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FELLOWSHIP FINAL REPORT

Phytotoxic and microbiological activities of soil-applied microencapsulated peppermint (*Mentha x piperita* L.) essential oil

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REPORT INFO

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Period of residence in region Centre-Val de Loire: September 2018 – September 2019

Keywords:
Botanical herbicide; growth inhibition; biochemical response; dose-response test; soil microbial activity; mycorrhizal symbiosis

ABSTRACT

During this fellowship I performed several greenhouse and laboratory experiments, aiming at assessing the phytotoxic and microbiological effects of microencapsulated peppermint (*Mentha x piperita* L.) essential oil (MPO). The different doses of MPO were applied in the pot experiments either into vermiculite or top layer of arable soils. As the acceptor-species, I used four maize cultivars, one cultivar of mustard and a weed– lambsquarters (*Chenopodium album* L.). The microbiological analyses were performed using i) commercial strain of arbuscular fungus *Rhizophagus irregularis* on maize roots by intersection method, and ii) natural soil-microbiota by FDA biotest. I have found, based on the ED50 analysis, that the phytotoxic effect of MPO is both dose and species as well as soil-medium dependent. The biochemical analyses revealed, that the plants' response to the application of MPO is typical for the allelopathic stress. The microbiological responses to the MPO applications were not clear enough to conclude and should be continued further.

1- Introduction

Essential oils (EOs) are multicomponent volatile compounds, produced by plants, of several biological functions [1]. Recently, there is a growing interest in applying EOs as so-called botanical (natural) pesticides [2]. Peppermint (*Mentha x piperita* L.) EO displays significant phytotoxic activities against germination and early growth of weeds, so it is a good candidate for a botanical herbicide, to control weeds [3, 4]. Moreover, peppermint EO is one of the most popular EOs in cultivation worldwide. It is utilized in many industries, either as a natural EO or its main compound - menthol [5].

Due to the unfavorable chemical properties of EOs namely water insolubility and volatility, their application is technically difficult. One of the methods to overcome these issues, is to encapsulate the EO within a solid carrier, composed of e.g. polysaccharide such as maltodextrin [6].

The aims of this project were to assess the effects of soil-applied microencapsulated peppermint oil on: i) the initial growth of four cultivars of maize (*Zea mays* L.), white mustard (*Sinapis alba* L.) and lambsquarters (*Chenopodium album* L.) and ii) the soil microbiota.

2- Experimental details

2.1. Physical and chemical analysis of the microencapsulated peppermint oil

The microencapsulated peppermint essential oil (MPO) was purchased from the Polish company Hoffmann Aroma (http://www.hoffarom.com). The producer encapsulated the essential oil in maltodextrin with a small addition of arabic gum, in the process of dry spraying.

The size and structure of MPO was analysed by the Scanning Electron Microscopy at a magnification of 2.10 K.

The content of peppermint essential oil (EO) in the microcapsules was assessed by a hydrodistillation method.

The EO was sent for further chemical analysis namely gas chromatography coupled with mass spectrometry, for the assessment of its components, to the University of Amiens.

2.2. Phytotoxic experiments

There were seven greenhouse pot experiments performed during the fellowship. The experiments were performed in two series and in three replications, in a totally randomised statistic design.

The phytotoxic effect of MPO was tested in the three different types of growth-media: i) mixture of small and medium sized vermiculite; ii) plough layer of a sandy soil and iii) plough layer of a loamy soil. The soils were collected in Autumn 2018 from the arable fields near Chartres. The physico-chemical analyses of both soils were performed in the Laboratoire d'Analyses Chambre d'Agriculture, Loiret.

The phytotoxic effect of MPO was performed according to the dose-response study [7], with five different doses of MPO. As a reference, pots containing no MPO addition and pots containing the carrier only, were used.

The tested plant species included: four cultivars of maize (*Zea mays* L.), cvs. Agrojanus, Severino, Kornfluens and KWS Stabil, (provided by KWS Maïs France), white mustard (*Sinapis alba* L., cv. Zlata) and a weed – lambsquarters (*Chenopodium album* L.), which was collected from the vegetable garden near Chartres during Autumn 2018.

The pot experiments were terminated when the tested plants reached the growth stage of 3 leaves for maize, and the stage of 2-3rd pair of true leaves for mustard and lambsquarters.

The biometric analyses of plant material included 1) time needed for maize emergence; 2) lenght of plants; 3) lenght of roots; 4) fresh mass of leaves; 5) fresh mass of stem; 6) fresh mass of roots; 7) dry mass of leaves; 8) dry mass of roots; 9) leaf area; 10) specific mass of leaves.

Methanolic extracts (50%) of lyophilized 2nd leaves and roots of maize, mustard and lambsquarters were prepared using ultrasound-assisted extraction (Prolabo Ultrasonic Bath). The biochemical analyses were performed in two technical repetitions and included spectrophotometrical analyses of 1) total chlorophyll content in leaves of maize; 2) leaf and root total phenolics content by means of Folin-Ciocalteu reagent [8]; 2) leaf and root total anthocyanins content using the pH differential method [9]; 3) antioxidant potential of leaf and root (ABTS and CUPRAC).

Accumulation of benzoxazinoids in plants was planned to be analysed by HPLC. The methanolic extracts of: i) maize (4 cultivars) leaves, ii) maize (4 cultivars) roots, iii) lambsquarters leaves, iv) lambsquarters roots and v) mustard leaves (465 samples in total) were evaluated by chromatographic technique, using a Varian (Les Ulis, France) HPLC instrument. The Host did not provide the details of this analysis.

2.3. Microbiological analysis of soil

2.3.1. The effect of MPO on arbuscular mycorrhizae on maize roots

This experiment was performed on roots of maize cv. Agrojanus, growing in the mixture of vermiculite and a commercial potting mixture (1:1 v/v), enriched with i) peppermint essential oil; ii) MPO; iii) carrier, and iv) control – no addition. The commercial strain of arbuscular mycorrhizal fungi *Rhizophagus irregularis* was added to the pots. The roots were stained in trypan blue and observed under light microscope for the collonization of fungi, using intersection method [10].

2.3.2. The effect of MPO on the overall soil microbial activity – laboratory experiment

This laboratory experiment was performed for five different agronomic soils, collected during Autumn 2018 from the plough-layer of arable fields near Chartres. The soils were air-dried, and next sieved through the laboratory mesh (4 mm mesh size), to remove all the macro-contaminations. There were three factors tested in this experiment: i) the effect of dose of MPO (5 levels), ii) soil humidity (3 levels) and iii) the temperature of soil incubation (3 levels) on soil microbiological activity. The overall microbial activity, based on the fluorescein diacetate hydrolytic activity was tested spectrophotometrically [11].

2.3.3. The effect of MPO on the soil microbial activity – pot experiment

In this experiment two soils, used in the pot experiment with maize, were prepared for the analyses. The soils were mixed with 5 doses of MPO, analogical doses of carrier and with no additions, then four cultivars of maize were planted to the pots. Next, the soils were collected (in total 192 soil samples) along with maize at the maize growth stage of 3 leaves. The soils were initially sieved through laboratory mesh (4 mm mesh size), placed in the plastic containers of 50 mL volume and stored in the -15 C for further analyses. The planned analyses included: fluorescein diacetate hydrolitic activity; soil alkaline and acid phosphatases; soil B-glucosidase [12, 13, 14, 15, 16]. The Host did not allow to carry out this experiment during the fellowship.

3- Results and discussion

3.1. Physical and chemical analysis of the microencapsulated peppermint oil

The MPO were composed of particles of variable sizes, with an average size of *ca.* 10 μm, and by about 9% of essential oil content.

The chemical composition of the microencapsulated peppermint oil was not provided by the Host.

3.2. Phytotoxic experiments

The phytotoxic experiments yielded interesting results, based on the statistical comparisons of values of effective doses, causing 50% of inhibition for the particular growth parameter (ED50) [9].

In brief:

- Type of medium influenced the susceptibility of the tested species to the different doses of MPO;
- There was a difference in susceptibility to MPO between the tested plant species. White mustard was the most tolerant species to the MPO;
- There was a variability in susceptibility to the MPO among the tested maize cultivars;
- Higher doses of MPO significantly slowed down the emergence of maize (in a cultivar dependent manner), but not that of white mustard nor lambsquarters. That observation is of significant ecological consequence, in relation to the competition between maize and lambsquarters [17];
- Higher doses of MPO completely inhibited growth and biomass accumulation of maize, and significantly reduced the both growth parameters for white mustard and lambsquarters.

The biochemical results (spectrophotometrical ones) were in accordance with the morphometric observations, and revealed stress reactions in the tested species, displayed by the increase of total phenolic compounds and anthocyanins in the leaves, in response to the presence of MPO in the medium. This is a confirmed phenomenon in plants, caused by the allelopathic stress-factor [4]. The biochemical changes in the roots were of a lower intensity, as compared to the leaves of the tested species.

3.3. Microbiological analysis of soil

3.3.1. The effect of MPO on arbuscular mycorrhizae

None of the applied treatments influenced significantly the performance of *Rhizophagus irregularis*. However, as a promising result, the growth of mycorrhizal fungus was not totally inhibited by the MPO treatment.

3.3.2. The effect of MPO on the overall soil microbial activity – laboratory experiment

The analysis revealed the effect of a soil type, in a combination with MPO, on the overall soil microbial activity. It was found, that the higher content of clay and loam fractions in the soil, the lower inhibitory effect of MPO. The effects of humidity and temperature were not significant.

3.3.3. The effect of MPO on the soil microbial activity – pot experiment

This analysis was not performed, due to the Hosts's decision.

4- Conclusion

The MPO displays phytotoxic effect, which is dose-dependent, but also depends on the type of soil-medium as well as the tested plant species. The biochemical response of the tested plants to the MPO is typical for the allelopathic type of stress and will be studied further. Also, the effect of MPO on soil microbiota is confirmed to some extent. However, the analyses performed during this fellowship did not allow me to sufficiently respond to the question of quantitative and qualitative shifts in the soil microbiota composition in response to the MPO and will be studied further.

5- Articles published in the framework of the fellowship

Kalemba, D., & Synowiec, A. (2020). Agrobiological Interactions of Essential Oils of Two Menthol Mints: Mentha piperita and Mentha arvensis. *Molecules*, *25*(1), 59.

6- Acknowledgements

This work was supported by the Le Studium, Loire Valley Institute for Advanced Studies, Orelans & Tours, France under Marie Sklodowska-Curie grand agreement no. 665790, European Commission.

I would like to acknowledge the Le Studium team for an excellent scientific environment during the Le Studium meetings.

I also would like to acknowledge Eng. Esmat Barakzoy for technical assistance in some the laboratory works.

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