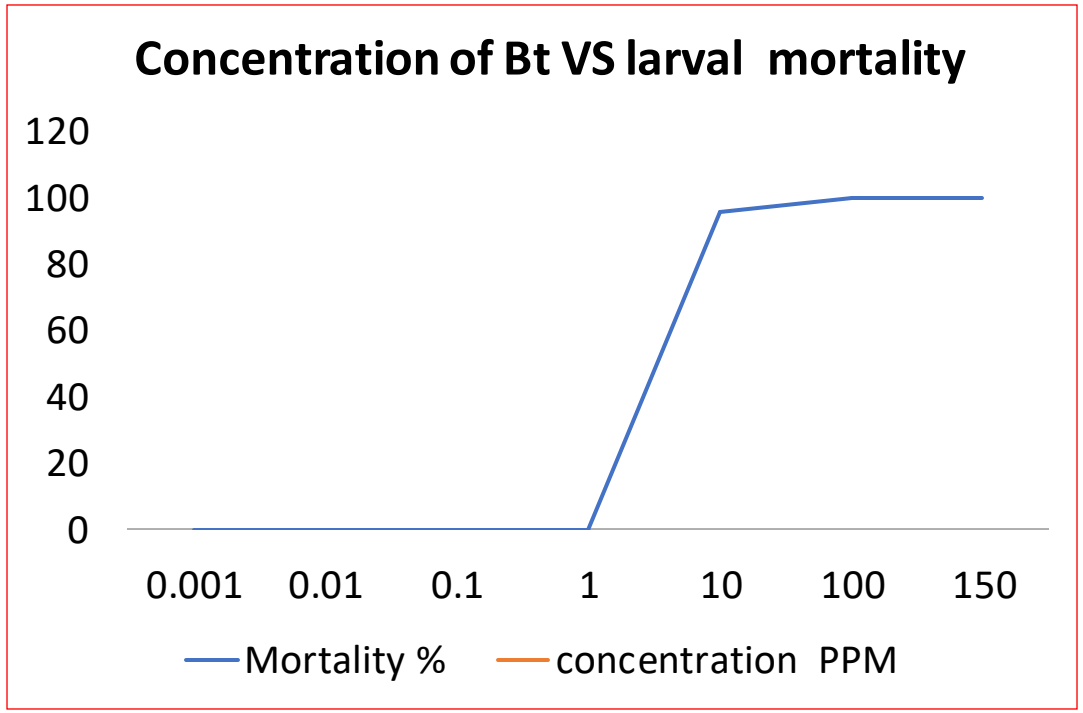


Fig: 3 concentration of Bt required to show 100% mortality.

Results and Discussion

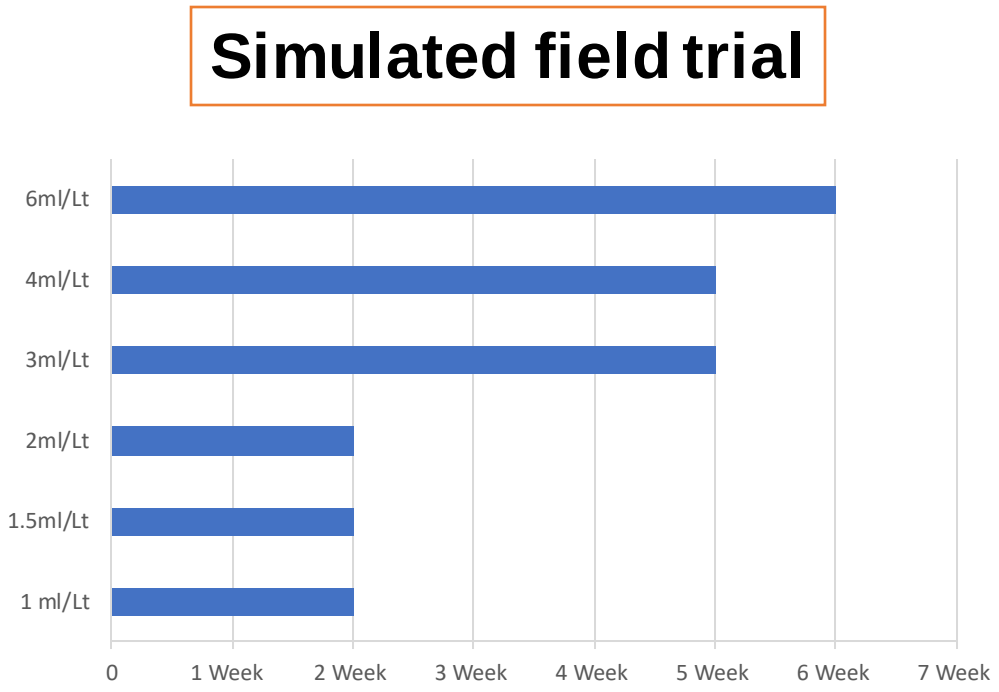


Figure 4. Simulated field trial of Bt-14P2A showing in100% mean mortality weeks

- Six ml bio-larvicide showed residual activity for 6 weeks with 100% mortality of the larvae.
- All the doses showed 100% mortality of larvae within 24 hours.

Results and Discussion

Open field trial

- Open field Tires 2ml/lt- residual effect for 2 weeks, 100% mortality.
- Shaded areas Tires 2ml /lt – residual effect 3-4 weeks with 100 mortality.

- All types of domestic containers residual effects with 100% mortality for > 6 weeks.
- Open field 5ml/lt replenishment with rain water 2 weeks 100% mortality.

Bio-larvicidal property is not reduced by plastic or metal containers.

Conclusion and Recommendation

- *Bacillus thuringiensis* Bt-14P2A, Bt-8P2A, Bt-7P2A identified as mosquito larvicidal bacteria.
- Laboratory assay and field trial results proved that the formulated bio-larvicide Bt-14P2A is effective in controlling mosquitoes at different breeding habitats.
- These isolates as future strategies for managing larval sources and combating mosquito-borne illnesses in Nepal.

Take away message

Let’s combat the dengue disease by using safe bio-larvicide Bt-14P2A Bti in our domestic containers or other aquatic habitats for LSM to break the transmission chain of the dengue virus.

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Thank you

Ganga G.C
Trichandra Multiple campus
gangagc2@gmail.com
9841204803

Bio

- Ganga Gharty Chhetri, Microbiologist, Lecturer of Trichandra Multiple campus. Academician and researcher. Interested to work in the field of biological control agents, Biofertilizers, biopesticides, and Antimicrobial resistance.