

SUPPLEMENTARY MATERIAL 1

Objective

This pre-experiment was designed to test the entomotoxic potential against *Macrosiphum euphorbiae* of a range of EOs 5 that are already known in the literature to be efficient against other insects/aphid species (Umpiérrez et al. 2012; Ikbal 6 & Pavela, 2019).

EO phytotoxic impact was also taken into consideration in the final selection.

Material and Methods

Biological material

Plants

All experiments were performed on Nano variety *Solanum lycopersicum* tomato plants. Plants used in bioassays were grown in a climatic chamber ($24 \pm 2^\circ\text{C}$, $40 \pm 10\%$ RH, 16:8 L.D.) for 4 to 5 weeks before being moved to the greenhouse ($25 \pm 6^\circ\text{C}$, $60 \pm 15\%$ RH, seasonal photoperiod). Leaflets used in experiments were 6 to 14 weeks old.

Insects

The aphids, *Macrosiphum euphorbiae*, were reared on Nano variety tomato plants in a greenhouse at INRAE, Sophia Antipolis, France ($24 \pm 5^\circ\text{C}$, $60 \pm 20\%$ RH, seasonal photoperiod). All experiments were performed on individuals in the second or third nymphal stages.

Chemical materials

Artemisia (*Artemisia vulgaris*), fennel (*Foeniculum vulgare*), green anise (*Pimpinella anisum*), and rosemary (*Rosmarinus officinalis*) EOs were provided by the university of Catania (Sicily) and lavender (*Lavandula angustifolia*) EO was obtained in Provence.

Experimental set-up

EOs were used in non-contact fumigation treatments where they were applied in their pure form to ashless Whatman™ filter paper. As a control treatment, water was applied to filter paper. EO concentrations tested were equal to $16 \mu\text{L}\cdot\text{L}^{-1}$ air. Bioassays were conducted under laboratory conditions ($22 \pm 3^\circ\text{C}$, $45 \pm 15\%$ RH, seasonal photoperiod). A single leaflet was suspended inside an inverted clear plastic cup (500 mL, height: 10 cm). Its stem was inserted through a hole in the middle of the cup's base and secured in a 1.5 mL Eppendorf tube filled with water. 10 aphids were then added to the experimental system. Six replicates were carried out per treatment. The system was then closed by means of a mesh square held by a rubber band. This system was placed on top of an open Petri dish (diameter: 9cm) containing a Whatman™ filter paper to which was applied one of the EOs tested or water for the control treatment. After EO application, the system was left in place for

24 hours, after which time the number of living and dead individuals in the system was counted and the mortality rate was calculated, according to the formula below.

Mortality rate=(*Number of dead individuals after 24 hours of EO/control treatment*) /*Total number of individuals*

Phytotoxicity was assessed visually.

Results

Preliminary entomotoxic screening was conducted at the leaflet scale. Mortality in rosemary, artemisia, anise and fennel EO treatments with a 16µl/L air concentration were significantly different from the control condition (**Figure S1**; df = 46, n = 6, p < 0.001). Mortality rate in the lavender EO treatment did not differ from the control condition (df = 46, n = 6, p= 0.92). Green anise and fennel were found to be the most efficient EOs against *M. euphorbiae*, killing 100% and 88.4% respectively of the population introduced in the assay.

Summary

The essential oils used were those found to have the greatest entomotoxic effect against *M. euphorbiae* without causing important phytotoxic damage to treated plants. Green anise EO and fennel EO were then selected for our large-scale study.

References

Umpiérrez, M., Lagreca, M., Cabrera, R., Grille, G. and Rossini, C. (2012). *Essential oils from Asteraceae as potential biocontrol tools for tomato pests and diseases*. Phytochemistry Reviews, 11, pp.339-350.

Ikbal, C. & Pavela R. J. (2019), *Essential oils as active ingredients of botanical insecticides against aphids*, Journal of Pest Science, pp. 1-16.

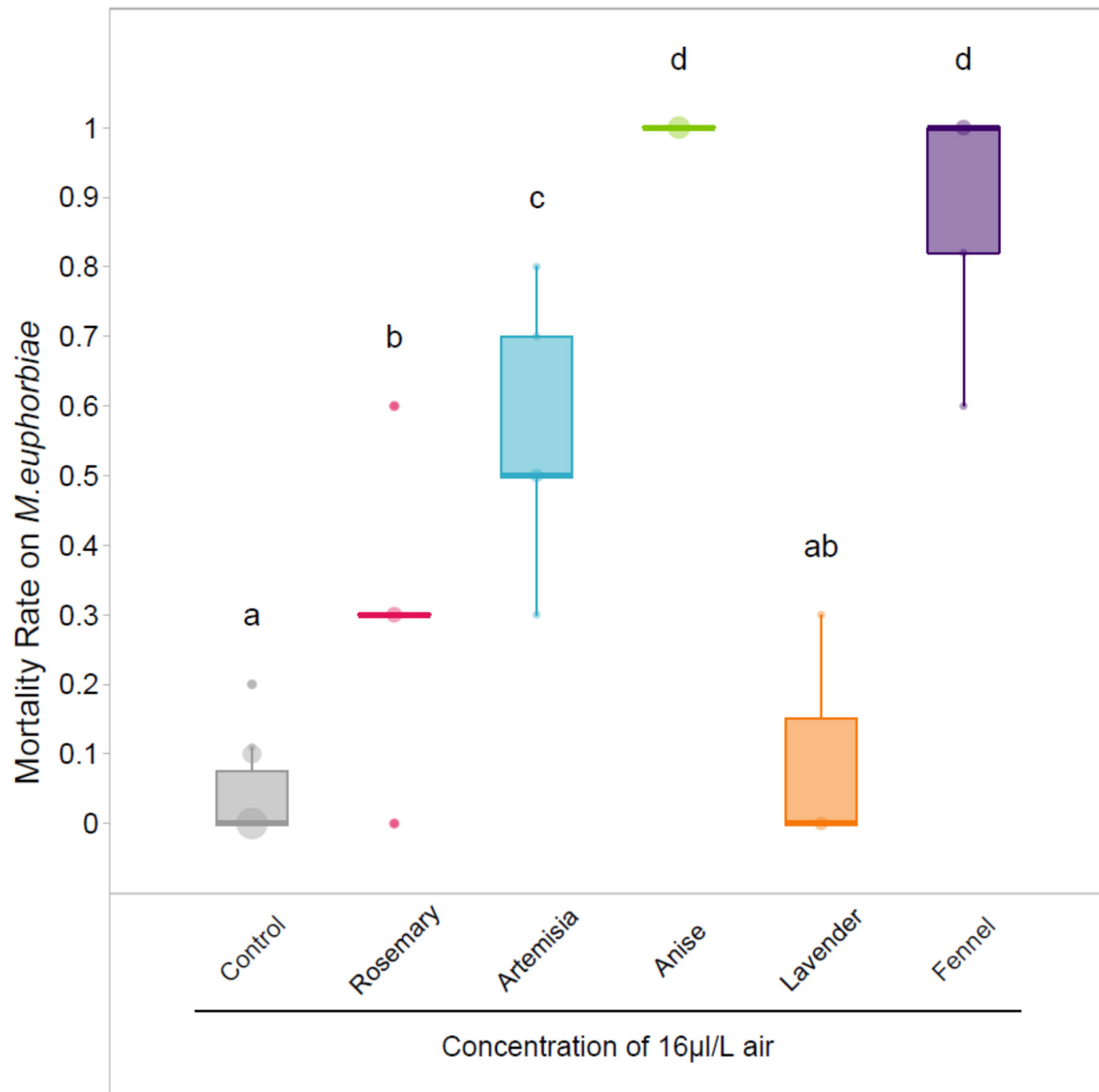


Figure S1. Mortality rate of *M. euphorbiae* in response to a 16µl/L air concentration of different EOs compared to the control treatment. Different letters indicate significant differences between groups ($F = 121.8$, $df = 5$, $n = 6$, p -value < 0.001).