In Vitro evaluation of Chitosan hydrochloride and COS (Chito-Oligosaccharides)-OGA (Oligo-Galacturonides) on phytopathogenic fungi and *Escherichia coli*

MAKAU S.M.[1], MOUMNI M. [1], LANDI L. [1], PIROZZI D. [2], SANNINO, F. [3], ROMANAZZI G.[1*]

[1]Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, 60131, Ancona, Italy. [2]Laboratory of Biochemical Engineering, Department of Chemical Engineering, Materials and Industrial Production (DICMaPI), University of Naples "Federico II", Piazzale Tecchio, 80, 80125 Naples, Italy. [3]Department of Agricultural Sciences, University of Naples "Federico II", Via Università 100, 80055 Portici, Naples, Italy. *g.romanazzi@staff.univpm.it

ABSTRACT

Use of novel alternative compounds in agriculture is being promoted to reduce synthetic pesticides. An *in vitro* study was conducted to evaluate antimicrobial and antioxidant activities of chitosan hydrochloride (CH) and COS (chito-oligosaccharides)-OGA (oligo-galacturonides). COS-OGA at 1% and 0.5% concentrations completely inhibited mycelial growth of *Alternaria alternata*, *Alternaria brassicicola*, *Botrytis cinerea*, *Monilinia laxa*, *Monilinia fructigena*, and *Monilinia fructicola*. Further, complete inhibition was observed with COS-OGA 0.25% against *M. fructigena* and *M. laxa*. Inhibition against *B. cinerea*, *M. fructicola*, *A. alternata*, and *A. brassicicola* by COS-OGA 0.25% was 86.75%, 76.31%, 69.73%, and 60.45%, respectively. *M. laxa* and *M. fructigena* were completely inhibited by CH concentrations of 1–0.25% and *M. fructicola* by concentrations of 1–0.5%. At CH 0.25%, inhibition for *M. fructicola*, *A. brassicicola*, *A. alternata*, and *B. cinerea* was 93.99%, 80.99%, 69.73%, and 57.23%, respectively. CH showed effective antibacterial activity against foodborne *Escherichia coli*. COS-OGA had higher antioxidant activity than CH assessed by DPPH and hydroxyl radical scavenging assays. Our findings offer insights into the antimicrobial efficacy and mechanisms of action of these novel compounds, which have the potential to serve as alternatives to synthetic pesticides. *In vivo* investigations are required to validate the prospective application of these treatments for pre- and postharvest disease management.

MATERIAL AND METHODS

In vitro antifungal activities of chitosan hydrochloride (CH; 100%) and COS (chito-oligosaccharides)-OGA (oligo-galacturonides (COS-OGA; 1.25%) were evaluated by monitoring mycelial growth of *Alternaria alternata*, *A. brassicicola*, *Botrytis cinerea*, *Monilinia laxa*, *M. fructigena*, and *M. fructicola* at concentrations of 1, 0.5, 0.25, 0.1, 0.05, and 0.025%. Orthogonal diameters of the colonies were measured daily. Fungicidal and fungistatic activities of CH and COS-OGA were observed on fresh, unamended PDA. Antibacterial activity of CH was assessed against *E. coli* in the presence and absence of light. *In vitro* antioxidant activity of CH and COS-OGA were assessed by DPPH and hydroxyl radical scavenging ability assays at 20 and 30 mg/mL concentrations. Antifungal data were subjected to Welch analysis of variance (ANOVA) and means were separated using Games-Howell test at $p \leq 0.05$. Package '*heatmaply*' was used to prepare the fungal mycelia growth heatmap figure with RStudio version 2023.03.0+386. A t-test was carried out to compare bacterial growth in the presence and absence of CH.

RESULTS

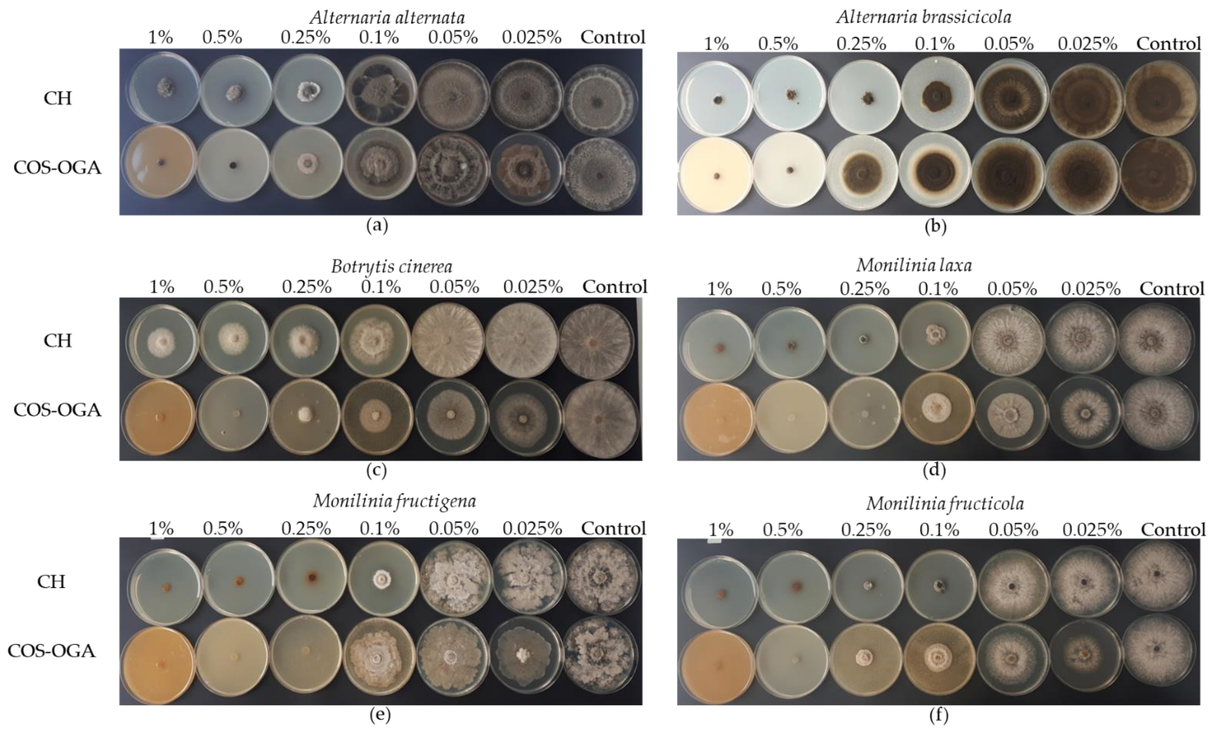

Figure 1. Representative mycelial growth of **(a)** *Alternaria alternata*, **(b)** *Alternaria brassicicola*, **(c)** *Botrytis cinerea*, **(d)** *Monilinia laxa*, **(e)** *Monilinia fructigena*, and **(f)** *Monilinia fructicola* treated with chitosan hydrochloride (CH; 100%) and COS (chito-oligosaccharides)-OGA (oligo-galacturonides) (COS-OGA; 1.25%) at 1%, 0.5%, 0.25%, 0.1%, 0.05%, and 0.025% in comparison to untreated control after 5-17 days post-incubation at 20 ± 1 ° C.

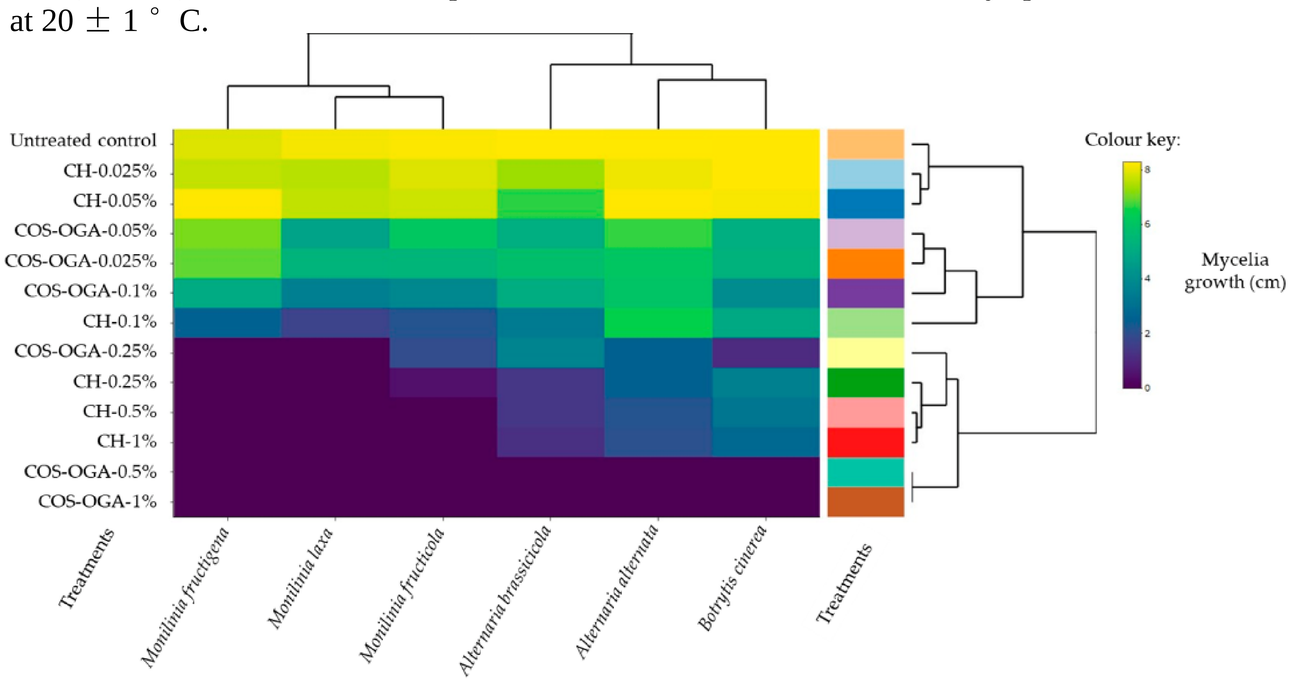

Figure 2. Heatmap of the mycelia growth (cm) of *Monilinia fructigena*, *Monilinia laxa*, *Monilinia fructicola*, *Alternaria brassicicola*, *Alternaria alternata*, and *Botrytis cinerea* treated with chitosan hydrochloride (CH) and COS (chito-oligosaccharides)-OGA (oligo-galacturonides; COS-OGA) after 5–17 days post- inoculation.

Table 1. Fungicidal and fungistatic activities of chitosan hydrochloride and COS (chito- oligosaccharides)-OGA (oligo-galacturonides) against *Alternaria alternata*, *Alternaria brassicicola*, *Botrytis cinerea*, *Monilinia laxa*, *Monilinia fructigena*, and *Monilinia fructicola* after 7 days of incubation at 20 ± 1 ◦C.

Fungus	Treatments [a]					
	CH 1%	CH 0.5%	CH 0.25%	COS-OGA 1%	COS-OGA 0.5%	COS-OGA 0.25%
Alternaria alternata	-	-	-	+	+	-
Alternaria brassicicola	-	-	-	+	+	-
Botrytis cinerea	-	-	-	+	+	-
Monilinia laxa	*	*	-	+	+	+
Monilinia fructigena	*	*	*	+	+	+
Monilinia fructicola	*	*	-	+	*	-

[a] CH=chitosan hydrochloride; COS-OGA=COS (chito-oligosaccharides)-OGA (oligo-galacturonides); + Fungicidal; * Fungistatic; - Not tested.

Table 2. Antibacterial activity of chitosan hydrochloride (100%).

Condition	Log CFU/mL In the absence of chitosan	Log CFU/mL In the presence of chitosan
In the dark	6.0 ± 0.4	4.9 ± 0.5
Exposed to sunlight	6.1 ± 0.5	5.0 ± 0.5

Table 3. *In vitro* antioxidant activity by DPPH- and Hydroxyl- radical scavenging assay.

Active ingredient	Concentration (mg/mL)	Antioxidant activity (%)
CH	20	5.3 ± 0.7
COS-OGA	20	22.1 ± 1.7
COS-OGA	30	29.9 ± 2.5

CONCLUSIONS

Chitosan and its derivatives have been shown to possess substantial antimicrobial efficacy against various microorganisms. Our results demonstrated antimicrobial efficacy of these compounds and their potential use as innovative sustainable compounds in plant protection. These compounds have a direct impact on plants, thus risk of resistance development by microbial organisms is limited. The confirmation of their antimicrobial properties and antioxidant capacity offers the potential to repurpose waste generated during their production. Our *in vitro* findings provide the foundation for subsequent *in vivo* investigations aimed at confirming the viability of using these two novel elicitors in pre- and postharvest management strategies.

ACKNOWLEDGMENTS

This work was conducted within the framework of the PSR ZeroSprechi and of PRIMA StopMedWaste project, which is funded by PRIMA, a programme supported by the European Union. www.stopmedwaste.net

REFERENCE

Makau, S.M., Moumni, M., Landi, L., Pirozzi, D., Sannino, F., Romanazzi, G. 2023. *In vitro* evaluation of chitosan hydrochloride and COS (Chito-Oligosaccharides)-OGA (Oligo-Galacturonides) on phytopathogenic fungi and *Escherichia coli*. *Horticulturae*, *9*, 1275. https://doi.org/10.3390/horticulturae9121275