Possibilities of Using Supercritical CO_2 Extracts from Oregano as Eco-friendly Solution in Sustainable Agri-Food Safety Management

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Introduction

Ensuring food safety and consumer health is currently a challenge requiring extensive action, hence the emphasis is on sustainable management of the safety of agri-food products. Currently, one of the most important problem is the search for environmentally friendly solutions that will constitute an alternative to the chemical compounds commonly used in both agriculture and food industry. Such an approach may be the use of plant extracts containing various biologically active compounds and exhibiting antimicrobial activity, which are of interest due to the possibility of their use at various stages of the food chain.

Aim of Study

The aim of the study was to assess the biological activity of extracts obtained from *Origanum vulgare* L. by supercritical CO_2 extraction using various extraction conditions.

Materials and Methods

The subject of the study were three CO_2 extracts obtained from the dried herb *O. vulgare*. CO_2 extraction was carried out at temperatures of 40, 50 and 60°C and pressure of 250 bar, using methanol as a solvent. Samples of the extracts for analysis were stored at -20°C.

The antibacterial activity was determined against Gram-positive (*Micrococcus luteus* ATCC 10240, *Staphylococcus aureus* ATCC 33862, *Enterococcus faecalis* ATCC 19433) and Gram-negative bacteria (*Escherichia coli* ATCC 25992, *Pseudomonas aeruginosa* ATCC 9027, *Campylobacter jejuni*, *Salmonella* Enteritidis ATCC 13076), MIC and MBC values were determined by using the microdilution method.

The antibiofilm activity The antibiofilm activity of *O. vulgare* extracts was examined in two ways: as a factor preventing biofilm formation and as a biofilm removal factor in flat-bottom 96-well microtiter plates. The final concentrations of the tested extracts were equal to the MIC values. Biofilm biomass was determined using the modified crystal violet method

The antifungal activity was determined against several species of toxigenic fungi of the *Fusarium* genus (*F. culmorum* KZF 5, *F. graminearum* KZF 1, *F. poae* KZF 181, *F. equiseti* KZF 6, *F. avenaceum* KZF 3), responsible for serious plant diseases in crops. The MIC, as well as the MBC/MFC, of the tested extracts were determined using the microdilution method.

Morphology observations were made with a light microscope at 400× magnification. Fungal samples treated with sterile water were used as control samples.

The viability of *Fusarium* cells was assessed by the fluorescence microscopy using fluorescein diacetate (FDA) and propidium iodide (PI).

Cell membrane damage was assessed spectrophotometrically by determining the release of intracellular material.

Each assay was repeated in triplicate.

Results

The results showed that all tested *O. vulgare* extracts showed antimicrobial activity against tested Gram-positive and Gram-negative bacteria and filamentous fungi (Table 1). It was observed that the lower extraction temperatures (40 and 50°C) resulted in higher antibacterial properties towards *S. aureus, M. luteus, E. faecalis* and *C. jejuni*. For the rest of the tested bacteria, the influence of the extraction conditions on antibacterial activity was not observed. The extracts exhibited fungistatic activity towards the tested *Fusarium* fungi; however, this activity was dependent mainly on the fungal species, whereas the extraction conditions generally did not affect the activity of the extracts. An exception was the extract obtained at 60°C which demonstrated activity against *F. equiseti* (MIC and MFC values were 1.9 mg/mL), whereas extracts obtained at 40 and 50°C (MIC/MBC 7.5 mg/mL)

Table 1. Antimicrobial activity of *O. vulgare* extracts

Microorganism	MIC/ MBC/MFC	*O. vulgare* extracts (extraction temp.)		
		40°C	50°C	60°C
Gram-positive bacteria				
***S. aureus* ATCC 33862**	MIC	0.25	0.25	0.5
	MBC	0.25	0.25	0.5
***M. luteus* ATCC 10240**	MIC	0.25	0.25	0.5
	MBC	0.25	0.25	0.5
***E. faecalis* ATCC 19433**	MIC	0.25	0.25	0.25
	MBC	0.25	0.25	0.5
Gram-negative bacteria				
***P. aeruginosa* ATCC 9027**	MIC	0.5	0.5	0.5
	MBC	0.5	0.5	0.5
***S.* Enteritidis ATCC 13076**	MIC	0.5	0.5	0.5
	MBC	0.5	0.5	0.5
***E. coli* ATCC 25**	MIC	0.5	0.5	0.5
	MBC	0.5	0.5	0.5
***C. jejuni* ATCC**	MIC	0.5	0.5	1.0
	MBC	0.5	0.5	1.0
Filamentous fungi				
***F. culmorum* KZF 5**	MIC	15	15	15
	MFC	15	15	15
***F. graminearum* KZF 1**	MIC	7.5	7.5	7.5
	MFC	7.5	7.5	7.5
***F. poae* KZF 181**	MIC	15	15	15
	MFC	15	15	15
***F. equiseti* KZF 6**	MIC	7.5	7.5	1.9
	MFC	7.5	7.5	1.9
***F. avenaceum* KZF 3**	MIC	7.5	7.5	7.5
	MFC	7.5	7.5	7.5

The antibiofilm activity of the tested *O. vulgare* extract, expressed as a percentage of inhibition of biofilm formation or biofilm removal on the example of two bacteria: *S. aureus* and *P. aeruginosa*, is presented in Fig. 1-2. Extract obtained in temp. 50ºC has been chosen due to similar results of antibacterial activity of all tested extracts. The inhibition of biofilm formation by *O. vulgare* was more effective in case of *S. aureus* (Fig. 1). The results of the removal of biofilm were more effective in case of *P. aeruginosa* with the exception of the extract used at concentration 0.5 MIC. The antibiofilm activity depended on the extract concentration.

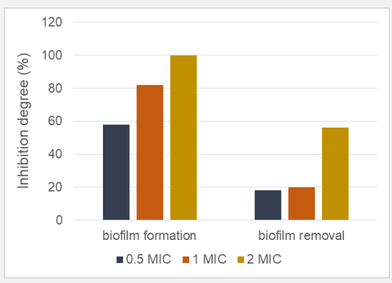

Fig.1. The effect of extract obtained at temp. 50ºC on biofilm formation by *S. aureus* and biofilm removal

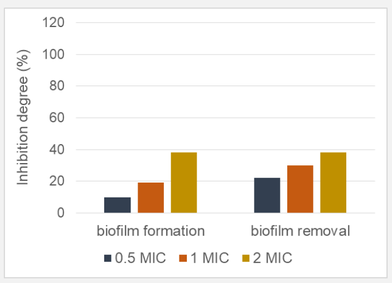

Fig.1. The effect of extract obtained at temp. 50ºC on biofilm formation by *P. aeruginosa* and biofilm removal

The results of FDA/ PI staining of *F. culmorum* treated with *O. vulgare* extracts are shown in Photographs 1 – 5. Untreated, control hyphae (Photo. 1) show strong green fluorescence demonstrating high viability, with only some single cells stained in red. In the samples treated with CO_2 extracts, the viability of fungal cells depended mainly on the time of incubation.

The spectrophotometric analysis suggest that extracts probably do not cause a large leakage of intracellular substances.

Conclusion

Based on the obtained results it can be stated that all extracts demonstrated antimicrobial and antibiofilm activity, depending on the extraction conditions.

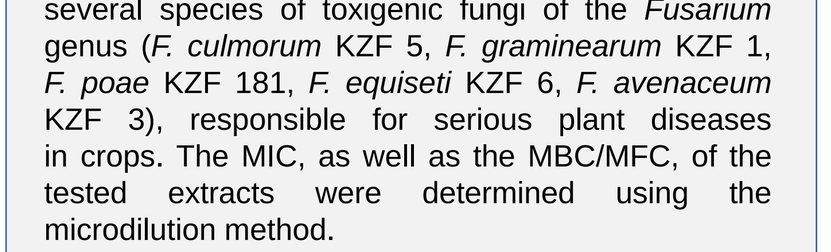

Photo 1. Morphology of untreated *F. culmorum* hyphae (a) unstained (b) stained with FDA; (c) stained with PI

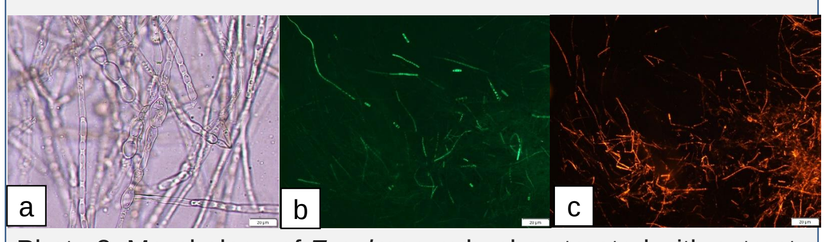

Photo 2. Morphology of *F. culmorum* hyphae treated with extract obtained at temp. 40ºC by 1 h (a) unstaied, (b) stained with FDA; (c) stained with PI

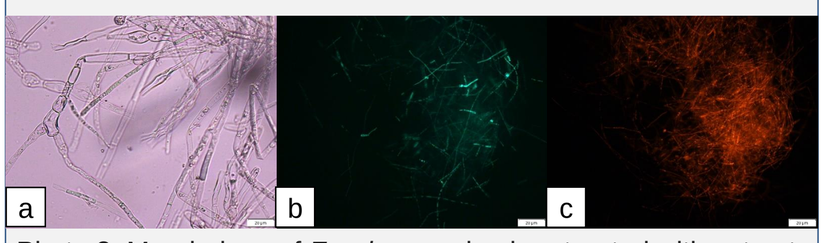

Photo 3. Morphology of *F. culmorum* hyphae treated with extract obtained at temp. 40ºC by 2 h (a) unstaied, (b) stained with FDA; (c) stained with PI

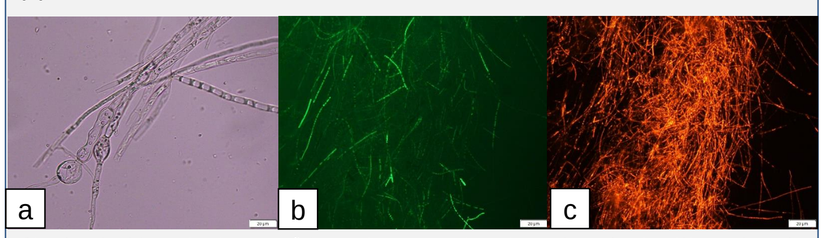

Photo 4. Morphology of *F. culmorum* hyphae treated with extract obtained at temp. 50ºC by 1 h (a) unstaied, (b) stained with FDA; (c) stained with PI

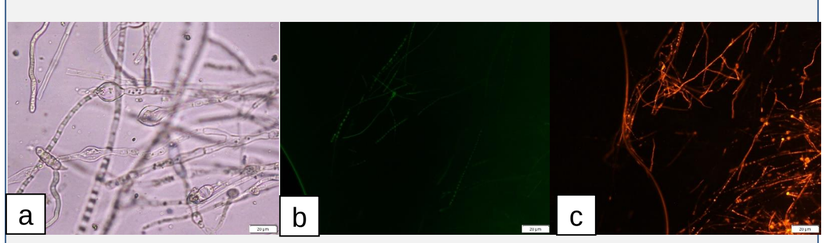

Photo 5. Morphology of *F. culmorum* hyphae treated with extract obtained at temp. 50ºC by 2 h (a) unstaied, (b) stained with FDA; (c) stained with PI

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