

## IPM-4-Citrus, Citrus disease

### Integrated Pest Management: from Research to Market

#### Environmental toxicity assessment of *NEW Bacillus thuringiensis kurstaki* - based biopests on non-target organisms using ISO Standards and OECD guidelines.

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**Background and aim:** *Bacillus thuringiensis* (Bt) was the first biopesticide widely used since 1957 against lepidopteron pests. The impacts on human and environment have to be evaluated, using toxicity assays according to the European guidelines. The aim of our research is to assess the biosafety of two new formulated Bt-based biopests on non-target organisms (i.e. Bt kurstaki BLB1 and LIP strains) by : (1) establishment of linear dependence of inhibition (I%) versus pesticide concentration, and (2) determination of the EC50 database for Bt-Lip concentrated growth culture.

#### ALGALTOXKIT F FRESHWATER TOXICITY TEST WITH MICROALGAE

*Selenastrum capricornutum* (presently as *Pseudokirchneriella subcapitata*) .Measured concentration of 0, 6.25, 12.5, 25, 50, and 100 mg/L of  $\delta$ -endotoxins  $\rightarrow$  72h ErC50 deduced using Macro REGTOX.

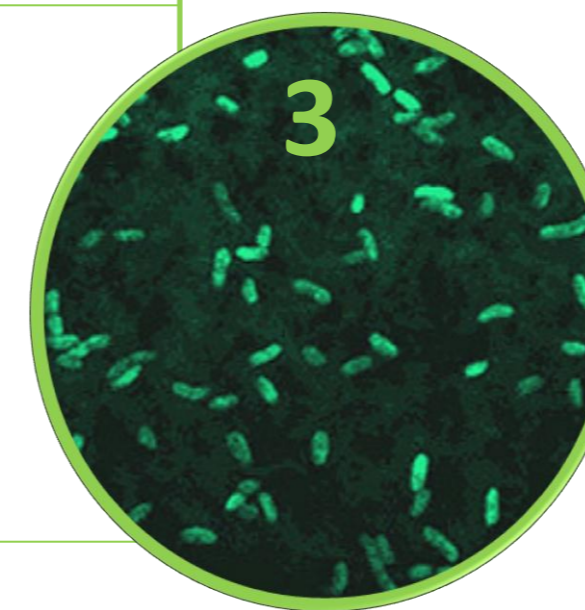
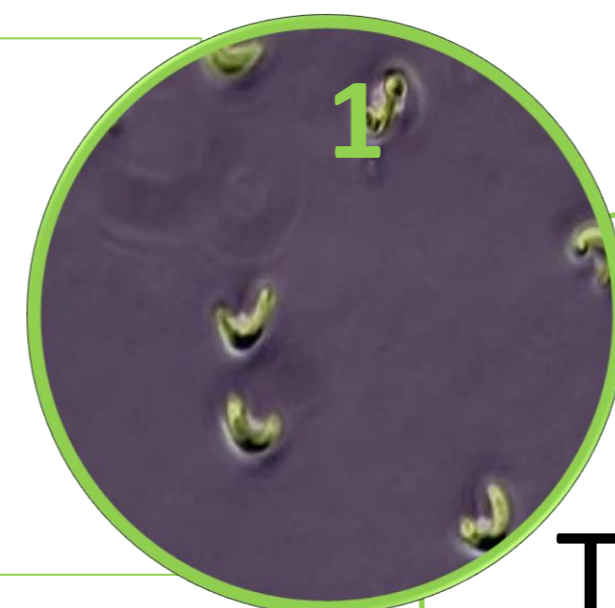
#### DAPHTOXKIT F TM MAGNA

Acute toxicity Test using **Newly hatched *Daphnia magna* larvae**, 3-day static renewal. Twenty individuals (4 replicates) were tested for each concentration : 0, 6.25, 12.5, 25, 50, and 100 mg/L of LIP vs Delfin WG<sup>TM</sup> (HD1)  $\delta$ -endotoxins  $\rightarrow$  48h ErC50 deduced using Macro REGTOX.

#### ALIIVIBRIO FISCHERI Bioluminescence Method

Toxicity Test using ***Vibrio Fischeri* luminescent bacteria** . All measurements were done on B.t.k LIP at 1000,500, 250, 125,62 and 31mg/L of LIP culture- $\delta$ -endotoxins. Bioluminescence measurements performed after 5 and 15 min exposure.

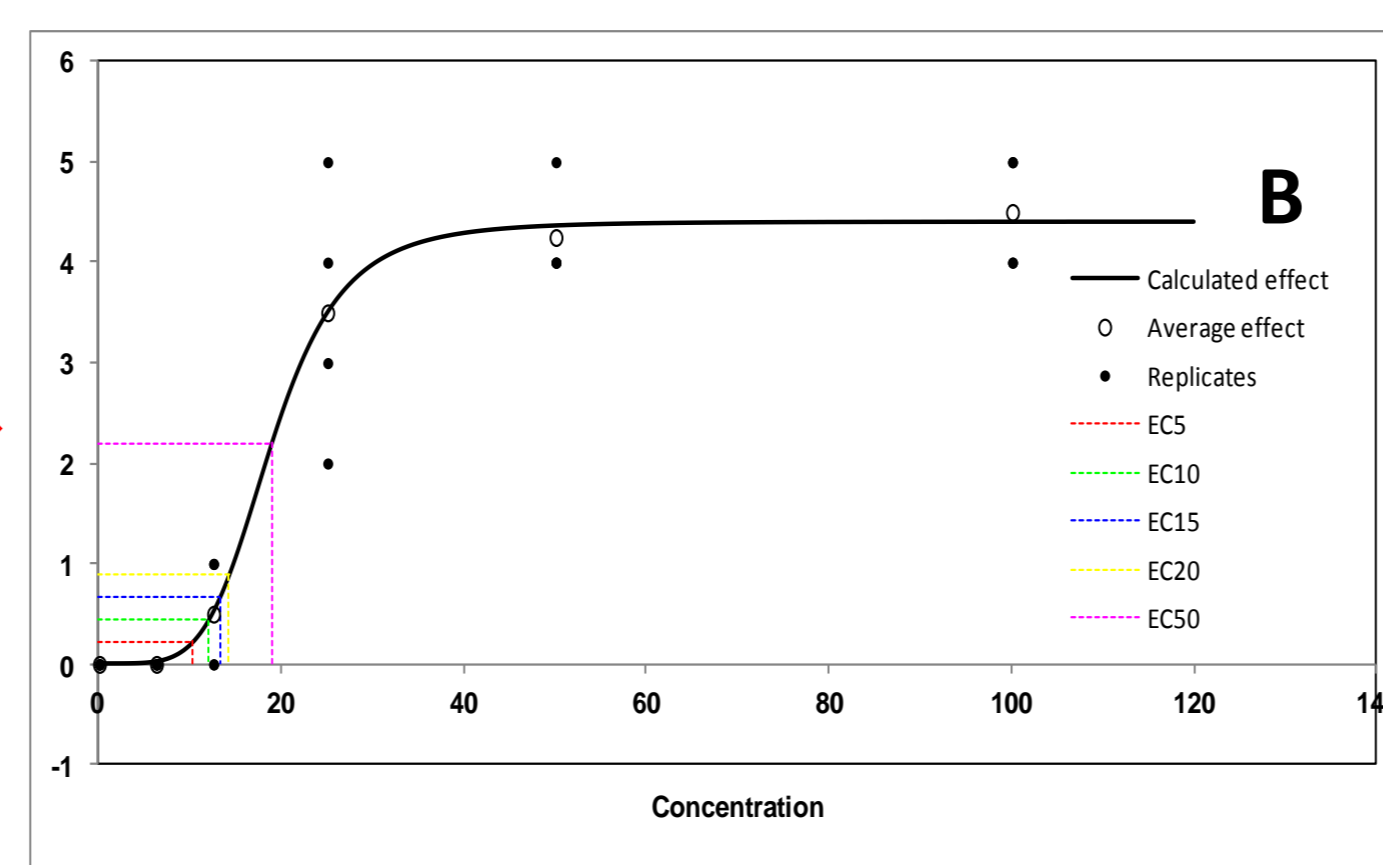
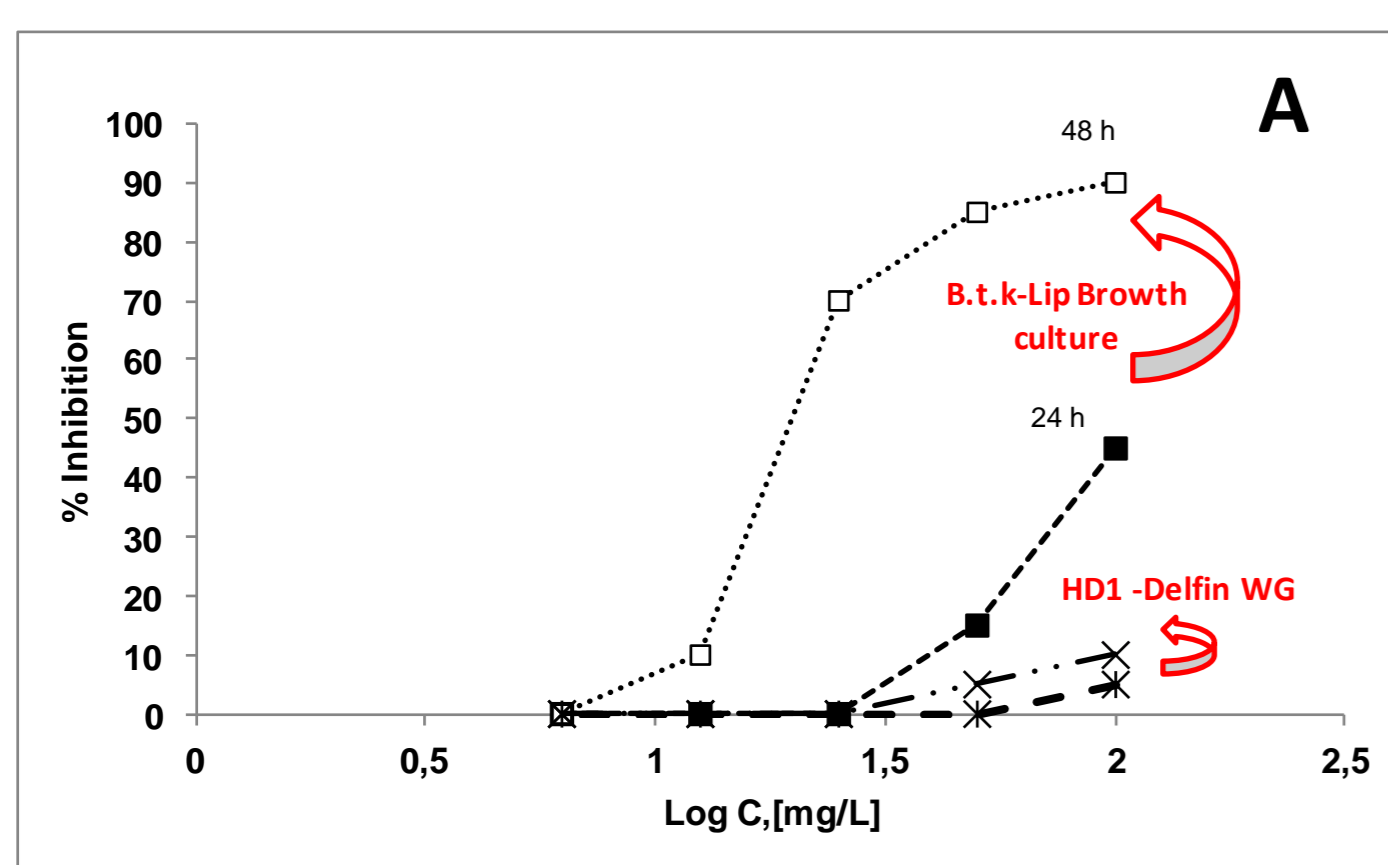
#### Material and methods



Toxicity of B.t.k based biopest (LIP growth culture versus commercial formulation HD1-Delfin WG) on non-target organisms

#### Results and Discussion:

#### Relationship between B.t.k-based biopesticide and percentage inhibition of *Daphnia magna* mobility in test lasting 24 and 48 hours

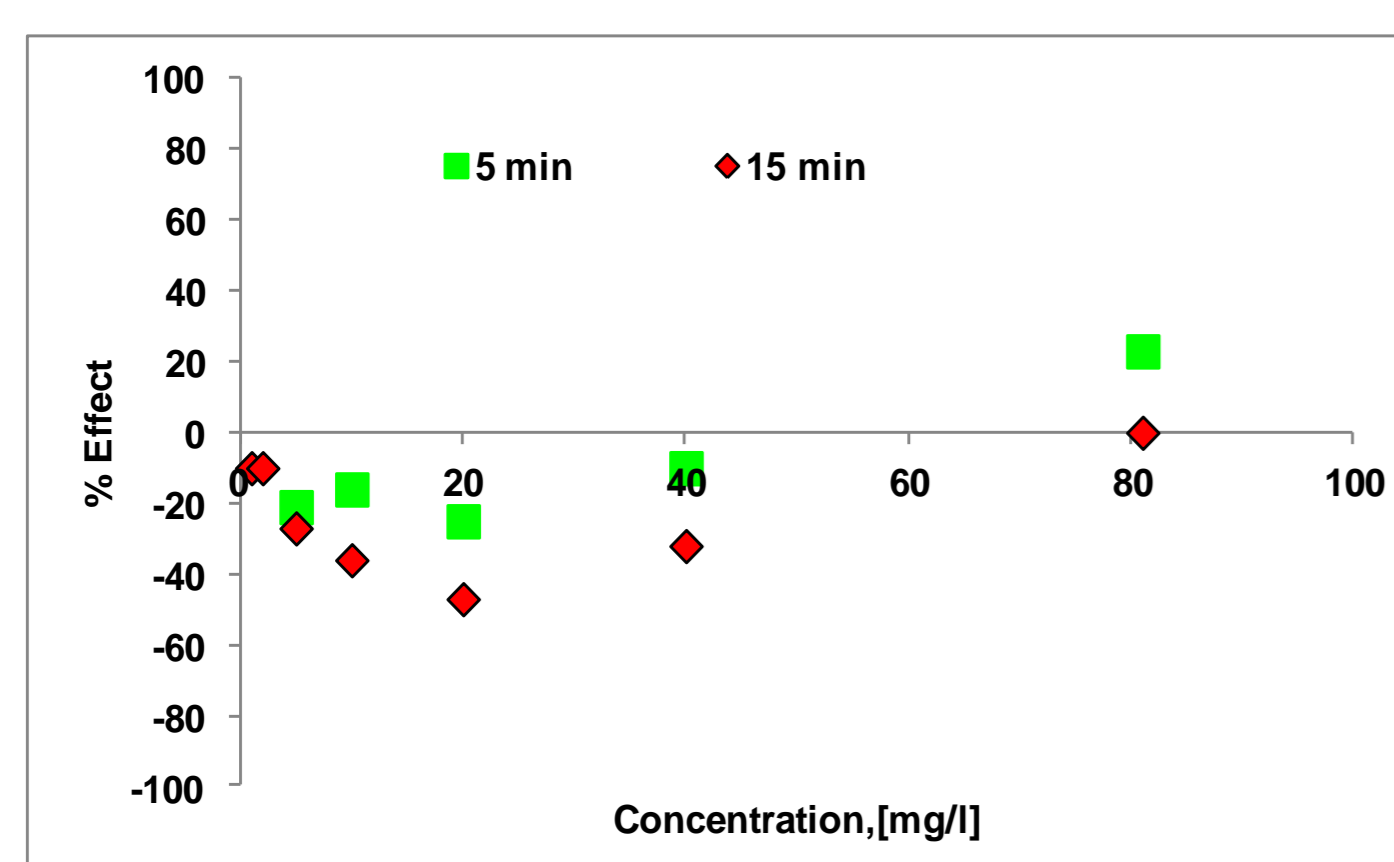


#### ❖ For D. Magna Acute toxicity test:

- ✓ The 48h ErC50 of Lip Growth culture: is estimated to **18,98 mg/L**.
- ✓ The 48h ErC50 of HD-1 commercial formulation (Dipel<sup>TM</sup>) with is estimated to **68.01mg/L** .
- ✓ the chronic daphnid no-observed effect concentrations , **NOEC is to 1,4 mg/L**.

$\rightarrow$  The Lip Growth culture is classified as “**moderately toxic**” to daphnids compared to HD-1 commercial formulation (Dipel<sup>TM</sup>) with a 21 day EC50 for immobilization of 14 mg/L and 10 d EC50 of 12 mg Bta/L for *B. thuringiensis* subsp. Aizawa (NOEC: 5.9 mg/ L (10-day)).

#### Lip toxicity effect on AliiVibrio Fischeri Bioluminescence after 5 and 15 min of exposure



#### ❖ For Alii Vibrio Fischeri Toxicity test:

Linear dependence between the Inhibition (%) and Lip-endotoxins concentrations but the test must be reproduced.

#### ❖ For Freshwater, microalgae Toxicity test:

The 72h Ec50 of Lip Growth culture is estimated to **81,80mg/L**

$\rightarrow$  Higher than the EC50 values for *B. thuringiensis* subsp. Aizawai (72 h EC50 of 64.3 mg Bta/L; NOEC of 56.5 mg/L , Endpoint used for risk assessment.

**Conclusion:** Most organisms seem relatively tolerant even to concentrations of B.t.k. in water that are up to 200,000 times higher than expected environmental concentrations (Christensen 1991f). The results demonstrate that Cry1C protein has no lethal or sublethal effects on this non-target species.



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