SCREENING OF NATURAL AND ECO-FRIENDLY COMPOUNDS FOR THEIR ANTIMICROBIAL ACTIVITY AGAINST XYLELLA FASTIDIOSA

Carmine Del Grosso, Stefania Zicca, Giuseppe Altamura and Giuseppe Lima
Plant Pathogenic Bacteria: «Control Strategies»

Preventive Measures

- Quarantine
- Eradication
- Sanitation
Traditional Plant Pathogenic Bacteria Control

- Copper based products (e.g. copper hydroxide, copper sulphate, copper oxychloride, etc.);
- Others chemical (Fosetil-Al, probenazole, potassium phosphite, etc.);
- Antibiotics (banned in agriculture in the EU);

EU Pesticides Database – Bactericide
Active Substances Status under Reg. (EC) No 1107/2009
(https://ec.europa.eu/food/plant/pesticides/eu-pesticides-db_en)
Modern Trends of Plant Pathogenic Bacteria Control

❖ Biological Control «sensu stricto»

Antagonists or Biocontrol Agents:
- Plant Growth-Promoting Rhizobacteria (PGPR);
- Mycorrhizal Activity;
- Bacteriophages;

❖ Biological Control «sensu lato»

- Natural compounds:
  - Plant-derived compounds (plant extracts, essential oils, etc.);
  - Microbial-derived compounds (bacteriocins, peptides, etc.);
  - and so others;
- Resistance inducers;
- Biological soil improver;
- Bio-fertilizer (complex or mineral derived);
- Biological activators.

❖ Integrated Pest Management
Strategies to control *Xylella fastidiosa*

to evaluate and set up a sustainable approach to counteract the bacterium on plant.

We evaluated:

i) The antimicrobial activity of some natural and eco-friendly compounds;

ii) The antibacterial activity against different strains and subsp. of *Xylella fastidiosa*;

iii) The capability of the selected compounds to reduce *Xylella* symptoms and pathogen colonization in olive plants.

<table>
<thead>
<tr>
<th>ID</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWP</td>
<td>Seaweed + polyphenols, water extract.</td>
</tr>
<tr>
<td>SWP-ETOH</td>
<td>Seaweed + polyphenols, ethanolic extract.</td>
</tr>
<tr>
<td>PB1</td>
<td>Polyphenolic biomolecules.</td>
</tr>
<tr>
<td>PB2</td>
<td>Polyphenolic biomolecules.</td>
</tr>
<tr>
<td>PB3</td>
<td>Polyphenolic biomolecules.</td>
</tr>
<tr>
<td>PB4</td>
<td>Polyphenolic biomolecules.</td>
</tr>
<tr>
<td>PB5</td>
<td>Polyphenolic biomolecules.</td>
</tr>
<tr>
<td>Si</td>
<td>Complex of silicon and micro-elements (including zinc).</td>
</tr>
<tr>
<td>Ki</td>
<td>Complex of chitosan and micro-elements.</td>
</tr>
<tr>
<td>Zn-F</td>
<td>Zinc Formulate</td>
</tr>
<tr>
<td>Cu-F</td>
<td>Copper Formulate</td>
</tr>
<tr>
<td>Zn+Cu_F</td>
<td>Mixture of ¾ of Zn-F and ¼ Cu-F formulates</td>
</tr>
</tbody>
</table>
i) Antimicrobial activity of natural and eco-friendly compounds

Antibacterial
Antibacterial activity against different strains and subsp. of *Xylella fastidiosa*.

**DISK DIFFUSION ASSAY**
Inhibition halo (in mm) of different concentration of Seaweed + Polyphenols mixture (SWP) by the disc diffusion assay against four strains of *Xylella fastidiosa*. Drug reference (Ampicillin + Streptomycin) was a positive control. Sterile Distilled Water + 8% ETOH was a negative control (0%). Bars indicate the mean and standard deviation of three replicates.
Inhibition halo (in mm) of different concentration of Polyphenolic Biomolecules (PB) by the disc diffusion assay against four strains of *Xylella fastidiosa*. Drug reference (Ampicillin + Streptomycin) was a positive control. Sterile Distilled Water (0%) was a negative control. Bars indicate the mean and standard deviation of three replicates.
Inhibition halo (in mm) of different concentration of Polyphenolic Biomolecules (PB) by the disc diffusion assay against four strains of Xylella fastidiosa. Drug reference (Ampicillin + Streptomycin) was a positive control. Sterile Distilled Water (0%) was a negative control. Bars indicate the mean and standard deviation of three replicates.
Inhibition halo (in mm) of different concentration of Silicon (Si) and Chitosan (Ki) complex by the disc diffusion assay against four strains of *Xylella fastidiosa*. Drug reference (Ampicillin + Streptomycin) was a positive control. Sterile Distilled Water (0%) was a negative control. Bars indicate the mean and standard deviation of three replicates.
Inhibition halo (in mm) of different concentration of Zinc (Zn-F), Copper (Cu-F) and Zinc + Copper (Zn+Cu_F) formulate by the disc diffusion assay against four strains of Xylella fastidiosa. Drug reference (Ampicillin + Streptomycin) was a positive control. Sterile Distilled Water (0%) was a negative control. Bars indicate the mean and standard deviation of three replicates.
# Experiments on Greenhouse Pot Grown Olive Plants

Evaluation of most effective compounds against *Xfp* symptoms and plant colonization

## EXPERIMENT 1 - SUSCEPTIBLE HOST (CV. OGLIAROLA)

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>TREATMENT</th>
<th>PLANTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-</td>
<td>NEGATIVE CONTROL <em>(uninocul. - untreat. plants)</em></td>
<td>3X3</td>
</tr>
<tr>
<td>C+</td>
<td>POSITIVE CONTROL <em>(inocul. - untreat. plants)</em></td>
<td>3X3</td>
</tr>
<tr>
<td>PB1</td>
<td>FOLIAR SPRAY 1% (v/v)</td>
<td>3X3</td>
</tr>
<tr>
<td>PB3</td>
<td>FOLIAR SPRAY 1% (v/v)</td>
<td>3X3</td>
</tr>
<tr>
<td>PB4</td>
<td>FOLIAR SPRAY 1% (v/v)</td>
<td>3X3</td>
</tr>
<tr>
<td>Zn-F</td>
<td>FOLIAR SPRAY 1,2% (v/v)</td>
<td>3X3</td>
</tr>
<tr>
<td>Cu-F</td>
<td>FOLIAR SPRAY 1,2% (v/v)</td>
<td>3X3</td>
</tr>
<tr>
<td>SWP1</td>
<td>SOIL TREATMENT PREVENTIVE 2% (v/v)</td>
<td>3X3</td>
</tr>
<tr>
<td>SWP2</td>
<td>SOIL TREATMENT CURATIVE 2% (v/v)</td>
<td>3X3</td>
</tr>
</tbody>
</table>
Evaluation of the disease severity using a Disease Index (DI%) scale and monitoring the bacterium in plant using q-PCR.

Disease index, DI (%), calculated by using an empirical scale from 0 (no symptoms) to 5 (100% symptoms or dead plant), of different treatments on pot grown olive plants inoculated with *Xylella fastidiosa* subsp. *pauca* ST53. C- = Negative Control (uninoculated - untreated plants); C+ = Positive Control (inoculated - untreated plants); PB1, PB2, PB3 and PB4 = plant inoculated with Xfp and treated with different Polyphenolic biomolecules using foliar spraying; Zn-F, Cu-F and Zn+Cu_F = plant inoculated with Xfp and treated with Zinc, Copper and Zinc+Copper formulated by foliar spraying; SW1 and SW2 = preventive and curative soil treatments, respectively, on inoculated plants with Xfp. Bars indicate the mean and standard deviation of the DI (%) calculated on three replicates of three plants per replication. The dot (●) represents the bacterial concentration (CFU/mL) for each plant analysed by q-PCR. Data reported were assessed on December 2020.
Representative photos of different treatments on pot grown olive plants inoculated with *Xylella fastidiosa* subsp. *pauca* ST53. C- = Negative Control (uninoculated - untreated plants); C+ = Positive Control (inoculated - untreated plants); PB1, PB2, PB3 and PB4 = plant inoculated with Xfp and treated with different Polyphenolic biomolecules by foliar spraying; Zn-F, Cu-F and Zn+Cu_F = plant inoculated with Xfp and treated with Zinc, Copper and Zinc+Copper formulate by foliar spraying; SW1 and SW2 = preventive and curative soil treatments, respectively, on inoculated plants with Xfp. The images were taken on January 2021.
Conclusion

❖ **In vitro**, all the tested compounds show an interesting large spectrum antimicrobial activity.

Some of them were highly effective against *Xylella fastidiosa* too;

However, we have to investigate further aspects regarding the antimicrobial activity.

❖ In the **in vivo** experiments, although no significative differences were observed on *Xfp* concentration inside the plants, treatments with PB1, PB2, PB3 and Zn were able to reduce temporarily *Xfp* symptoms.

To this end, we must perform further experiments also using a simplified pathosystem (e.g. an herbaceous host) that is more easily manageable than the olive tree.
Thank you for your attention!

Acknowledgments

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