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Projeto 04/SIAC/2015 – SIAC 16159

Medicinal and aromatic plants (MAP) as potential antibiotics to control fire blight

Teresa Costa, João Pedro Luz, Conceição Amaro, Susana Dias, Fernanda M. Ferreira, Paula Castro and Cristina Galhano

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PROBLEM

- ***Erwinia amylovora***
  (EPPO A2 List)

- One of the "top ten" bacteria in molecular plant pathology

- **Fire blight**

PROBLEM

Erwinia amylovora hosts

Rosaceae family:

Pear tree (*Pyrus* spp.)

Apple tree (*Malus* spp.)

Quince (*Cydonia oblonga*)

Ornamental species

(*Rubus* spp. – blackberry, raspberry,

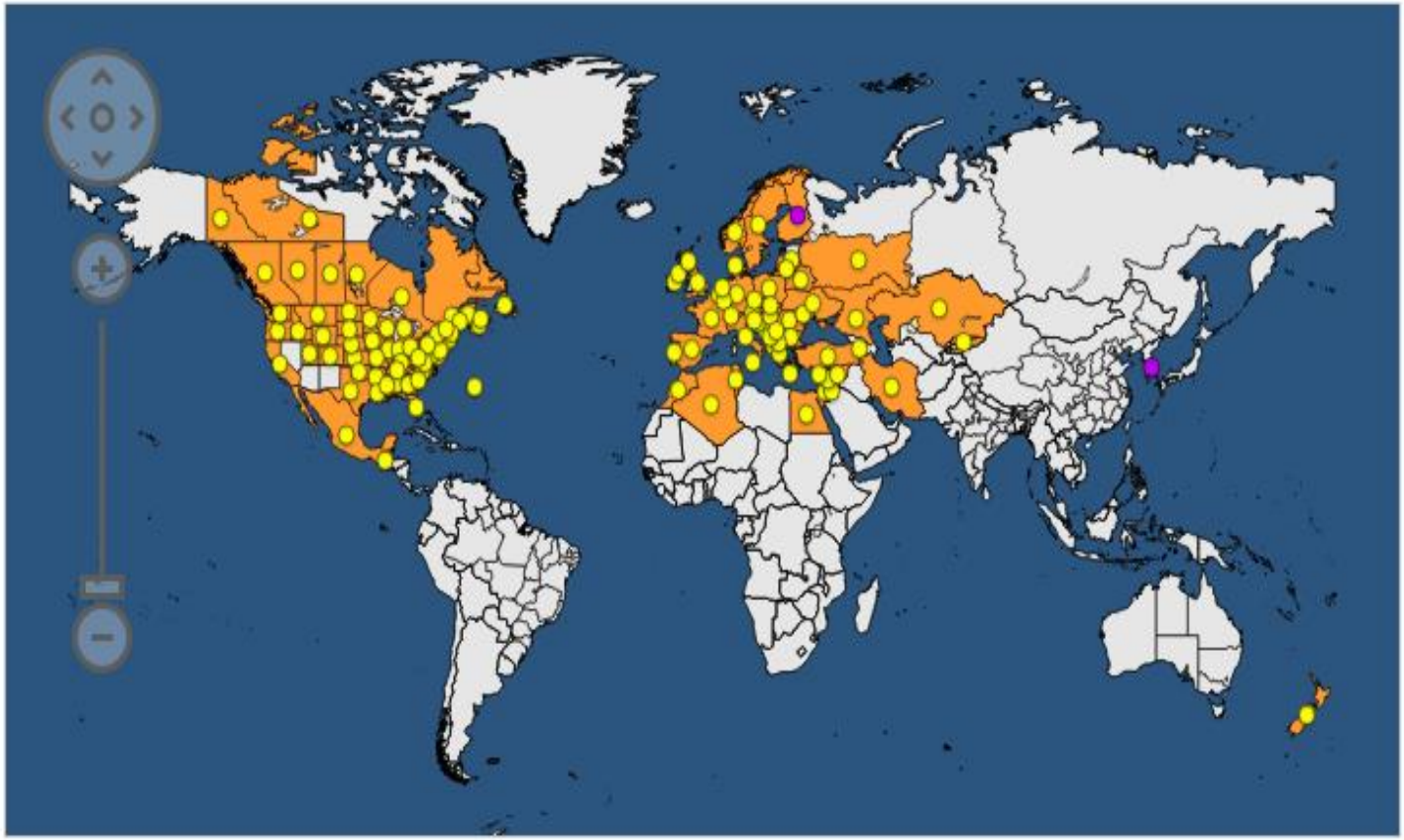
Crataegus spp. - hawthorn

Pyracantha spp. - firethorn

Rosa rugosa - rose)

PROBLEM

Erwinia amylovora distribution



Legend: Present Transient

(Source: EPPO)

PROBLEM

Erwinia amylovora → Fire blight

- Bacterium
- Gram-negative
- Rod
- Facultative anaerobe
- Taxonomy:

  Kingdom: Bacteria

  Phylum: Proteobacteria

  Class: Gammaproteobacteria

  Order: Enterobacteriales

  Family: Enterobacteriaceae

- Severe economic losses: **directly** in crop productivity **+** establishment of eradication and quarantine programs entailing high costs
- Rapid propagation
- **NO EFFECTIVE control methods**

Current SOLUTIONS

- ✓ Integrated strategies
- ✓ Combination of cultural practices
- ✓ Resistance of rootstocks
- ✓ Chemical treatments (copper, antibiotics as streptomycin, or products that stimulate the defence mechanisms of plants)
- ✓ Products based on authorized microorganisms (derivative products from the fungus *Aureobasidium pullulans* and from the bacterium *Bacillus subtilis*, act preventively by colonizing the open flowers, and competing directly for nutrients and space with fire blight)

OUR WORK

Medicinal and Aromatic Plants (MAP) as potential antibiotics to control fire blight

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GOAL

To assess the biocidal potential of various Medicinal and Aromatic Plants (MAP) in relation to *Erwinia amylovora*

More specifically, it was evaluated the *in vitro* effect of aqueous extracts and essential oils from various MAP on *E. amylovora*

MATERIAL AND METHODS …

Table 1. Plants used in the study of the potential antibacterial effect on *Erwinia amylovora*.

Species	Common name	Family	Extract	Essential oil
Acacia delbata	Mimosa/Silver wattle /Blue wattle	Fabaceae	x	
Allium sativum	Garlic	Amaryllidaceae	x	
Cinnamomum camphora	Camphor tree/ Camphorwood	Lauraceae	x	x
Eucalyptus globulus	Eucalyptus	Myrtaceae	x	x
Laurus nobilis	Laurel	Lauraceae	x	
Origanum vulgare	Oregano	Lamiaceae	x	x
Ricinus communis	Castor bean	Euphorbiaceae		x
Rosmarinus officinalis	Rosemary	Lamiaceae	x	x
Thymus mastichina	Mastic thyme	Lamiaceae	x	x
Thymus vulgaris	Common thyme	Lamiaceae	x	x

MATERIAL AND METHODS (cont.) …

9 MAP species

Aqueous extracts obtained by maceration (MAE)

5 g of ground dried plant + **30 mL** water (24 hours, dark conditions)

↓

Centrifuge (4800 rpm, 5 min)

↓

Filtered through microfilters to Eppendorf tubes

7 MAP species

Essential oils (EOs):

Purchased from a Portuguese distributor

Mastic thyme EO – provided by Fernanda Ferreira (ESAC)

MATERIAL AND METHODS (cont.) …

Portuguese strain of *E. amylovora*

provided by João Pedro Luz (Polytechnic Institute of Castelo Branco)

✓ peaked and kept in Petri dishes with King B medium refrigerated at 4°C

✓ to obtain the culture in the *lag phase* of growth, when necessary, the bacterium was peaked to a new medium

MATERIAL AND METHODS (cont.) …

In vitro bioassay: Antibiograms

- ✓ Mother suspension: 10^8 CFU.mL^{-1}
- ✓ Preparation of Petri dishes with King-B medium
- ✓ 4 filter paper discs /Petri dish
- ✓ On each disc 20 µL of: EO/MAE/ positive control/negative control
- ✓ positive control: streptomycin negative control: distilled water
- ✓ 6 replicates
- ✓ Incubation - 38 hours, at 25°C

Observations

- ✓ Measurement of the diameter of inhibition were measured and recorded
- ✓ Efficiency:

  Comparison of the EO/EAM inhibition diameters with that of the positive control

  Calculation of index of antimicrobial activity (IAA):

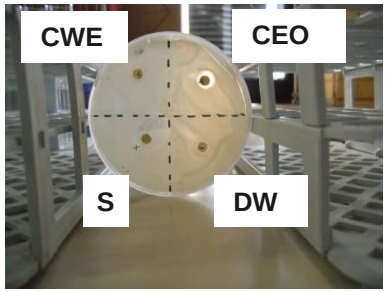
$$\mathbf{IAA\ (\%) = \{-1x\ [(C-T) / (C + T)]\}\ x100}$$

C - average inhibitory zone (cm) of streptomycin

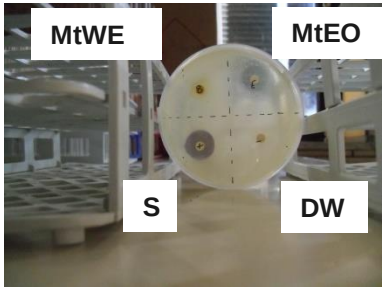
T - average inhibitory zone (cm) of the tested essential oil

RESULTS …

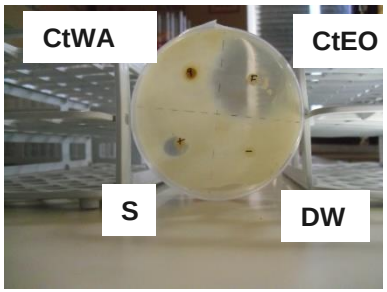
- **All the Essential Oils** had an **inhibitory effect** on *Erwinia amylovora* growth; the same was observed in the positive control, the antibiotic streptomycin
- The **Maceration Aqueous Extracts** (5 g of dry plant/30 mL concentration), had **no antibacterial effect**; similar behaviour for the negative control, distilled water.



CWE = Camphor Water Extract;
CEO = Camphor Essential Oil;



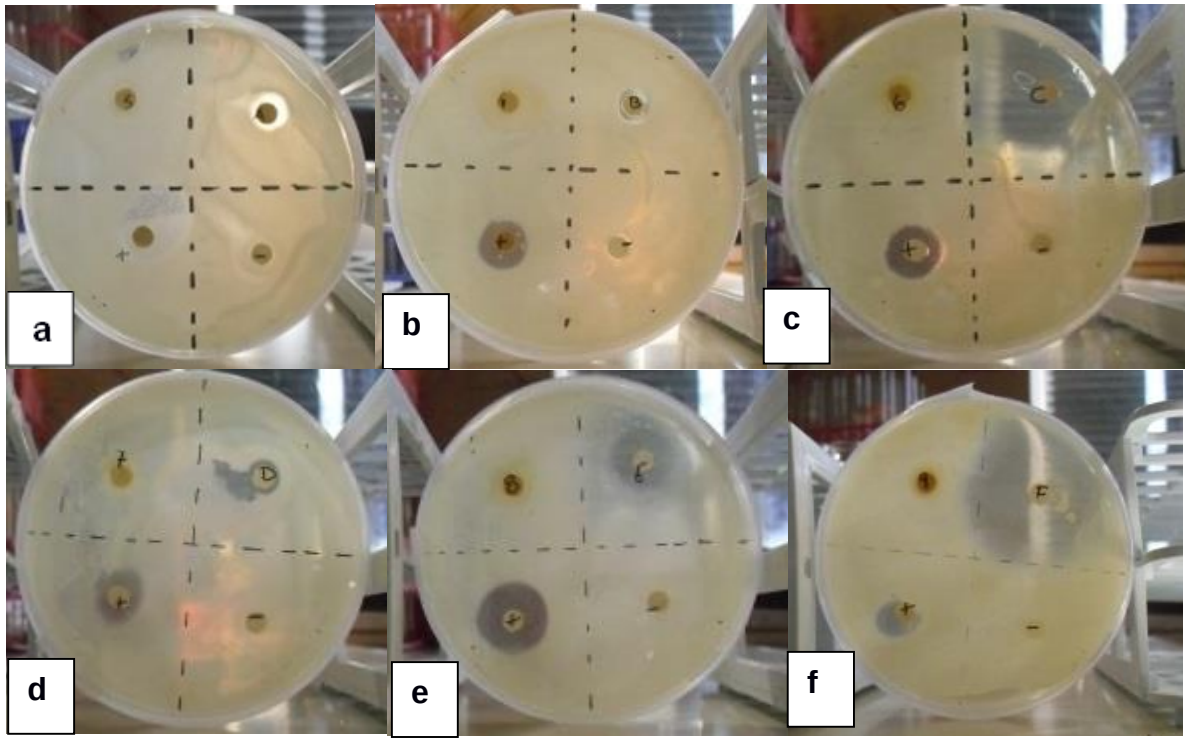
MtWE = Mastic thyme Water Extract;
TmEO = Mastic thyme Essential Oil;



CtWE = Common thyme Water Extract;
CtEO = Common thyme Essential Oil;

S = Streptomycin; **DW** = Distilled Water

RESULTS …



Antibiograms with the inhibition zone, of the essential oils of **(a)** ***Cinnamomum camphora*****; (b)** ***Eucalyptus globulus***; **(c)** ***Origanum vulgare*****; (d)** ***Rosmarinus officinalis*****; (e)** ***Thymus mastichina***; and (f) ***T. vulgaris***. Extracts from the same plants, the antibiotic streptomycin (0.02%) (positive control), and sterilized distilled water (negative control).

RESULTS …

Table 2. Inhibition growth zone (cm) of *Erwinia amylovora* due to the plants under study (aqueous extract and essential oils), compared to the positive control, streptomycin, and to the negative control, sterilized distilled water.

Plant species	EO (cm)	WE (cm)	Positive Control (cm)	Negative Control (cm)
Acacia dealbata	Not tested	n.d.	1.60±0.580	n.d.
Allium sativum	Not tested	n.d.	1.60±0.580	n.d.
Cinnamomum camphora	0.92±0.360[a]	n.d.	1.55±0.745[b]	n.d.
Eucalyptus globulus	1.07±0.266[a]	n.d.	1.42±0.279[a]	n.d.
Laurus nobilis	Not tested	n.d.	2.08±0.264	n.d.
Origanum vulgare	3.33±1.633[a]	n.d.	1.77±1.294[b]	n.d.
Ricinus communis	n.d.	Not tested	1.00±1.112	n.d.
Rosmarinus officinalis	2.37±1.227[a]	n.d.	1.40±0.518[a]	n.d.
Thymus mastichina	2.58±0.736[a]	n.d.	2.18±0.204[a]	n.d.
Thymus vulgaris	3.17±2.562[a]	n.d.	1.13±0.920[b]	n.d.

[(**n.d.** – non-detectable inhibition halo value. Results are presented as mean ± standard deviation of six replicates. Different letters indicate statistically significant differences ($p < 0.05$)].

RESULTS …

EOs with the most relevant antibacterial effect:

Origanum vulgare: inhibition zone - 3.33 cm

Thymus vulgaris: inhibition zone - 3.17 cm

T. mastichina: inhibition zone - 2.58 cm

Rosmarinus officinalis: inhibition zone - 2.37 cm

EOs of ***Origanum vulgare*** (oregano) and ***T. vulgaris*** (common thyme) showed a **bactericidal effect** statistically **superior to that** of **streptomycin**

EOs of ***Eucalyptus globulus*** (eucalyptus), ***Rosmarinus officinalis*** (rosemary) and ***Thymus mastichina*** (mastic thyme) had a **bactericidal effect** statistically **comparable to that** of **streptomycin**

EOs of ***Cinnamomum camphora*** (camphor) had a statistically **lower bactericidal effect** than **streptomycin**

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RESULTS …

Table 3. Index of antimicrobial activity (IAA) of the tested essential oils in relation to *Erwinia amylovora*. Results are calculated based on means of six replicates.

Essential Oil	**IAA (%)**
Cinnamomum camphora	-25.8
Eucalyptus globulus	-14.1
Origanum vulgare	30.7
Rosmarinus officinalis	25.7
Thymus mastichina	8.4
Thymus vulgaris	47.3

$$IAA\ (\%) = \{-1x\ [(C-T) / (C + T)]\}\ x100$$

FINAL REMARKS …

- The **extracts** of the studied plants, in the concentration of 5 g of plant/30 mL of water, **did not inhibit** the ***E. amylovora* growth**;
- All the essential oils considered, except castor oil, inhibited the growth of *E. amylovora*;
- *Origanum vulgare* EO presented the highest inhibition zone;
- *Cinnamomum camphora* EO presented the lowest inhibition zone;
- The IAA showed that the essential oils of *O. vulgare, Rosmarinus officinalis, Thymus mastichina* and *T. vulgaris* had a higher effect than streptomycin.

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FINAL REMARKS

This work added relevant information about the biocidal potential of some MAP aqueous extracts and essential oils in relation to this bacterium

- In a context characterised by unprecedented evidences of climatic change it is indisputable that farmers are facing serious plant protection issues and phytosanitary risks which seriously affect economic dynamism. The high vulnerability of modern agrosystems to crop damage by pests and diseases leads to the crucial development of innovative management systems which inevitably foster the search for sustainable and healthier alternatives to synthetic chemicals;

- *O. vulgare*, *R. officinalis* and *Thymus* spp. essential oils have shown promising results in this challenging fight

- Further *in vitro* and *in vivo* bioassays will be required to fully evaluate these plant's potential to control *Erwinia amylovora*

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Thank
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