

# Compounds of natural origin for seed treatment

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## Introduction

In organic agriculture, the use of synthetic agents during seed production- and processing, to control seed transmissible diseases, is not allowed. We are developing a combination therapy, by bringing together physical treatments, treatments with compounds of natural origin and microbial buffering of disinfected seed with antagonists. Here we show the potential value of formulated thyme oil for control of seed transmissible bacterial and fungal diseases on seed.

## Materials and methods

**In vitro tests.** Antimicrobial activity of essential oils was tested in 96 wells plates. Bacterial growth was determined using resazurin, which is only converted from pink to blue by metabolically active bacteria (Fig. 1A). Fungal growth was determined using fluorescein diacetate, which is only converted from colorless to yellow by metabolically active fungi (Fig. 1B), or by measuring of radial growth on agar media in a 24 wells plate (Fig. 1C).

**Seed treatments.** The effect of essential oils on seed germination was determined in a blotter test using the Seed Calculator 2.2 (Plant Research International).

## Results and conclusions

- Thyme and clove oil were the most active essential oils against *Xanthomonas campestris* pv. *campestris* (Gram-) and *Clavibacter michiganensis* subsp. *michiganensis* (Gram+) (see table 1)
- Thyme oil was also effective in *in vitro* assays against *Alternaria brassiciola*, *A. alternata*, *Colletotrichum capsici* and *Phoma lingam* in a concentration of  $\leq 0.15\%$ .
- Formulation of thyme oils with a chelator and a detergent decreased the minimum inhibitory concentration for bacteria from 0.6% to less than 0.01%.
- Cabbage seed treated with formulated thyme oil at a

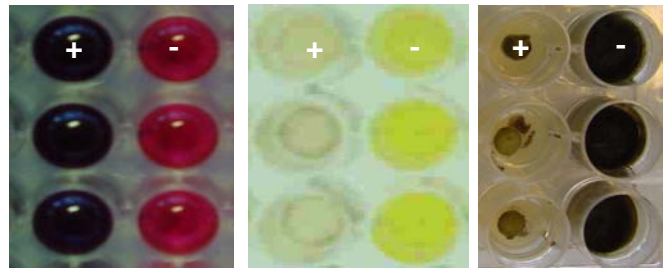


Fig. 1. *In vitro* assays for determining bacterial growth (1A) or fungal growth (1B and 1C) in the presence (+) and absence (-) of essential oils.

Essential oil	Xcc	Cmm	Essential oil	Xcc	Cmm
Carvacrol	5	>10	Anise	ND	>10
Thyme	0.6	0.6	Citron	ND	>10
Basil	10	2.5	Pine	ND	10
Tea tree oil	>10	10	Geranium	ND	10
Eucalyptus	>10	>10	Junipers	ND	>10
Lavender	>10	10	Camille	ND	>10
Clove	1.25	0.6	Marjoram	ND	>10
Castor	>10	>10	Cypress	ND	>10

Table 1. Minimum inhibitory concentrations (in %) of essential oils in *in vitro* assays for *Xanthomonas campestris* pv. *campestris* and *Clavibacter michiganensis* subsp. *michiganensis*

concentration exceeding 0.25% oil, affected germination adversely.

- Formulated thyme oil strongly reduced the growth of (saprophytic) fungi in a blotter test (Fig. 2)

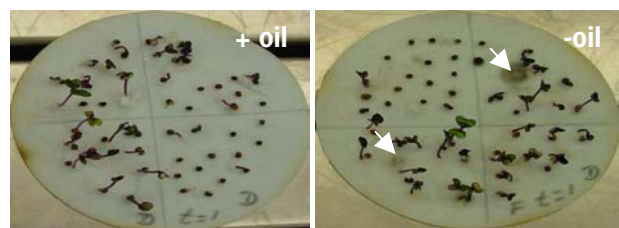


Fig. 2. Cabbage seed treated with formulated thyme oil (left) and with water (right). Fungi in water-treated samples are indicated with a white arrow.

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