A new insecticide based on *Clitoria ternatea* extract

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Clitoria ternatea L. - butterfly pea

- Fabaceae family
- Excellent forage legume (very good regrowth and yields)
- Cover crop
- Edible plant (young and tender parts of the plant, shoots, leaves, flowers and pods)
- Pharmacological properties
Insecticide properties

- Recent studies indicate that *C. ternatea* has insecticidal effects (cyclotides, flavonyl glycosides, proteins)
- Complex mechanism: ingestion, partially contact

Cyclotides: molecules composed of 28-37 amino acids in a head-to-tail cyclic backbone with three interlocking disulfide cystine bonds, mainly produced by plants as defence proteins
Insecticide properties

- **Cyclotides** are only found in four families (rubiaceae, violaceae, rabacae and cucurbitaceae), but they are widespread
  - extremely water soluble (around 1–10 g/L)
  - not readily degraded except in sunlight over weeks when the amino acid tryptophan is present
- Thousands of **flavonoids** present in plants: six are identified in *C. ternatea* extract
  - kaempferol, quercetin, myricetin, astragalin, kaempferol 3-neohesperidoside and clitorin
  - low volatility, high water solubility and ready biodegradability
  - unlikely to persist or bioaccumulate
Toxicology

- **Non toxic**: low acute oral (LD50 > 2000 mg/kg bw) and dermal (LD50 > 2000 mg/kg bw) toxicity in rats
- **Not a skin irritant** in rabbits and the results of a skin sensitization study (local lymph node assay) did not provide evidence of a sensitisation potential
- The acute **inhalational toxicity** is likely to be **low**
• **Non-toxic to mammals** on an acute basis: no signs of toxicity when mammals were exposed to the highest level tested (oral and dermal routes)

• **Fish, crustaceans, algae and aquatic plants:** practically *non-toxic* an acute basis

• **Bees:** some sensitivity when tested *at the highest* concentrations

• **Earthworms:** some sensitivity for acute exposure *at the highest* concentrations in soils tested

• **No phytotoxicity** observed on tested crops
Possible uses of *C. ternatea* ethanolic extract against a wide range of phytophagous insects
Materials and methods

• Small scale trials under controlled conditions (lab, greenhouse)
• Concentration of the active ingredient in the formulated product: 400 g/l
• Dosage of the formulated product: 20 ml/l
• Untreated control (UTC): water
• Chemical standard reference
• Experiment carried out at least twice with 5 replicates/treatment
• Data of the experiments were pooled
• Statistics: ANOVA, Tukey’s test (α = 0.05)
Target pests and chemical standard references and related dosages

<table>
<thead>
<tr>
<th>Target</th>
<th>Reference Product 1</th>
<th>Reference Product 2</th>
<th>Reference Product 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Active Ingredient</td>
<td>Dosage (ml or g/l)</td>
<td>Active Ingredient</td>
</tr>
<tr>
<td>Aphis gossypii</td>
<td>Flonicamid</td>
<td>0,14</td>
<td></td>
</tr>
<tr>
<td>Antispila oinophylla</td>
<td>Acetamiprid</td>
<td>2,00</td>
<td></td>
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<tr>
<td>Drosophila suzukii</td>
<td>Spinosad</td>
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<td>Deltamethrin</td>
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<tr>
<td>Frankliniella occidentalis</td>
<td>Abamectine</td>
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<tr>
<td>Halyomorpha halys</td>
<td>Acetamiprid</td>
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<td>Clorantraniliprole</td>
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<tr>
<td>Lobesia botrana</td>
<td>Emamectine Benzoate</td>
<td>1,50</td>
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</tr>
<tr>
<td>Scaphoideus titanus</td>
<td>Flonicamid</td>
<td>0,14</td>
<td></td>
</tr>
<tr>
<td>Trialeurodes vaporariorum</td>
<td>Abamectine</td>
<td>0,13</td>
<td></td>
</tr>
</tbody>
</table>
Antispila oinophylla

Mortality of *A. oinophylla* larvae, 40 hours after the treatment, was similar to chemical reference.

Treatments (drops) were applied on leaves, above the mines containing active larvae inside, and incubated in Petri dishes; two experiments pooled.
Trialeurodes vaporariorum

Mortality of nymphs, recorded as the number of specimens that did not emerge from the ‘pupa’ in the three weeks following the treatment.

Treatments were carried out on tobacco leaf disks infested by nymphs; two experiments pooled.
**Frankliniella occidentalis**

Mortality of nymphs, 48 hours after the treatment

Treatments were carried out on insects placed on bean leaf disk and then incubated in Petri dishes; two experiments pooled
Insecticide efficacy calculated on total number of insects: adults and larvae (Abbott’s for.)

- Karate Zeon 100 mL/ha BC
- Laser 200 mL-g/ha BC
- Clitoria extract 0,5 L/ha ABCD
- Clitoria extract I 1 L/ha ABCD
- Clitoria extract 2 L/ha ABCD
- Clitoria extract I 2 L/ha ABCD (300 L/ha)
- Clitoria extract 2 L/ha + Silwet Fastex 50 ml/100 L ABCD
- BPA044I 4 L/ha ABCD

UTC

A At presence of first adults
B 7 days after A
C 7 days after B
D 7 days after C
Drosophila suzukii

Mortality of adults 48 hours after the treatment

Treatments were sprayed directly on insects, which were then incubated in Petri dishes; some variability in the efficacy between trials (exp1 and 2) was noticed.
Aphis gossypii

Mortality of nymphs, 4 days after the treatment, was lower than chemical reference, but higher than UTC.

Treatments were carried out on insects placed on zucchini leaf disk in Petri dishes; two experiments pooled.
**Scaphoideus titanus**

Mortality of nymphs, 7 days after the treatment

Treatments were carried out on grapevine leaf disks, insects were then placed on the leaf disks and incubated in Petri dishes.
Halyomorpha halys

Mortality of adults up to 14 days after the treatment

Treatments were carried out on crabapples, insects were then placed on the apples and incubated in boxes; two experiments pooled
Conclusions

• No toxicity for mammals
• Almost no toxicity for the environment
• Good persistency and good efficacy against several target insect species (but not all)
• Selectivity (expected)
• No phytotoxicity
Thank you for your attention!

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Sandro Frati