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Vienna, Austria

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Evaluation of the efficacy of a spray-dried biopesticide based on *Bacillus thuringiensis* subspecies *kurstaki* (strain Lip) on a wheat bran-based complex media

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PRIMA
IN THE MEDITERRANEAN AREA

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Little story

Previous project: IPM-4-Citrus

Aims:

- ✓ Develop two new bio-pesticides (Bt LIP & BLB1) active against citrus pests and scale them up from lab to market.
- ✓ Strengthen collaborations between academic and non-academic partners.

Table 1: *Scientific achievements of IP-4-Citurs*

Article Name	Authors & DOI
Biopesticides production by *Bacillus thuringiensis* serovar *kurstaki* on a wheat bran complex medium: from lab to field	Nasreddine,R.,Barssoum,R., et al. (2024) pending
Physical Limitations in Wheat Bran-Based Medium for Biopesticides Production	Barssoum, R., Nasseredine, R., et al. (2023) DOI:10.1051/matecconf/202337905003
Complete Genome Sequences of *Bacillus thuringiensis* Serovar *Kurstaki*	Fayad, N., Barssoum, R., Marsaud, N., et al. (2023), DOI:10.1128/MRA.00060-23
Biochemical Limitations in Biopesticides Production	Barssoum, R., Al Kassis, G., Nasseredine, R., et al., DOI:10.1016/j.resmic.2023.104043
Optimization of Culture Conditions for Biopesticides Production	Fayad, N., Abboud, J., Driss, F., et al. (2022), DOI:10.3390/fermentation8120666
Dynamic Model for Biomass and Proteins Production	Monroy, T. S., Abdelmalek, N., Rouis, S., et al. (2021), DOI:10.3390/pr9122147
Review on Biopesticide Production Parameters	Jallouli, W., Driss, F., Fillaudeau, L., Rouis, S. (2020), DOI:10.1016/j.procbio.2020.07.023

SCAN ME

Project overview

Alternative Biopesticides For SAFe Integrated Pest And WAter Management Around Mediterranean.

1. Developing a new competitive alternative based on *Bt* biopesticides.
2. Validating large scale production and formulation of the new biopesticide.
3. Reducing the use of harmful chemicals by enhancing the use of biopesticides.
4. Implementing good practices in farming activities.
5. Rising awareness among farmers about good practices impact on water and land safety.

11 PARTNERS

7 COUNTRIES

3 YEARS DURATION

STRAIN USED:

Bacillus thuringiensis kurstaki

Lip & Blb1

TARGET INSECT PESTS:

Prays oleae / Olive

Spodoptera frugiperda / Corn

Tuta absoluta / Tomato

Ectomyeloïs ceratoniae / Pomegranate

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Project overview

Strain & media

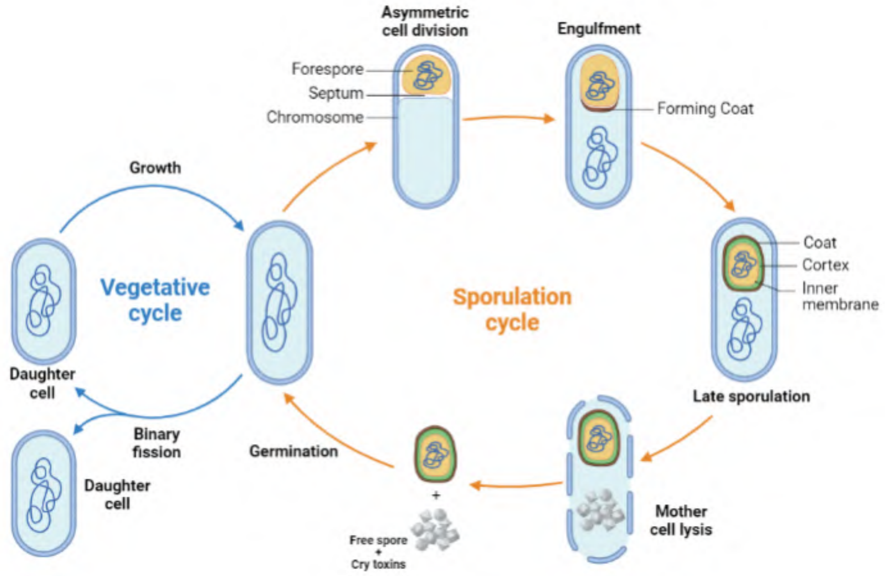


Figure 1. *Growth cycle of Bacillus thuringiensis spp.*

Btk Lip

- ✓ 12 plasmids
  - ✓ pLip97 (CP116324) -> 2: Cry1Ab; Cry1Ac
  - ✓ pLip 300 (CP116317) -> 5: Cry1Aa; Cry1Ac; Cry1Ia; Cry2Aa; Cry2Ab

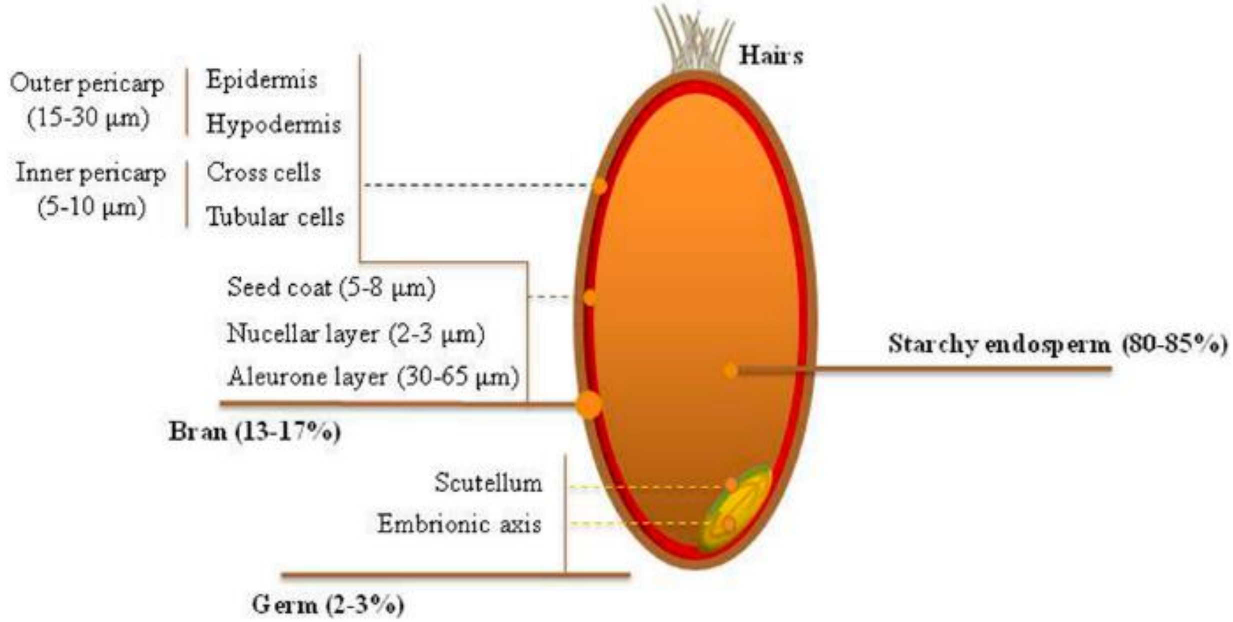


Figure 2. *Schematic representation of wheat grain fractions.*

Fayad, N., Barssoum, R., Marsaud, N., Nasseredine, R., Abdelmalek, N., Rouis, S., Teste, M. A., Pailler, V., Gautier, V., Belmonte, E., Aceves Lara, C. A., Cescut, J., Fillaudeau, L., & Kallassy Awad, M. (2023). Complete genome sequences of two *Bacillus thuringiensis* serovar *kurstaki* strains isolated from Lebanon and Tunisia, highly toxic against lepidopteran larvae. *Microbiology resource announcements*, *12*(9), e0006023. https://doi.org/10.1128/MRA.00060-23

Laddomada B, Caretto S, Mita G. Wheat Bran Phenolic Acids: Bioavailability and Stability in Whole Wheat-Based Foods. *Molecules*. 2015; 20(9):15666-15685. https://doi.org/10.3390/molecules200915666

Barssoum, R., Nasseredinne, R., Chalbi, K. M., Cescut, J., Kallassy, M., Aceves-Lara, C., & Fillaudeau, L. (2023). Physical limitations induced by a medium based on wheat bran for the production of biopesticides by Bacillus thuringiensis spp kurstaki. MATEC Web of Conferences, 379, 05003. https://doi.org/10.1051/matecconf/202337905003

Objectives

Alternative drying technique

- Proof of spray-drying technique concept

Quality assessment

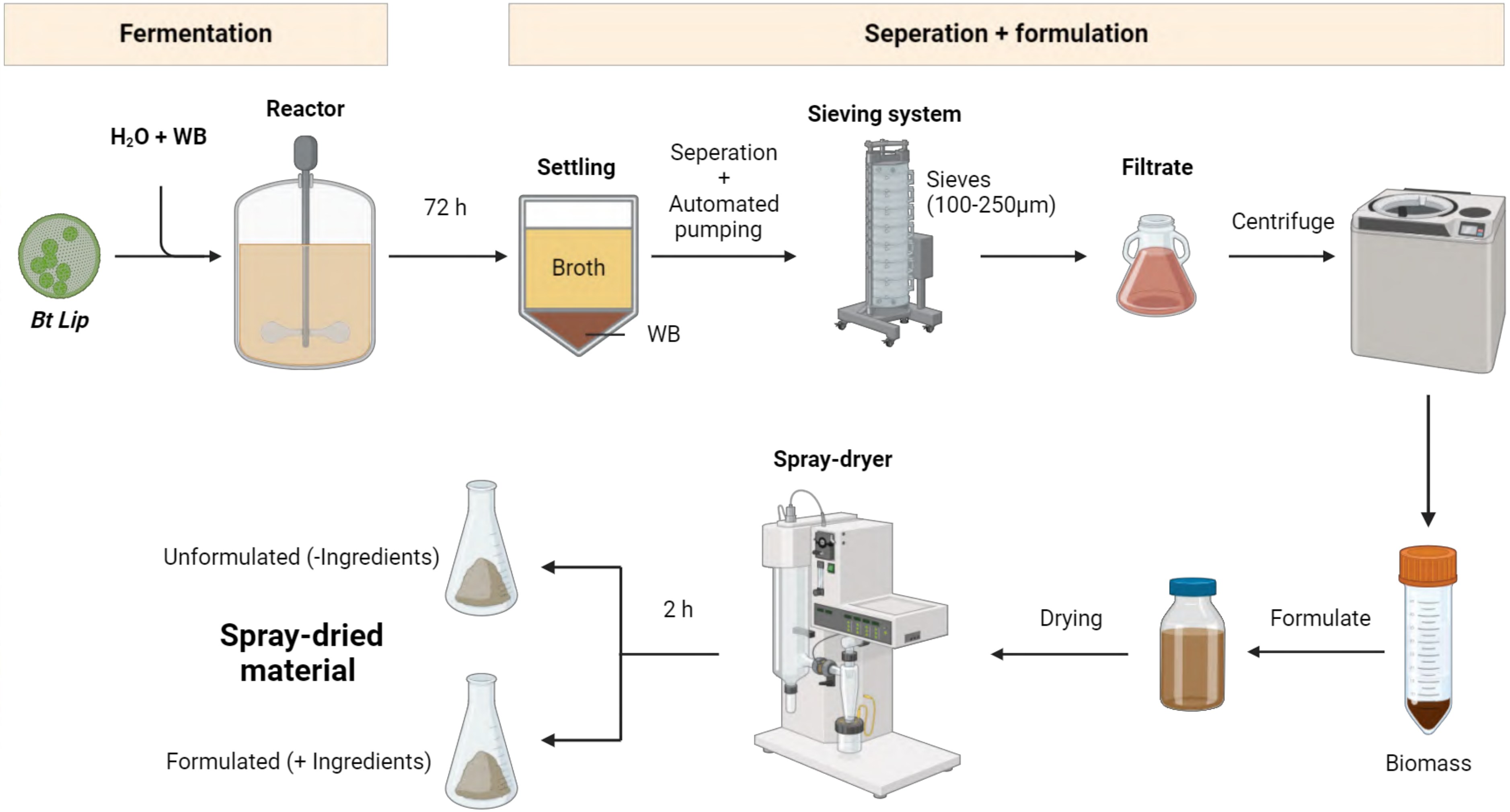
- Protein content
- LC_{50} vs *S. frugiperda*
- Viable spore concentration
- Particle size

Efficacy tests

- Lab assays & Greenhouse assay: *Tuta absoluta*

Alternative drying technique: DSP

Down stream process: Description of the production chain

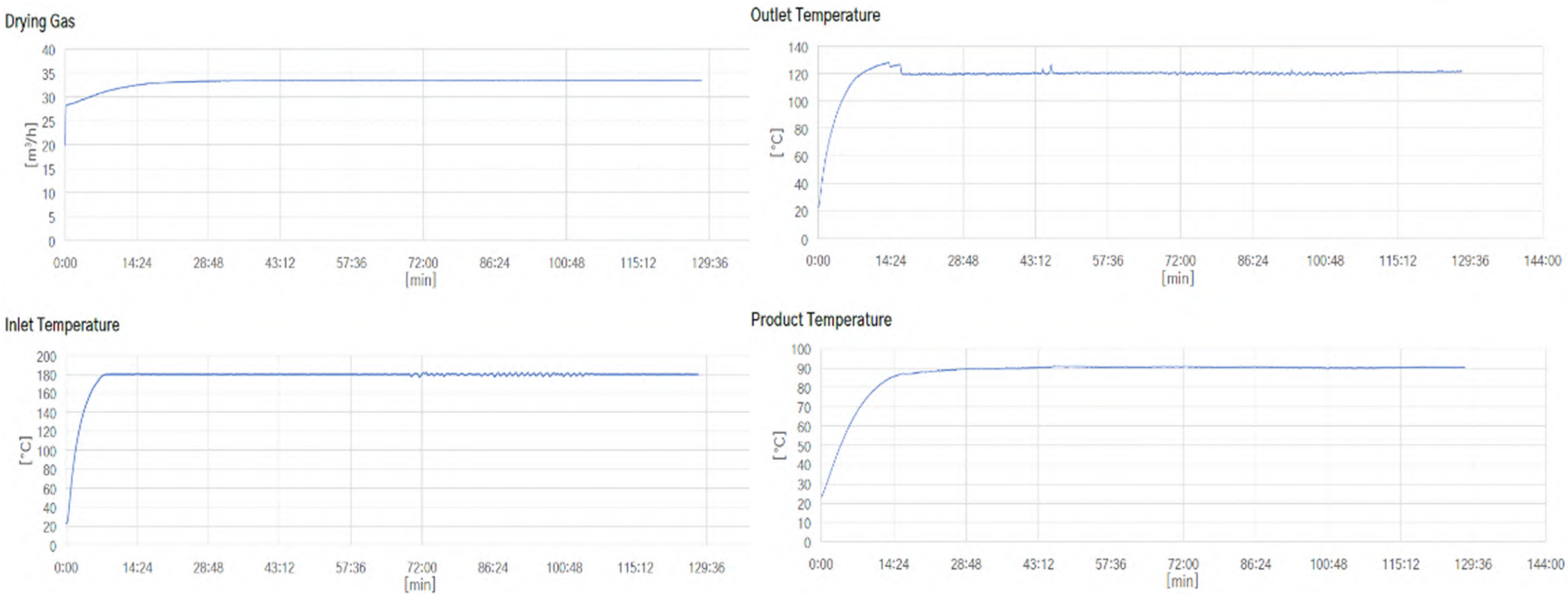


Per batch:

Biomass = 38 g- 42g
Dry weight= 14%-22%
Powder weight = 7-10 g

Lip_Unformulated_MIX
Lip_Formulated_MIX

Alternative drying technique: Spray-drying monitoring



Drying Gas: 35 m³/h, **Inlet Temperature:** 180 °C, **Spray Gas:** 750 L/h, **Pump:** 2.5 mL/min, **Outlet Temperature:** 120 °C

Product Temperature: 80-90 °C

Quality assessment

Table 3: *Various quality experiments describing and comparing the three products*

Products	Protein (mg/g)	LC_{50} (mg/ml) vs. *S. frugiperda*	95 % confidential interval		Viable spore concentration (CFU/ml)	Coarse (Dec>30 µm)
Delfin	116.9	1.10	0.25	2.72	1.2e+13	562
Lip_U_Mix	144.4	0.77	0.22	1.38	2.1e+13	273
Lip_F_Mix	120.8	0.66	0.16	1.095	2.4e+13	291

Higher protein content

Lower LC_{50}

Higher viable spore concentration

Smaller particle size

Efficacy tests

Tuta absoluta lab assay

Figure 3. Infected *Tuta absoluta* 2nd *stage larvae.*

Lab assay design

	0.125 mg/ml	0.25 mg/ml	0.5 mg/ml	0.75 mg/ml	1 mg/ml
LIP_F	10 larvae	10 larvae	10 larvae	10 larvae	10 larvae
LIP_U	10 larvae	10 larvae	10 larvae	10 larvae	10 larvae
DELFIN	10 larvae	10 larvae	10 larvae	10 larvae	10 larvae
CONTROL (Water)	10 larvae	10 larvae	10 larvae	10 larvae	10 larvae

Tp=25 °C, 96h

- 50 larvae per product
- 200 larvae per assay

600 larvae in total

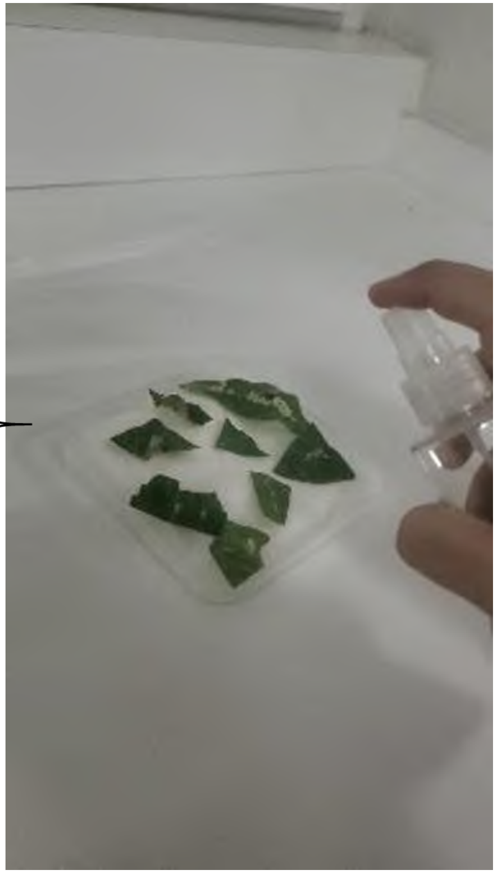
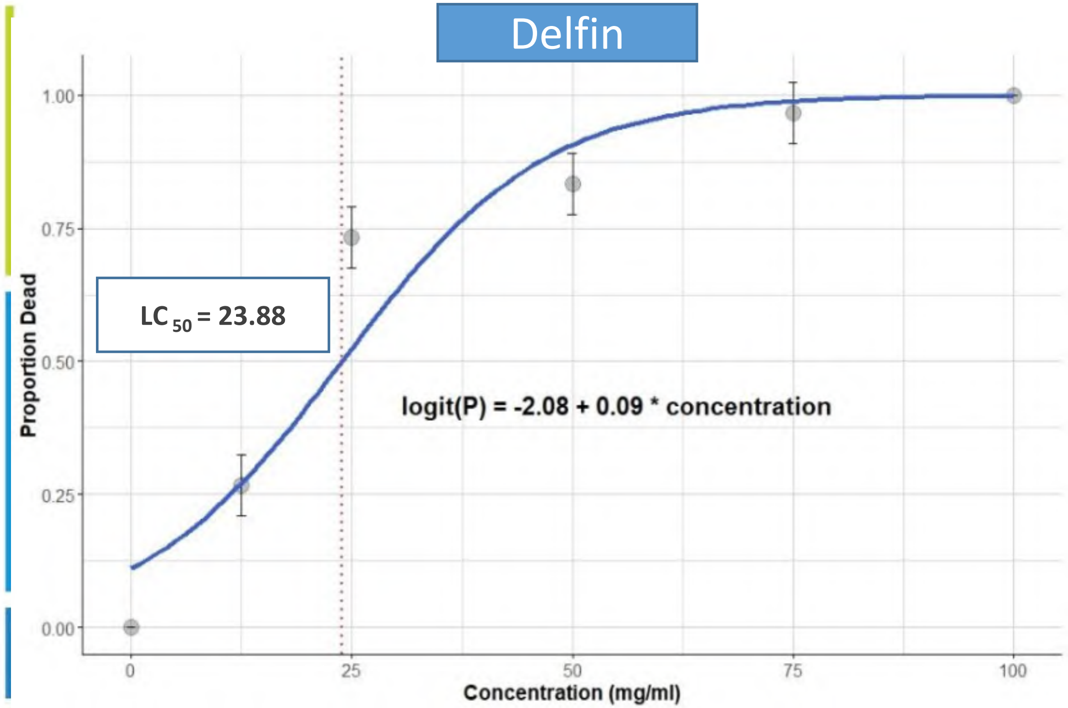
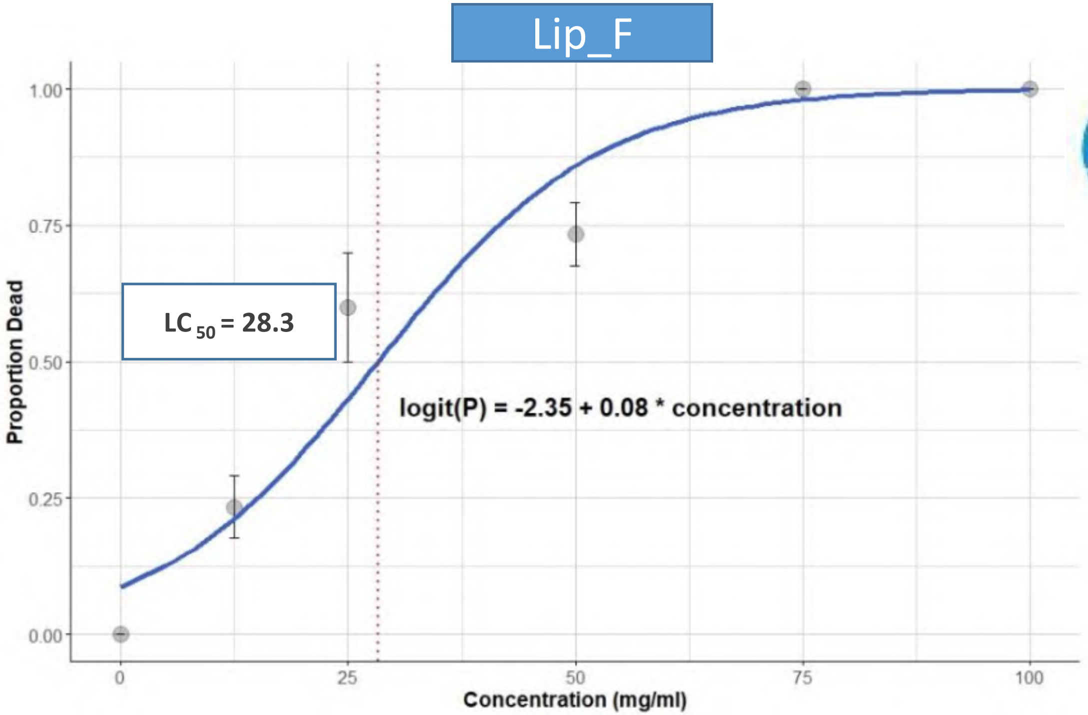


Figure 4. *Bioassay design of Tuts absoulta.*





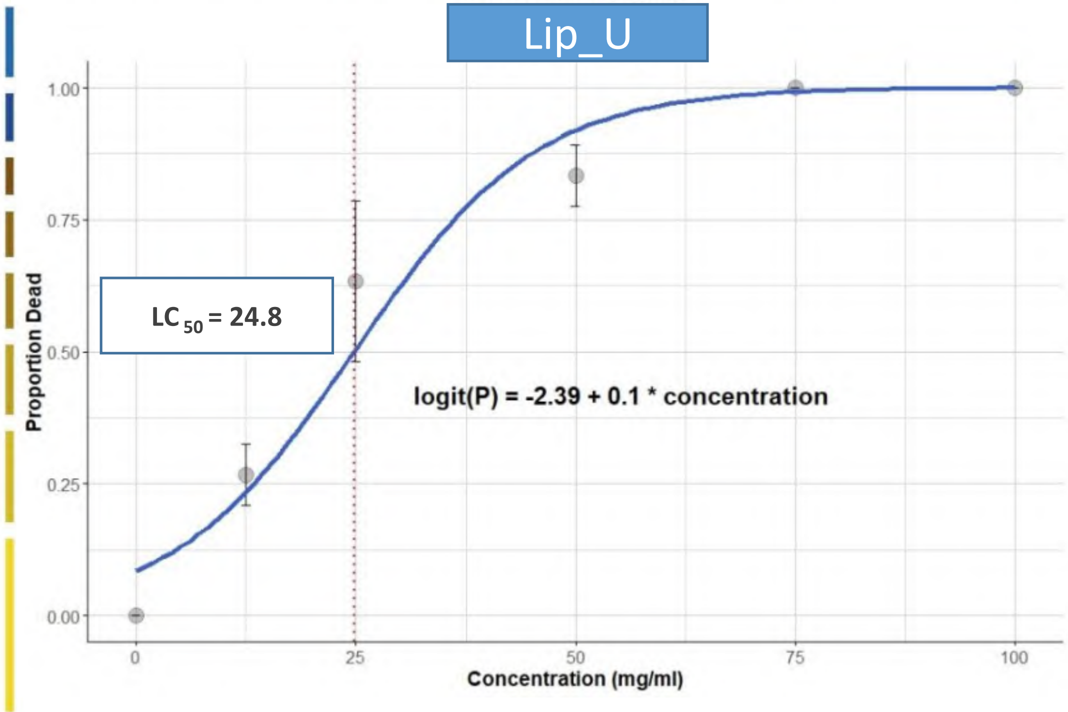


Figure 4. *Logisitc regression curve of Delfin, Lip_U and Lip_F indicating the lethal concentration of 50 %.*

After 48 h:

LC_{50}: Delfin < Lip_U < Lip_F

Efficacy tests

Tuta absoluta Greenhouse assay

Randomized Complete Block Design (RCBD)

- ❑ 4 treated blocks
- ❑ 4 iterations for each treatment
- ❑ Barrieres to prevent edge effects
- ❑ Gaps to prevent cross-contamination
- ❑ 160 plants

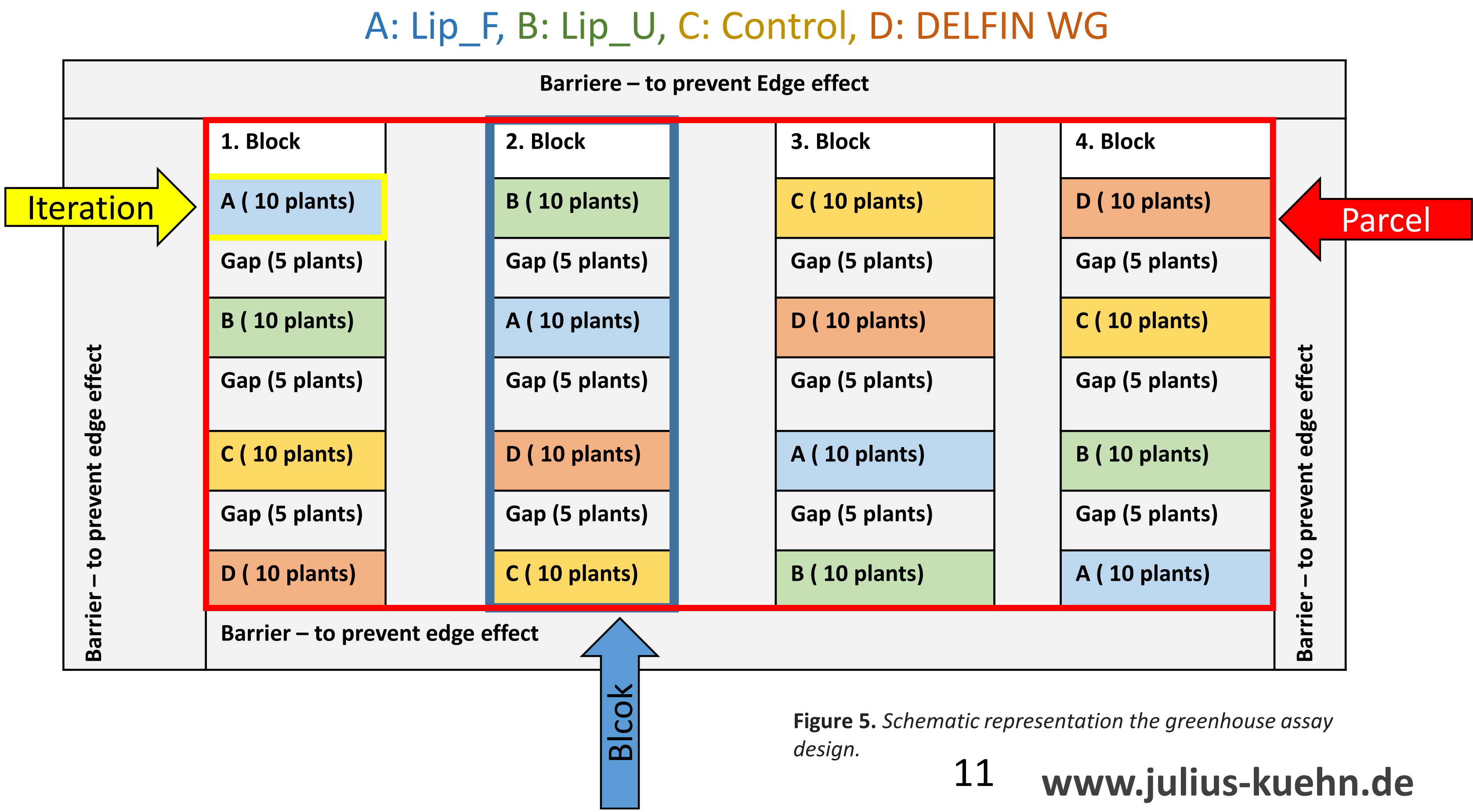


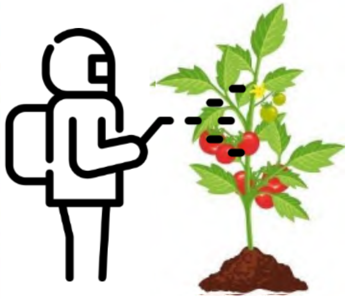
Figure 5. *Schematic representation the greenhouse assay design.*

Efficacy tests

Tuta absolута Greenhouse assay

Day 0

100 leaves per product



- Sampling before spraying
- Spraying
- Sampling after spraying

1st day

100 leaves per product

- Sampling
- Counting

3rd day

100 leaves per product

- Sampling
- Counting

5th day

100 leaves per product

- Sampling
- Counting

7th day

100 leaves per product

- Sampling
- Counting

14th day

100 leaves per product

- Sampling
- Counting

Efficacy tests

Tuta absoluta Greenhouse assay

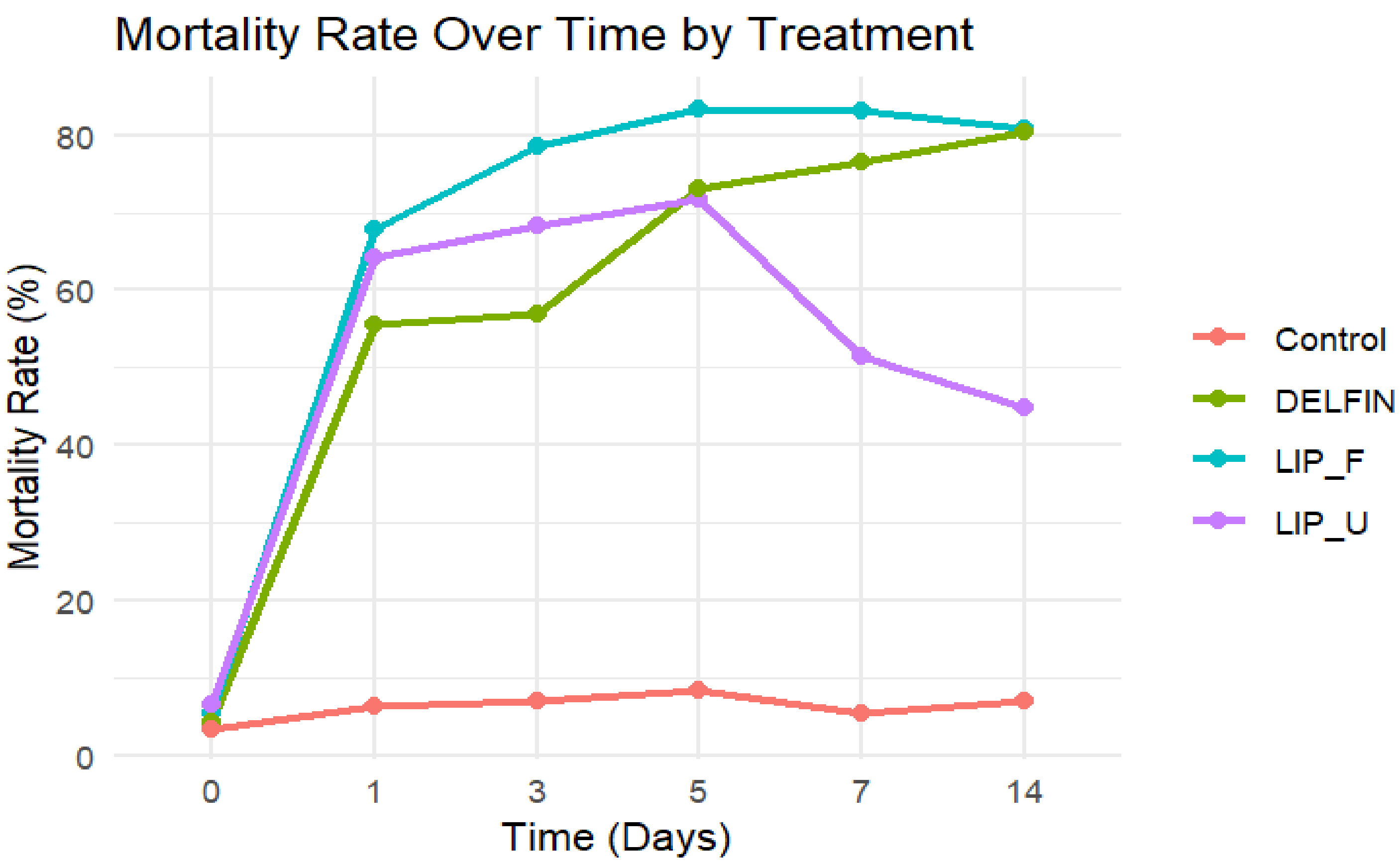


Figure 6. *Percentage of mortality rate of Tuta absoluta over time of each treatment in the greenhouse*

Descriptive plot :

LIP_F was more effective than Delfin

The absence of co-ingredients made LIP_U less effective for 14 days.

Conclusion

Alternative drying technique

- ✓ Spores and crystals can resist high temperature
- ✓ Spray drying concept approved

Quality assessment

- ➢ Lip_F:
  - [Prot]: **120.2 mg/g**
  - [Spore]: **2.4e+13 Cfu/ml**
  - Coarse: **291 µm**

Efficacy tests

- ✓ Proved efficacy of LIP_F against two model insects.
- ✓ Importance of using UV protectant in the formulation

Perspectives

1. Enhancing our fermentation process
   - Overcoming physical and biochemical limitations
   - Computational modelling (JKI-TBI)

2. Optimizing the spray drying technique and the formulation
   - Protective agents, bioorganic ingredients

3. Re-assessing our final product
   - *Field assays following EPPO guidelines*
     - *Prays oleae* / Olive
     - *Ectomyeloïs ceratoniae* / Pomegranate
   - UV stability
   - Solubility and Suspendability

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Thank you !

SAFWA Prima

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