Essential oils from oregano and thyme plants organically cultured in Lemnos island (Greece) present strong antimicrobial action against some important foodborne bacterial pathogens

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INTRODUCTION

- The growing antimicrobial resistance of many pathogenic microorganisms to the known antimicrobial agents, together with the rising environmental sustainability issues, have shifted the search for new antimicrobials to those derived from natural sources, such as plants [1].
- **Oregano** and **thyme** are both well-known aromatic plants that belong to the Lamiaceae family. Their essential oils (EOs) have been extensively studied for their bioactivity which is attributed their rich content in secondary metabolites, especially terpenoids such as carvacrol and thymol [2,3].
- In this study, EOs from oregano (***Origanum vulgare* subsp. hirtum**) and thyme (***Thymus capitatus***) plants organically cultured in Lemnos island (north-eastern Greece) were investigated for their antimicrobial actions against three important foodborne pathogenic bacterial species (i.e. *Salmonella enterica* ser. Typhimurium, *Listeria monocytogenes* and *Yersinia enterocolitica*).

AIMS

➢ We tested the action of each EO against the **planktonic** and **biofilm** growth of each bacterial pathogen.

METHODS

- The **minimum inhibitory concentrations (MIC)** of each EO were determined by **the broth microdilution method** (using Bioscreen C) as its lowest concentration inhibiting the visible **planktonic growth** (i.e., no increase in absorbance).
- The **minimum biofilm inhibitory concentration (MBIC)** of each EO was defined as its lowest concentration inhibiting **biofilm growth** (>90%).
- Before the calculation of the MBICs, the optimal conditions for biofilm formation by each target microorganism were determined using **96-well polystyrene microplates as the growth substrata**.
- The accumulated biofilm biomasses at each condition were quantified following **crystal violet (CV) staining** and absorbance measurements (**Fig. 1**).

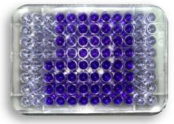


Fig 1. Stained biofilm biomasses through the crystal violet assay.

RESULTS

- The MIC (% v/v) and MBIC (% v/v) of each EO against each target pathogen are presented in **Table 1**.

Table 1

Bacterial species	Oregano EO		Thyme EO	
	MIC	MBIC	MIC	MBIC
L. monocytogenes	0.06	0.031	0.031	0.031
Y. enterocolitica	0.06	0.063	0.031	0.031
S. Typhimurium	0.13	0.125	0.063	0.125

- The optimum biofilm-forming conditions for each target pathogen are presented in **Table 2**.

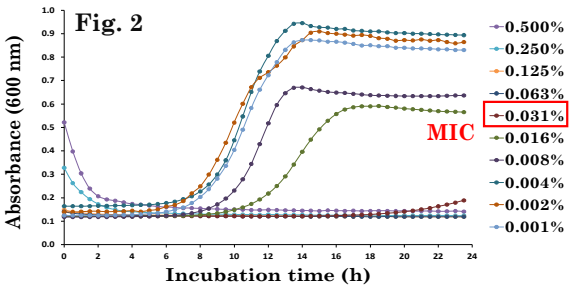
Table 2

Bacterial species	Strain code	Broth	Temperature (°C)	Incubation time (h)*
L. monocytogenes	AAL 20107	BHI	37	96
Y. enterocolitica	DSM4790	TSB	20	96
S. Typhimurium	FMCC_B137	TSB (1/10)	20	96

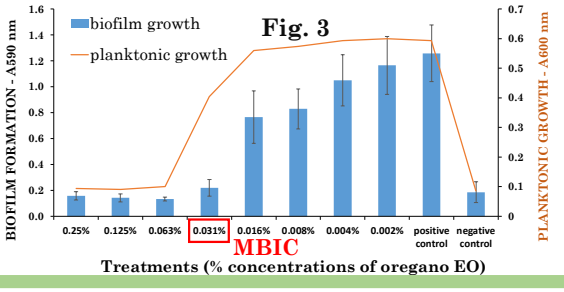
FMCC: Food Microbiology Culture Collection, Laboratory of Microbiology and Biotechnology of Foods, Dept. Food Science and Human Nutrition, Agricultural University of Athens, Greece
AAL: Athens Analysis Laboratories SA, Microbiology Laboratory in Metamorfosi, Greece
DSM: DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Leibniz Institute, Braunschweig, Germany

*** Renewal of broths took place at 48 h of incubation**

- **Fig. 2** presents (as an example) the **planktonic growth** of *L. monocytogenes* in the presence of 10 different concentrations of **thyme EO** (0.5 – 0.001% v/v) (Bioscreen C absorbance growth curves).



- **Fig. 3** presents (as an example) the **biofilm growth** of *L. monocytogenes* in the presence of 8 different concentrations of **oregano EO** (0.25 – 0.002% v/v) (CV staining and absorbance measurements). The planktonic growth is also shown.



CONCLUSIONS

The results demonstrated that the EOs of two endemic organic plants of a Greek island both present strong antibacterial action and could be further exploited as natural antimicrobials for food and health applications.

REFERENCES

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