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Short communication

Allelopathy, cardiotoxicity and lethality bioassays of essential oils

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Introduction

Essential oils are widely targeted for their properties, such as antimicrobial, antiparasitic, insecticidal and repellent actions, among other therapeutic applications on which studies have been based (Tidori Miura et al., 2021)

Allelopathy is defined as the effect(s) of one plant on other plant (s) through the release of chemical compounds (allelochemicals) in the environment by leaching, exudation, volatilization (release of essential oils), or decomposition (El Sawia et al., 2019)

For this study were used the commercial essential oils of *Origanum Vulgaris* and *Thymus Vulgaris***.** The purpose of this study was the evaluation of the ecotoxicological activity of these essential oils.

Materials and methods

Commercial essential oils of oregan (*Origanum vulgaris*) and thymus (*Thymus vulgaris*) were purchased from the farm company "Giardino Officinale", Morro D'Oro, Province of Teramo (Italy). The main components of organ essential oil were: carvacrol, thymol, linalool, borneol. The main components of thymus essential oil were: cineol, borneol, geraniol, linalool, bornile acetate, linalile acetate, cymene, terpinene.

Allelopathy assay

Commercial seeds of *Dicondra repens* and *Cichorium intybus* were selected for the test due to their fast germination rate and sensitivity. Seeds were distributed on paper beds in a Petri capsule imbibed with the essential oils 3.9-1000 µg/mL. The emergence of at least 2 mm length with the typical geotropic curvature of the radicle was used as the criterion to define the presence/absence of germination. The seeds that showed false germination by soaking were not accounted for. The germination was recorded at 96 h by counting the number of germinated seeds and was expressed as the mean value of at least triplicate experiments.

Artemia salina lethality and Daphnia magna toxicity assays

The biocompatibility limits of the extracts were predicted through the *Artemia salina* and *Daphnia magna* toxicity assays.

For the *A. salina* assay, the essential oils were tested in the concentration range 3.9–1000 µg/mL. *Artemia salina* cysts were hatched in oxygenated artificial sea water (1 g cysts/L). Ten larvae per well were incubated at 25–28 °C for 24h. After 24 h, the number of living napulii were counted under light microscope and compared to control untreated group. Results were expressed as percentage of mortality calculated as: ((T– S)/T)*100. T is the total number of incubated larvae and S is the number of survival napulii.

In the *Daphnia magna* model, the cardiotoxicity was induced by ethanol 10% solution. The details about these tests are fully reported in previous studies (Ferrante et al., 2019; Chiavaroli et al., 2021; Orlando et al., 2021).

Results and discussion

In the present study, commercial oregan and thymus essential oils were tested in different eco-toxicological models, namely allelopathy assay, brine shrimp (*Artemia salina*) lethality assay, and *Daphnia magna* cardiotoxicity assay, in order to define the limits of biocompatibility.

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In the allelopathy assay, the essential oils were tested in a wide concentration range 3.9-1000 µg/mL and the effects of the essential oils were monitored against the seedling germination of *Dicondra repens* and *Cichorium intybus*, which are characterized by a high rate of germination.

Specifically, the essential oils determined a total loss in seedling germination at the highest concentration range, whereas an acceptable profile of tolerability was observed in the range 3.9-62.5 µg/mL. In this narrow range of concentrations, the seedling germination *of C. intybus* and *D. repens* was always higher than 70%, compared with non-treated control group (Ctrl); thus, indicating a complete profile of biocompatibility.

The concentration range 3.9-62.5 µg/mL was of reference for the following tests against brine shrimp and *D. magna*. In the brine shrimp lethality assay, the essential oils of oregan and thymus showed a very close profile of toxicity, with LC50 values in the range 20-25 µg/mL. These values were in turn reference for a further experiment in the *D. magna* cardiotoxicity assay, and the essential oils were tested at the concentration 20 µg/mL.
D. magna crustaceans were exposed to the essential oils, both in absence and in presence of ethanol 10% solution, this last representing the reference cardiotoxicity stimulus able to drastically reduce heart rate (Chiavaroli et al., 2021).

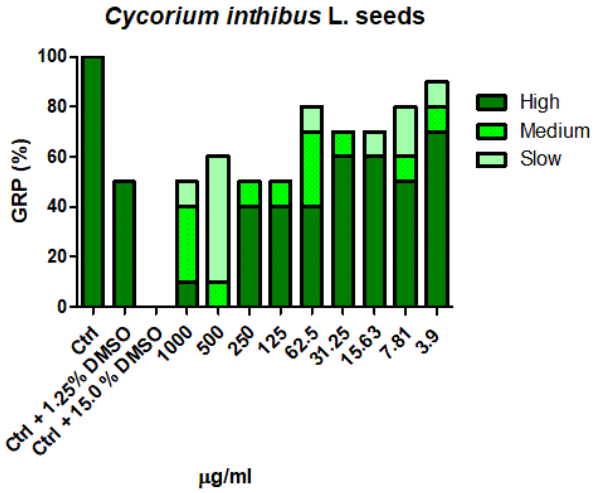

Fig. 1. Effects of oregan essential oil 3.9-1000 µg/mL on *Cycorium intybus* seedling germination.

Conclusion

Interestingly, the essential oils did alter heart rate, in basal conditions; additionally, the growth medium supplementation with the essential oils determined a blunting effect on ethanol-induced reduction in heart rate; thus, suggesting cardioprotective effects which also scale back the intrinsic toxicity showed against brine shrimps, at the same concentrations.

However, considering the different sensitivity of the abovementioned eco-toxicological models to oregano and thymus essential oils, it is recommended at least a ten-fold reduction of the dosage of the essential oils (Ferrante et al., 2019), for future experiments in human cell and tissue models, not only for defining biocompatibility, but also for unravelling potential applicability as health-promoting agents.

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