



# The screening of new active ingredients against *Varroa destructor*

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# The Véro-pharma R&D strategy



# What weapons do we have today to fight varroa mites?

**All the veterinary based medicines are based on 7 active ingredients (= molecules):**

- ▶ Amitraz (Apitraz, Apivar)
- ▶ Oxalic acid dihydrate (ApiBioxal, Oxybee, VarroMed\*, Dany Bienenwohl)
- ▶ Formic acid (MAQS, Formic Pro, VarroMed\*)
- ▶ Thymol (Apiguard, Thymovar, Apilife Var)
- ▶ Tau-fluvalinate (Apistan)
- ▶ Flumethrin (Polyvar Yellow, Bayvarol)
- ▶ Coumaphos (Checkmite)

# What is the Vétô-pharma strategy for new solutions?

## Reformulation of existing active ingredients



- ▶ To improve efficacy and reduce variation in treatment outcome
- ▶ To simplify the use and increase safety for the beekeeper
- ▶ Minimize the negative impact of temperature variations (e.g. formic acid, thymol)
- ▶ To propose a better management of the varroa infestation throughout the year (in many situations, a single treatment is not enough)

## Research of new active ingredients



- ▶ Synthetic and organic
- ▶ To enable beekeepers to develop a real strategy of integrated pest management (rotation, prevention of potential resistance ...)

=> « Varroa 2.0 » project



## How do we do that?



### A galenic and analytical development team

- ▶ Reformulation of existing actives
- ▶ Formulation of new actives
- ▶ Verification of the product stability and quality



### A « bee lab »

- ▶ Safe in-vitro testing of new actives or new formulations



### An experimental apiary with 250 colonies (Dadant 10 frames) and 2 beekeepers

- ▶ Sampling for in-vitro testing
- ▶ Efficacy monitoring of vet medicines
- ▶ Efficacy and tolerance monitoring (on development, brood, adult bees...) of new formulations

Reactivity



A microscopic view of a honeycomb cell containing several Varroa mites. The mites are small, reddish-brown, oval-shaped organisms with visible legs and internal structures. They are clustered together on the surface of the honeycomb cell. The background is a warm, golden-brown color, likely due to the lighting of the honeycomb.

## The « Varroa 2.0 » project

# Objective and methods

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# Objectives?

## Identify new active ingredients to fight varroa mites:

- ▶ Organic or conventional (chemical)
- ▶ Effective
- ▶ Non-toxic for bees
- ▶ Non-toxic for beekeepers
- ▶ Respecting the quality of the honey (residues)

## Three measured criteria:

- ▶ Varroa mite mortality over time (up to 24 hours)
- ▶ Bee mortality over time (up to 24 hours)
- ▶ Potential behavioral changes



About 40  
molecules have  
been screened  
over the past  
years

# Actions in the apiary



**Varroa mites sampling  
in the « mite concentrators »**

Sampling of 300 bees  
with powdered sugar



**Sampling of bees  
for screening**

## Actions in the Bee lab



Separation of varroa mites from powdered sugar after mite sampling



Collection of 10 mites per petri dish

# Intoxication of bees



Anesthesia of  
bees with CO<sub>2</sub>



Thoracic deposit of  
the active ingredient  
in solvent



Deposit  
of « intoxicated » bees  
in the boxes containing  
the mites



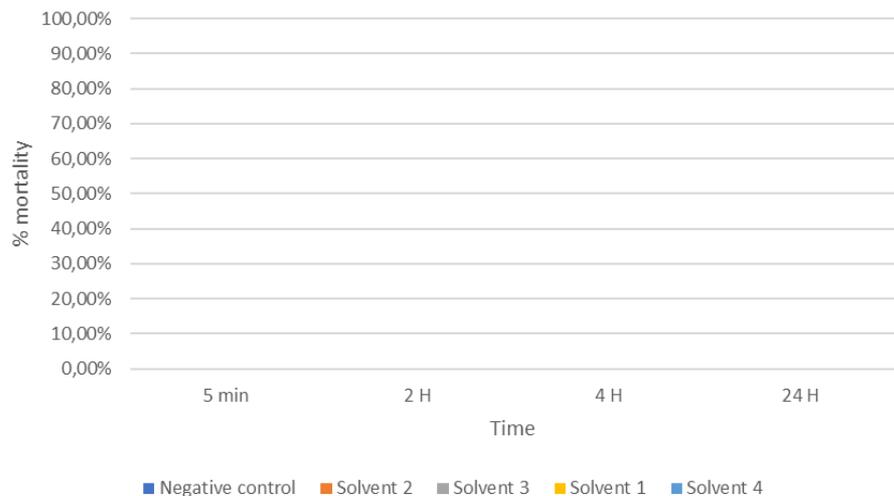
Incubator

# Protocol validation

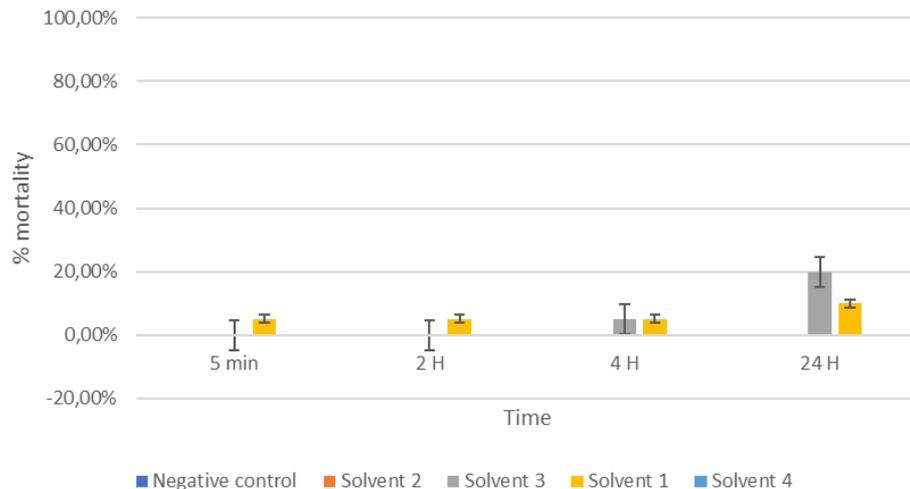
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# Choosing the right solvent

Bee mortality



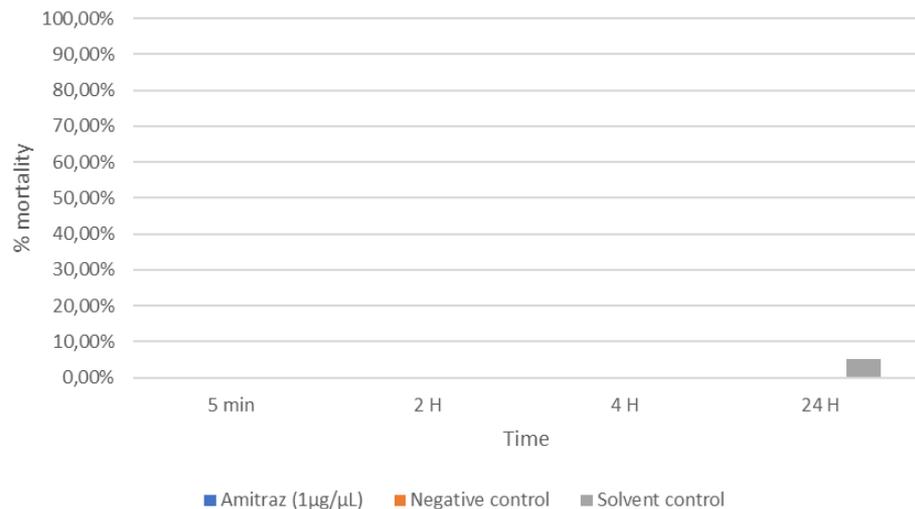
Varroa mite mortality



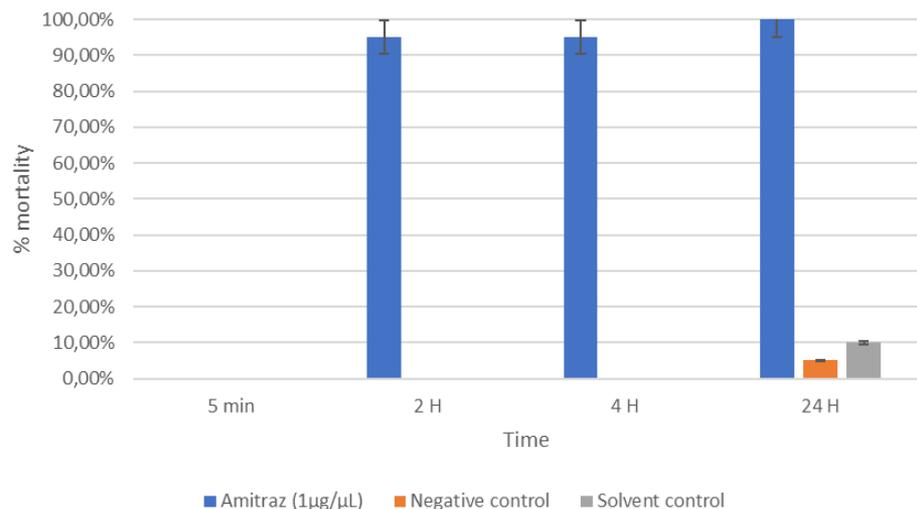
- ▶ Compatibility with the active to test
- ▶ Non-toxic for varroa mites (<10%)
- ▶ Non-toxic for bees (<10%)
- ▶ Possible application on the thorax of the bees
- ▶ Fast evaporation

# Positive control: amitraz

Bee mortality



Varroa mite mortality

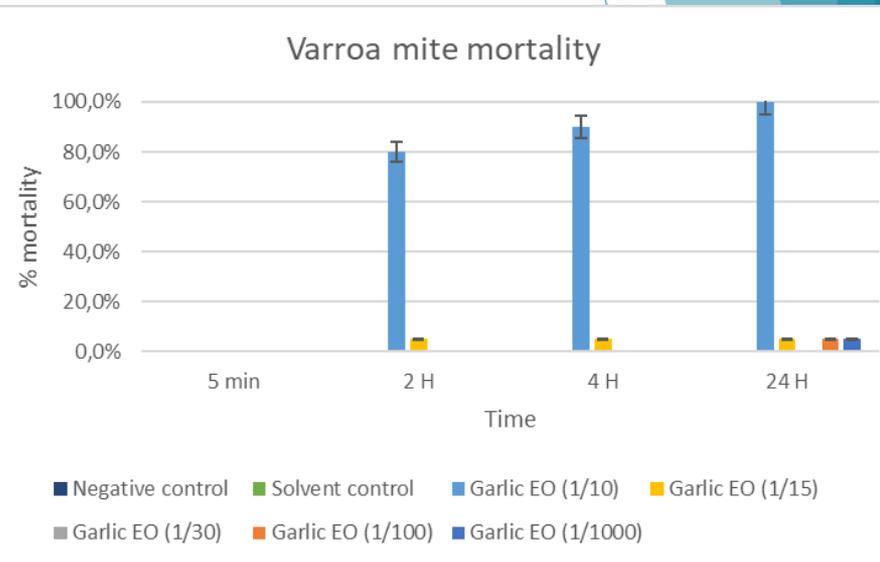
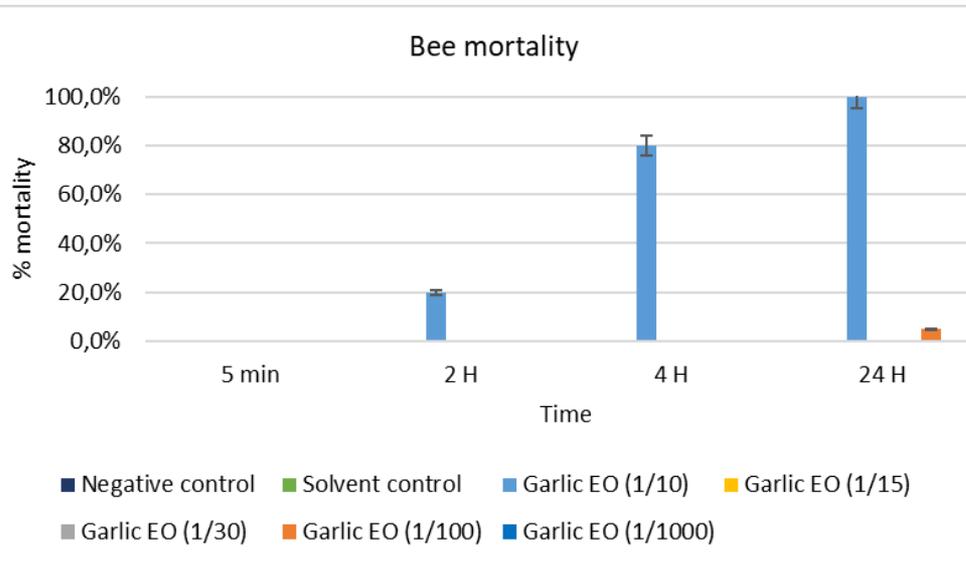


**Rapid efficacy of amitraz on varroa mite populations and non-toxicity for bees.**

# “Excluding” and “encouraging” results

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# Molecules to avoid (Garlic Essential Oil)

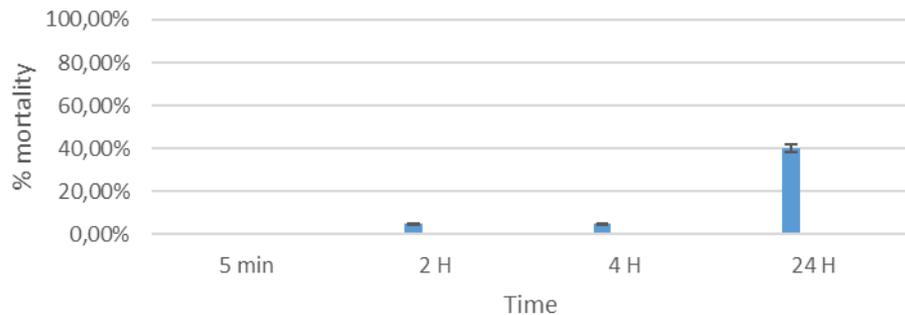


**Very effective against varroa mite but very high toxicity for bees.  
The effectiveness decreases quickly with dilution.**

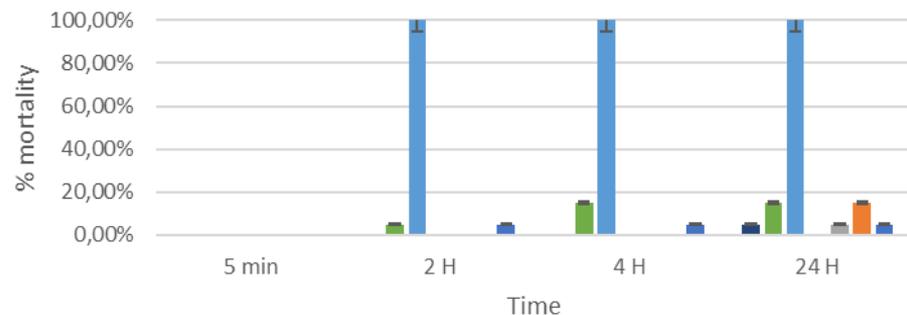
EO = Essential oil

# Molecules to avoid (Clove Essential Oil)

## Bee mortality



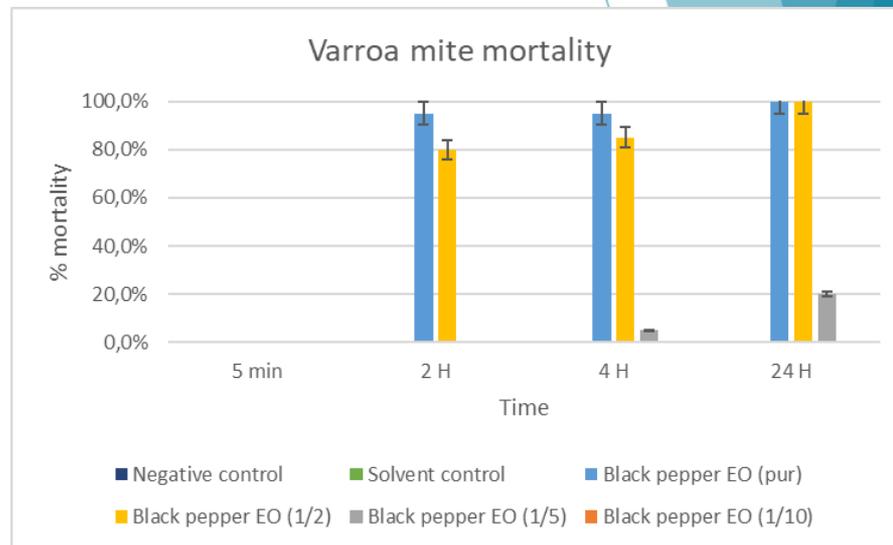
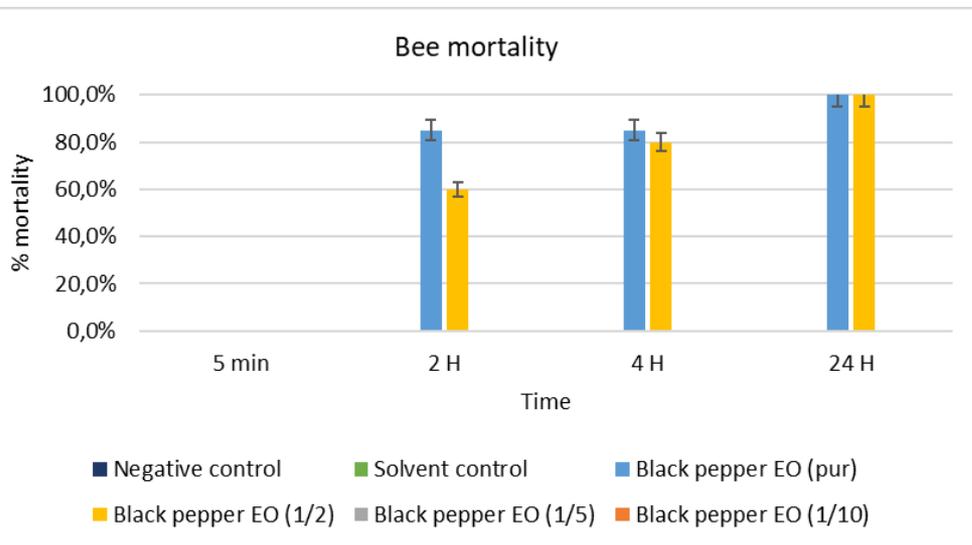
## Varroa mite mortality



**Very effective against varroa mite but very high toxicity for bees.  
The effectiveness decreases quickly with dilution.**

EO = Essential oil

# Molecules to avoid (Black pepper Essential Oil)

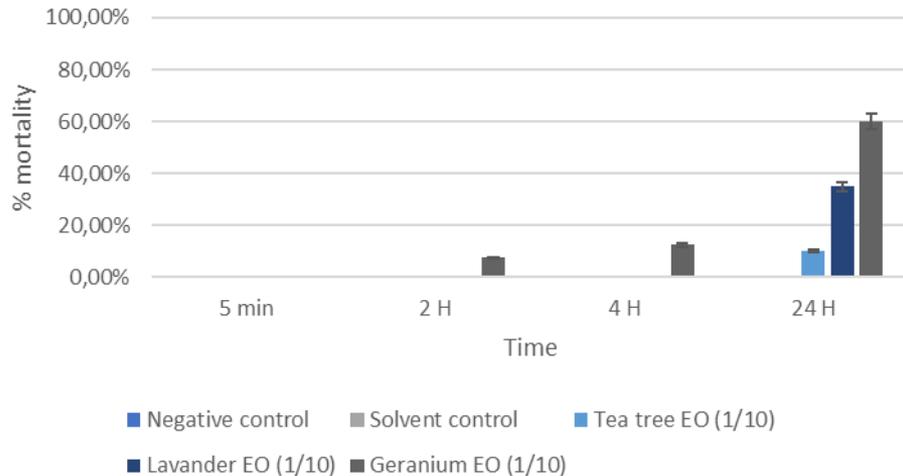


**Very effective against varroa mite but very high toxicity for bees.  
The effectiveness decreases quickly with dilution.**

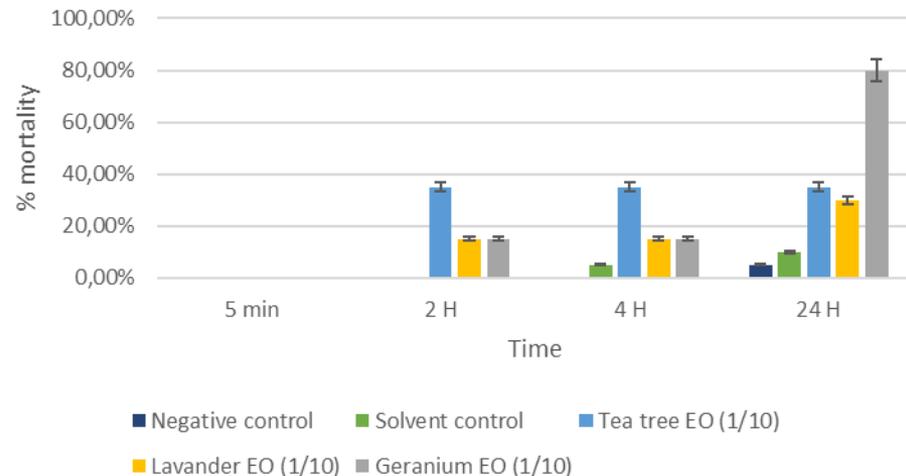
EO = Essential oil

# Molecules to avoid (Tea tree, Lavender and Geranium Essential Oils)

Bee mortality



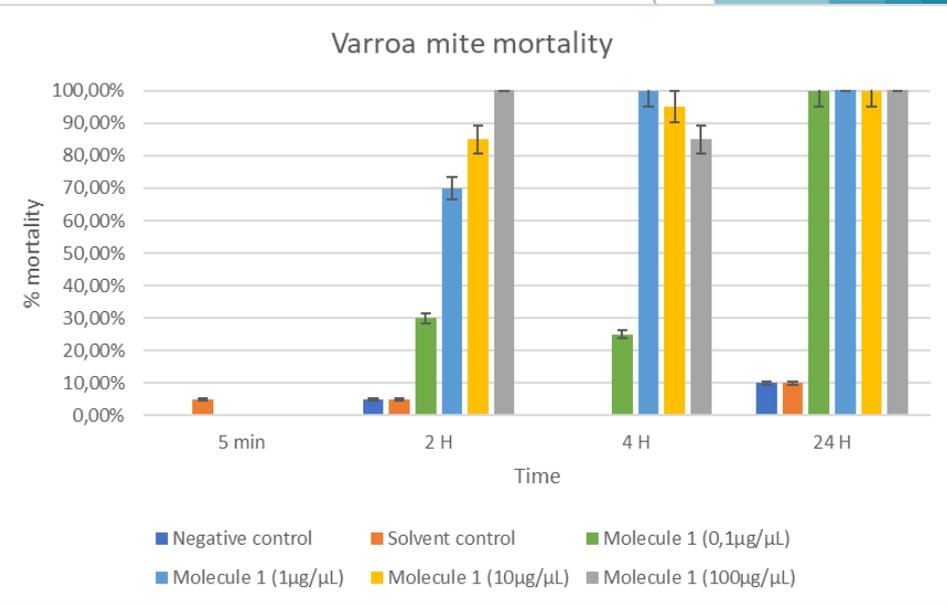
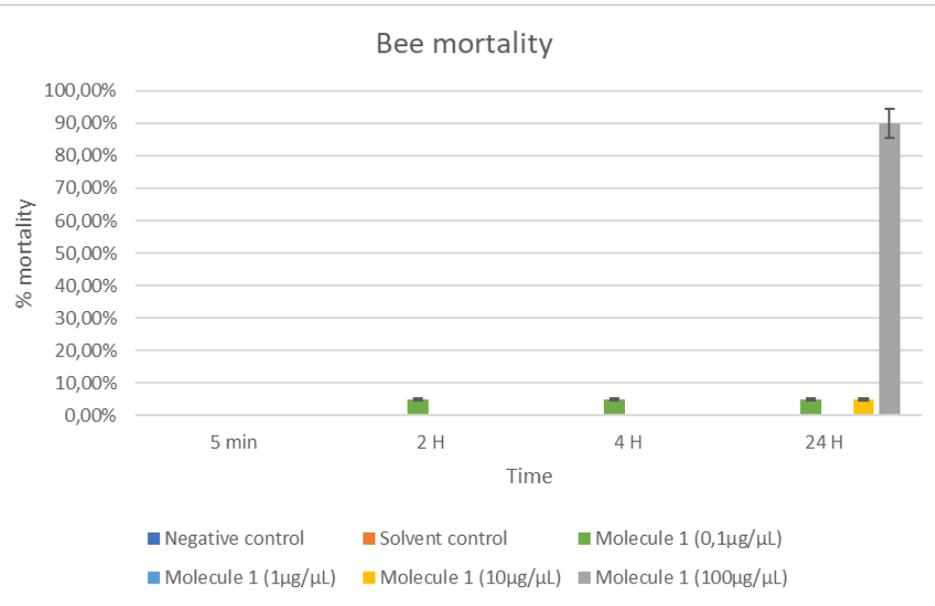
Varroa mite mortality



**Geranium EO is very effective against varroa mite but very toxic for bees.  
The two others are toxic as well and less effective.**

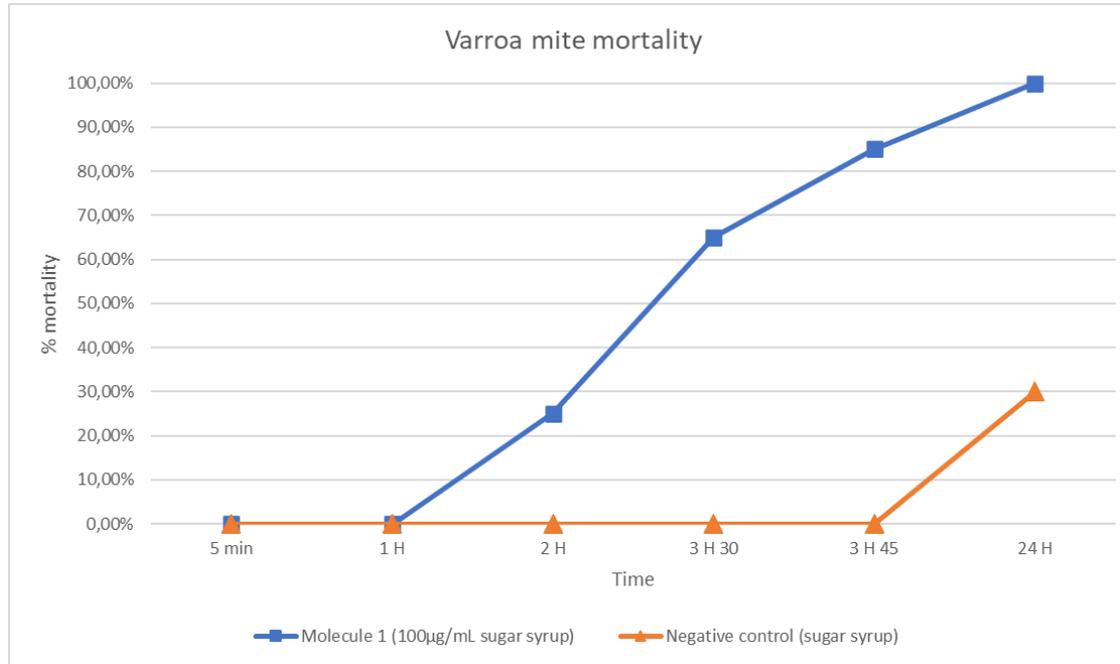
EO = Essential oil

# Promising molecules (molecule 1 - thoracic)



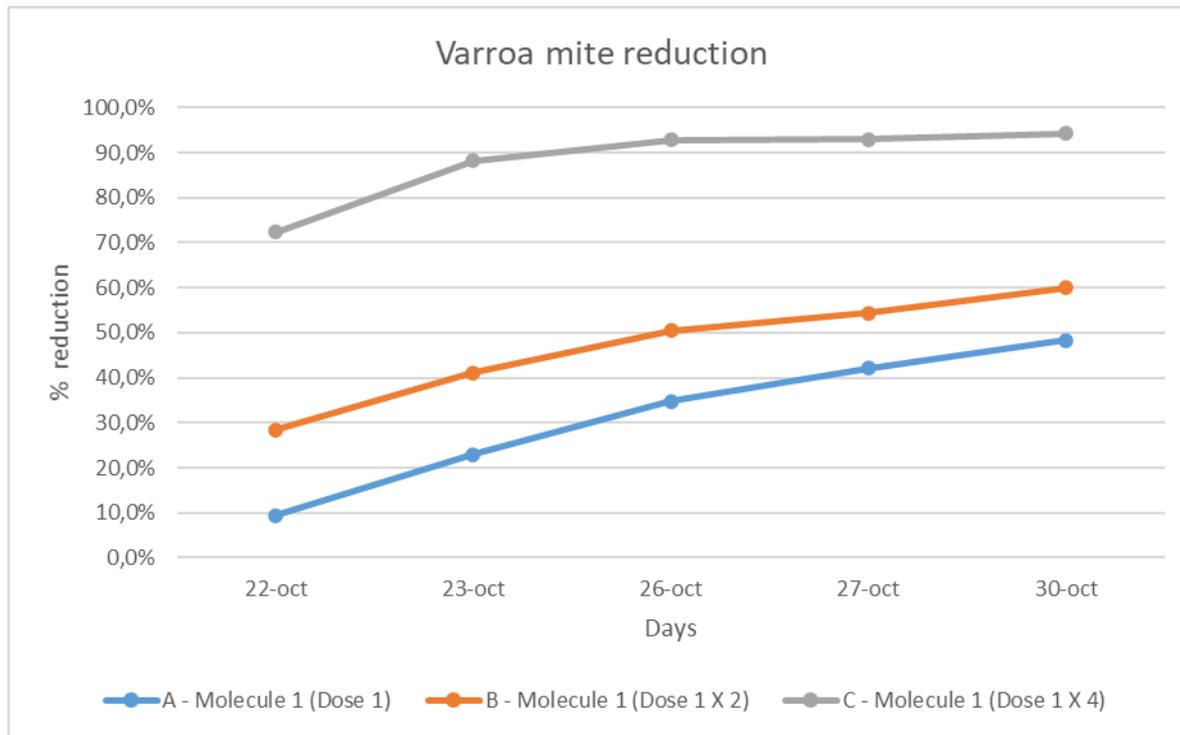
Same efficiency than amitraz (same kinetic).  
Very low toxicity for bees except for the higher concentration.

# Promising molecules (molecule 1 - ingestion)



**No difference between bee mortality in the two groups.  
Could the ingestion of the molecule lead to residues in the hive products  
or brood toxicity?**

# Promising molecules (molecule 1 – in hive - broodless)

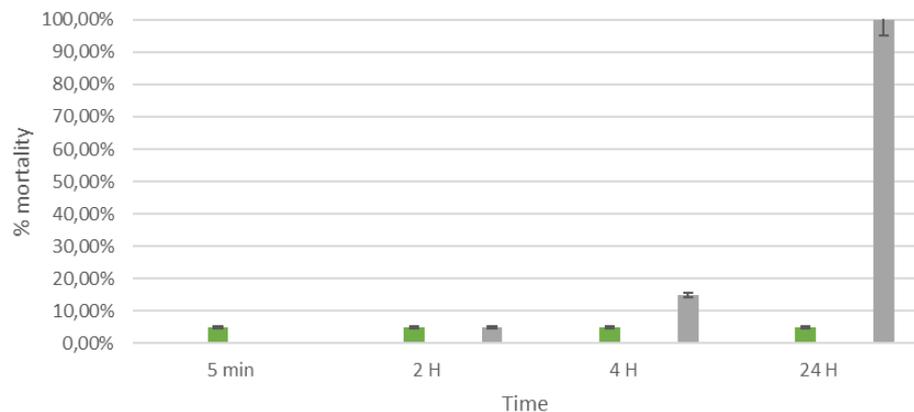


**94.3% varroa mite reduction after 10 days in broodless colonies.  
Only 3 hives per group.**

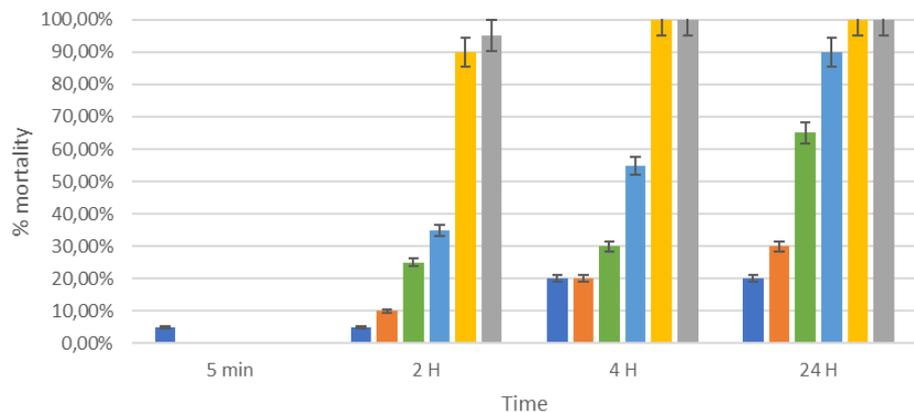
# Promising molecules

(molecule 3 = chemically related to molecule 1)

### Bee mortality



### Varroa mite mortality



Efficiency a little bit lower than molecule 1.  
Very low toxicity for bees except to the higher dose.

# Still some questions...and improvements...

- ▶ The way of intoxication on bees seems to have an impact on the results (thoracic / abdominal / feeding).
- ▶ What could be the effect of mixing different active ingredients?
  - ▶ Reduction of the toxicity for bees?
  - ▶ Reduction of efficiency against varroa mites?
- ▶ Test of new in-vitro trial with different ways of intoxication.
- ▶ Increase the observation time (some active ingredients could be very effective but with a slow kinetic).
- ▶ Understand the mechanism of action from a molecular point of view.

Identification of these molecules?

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## Numerous sources



### Partnerships with:

- ▶ Universities (France and abroad)
- ▶ Researchers (France and abroad)
- ▶ Independent and private structures (France and abroad)
- ▶ Beekeepers-researchers
- ▶ In all cases: **under confidentiality agreement** for protection of the inventor's data
- ▶ During congresses, conferences, mail, telephone...



### Internal resources:

- ▶ Bibliographic studies
- ▶ Internal thinking



### The importance of the project:

- ▶ Compare external results with an approved protocol
- ▶ Reproducibility of results



# Next steps

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# Preclinical development

## Identify the formulation of the new active

- ▶ In what form?
- ▶ With which support?
- ▶ Verification of the harmlessness for the bees
- ▶ Verification of the absence of residues
- ▶ Verification of product effectiveness
- ▶ Tolerance check
- ▶ Complete file on the molecule itself



# Clinical development

## Clinical trials

- ▶ Confirmation of veterinary medicine properties under normal conditions of use and in the field (total infestation, colony size, etc.).
- ▶ Provides the desired therapeutic effect
- ▶ Tests performed by an independent structure and subject to good laboratory practice (GLP) are also conducted for subsequent use of the data.

# Registration (market authorisation)

## Market authorisation

- ▶ Compilation of all data from preclinical and clinical trials
- ▶ Submission to the authorities:
  - ▶ National Agency for Veterinary Medicines (ANSES-ANMV) for France
  - ▶ Other national agencies
  - ▶ European Medicines Agency (EMA)
    - ▶ Centralized MAs for the European Union
- ▶ Evaluation of the benefit/risk ratio



Source: MSD Santé animale



# Véto-pharma

Committed to apiculture

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Merci !  
Thank you!  
Danke!  
¡Gracias!

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