Optimisation of slow-release formulations as biological control devices

SOLAPHID (Wallo2 convention RW/FSAGX 061/6287 et RW/UCL 051/6067)

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Introduction

Eβ-Farnesene, the alarm pheromone of many aphid species and β-caryophyllene, recently identified as one of the possible component of the aggregation pheromones of the Asian ladybeetles Harmonia axyridis Pallas, are considered as two sesquiterpenes attractive for aphids’ predators and parasitoids.

In the present research, alginate gel beads formulations were optimised as semiochemical slow-release devices. The formulations were evaluated in terms of volatiles release capacity, protection efficiency of sesquiterpenes and biological activity towards Episyrphus balteatus and Aphidius ervi. The sesquiterpenes used in the formulations were obtained from natural sources. They were purified by fast chromatography fractionation of essential oils.

Experimental

Purification of semiochemicals by essential oils flash chromatography

Matricaria chamomilla L. The purities of the compounds were determined by fast GC optimised method

<table>
<thead>
<tr>
<th>Compound</th>
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<td>Eβ-Farnesene</td>
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Nepeta cataria L. The purities of the compounds were determined by fast GC optimised method

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Chromatographic conditions

Ultra Fast Module: F/M = 0.1 μm film thickness, 75 mm x 0.1 mm I.D.

Carrier gas: He 95 min

Split ratio: 1:100

Oven:

Initial T°: 40°C, 0.10 min

Ramp 1: 30°C/min → 95°C

Ramp 2: 35°C/min → 155°C

Ramp 3: 200°C/min → 280°C; 0.5 min

Oven run time: 4.78 min

Alginate gel beads formulations

Optimisation of formulations density and encapsulation capacities considering the following parameters:

- Alginate type: M/G ratio determined by NMR
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- Reaction time in CaCl₂

Slow release autocontrol with relative humidity

Preliminary field and lab tests have demonstrated the stop of release at high relative humidities (ΔH). This observation was confirmed by confocal microscopy.

Physical and biological efficiency of formulations

Sesquiterpenes protection

Slow release study

A mathematical modelling of semiochemicals release is presently in study. The influence of physical parameters is measured (T°, relative humidity, wind speed) for Eβ-farnesene and β-caryophyllene formulations.

First assays have demonstrated that diffusion doesn’t depend on wind speed.

Laboratory controlled conditions

- Temperature: 20°C
- Sampling air flow: 0.5 L/min
- Relative humidity: 45 %

Biological tests

Episyrphus balteatus De Geer

Field experiments: verification of the attraction efficiency of formulations towards Syrphididae. The data were treated with MINITAB (Anova : GLM). Eβ-Farnesene and β-caryophyllene beads were compared to beads without semiochemicals.

- Eβ-Farnesene: P = 0.0200 (< 0.05) significant difference
- β-caryophyllene: P = 0.0064 (< 0.01) highly significant difference

Electron microscopy: significant presence with the semiochemicals.

Fossilisation tests: more eggs laid with the semiochemicals than with the blanco.

Wind tunnel: lab scale attraction: in progress.

Aphidius ervi Haliday

Olfactometry (2 ways):

Almost 90% of the parasitoids were attracted by the two semiochemicals compared to only 15% for the blanco (beads without semiochemical). However, a quantity of beads three times higher is necessary for the Eβ-farnesene formulation.

Conclusions

- Optimisation of slow release formulations in terms of encapsulation capacities.
- Efficiency of the formulations in biological tests towards predators and parasitoids of aphids.
- Protection properties of alginate beads, adequate slow release devices depending on temperature and relative humidity.
- Other advantages of the formulations are their naturally occurring components and their biodegradability.