Università degli Studi del Molise

Institute for Sustainable Plant Protection
National Research Council of Italy

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

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3rd European conference on Xylella fastidiosa 2021

XF ACTORS

Xylella Fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy

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)

Substance	
4-t-Pentylphenol	
Alkyltrimethyl ammonium chloride	
Aluminium sulphate	
Aureobasidium pullulans (strains DSM 14940 and DSM 14941)	
Bacillus amyloliquefaciens (formerly subtilis) str. QST 713	
Benzoic acid	
Bronopol	
Chlorhydrate of poly(iminino imido biguanidine)	
Chlorophylline	
Dioctyldimethyl ammonium chloride	
Ethylhexanoate	
Flumequine	
Gentian violet	
Glutaraldehyde (aka glutardialdehyde)	
Kasugamycin	
Lauryldimethylbenzylammonium bromide	
Octyldecyldimethyl ammonium chloride	
Oxytetracycline	
Potassium permanganate	
Probenazole	
Propionic acid	
Sodium dichlorophenate	
Sodium hypochlorite	
Sodium lauryl sulfate	
Sodium p-t-amylphenate	
Sodium-p-toluene-sulfonchloramid	
Streptomyces lydicus WYEC 108	
Streptomycin	
Yucca Schidigera extract	

Not approved

Pending

Approved

Modern Trends of Plant Pathogenic Bacteria Control

❖ Biological Control «sensu stricto»

Antagonists or Biocontrol Agents:

- Plant Growth-Promoting Rhizobacteria (PGPR);
- Mycorrhizal Activity;
- Bacteriophages;

❖ Integrated Pest Management

❖ Biological Control «sensu lato»

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

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;

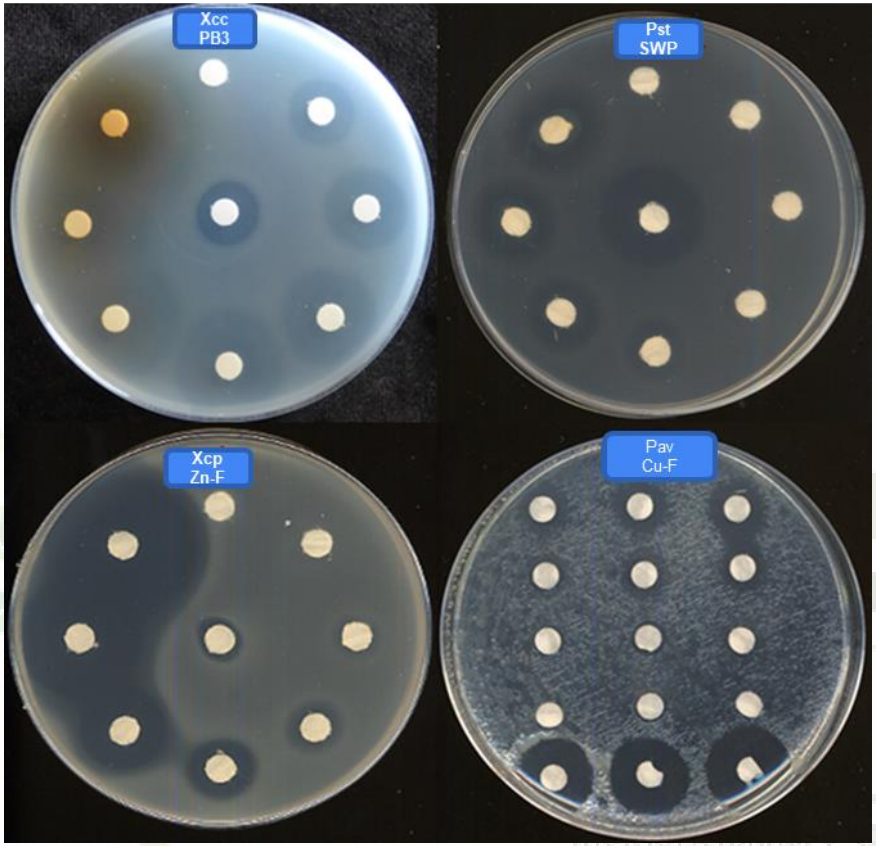
ii) The antibacterial activity against different strains and subsp. of *Xylella fastidiosa*;

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

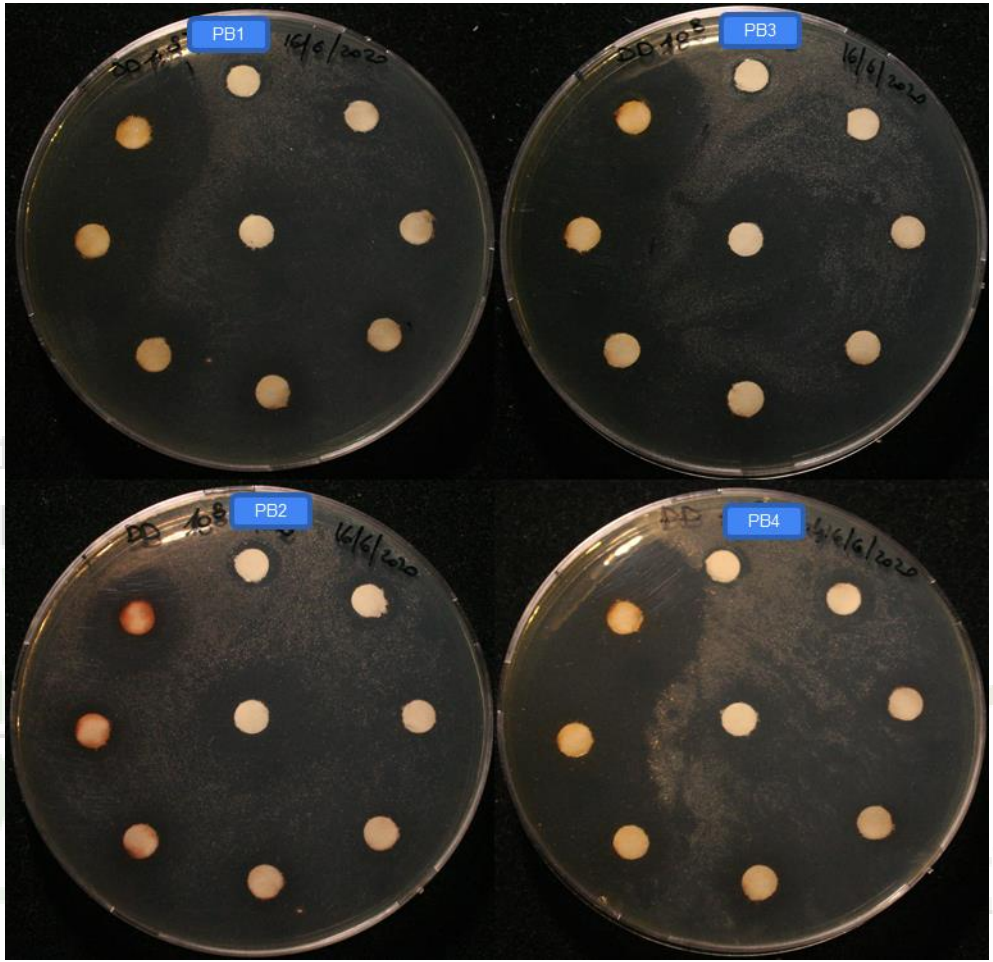
ID	Description
SWP	Seaweed + polyphenols, water extract.
SWP-ETOH	Seaweed + polyphenols, ethanolic extract.
PB1	Polyphenolic biomolecules.
PB2	Polyphenolic biomolecules.
PB3	Polyphenolic biomolecules.
PB4	Polyphenolic biomolecules.
PB5	Polyphenolic biomolecules.
Si	Complex of silicon and micro-elements (including zinc).
Ki	Complex of chitosan and micro-elements.
Zn-F	Zinc Formulate
Cu-F	Copper Formulate
Zn+Cu_F	Mixture of ¾ of Zn-F and ¼ Cu-F formulates

i) Antimicrobial activity of natural and eco-friendly compounds

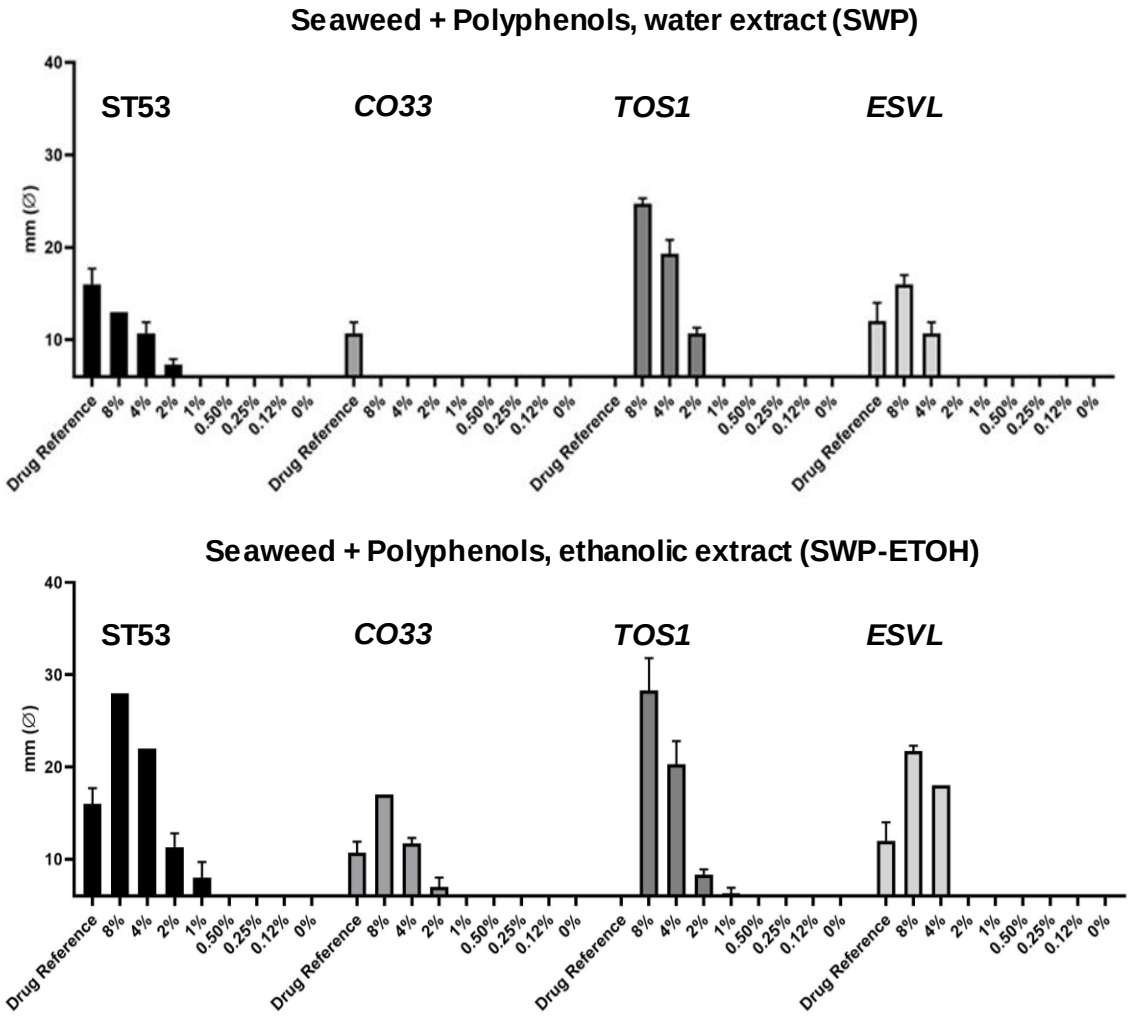
Antibacterial



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

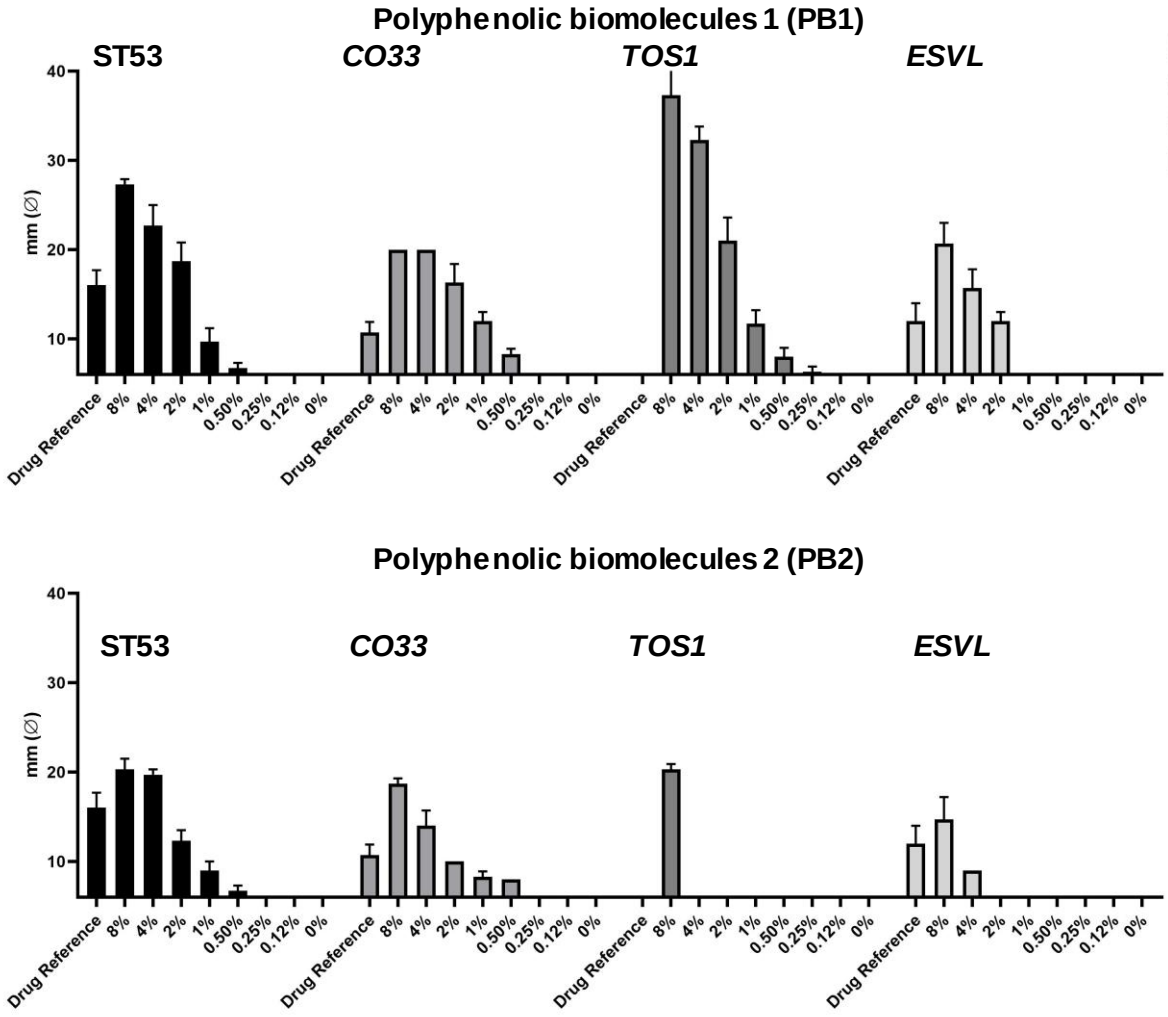


DISK DIFFUSION ASSAY



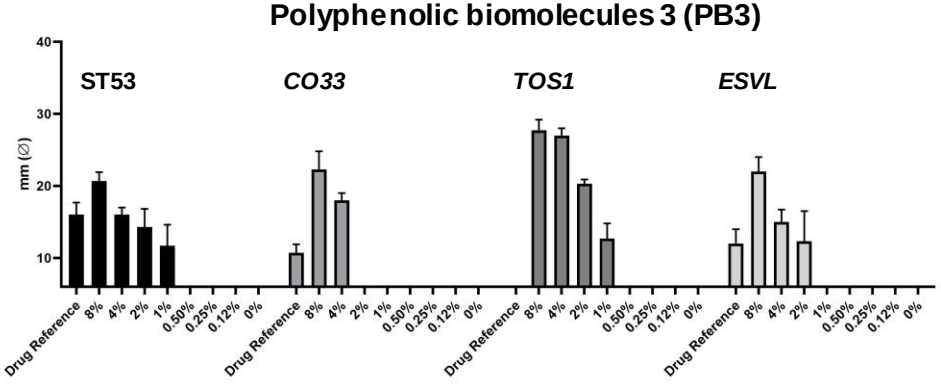
Inhibition halo (in mm) of different concentration of Seaweed + Polyphenols mixture (SWP) by the disc diffusion assay against four strains of *Xylella fastidiosa*. Drug reference (Ampicillin + Streptomycin) was a positive control. Sterile Distilled Water + 8% ETOH was a negative control (0%).

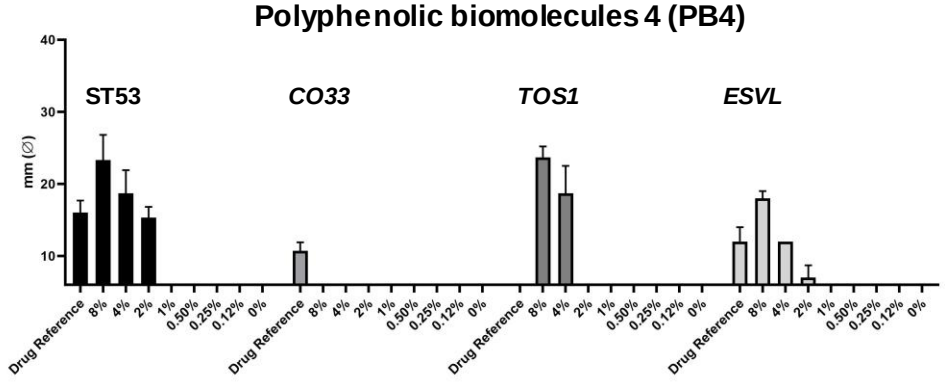
Bars indicate the mean and standard deviation of three replicates.

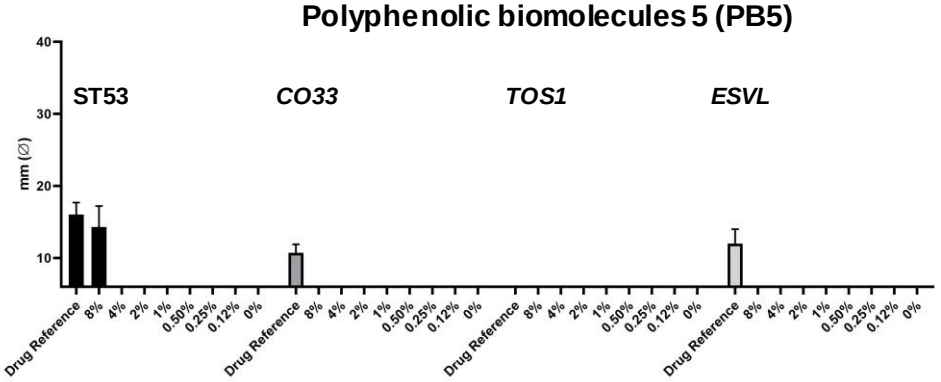


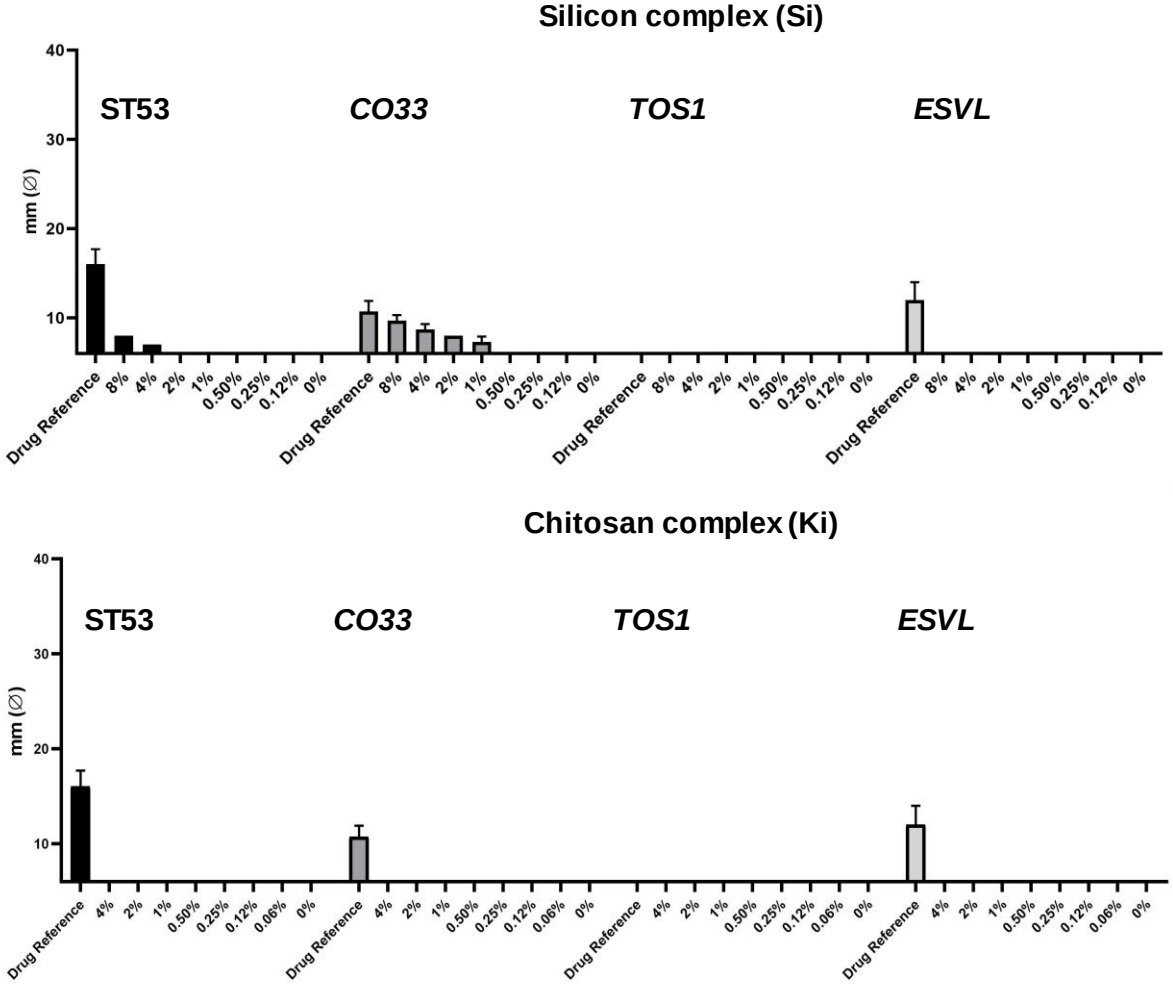
Inhibition halo (in mm) of different concentration of Polyphenolic Biomolecules (PB) by the disc diffusion assay against four strains of *Xylella fastidiosa*. Drug reference (Ampicillin + Streptomycin) was a positive control. Sterile Distilled Water (0%) was a negative control. Bars indicate the mean 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.



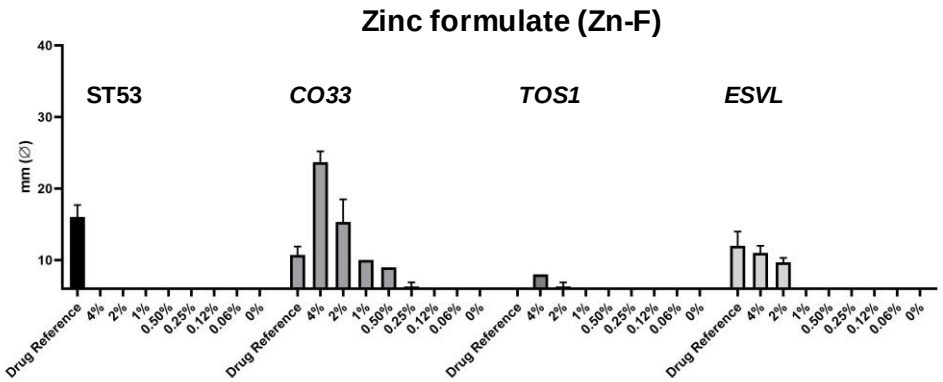


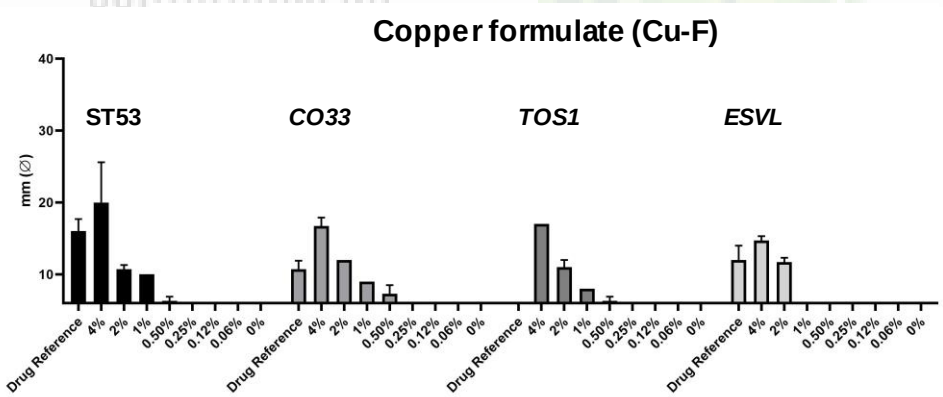


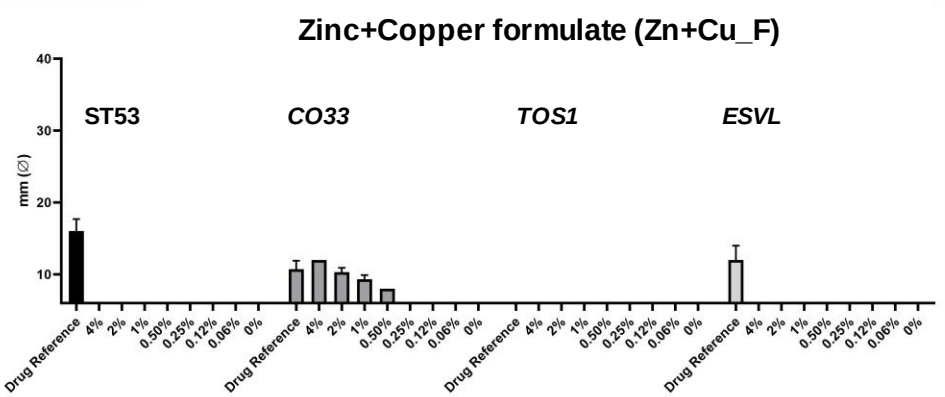


Inhibition halo (in mm) of different concentration of Silicon (Si) and Chitosan (Ki) complex by the disc diffusion assay against four strains of *Xylella fastidiosa*. Drug reference (Ampicillin + Streptomycin) was a positive control. Sterile Distilled Water (0%) was a negative control. Bars indicate the mean and standard deviation of three replicates.

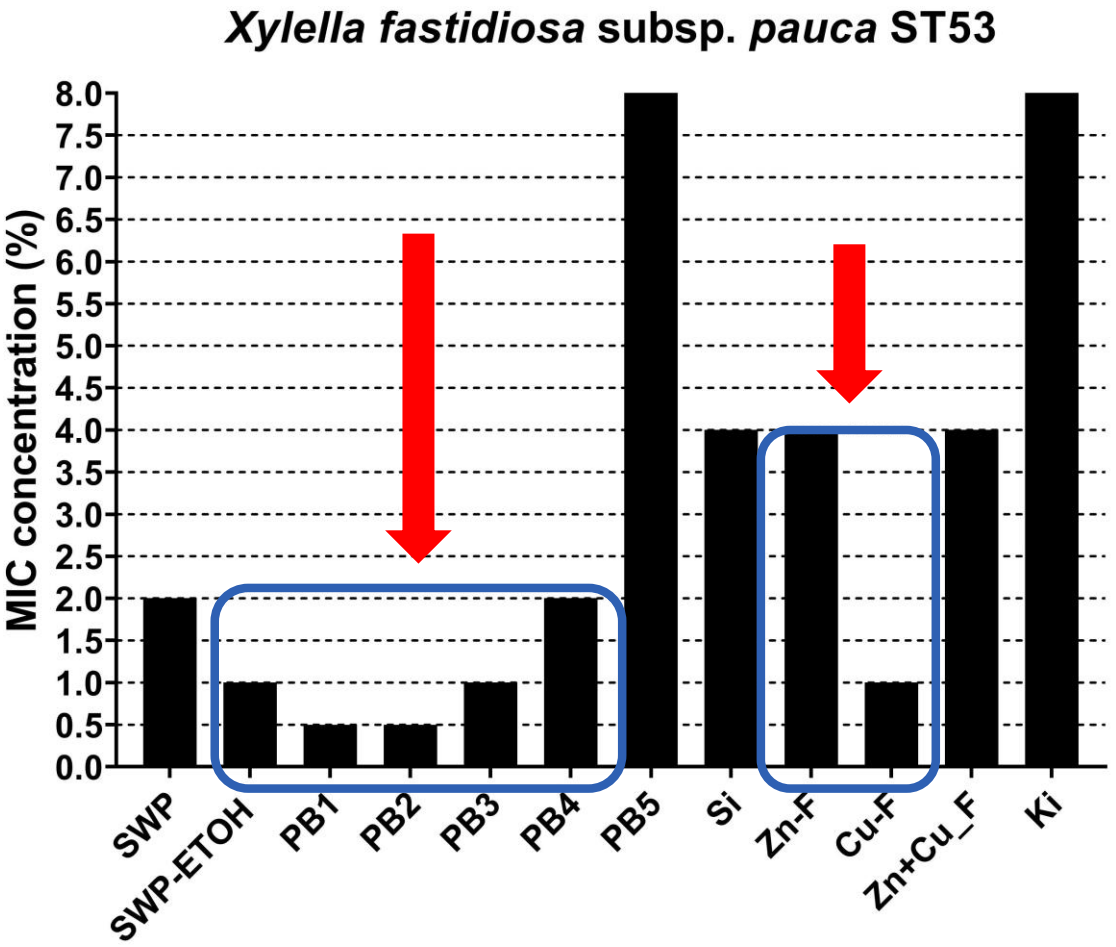
Inhibition halo (in mm) of different concentration of Zinc (Zn-F), Copper (Cu-F) and Zinc + Copper (Zn+Cu_F) formulates by the disc diffusion assay against four strains of *Xylella fastidiosa*. Drug reference (Ampicillin + Streptomycin) was a positive control. Sterile Distilled Water (0%) was a negative control. Bars indicate the mean and standard deviation of three replicates.





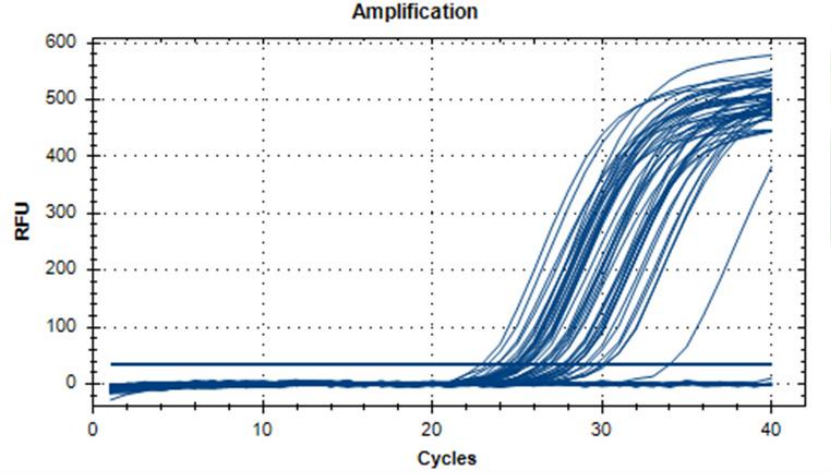


MIC (Minimum Inhibitory Concentration)



Experiments on Greenhouse Pot Grown Olive Plants

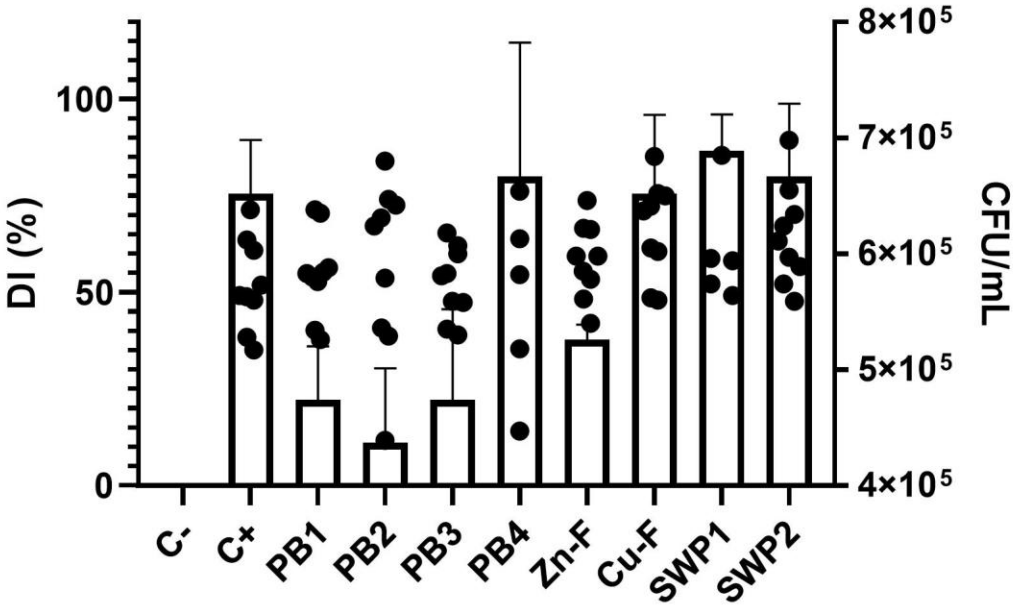
Evaluation of most effective compounds against *Xfp* symptoms and plant colonization



EXPERIMENT 1 - SUSCEPTIBLE HOST (CV. OGLIAROLA)

SAMPLE	TREATMENT	PLANTS
C-	NEGATIVE CONTROL (*uninocul.- untreat. plants*)	3X3
C+	POSITIVE CONTROL (*inocul.- untreat. plants*)	3X3
PB1	FOLIAR SPRAY 1% (v/v)	3X3
PB3	FOLIAR SPRAY 1% (v/v)	3X3
PB3	FOLIAR SPRAY 1% (v/v)	3X3
PB4	FOLIAR SPRAY 1% (v/v)	3X3
Zn-F	FOLIAR SPRAY 1,2% (v/v)	3X3
Cu-F	FOLIAR SPRAY 1,2% (v/v)	3X3
SWP1	SOIL TREATMENT PREVENTIVE 2% (v/v)	3X3
SWP2	SOIL TREATMENT CURATIVE 2% (v/v)	3X3

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 formulates 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 formulates 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!

Aknowledgments

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