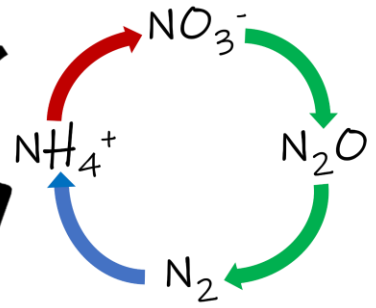
ACTION Nr

Research Action Network for Reducing Reactive Nitrogen Losses from Agricultural Ecosystems

Project No. 101079299

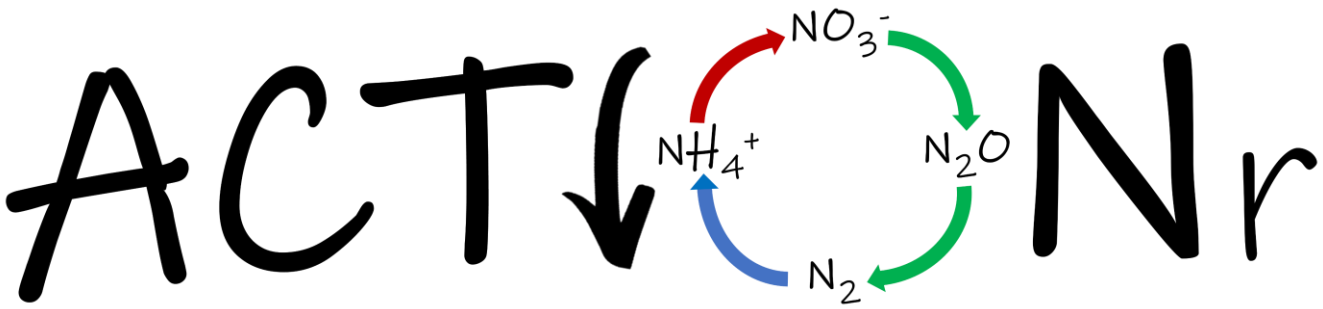
ACTION Nr



PhD summer school

"Biological Nitrification Inhibition: Integrating Microbial Functions, Plant Traits, and Technological Innovations for Sustainable Nitrogen Cycling"

University of Thessaly
12-16 May 2025

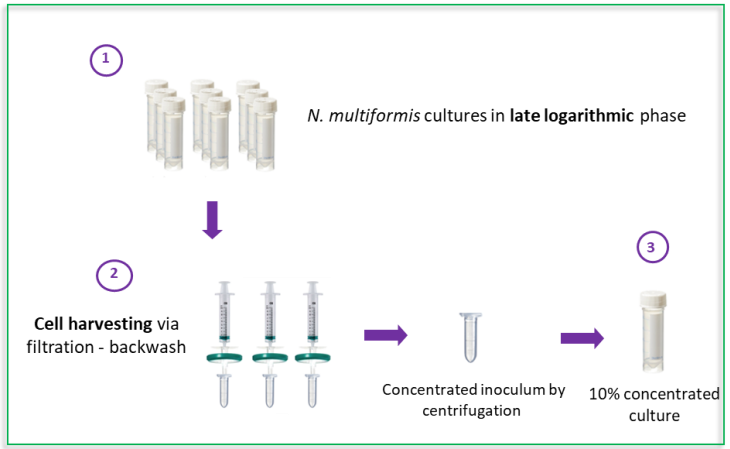


Screening and management of fast track assay data

Eleftheria Bachtsevani

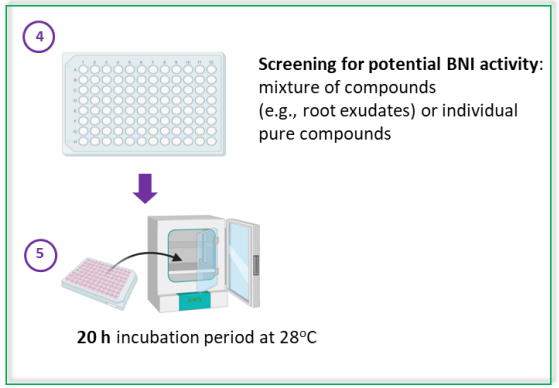
Screening of fast-track assay data

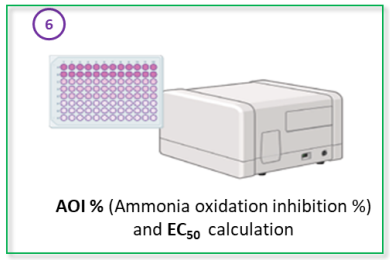
Stage 1: Cell harvesting & inoculation



Ghatak et al. Plant Biotechnology (submitted)

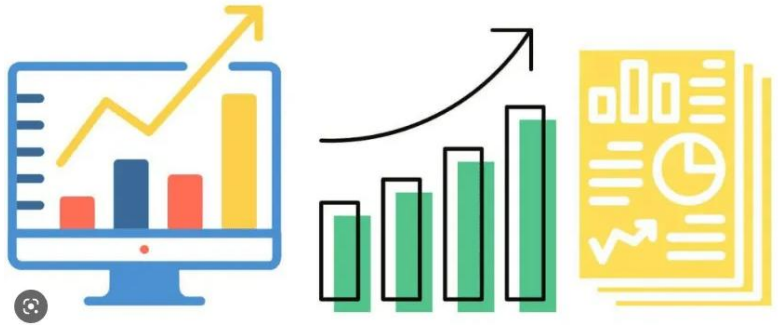
Stage 2: Cell plating & sample application





Stage 3: Colorimetric NO_2^- quantification

Stage 4: Management and evaluation of the NO_2^- data



Screening of fast-track assay data

❑ **We can use NO_2^- measurements for:**

- Assessing the activity of ammonia-oxidizing microorganisms
- Calculating inhibition percentages and generating inhibition curves
- Estimating of inhibition thresholds

Screening of fast-track assay data

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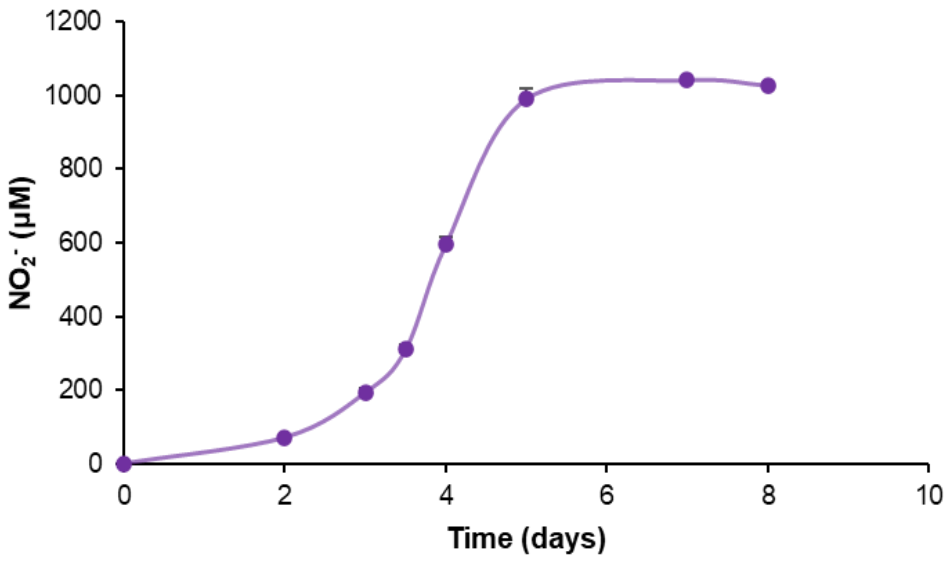
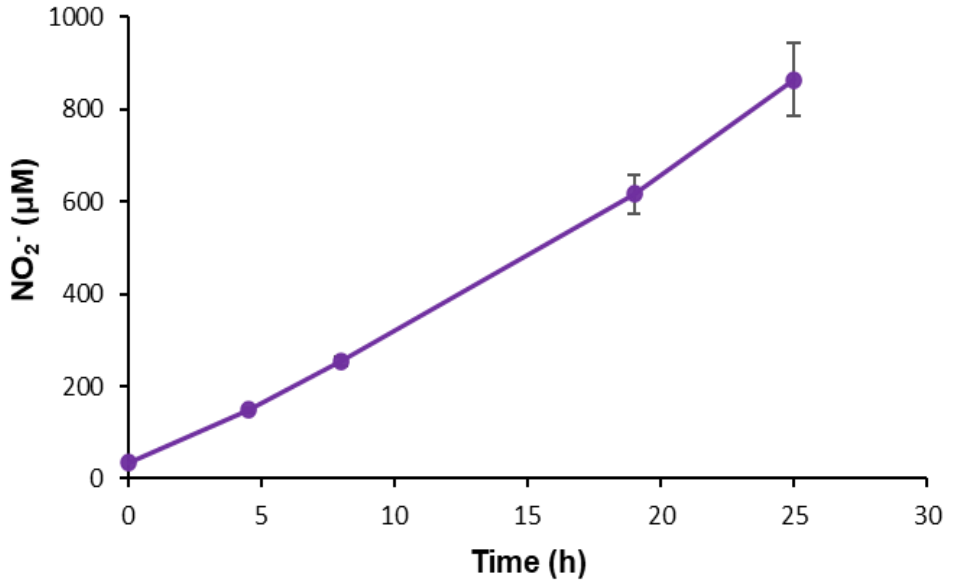
Assessing the activity of ammonia-oxidizing microorganisms

- **Activity curves of ammonia-oxidizing microorganisms in batch cultures vs fast track**

Batch culture



Fast track



Screening of fast-track assay data

❑ **We can use NO_2^- measurements for:**

- Assessing the activity of ammonia-oxidizing microorganisms
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Calculation of inhibition percentage

- **Formula for estimating inhibitory effects on ammonia oxidation**

$$AOI\ (\%) = \frac{a_{control} - a_i}{a_{control}}\ x\ 100\%$$

AOI (%): Ammonia Oxidizing Inhibition %
$a_{control}$: NO_2^- of Control
a_i: NO_2^- of sample

Calculation of inhibition percentage

- **Calculation of ammonia oxidation inhibition (AOI%) for a single time point**

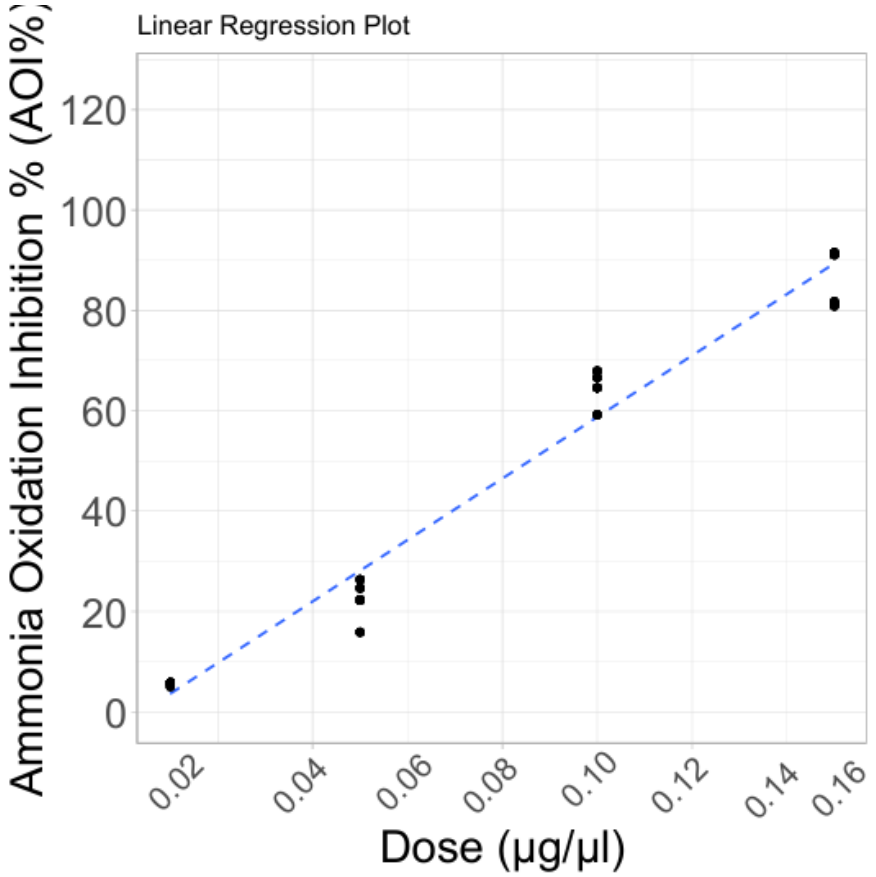
$$Normalised\ NO_{2_{control}} = \frac{NO_{2_{control}}}{Average\ NO_{2_{control}}}$$

$$Normalised\ NO_{2_{sample}} = \frac{NO_{2_{sample}}}{Average\ NO_{2_{control}}}$$

$$AOI\ \% = -(Normalised\ NO_{2_{sample}} - Normaised\ NO_{2_{control}})$$

Calculation of inhibition percentage

- **Inhibition curve of ammonia oxidizing activity**



Screening of fast-track assay data

❑ **We can use NO_2^- measurements for:**

- Assessing the activity of ammonia-oxidizing microorganisms
- Calculating inhibition percentages and generating inhibition curves
- Estimating of inhibition thresholds

Estimation of EC_{50} values

- ✓ **EC_{50} (Half Maximal Effective Concentration)** indicates the concentration of a compound that produces **50% of its maximum possible effect**
- ✓ EC_{50} is a key parameter in **dose-response curves**
- ✓ It helps compare **potency**: the lower the EC_{50}, the more potent the compound

Estimation of EC_{50} values

- **First approach for EC_{50} estimation: inhibition curve equation method**

1. Calculation of inhibition percentages

$$AOI\ (\%) = \frac{a_{control} - a_i}{a_{control}}\ x\ 100\%$$

$$Normalised\ NO_{2_{control}} = \frac{NO_{2_{control}}}{Average\ NO_{2_{control}}}$$

$$Normalised\ NO_{2_{sample}} = \frac{NO_{2_{sample}}}{Average\ NO_{2_{control}}}$$

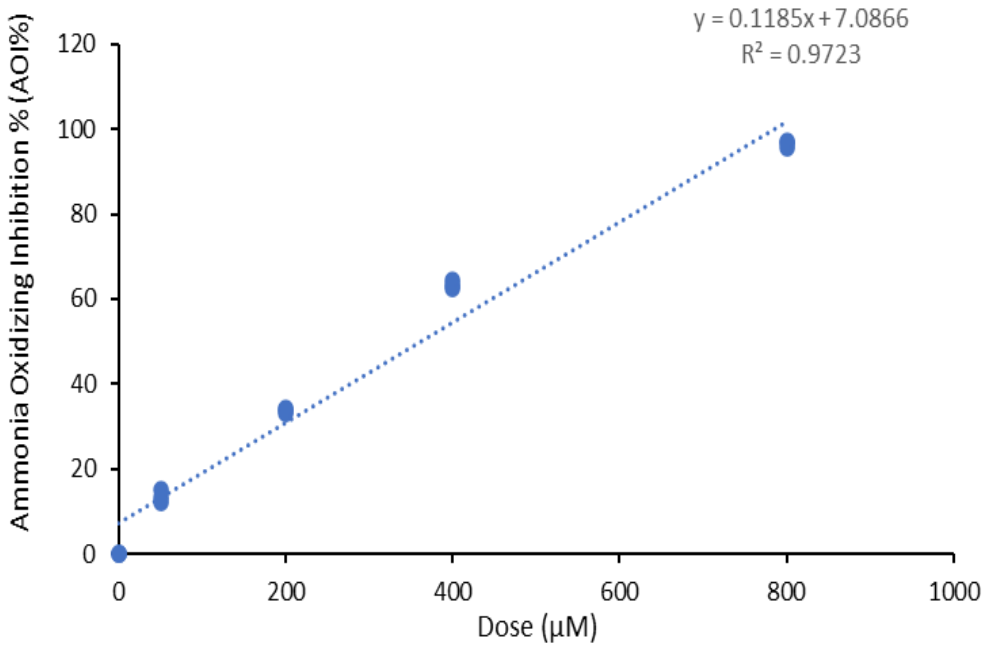
$$AOI\ \% = -(Normalised\ NO_{2_{sample}} - Normaised\ NO_{2_{control}})$$

Estimation of EC_{50} values

- **First approach for EC_{50} estimation: inhibition curve equation method**

2. Preparation of % inhibition curves

✓ Linear regression

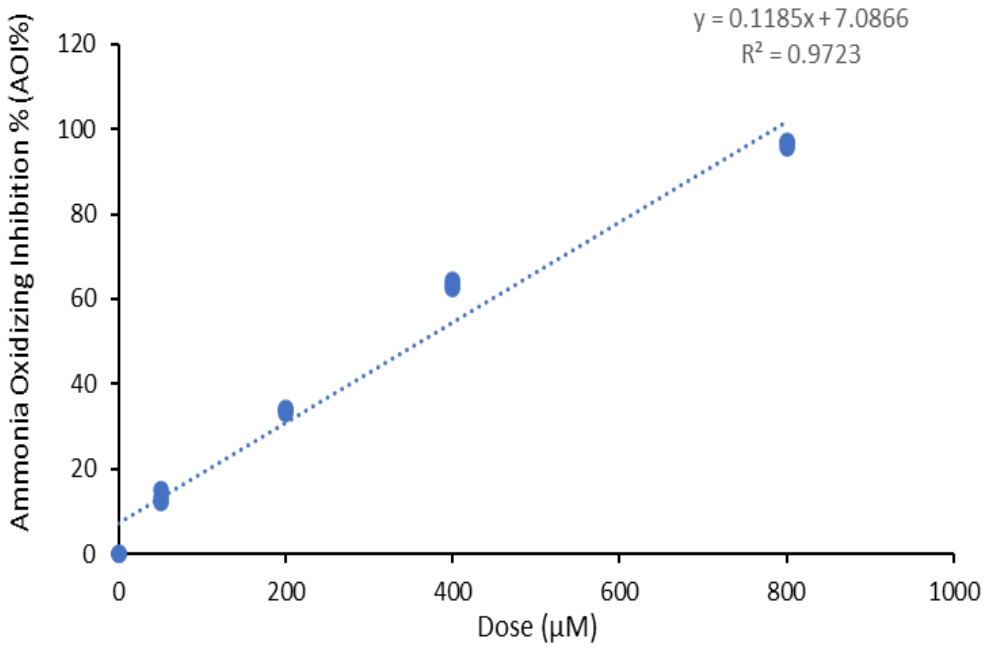


Estimation of EC_{50} values

- **First approach for EC_{50} estimation: inhibition curve equation method**

2. Preparation of % inhibition curves

✓ Linear regression



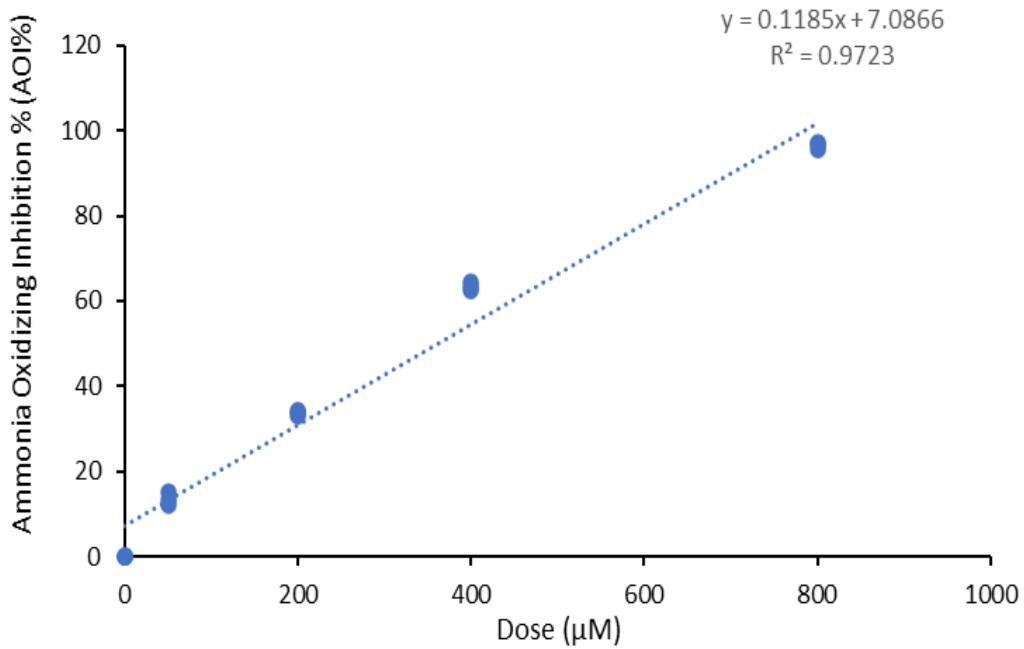
3. Calculation of EC_{50} value using the equation, for y = 50.

EC_{50}=362.14 µM

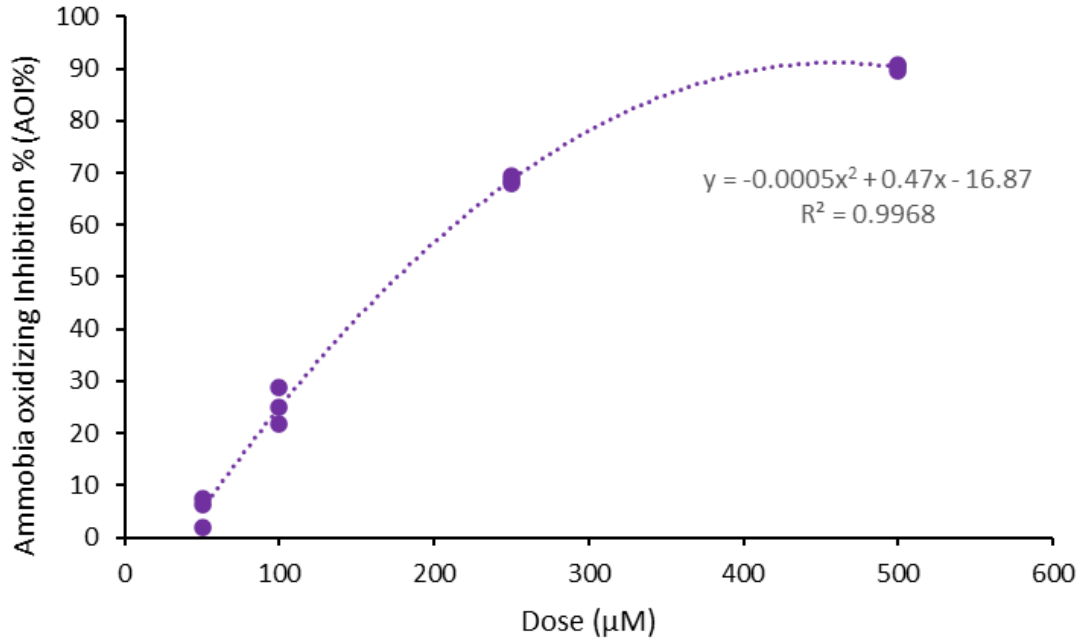
Estimation of EC_{50} values

- **First approach for EC_{50} estimation: inhibition curve equation method**

✓ Linear regression

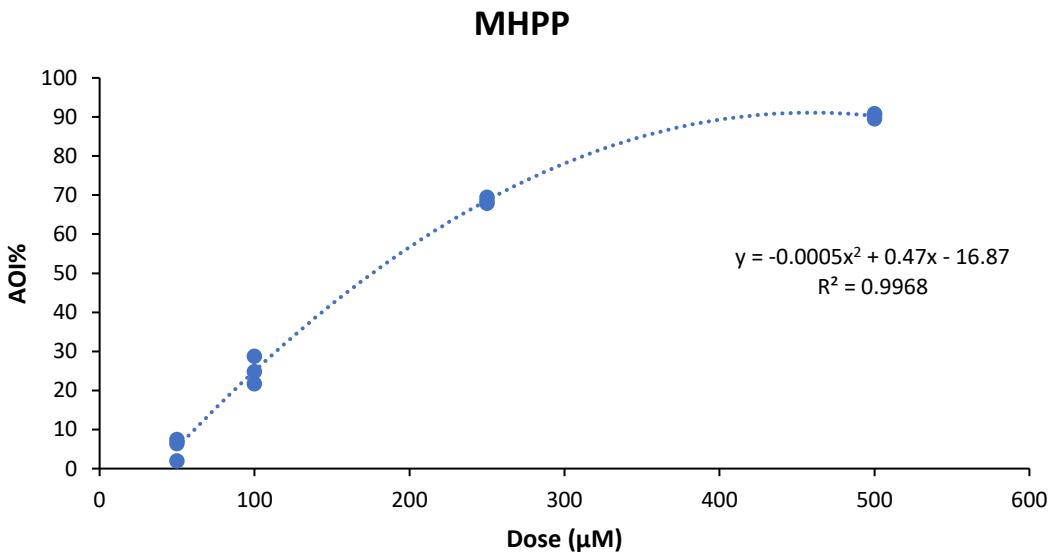


✓ Polynomial regression



Estimation of EC_{50} values

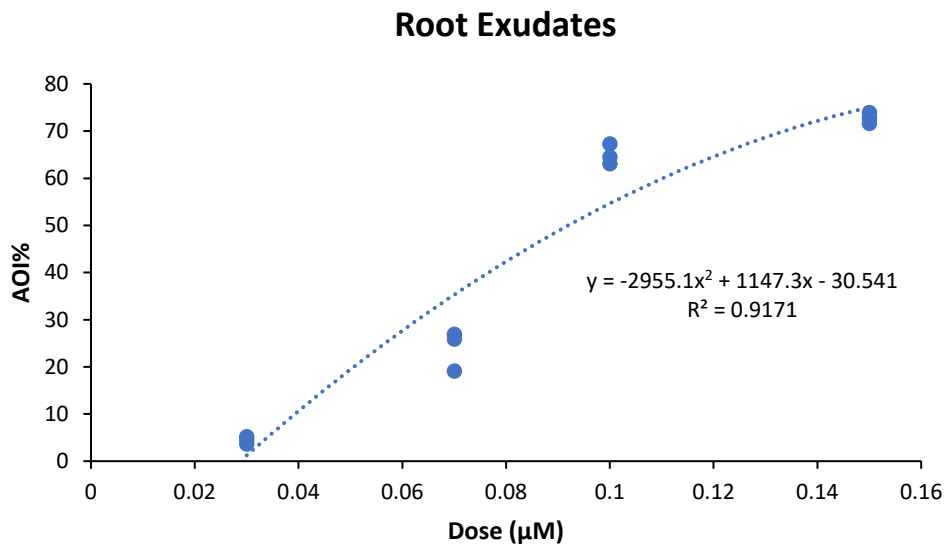
- **Calculation of EC_{50}-Practical course**



EC_{50}=174.8 µM

Estimation of EC_{50} values

- **Calculation of EC_{50}-Practical course**



EC_{50}=0.092 µM

Estimation of EC_{50} values

- **Second approach for EC_{50} estimation: dose response models**

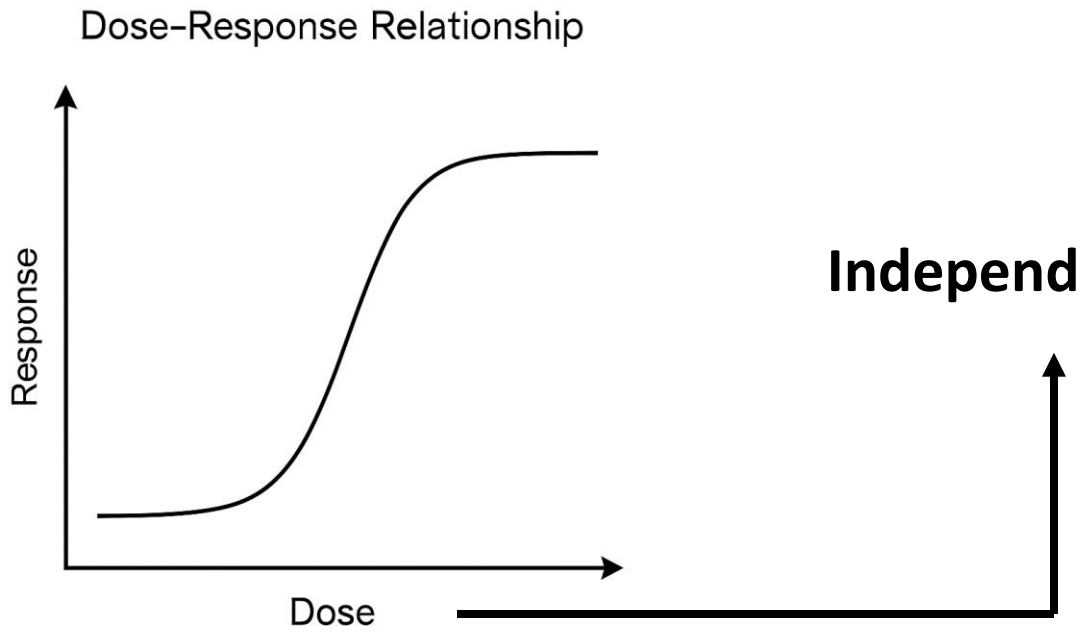
Dose-Response relationship → **Sigmoidal behavior of biological responses**

Estimation of EC_{50} values

- **Second approach for EC_{50} estimation: dose response models**

Dose-Response relationship → **Sigmoidal behavior of biological responses.**

Dependent variable: NO_2^- data ←



→ **Independent variable**

Estimation of EC_{50} values

- **Second approach for EC_{50} estimation: dose response models**

✓**Types of dose-response models**

1. **Log-logistic models**: widely used
2. Other models: Weibull, Gompertz, Log-normal

Estimation of EC_{50} values

- **Second approach for EC_{50} estimation: dose response models**

✓**Types of dose-response models**

1. **Log-logistic models**: widely used
2. Other models: Weibull, Gompertz, Log-normal

4-parameter log-logistic model

$$f(x,(b,c,d,e)) = c + \frac{d-c}{(1+\exp(b(\log(x)-\log(e))))}$$

b: slope
c: lower limit
d: upper limit
e: EC_{50} value

Estimation of EC_{50} values

- **Second approach for EC_{50} estimation: dose response models**

✓**Model selection and goodness of fit**

1. **Comparison of models using** AIC (Akaike Information Criterion)

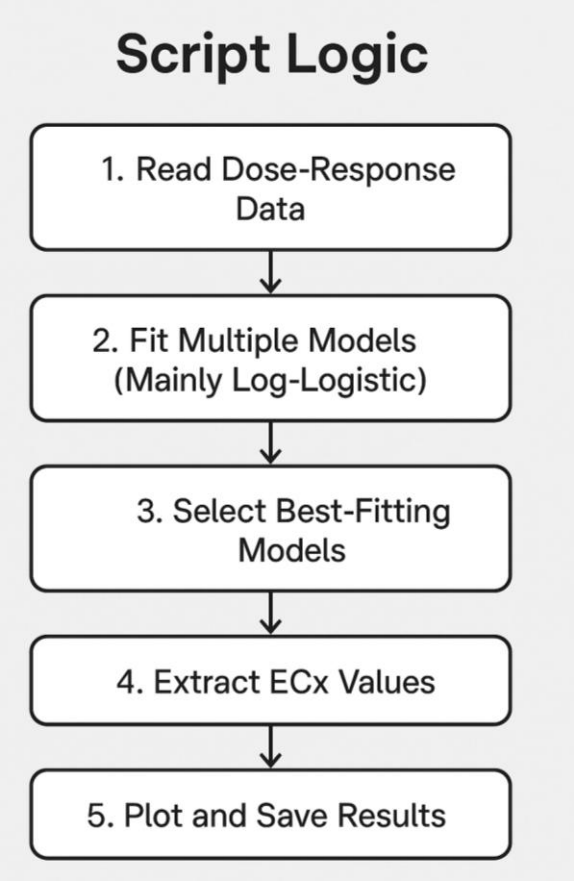
2. **Evaluating model performance:**
   - R^2
   - Residual standard deviation (ResSD)
   - P-values for parameters

Estimation of EC_{50} values

- **Second approach for EC_{50} estimation: dose response models**

Estimation of EC_{50} values

- **Second approach for EC_{50} estimation: dose response models**

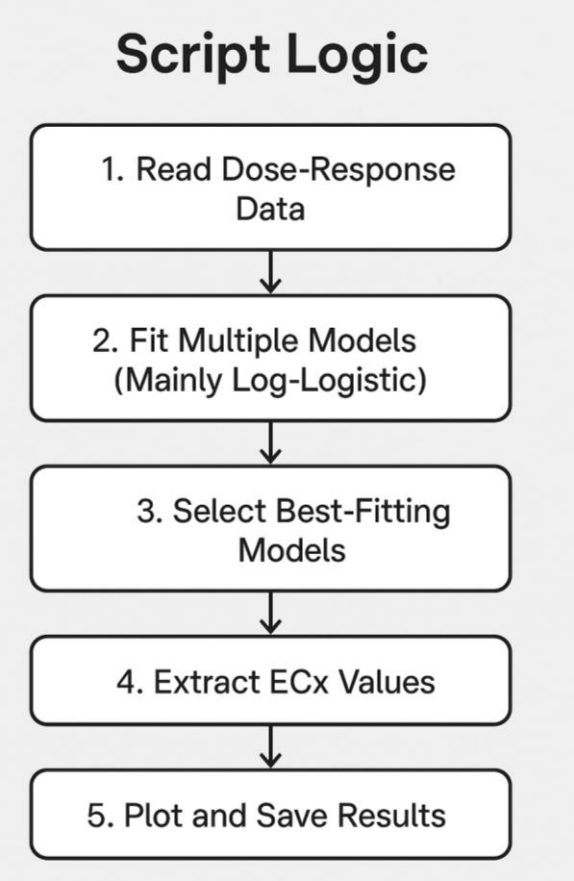


Tools and Packages

- R package: drc
- Key functions: drm(), ED(), AIC(), Rsq(), mselect()

Estimation of EC_{50} values

- **Second approach for EC_{50} estimation: dose response models**



→ Normalized NO_2^- data

Tools and Packages

- R package: drc
- Key functions: drm(), ED(), AIC(), Rsq(), mselect()

Estimation of EC_{50} values

- **Second approach for EC_{50} estimation: dose response models**

Script Logic

1. Read Dose-Response Data → Normalized NO_2^- data
2. Fit Multiple Models (Mainly Log-Logistic)
3. Select Best-Fitting Models
4. Extract ECx Values
5. Plot and Save Results → **EC_{50} values & dose response curves**

Tools and Packages

- R package: drc
- Key functions: drm(), ED(), AIC(), Rsq(), mselect()

Conclusion

Dose-response models, are generally a *good and accurate* method for calculating EC_{50}.

- ✓ **Biologically relevant**: Designed to model sigmoidal dose-response relationships typical in ecotoxicology and pharmacology.
- ✓ **Direct EC_{50} estimation**: EC_{50} is a model parameter, not just inferred from a trend line.
- ✓ **Accurate & robust**: Handles data variability and provides statistical confidence (e.g., AIC, R^2, residuals).

THANK YOU