



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



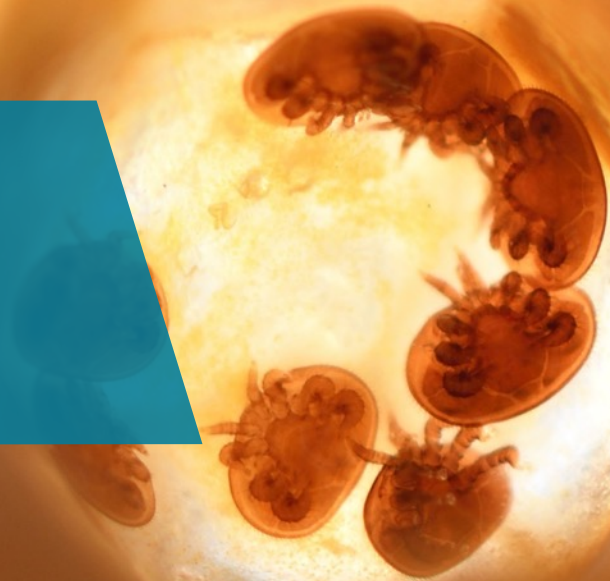
An experimental apiary with 250 colonies (Dadant 10 frames) and 2 beekeepers

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

Reactivity



The « Varroa 2.0 » project



Objective and methods

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

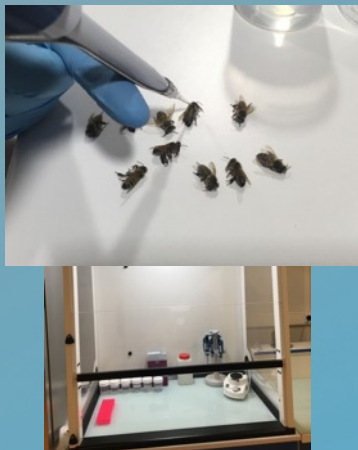


Collection of 10 mites per petri dish

Intoxication of bees



Anesthesia of
bees with CO₂



Thoracic deposit of
the active ingredient
in solvent



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

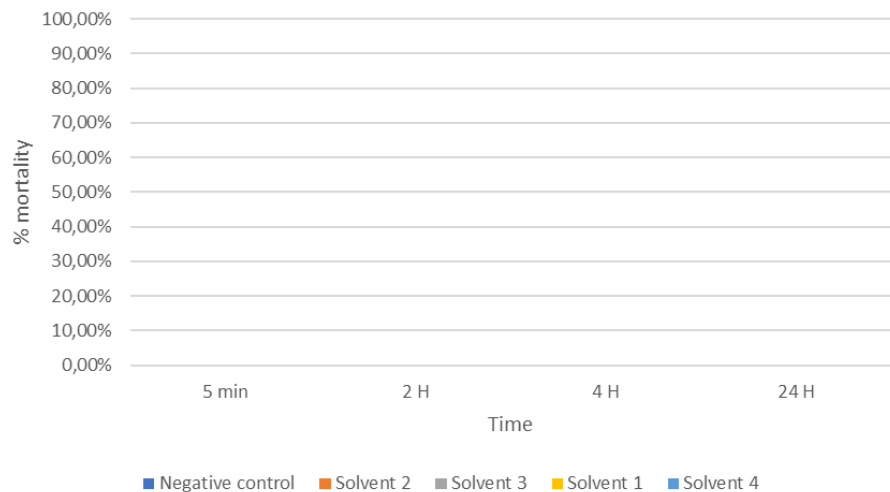


Incubator

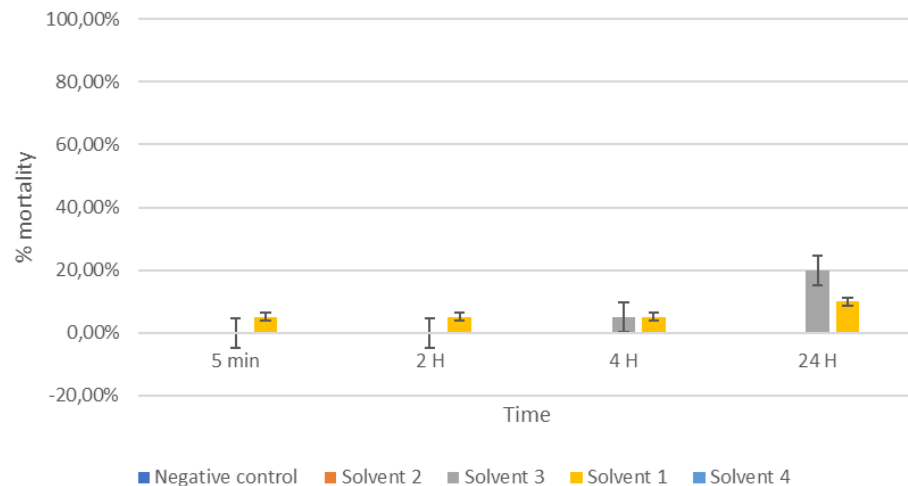
Protocol validation

Choosing the right solvent

Bee mortality



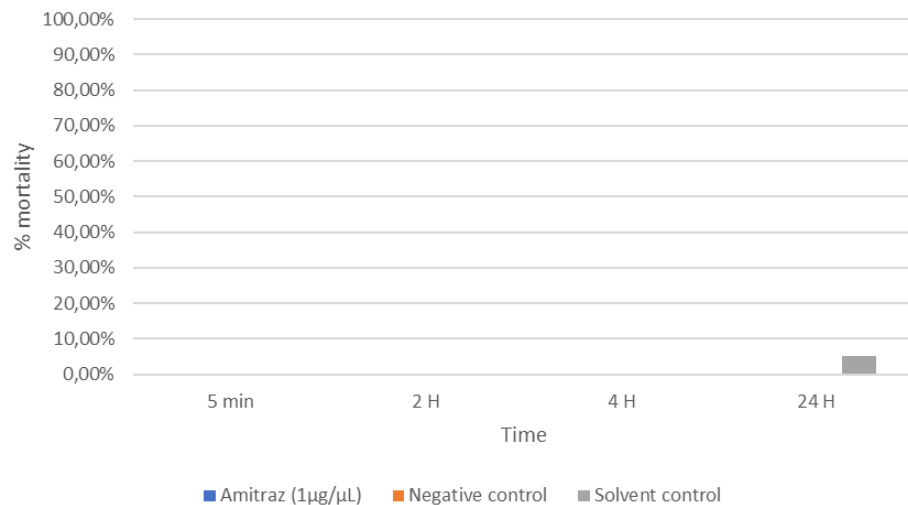
Varroa mite mortality



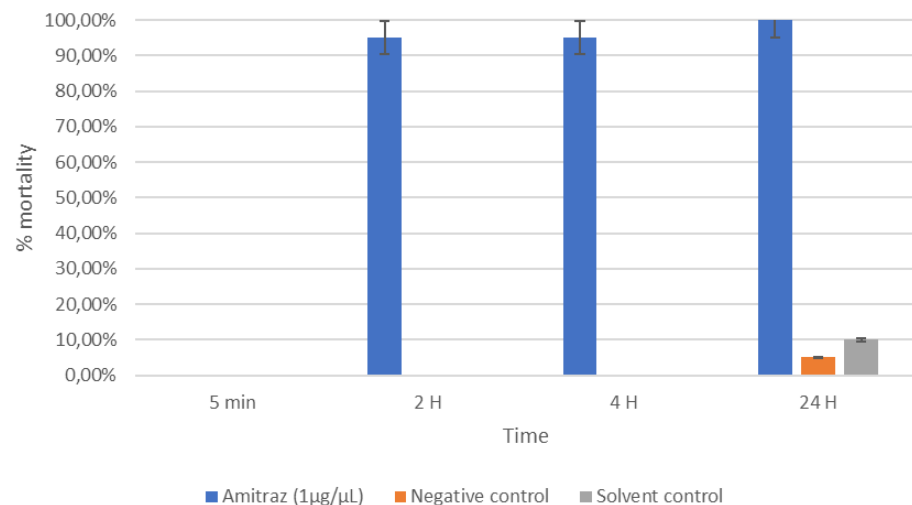
- Compatibility with the active to test
- Non-toxic for varroa mites (<10%)
- Non-toxic for bees (<10%)
- Possible application on the thorax of the bees
- Fast evaporation

Positive control: amitraz

Bee mortality



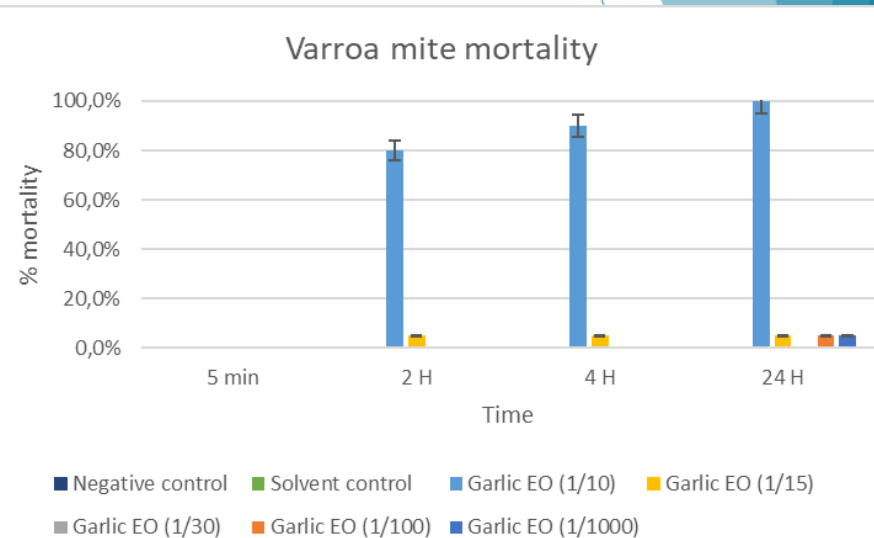
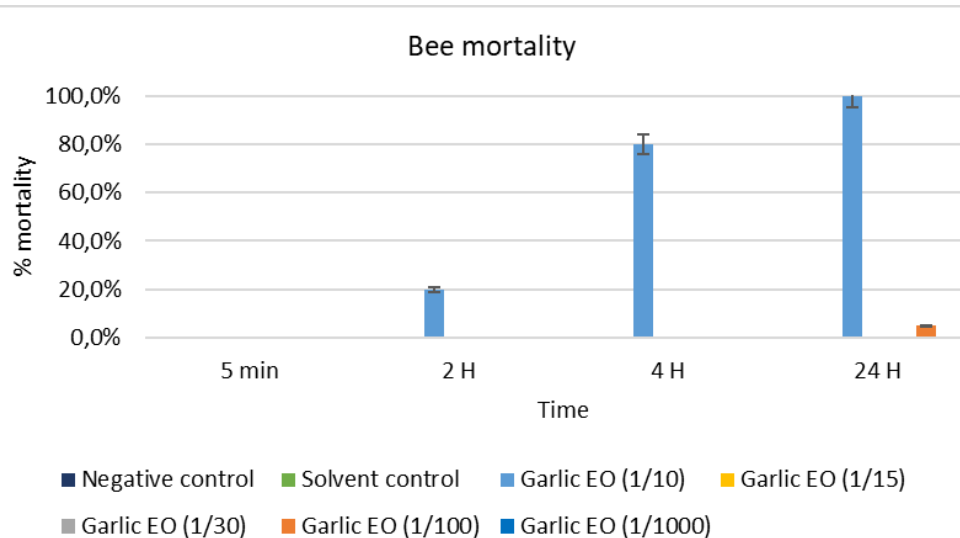
Varroa mite mortality



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

“Excluding” and “encouraging” results

Molecules to avoid (Garlic Essential Oil)

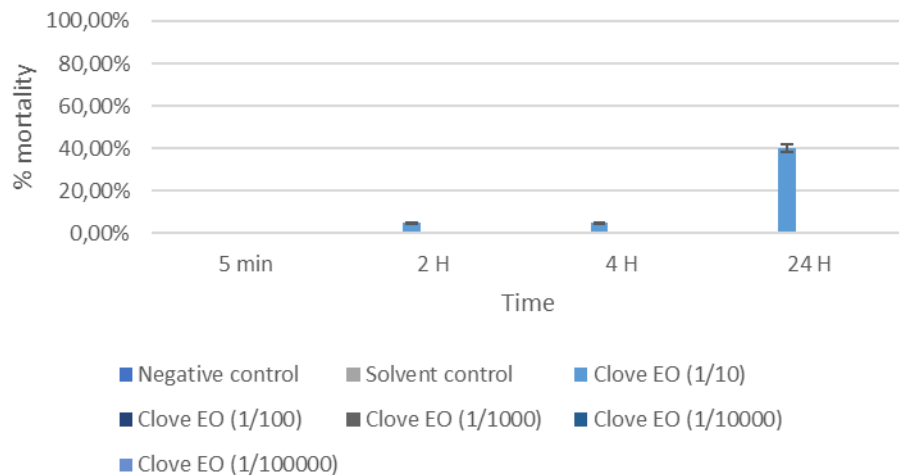


**Very effective against varroa mite but very high toxicity for bees.
The effectiveness decreases quickly with dilution.**

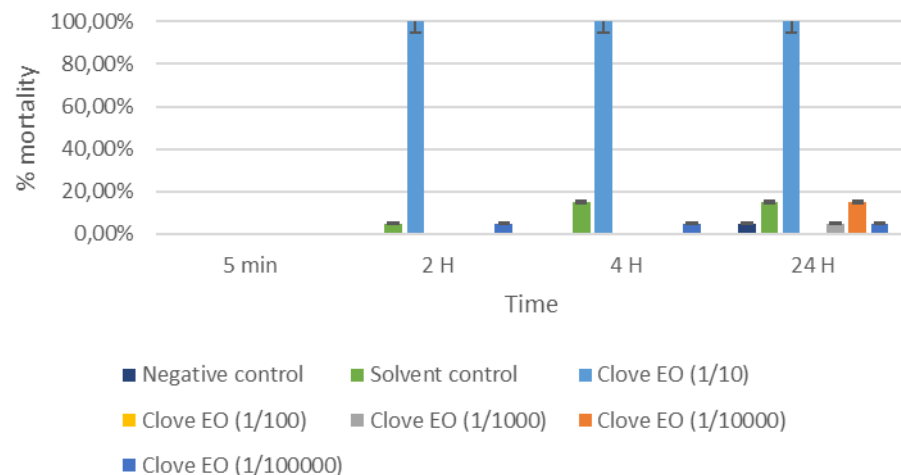
EO = Essential oil

Molecules to avoid (Clove Essential Oil)

Bee mortality



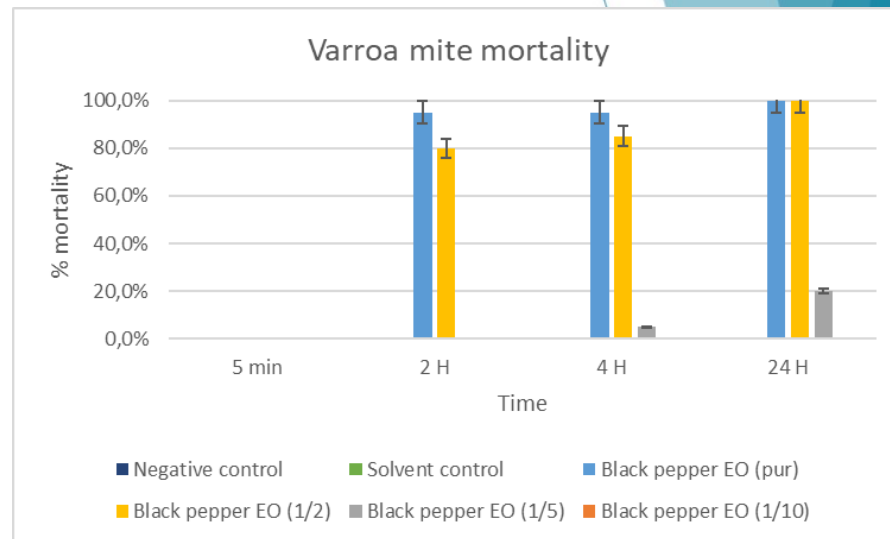
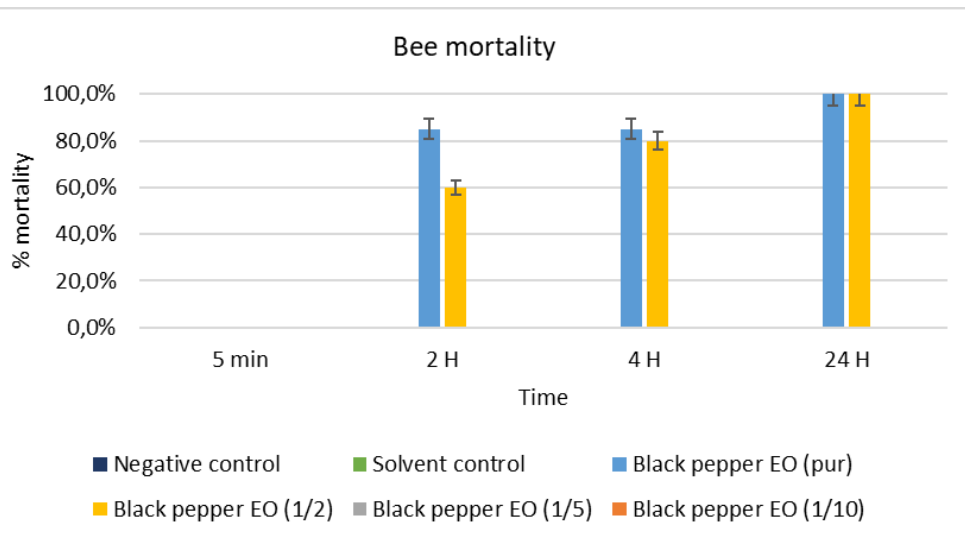
Varroa mite mortality



**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)

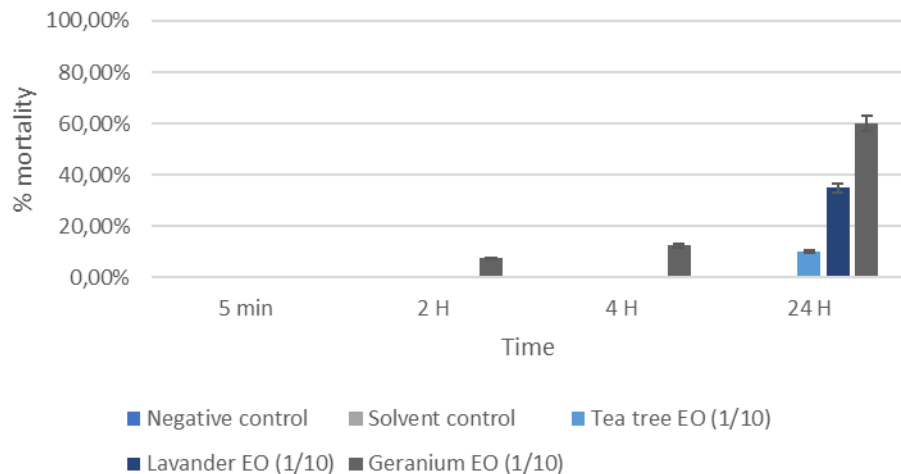


**Very effective against varroa mite but very high toxicity for bees.
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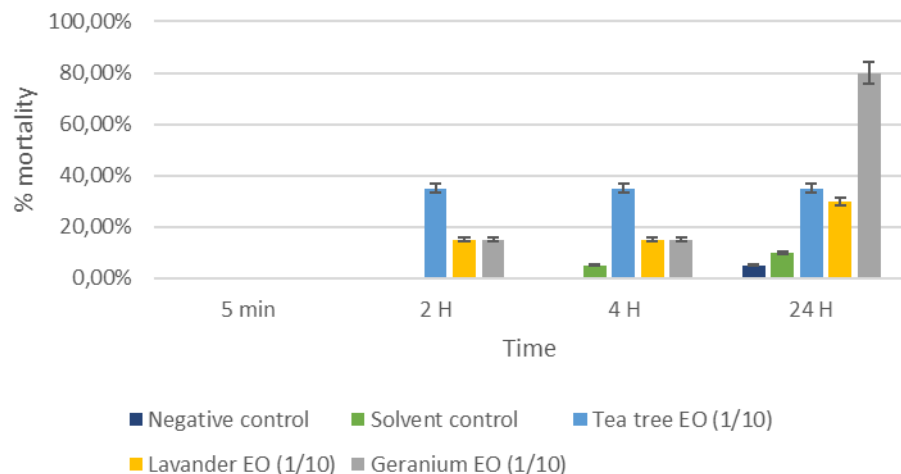
EO = Essential oil

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

Bee mortality



Varroa mite mortality

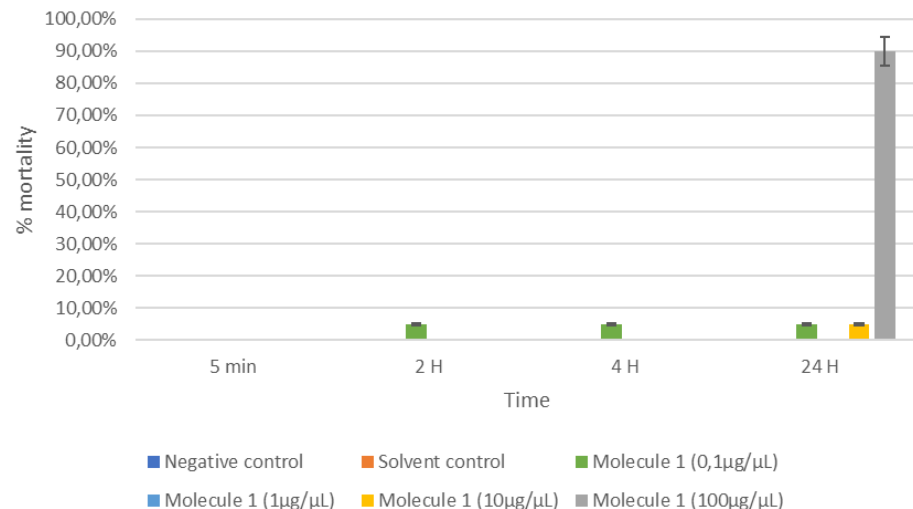


**Geranium EO is very effective against varroa mite but very toxic for bees.
The two others are toxic as well and less effective.**

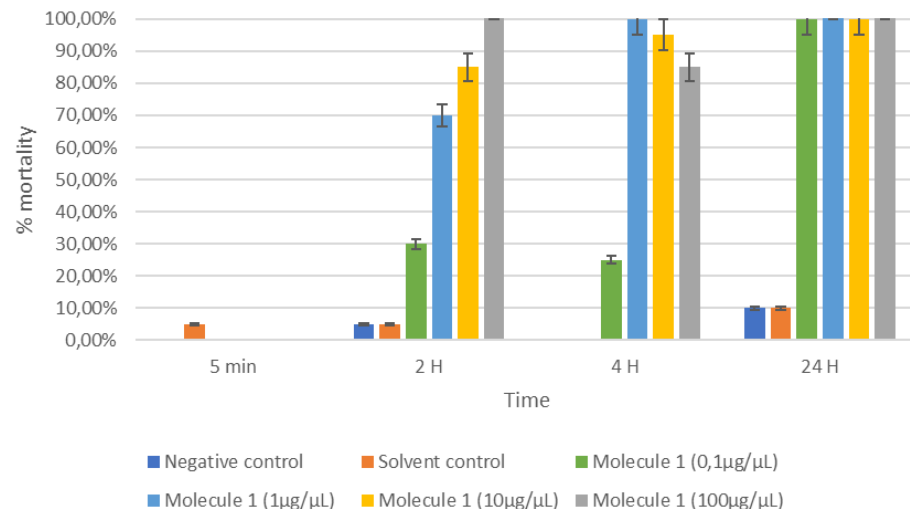
EO = Essential oil

Promising molecules (molecule 1 - thoracic)

Bee mortality

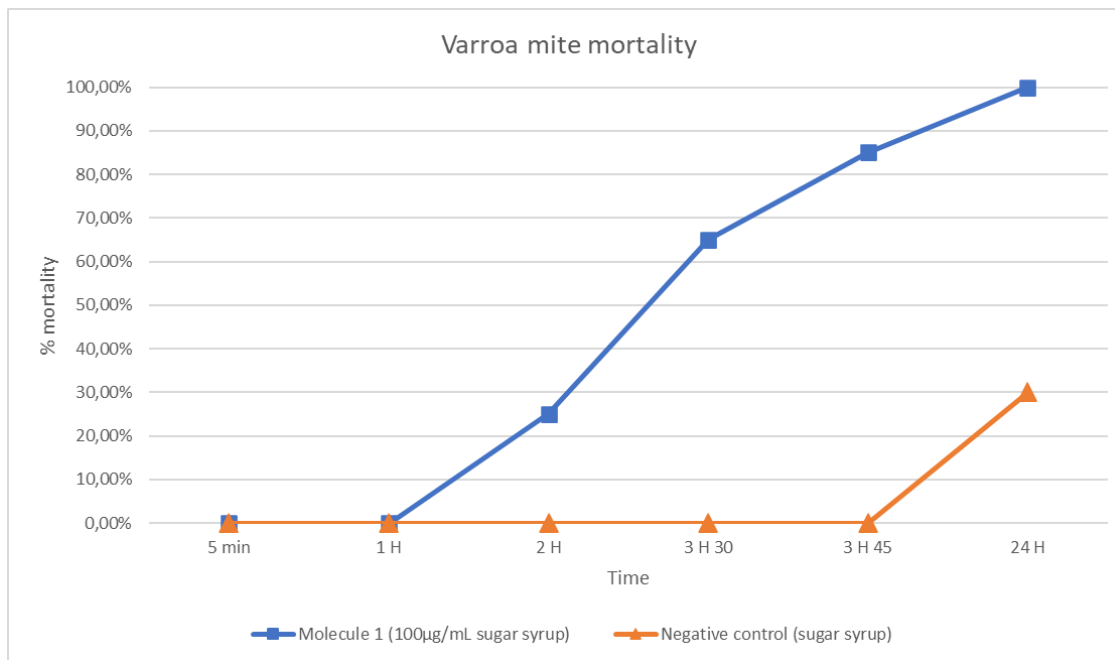


Varroa mite mortality



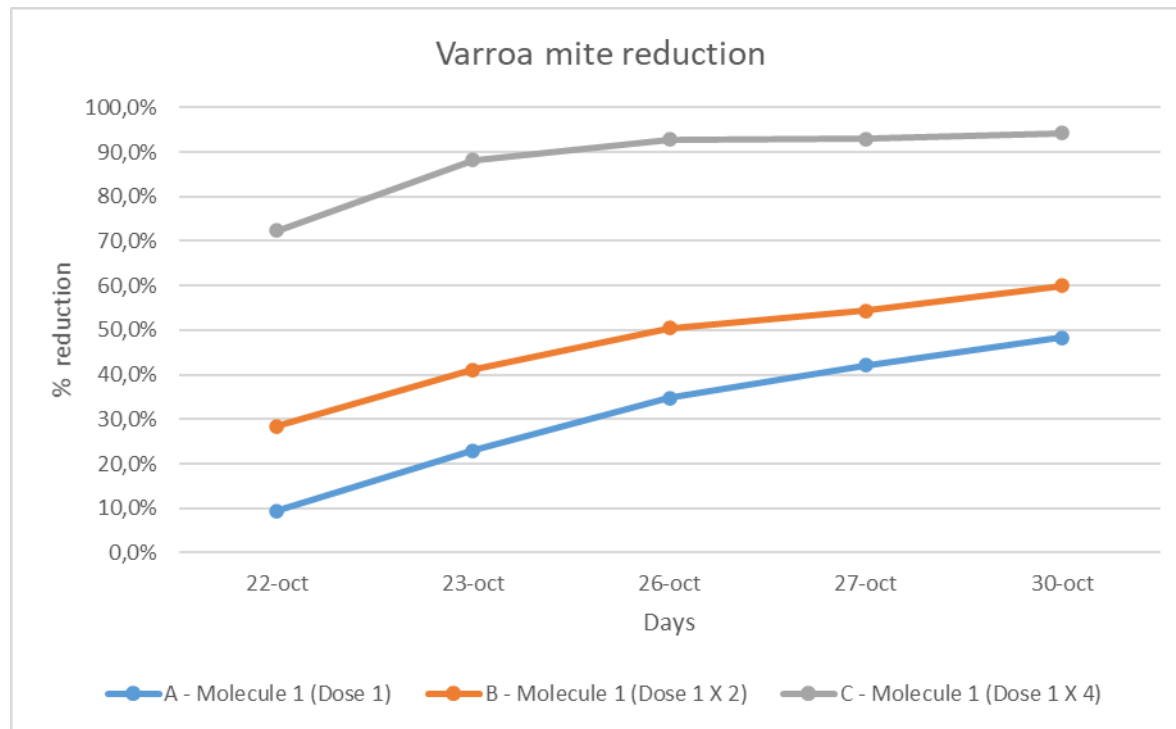
Same efficiency than amitraz (same kinetic).
Very low toxicity for bees except for the higher concentration.

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)

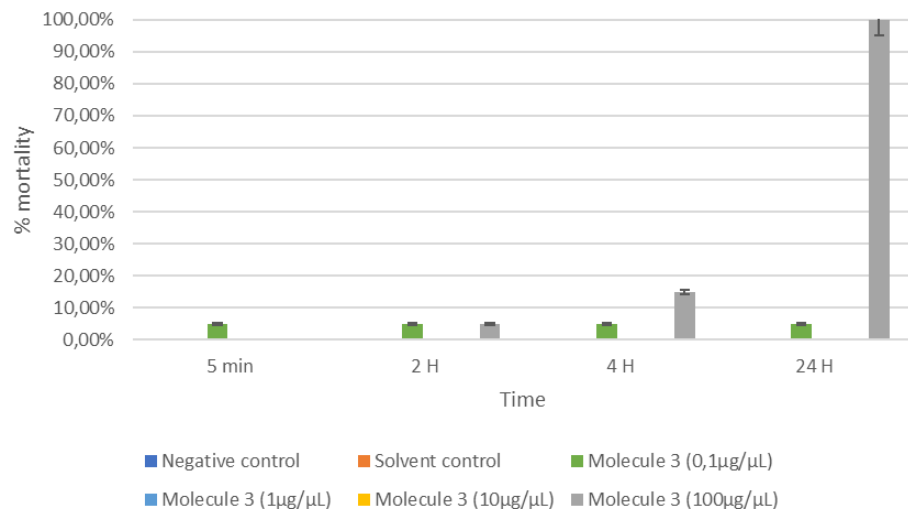


94.3% varroa mite reduction after 10 days in broodless colonies.
Only 3 hives per group.

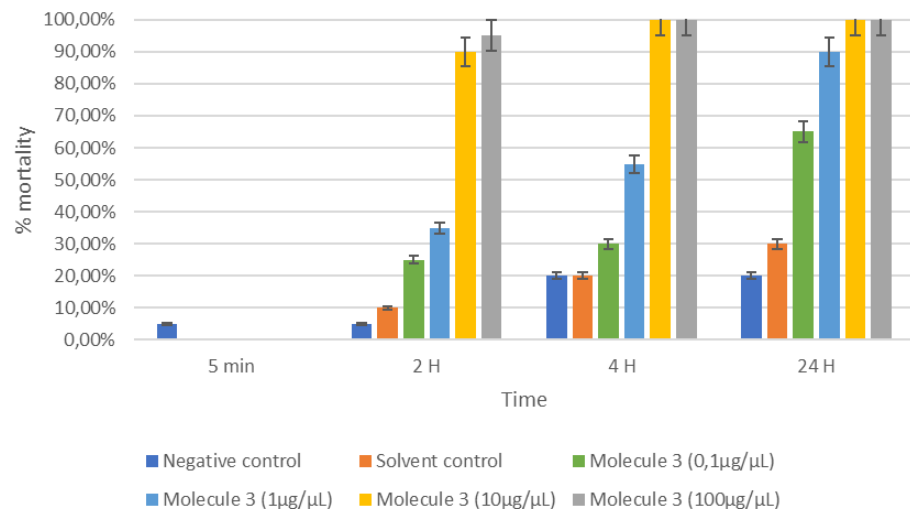
Promising molecules

(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?

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

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

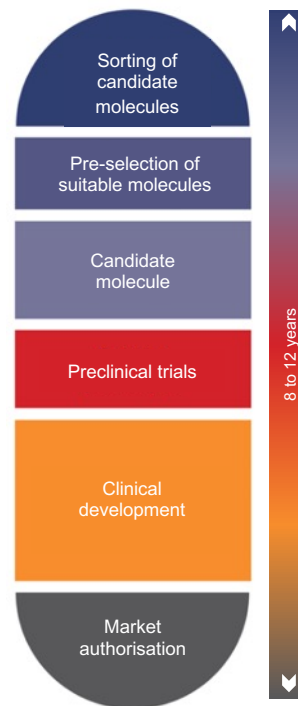
Clinical trials

- ▶ 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



Merci !
Thank you!
Danke!
¡Gracias!

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