IPM-4-CITRUS

From Research from Lab ...to Market ...to Field

Risk assessment and environmental impact

Environmental risk assessment on Non-target organisms

Presented by Dr. Rim EL JENI, IPT

Rim EL JENI, Sayda DHAOUADI, Hazar KRAIEM, Gul AYYILDIZ, Zakaria BENLASFAR, Zeynep YURTKURAN, Balkiss BOUHAOUALA-ZAHAR

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

IPM-4-Citrus, Final Meeting (19th & 20th December 2022, Nabeul & Hammamet, TUN)

IPM-4-CITRUS

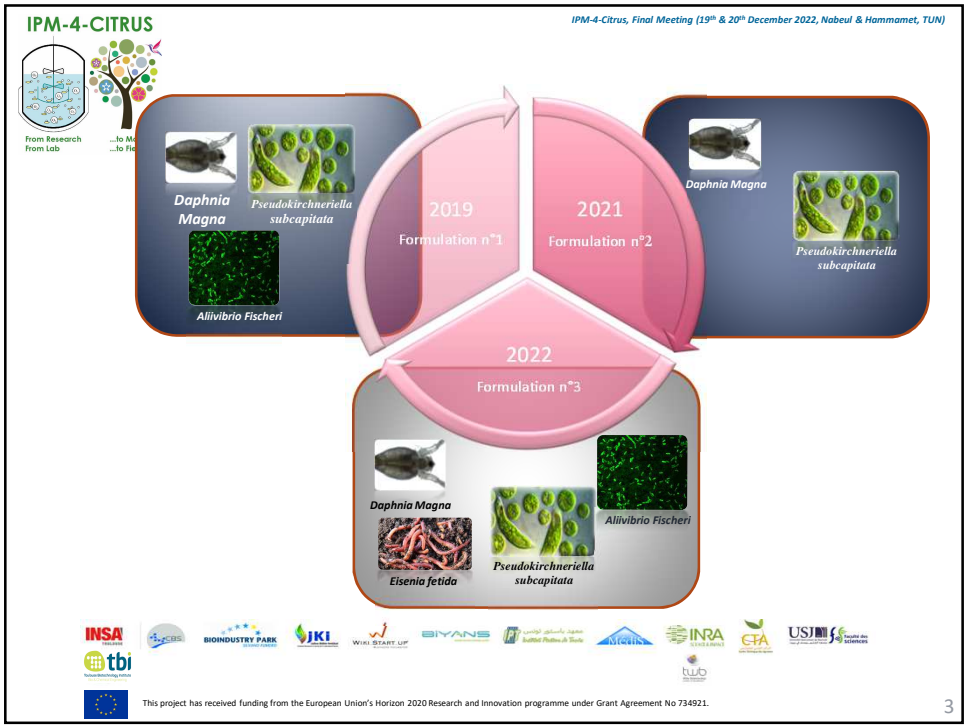
From Research from Lab ...to Market ...to Field

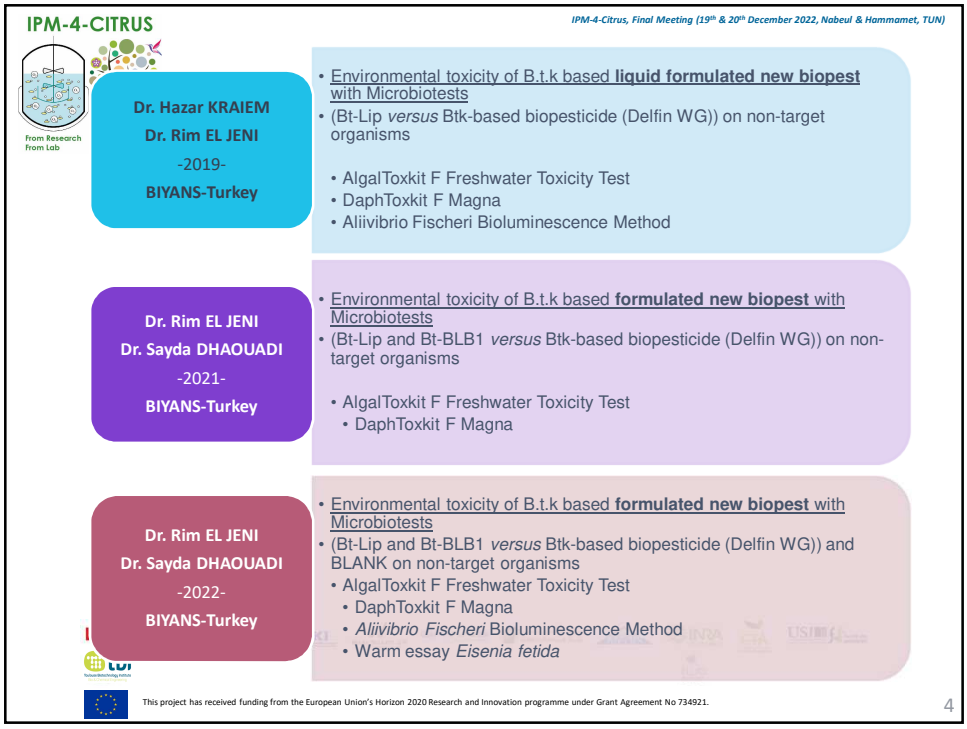
Secondment Specific Objectives: IPT →BIYANS

Objective 1: ToolBox preparation of risk assessment on non target organisms according to guidelines (ISO; OECD and EPA), regulations and procedures.
Objective 2: Setting up the general procedures of ecotoxicological studies
Objective 3:Non target organisms toxicity test for screening of formulated biopesticides potent toxicity.

	Tests		Faisability	Risk characterization/toxicity value
Environmental risk assessment	Aquatic organisms	Freshwater Daphnids (Daphnia magna)	✔	50% effect concentration (EC50)
		Aliivibrio Fischeri luminescent bacteria	✔	
		Fish. (Zebrafish)	No applicable	
	Aquatic plants	Freshwater Algae (Pseudokirchneriella subcapitata)	✔	
	Terrestrial invertebrates	Warm	✔	

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.


IPM-4-CITRUS
IPM-4-Citrus, Final Meeting (19th & 20th December 2022, Nabeul & Hammamet, TUN)
From Research from Lab
Daphnia Magna
Pseudokirchneriella subcapitata
Aliivibrio Fischeri
2019
Formulation n°1
2021
Formulation n°2
Daphnia Magna
Pseudokirchneriella subcapitata
2022
Formulation n°3
Daphnia Magna
Aliivibrio Fischeri
Eisenia fetida
Pseudokirchneriella subcapitata
This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.
3


IPM-4-CITRUS
IPM-4-Citrus, Final Meeting (19th & 20th December 2022, Nabeul & Hammamet, TUN)
From Research from Lab
Dr. Hazar KRAIEM
Dr. Rim EL JENI
-2019-
BIYANS-Turkey
• Environmental toxicity of B.t.k based liquid formulated new biopest with Microbiotests
• (Bt-Lip versus Btk-based biopesticide (Delfin WG)) on non-target organisms
• AlgalToxkit F Freshwater Toxicity Test
• DaphToxkit F Magna
• Aliivibrio Fischeri Bioluminescence Method
Dr. Rim EL JENI
Dr. Sayda DHAOUADI
-2021-
BIYANS-Turkey
• Environmental toxicity of B.t.k based formulated new biopest with Microbiotests
• (Bt-Lip and Bt-BLB1 versus Btk-based biopesticide (Delfin WG)) on non-target organisms
• AlgalToxkit F Freshwater Toxicity Test
• DaphToxkit F Magna
Dr. Rim EL JENI
Dr. Sayda DHAOUADI
-2022-
BIYANS-Turkey
• Environmental toxicity of B.t.k based formulated new biopest with Microbiotests
• (Bt-Lip and Bt-BLB1 versus Btk-based biopesticide (Delfin WG)) and BLANK on non-target organisms
• AlgalToxkit F Freshwater Toxicity Test
• DaphToxkit F Magna
• Aliivibrio Fischeri Bioluminescence Method
• Warm essay Eisenia fetida
This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.
4

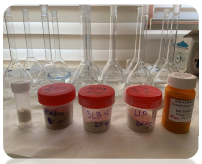
IPM-4-CITRUS

From Research from Lab ...to Market ...to Field

Training / Skills

ALGALTOXKIT F KIT

FORMULATED PRODUCTS



Batch-2022-

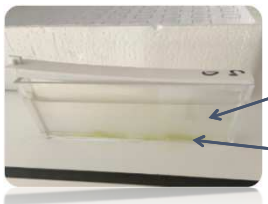
Batch-2021-

- **Endotoxin concentrations** based on Bradford essay
- **Whole product** mass concentration

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

IPM-4-CITRUS

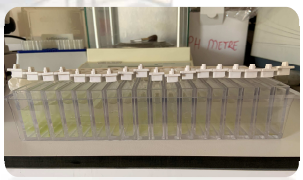
From Research from Lab ...to Market ...to Field



Turbidity related to bacteria growth

Algae growth

Symbiosis between Algae and bacteria growth



This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

IPM-4-CITRUS

From Research from Lab ...to Market ...to Field

2021-Secondment

Toxicity test of formulated unfiltered samples with microalgae

BLB1 and Delfin Biopesticides

Preliminaries result

Table 1. Formulated *Bacillus thuringiensis* BLB1 biopesticide on microalgae *S. capricornutum*

Concentration (mg/L)	Nb replicates	Average effect	Standard deviation	% growth
Control	3	100	173.2051	173.2051
6.25	3	2932.644	67.59008326	2.304749
12.5	3	3422.622	35.32717482	1.032167
25	3	3736.27	146.5959535	3.923591
50	3	4202.27	18.32483879	0.43607
100	3	4695.853	-	-

Table 2. Toxicity data of the formulated commercial product Delfin biopesticide on microalgae *S. capricornutum*

Concentration (mg/L)	Nb replicates	Average effect	Standard deviation	% growth
Control	3	100	3.832086	3.832086
6.25	3	258.4719	12.1296236	4.692821
12.5	3	307.3604	3.939721018	1.281792
25	3	353.041	2.767488399	0.47839
50	3	396.8418	2.509655322	0.632407
100	3	436.1724	0.755699215	0.173257

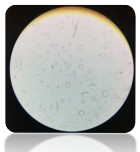
Toxicity test of filtered formulated sample with microalgae

LIP Biopesticides

Preliminaries result

Table 3. Toxicity data of the formulated *Bacillus thuringiensis* LIP biopesticide on microalgae *S. capricornutum*

Concentration (mg/L)	Nb replicates	Average effect	Standard deviation	% growth
Control	2	100	16.29874	16.29874
6.25	2	29.22175	4.394103769	15.0371
12.5	2	40.19435	11.12349294	27.67427
25	2	109.5072	7.718240477	7.048158
50	2	179.0633	1.778254354	0.993087
100	2	212.1565	0.804098594	0.379012



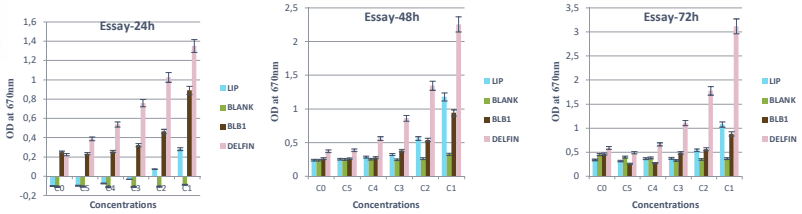
This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

IPM-4-CITRUS

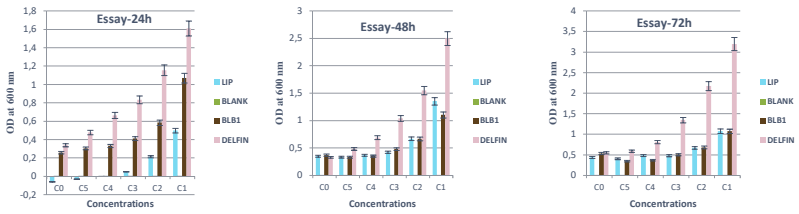
From Research from Lab ...to Market ...to Field

Main results-2022-

Algae growth detection at 670nm



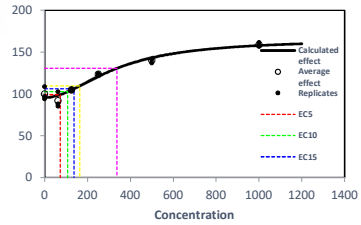
Bacteria growth detection at 600nm



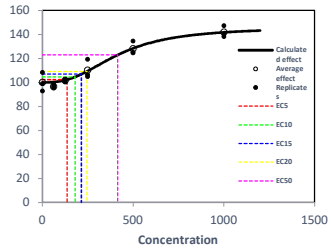
This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

IPM-4-CITRUS

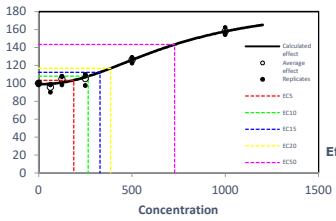
Main results-2022-



Effect of Formulated *Bt.* ***DELFIN*** biopesticide on microalgae *S. capricornutum.* (EC50 336,8129215)



Effect of Formulated *BT.* **BLB1** biopesticide on microalgae *S. capricornutum.* (EC50 415,4702746)



Effect of Formulated *Bt.* ***LIP*** biopesticide on microalgae *S. capricornutum.* (EC50 728,8094799)

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

9

IPM-4-CITRUS

Part II

Assessement of the acute toxicity of Btk-biopesticides on non-target organisms/ **The water flea Daphnia magna Straus**

Btk-biopesticides Monitoring toxicity purposes

The effects of various Btk-biopesticides concentrations on the sensitivity response of *Daphnia magna.*

Strategy I

Testing the Btk-biopesticides whole products taking into account **endotoxins concentration**

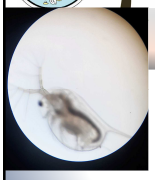
Strategy II

Testing the Btk-biopesticides taking into account whole products mass concentration

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

IPM-4-CITRUS

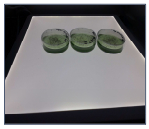
Training / Skills



Assessement of the acute toxicity of Btk-biopesticides on non-target organisms/ **The water flea Daphnia magna Straus**

- ✓ **Test Organism:** Acute toxicity test conducted with **Daphnia magna crustaceans.**
- ✓ **Regulatory framework:** Assay endorsed by ISO 6341 and OECD 1984/2004.
- ✓ **Microbiotest:** Daphtoxkit F magna

Hatching conditions of cryptobiotic eggs



Neonates hatched from dormant eggs.

Preparation of Btk-biopesticides serial concentrations.

Assessement endpoint: 24h/48h

Effect criterion: mortality/immobility

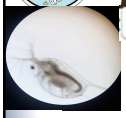
This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

IPM-4-CITRUS

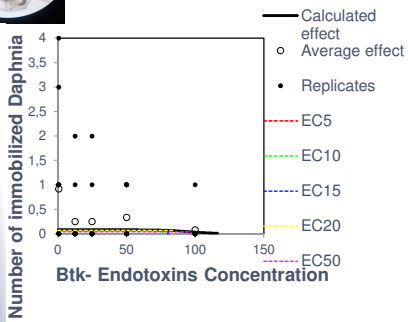
IPM-4-Citrus, Final Meeting (19th & 20th December 2022, Nabeul & Hammamet, TUN)

Main Results 2021

Determination of the EC50

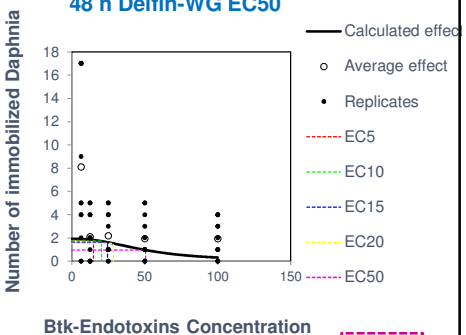


48 h Lip EC50



EC5	EC10	EC15	EC20	EC50
70,335639	76,286686	80,220877	83,316115	**96,865899**

48 h Delfin-WG EC50



EC5	EC10	EC15	EC20	EC50
14,855110	20,280975	24,592538	28,433580	**50,662879**

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

IPM-4-CITRUS

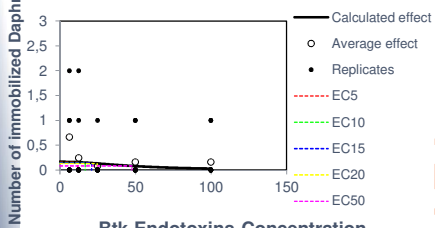
Main Results 2021

Strategy I

Assessement of the effects of various Btk-endotoxins concentrations (0,6.25,12.5,25,50,100 mg/L) on the sensitivity response of ***Daphnia magna after 48 h exposure time.***

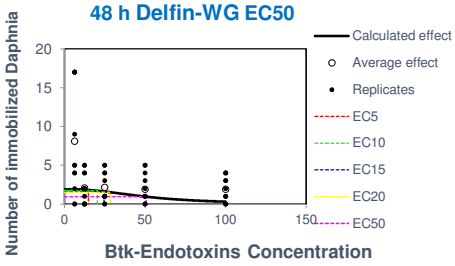
Determination of the EC50

48 h BLB1 EC50



EC5	EC10	EC15	EC20	EC50
53,936641	61,635778	66,943753	71,239757	91,25

48 h Delfin-WG EC50



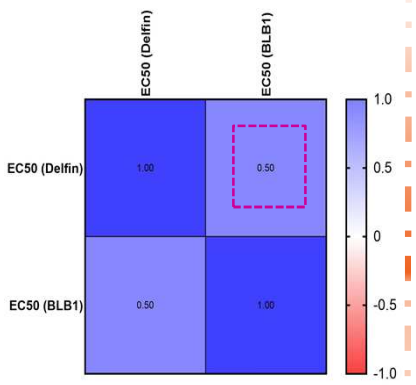
EC5	EC10	EC15	EC20	EC50
14,8551101	20,280975	24,592538	28,433580	50,6628794

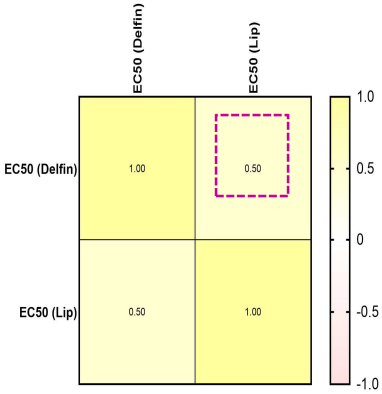
This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

IPM-4-CITRUS

Results 2021

The effects of various Btk-biopesticides concentrations on the sensitivity response of *Daphnia magna*.

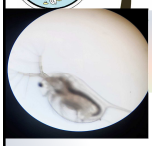




This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

IPM-4-CITRUS

Main Results 2022



Assessement of the effects of various formulated Btk-biopesticides concentrations on the sensitivity response of ***Daphnia magna***

The effects of various Btk-biopesticides concentrations on the sensitivity response of *Daphnia magna*.

Strategy II

Testing the **Btk-biopesticides** taking into account whole products mass concentration

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

IPM-4-CITRUS

Main Results 2022

Btk formulation-based biopesticides (LIP)

Alive Daphnia magna treated and exposed to the **Lip** for 48h

Immobilized Daphnia magna treated and exposed to the **Lip** for 48h

Non lethality/ non toxic effects.

➢ The inhibition of the mobility of few Daphnia magna straus is inherent to biopesticide tested powder.

Btk formulation-based biopesticides (BLB1)

Daphnia magna treated and exposed to **BLB1** for 48h.

Non lethality/ non toxic effects

Dead Daphnia magna treated an exposed to BLB1 for 48h.

Blank formulation

Immobilized Daphnia magna treated and exposed to the **Blank** for 48h.

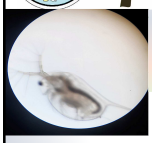
Non lethality/ non toxic effects.

Mortality is related to abiotic condition which has effects on viability.

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

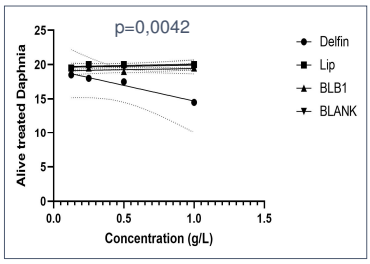
IPM-4-CITRUS

Main Results 2022



Assessement of the effects of various formulated Btk-biopesticides concentrations on the sensitivity response of ***Daphnia magna***.

The effects of various Btk-biopesticides concentrations on the sensitivity response of *Daphnia magna*.



After 48h exposure: **Daphnia magna is more sensitive to Delfin than Lip and BLB1**

Dose-response curves of Daphnia magna acute toxicity tests with Delfin, Lip, BLB1 and BLANK.

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

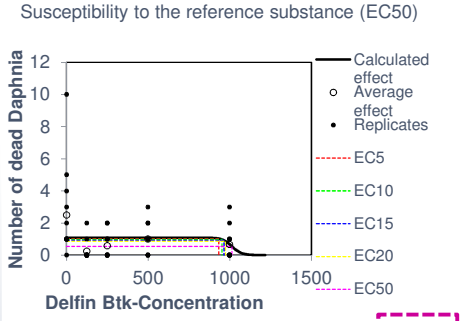
IPM-4-CITRUS

Main Results 2022

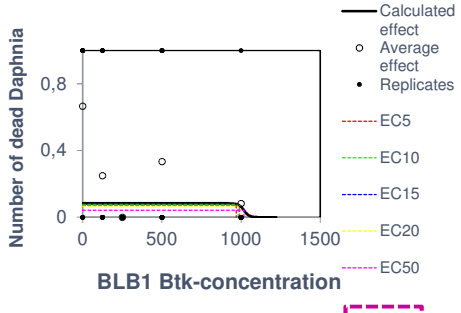
from Research from Lab ...to Market ...to Field

Determination of the EC50

Susceptibility to the reference substance (EC50)



EC5	EC10	EC15	EC20	EC50
932,341456	952,27555	964,83007	974,39158	1013,3956



EC5	EC10	EC15	EC20	EC50
969,5477	981,6233	989,2611	995,0193	1018,233

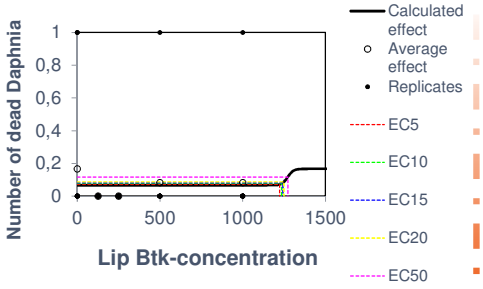
This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

IPM-4-CITRUS

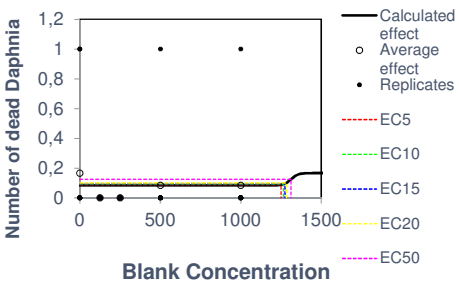
From Research from Lab ...to Market ...to Field

Main Results 2022

Determination of the EC50



EC5	EC10	EC15	EC20	EC50
1249,67	1265,14	1275,09	1282,559	1312,5



EC5	EC10	EC15	EC20	EC50
1249,67	1265,14	1275,09	1282,559	1312,5

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

IPM-4-CITRUS

From Research from Lab ...to Market ...to Field

Conclusions (PartII)

- **Abnormal behaviour was observed for Daphnia treated with Delfin:**
  - ✓ **Excessive increase of turnings----> Disturbances in movement coordination**
  - ✓ **Swiming behaviour disruption.**
  - ✓ **Circular movements (Movement track).**
  - ✓ **Twiching the whole body.**
- **No significant lethal effect was recorded for Daphnia treated with Lip, BLB1 and BLANK formulation**
- **Rate of immobilized Daphnia exposed to BLANK is 2%: These Daphnia lost their ability to swim as they are sticked and stucked into the Blank powder (same for Lip).**
- **Under progress (Dr,Sayda Dhaouadi Internship in progress) : Acute toxicity test on Fetida Eisenia worms.**
- **Planned to perform Acute toxicity test on Aliivibrio Fischeri**

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

IPM-4-CITRUS

From Research from Lab ...to Market ...to Field

A Strong scientific exchanges between **BIYANS** and **IPT:**
Partnership elaboration with public and private sectors (**2019-2022**)

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

IPM-4-CITRUS

From Research from Lab ...to Market ...to Field

THANKS to…

IPM-4 CITRUS-IPT Members -TUNISIA


Pr. Balkis BOUHAOUALA
Teamleader, NanoBioMedica, LBVAT


Dr. Hazar KRAIEM
Postdoctoral Researcher
IPT/MediS pharmaceutical Industry.


Dr. ZAKARIA BENLASFAR
Consultant Veterinary Doctor.


Dr. Rim EL JENI
Postdoctoral Researcher
IPT/INSTM


Dr.Sayda DHAOUADI
Post-Doctoral Researcher
IPT

IPM-4 CITRUS- BIYANS Members -TURKEY


Dr Zeynep Yurtkuran Ceterez
BIYANS CEO


Dr Gul Ayyildiz
BIYANS team member

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.

THANK YOU FOR YOUR ATTENTION

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.