IECHo 2025 Conference

The 2nd International Electronic Conference on Horticulturae

27–29 May 2025 | Online

MDPI

Smart Delivery of Biorationals: A Novel Strategy Against Root Crop Pathogens

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INTRODUCTION & AIMS

Horticultural crops, particularly sugar beet and carrot, face significant threats from fungal pathogens, causing up to 40% yield losses. Key pathogens include *Cercospora beticola* (leaf spot), *Rhizoctonia solani* (crown and root rot), *Sclerotinia sclerotiorum* (white mold), and *Botrytis cinerea* (grey mold).

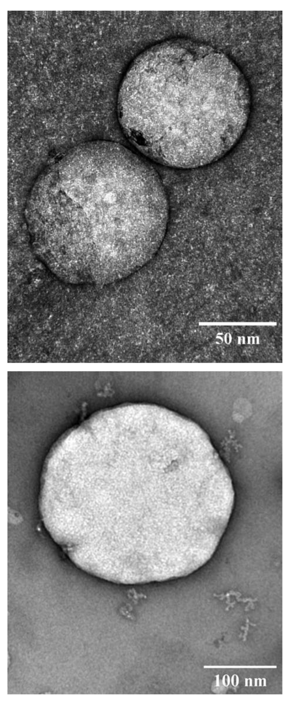
Environmental concerns and regulatory restrictions on conventional fungicides necessitate sustainable alternatives. Plant extracts offer promising antimicrobial properties but face challenges in field application due to rapid degradation and limited bioavailability.

This study aimed to:

- Evaluate chitosan-based nanocarrier (NC) systems loaded with extracts from *Rubia tinctorum* and *Uncaria tomentosa* against key pathogens
- Assess protective effects under controlled conditions
- Validate performance in sugar beet field trials

METHODS

Plant extracts & nanocarrier synthesis



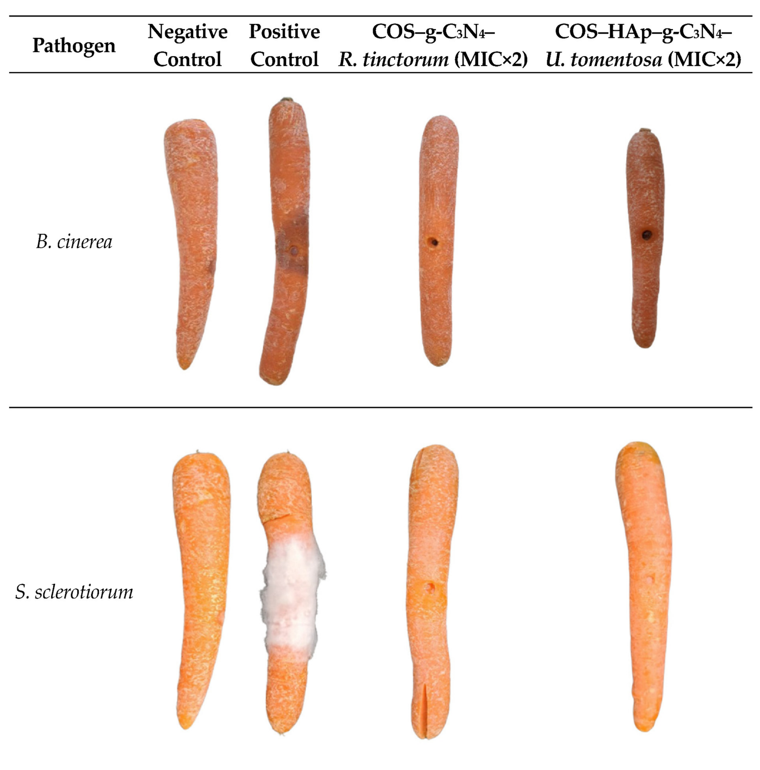
- *R. tinctorum*: Aqueous ammonia extract containing antifungal anthraquinones [1]
- *U. tomentosa*: Aqueous ammonia extract containing alkaloids and polyphenols [2]
- Two patented NC systems based on chitosan oligomers (COS) and carbon nitride (g-C_3N_4): COS-g-C_3N_4 and COS-HAp-g-C_3N_4 (with hydroxyapatite) [3,4]
- High encapsulation efficiency: 95-97% (*R. tinctorum*) and 82% (*U. tomentosa*)

Antifungal assessment

- *In vitro*: Agar dilution method
- *Ex-situ/In vivo*: Protection assays on artificially inoculated carrot roots and sugar beet plants
- Field trials: AIMCRA's experimental fields (Valladolid, Spain, 2024 season)
  - Randomized microplots with four replications per treatment
  - Disease severity assessed using KWS scale and SAUDPC calculations

Table 1. Minimum inhibitory concentrations (MIC, µg/mL) of unencapsulated plant extracts and nanocarrier formulations against horticultural pathogens. Values in brackets indicate the equivalent concentration of encapsulated plant extract corresponding to the nanocarrier MIC.

Treatment	Pathogen			
	B. cinerea	***C. beticola***	***R. solani***	***S. sclerotiorum***
R. tinctorum extract	93.75	93.75	93.75	93.75
U. tomentosa extract	375	93.75	93.75	500
COS-g-C_3N_4-*R. tinctorum*	375 (53.6)	125 (17.9)	187.5 (26.8)	187.5 (26.8)
COS-HAp-g-C_3N_4-*U. tomentosa*	250 (55.6)	375 (83.3)	500 (111.1)	187.5 (41.7)



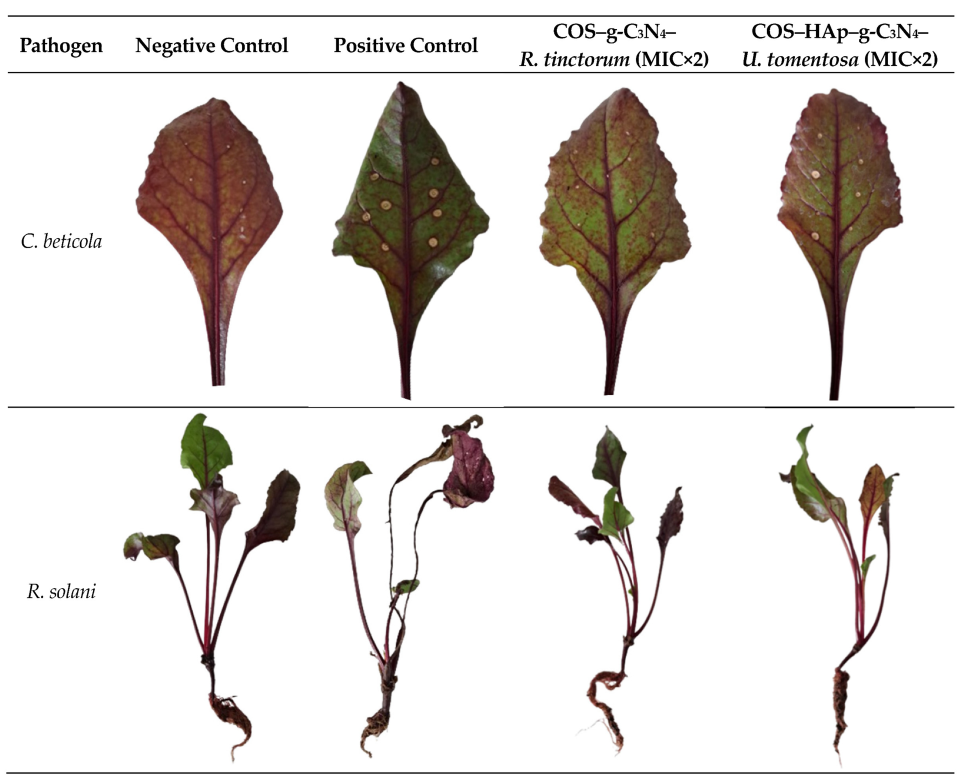


Figure 1. Results of NC protection efficacy *ex-situ* trials against *B. cinerea* and *S. sclerotiorum* on carrot roots (*left*), and results of NC protection efficacy *in vivo* experiments against *C. beticola* and *R. solani* on sugar beet plants (*right*).

RESULTS & DISCUSSION

In vitro antifungal activity (Table 1)

- NC-delivered extracts showed enhanced efficacy compared to unencapsulated forms
- COS-g-C_3N_4-*R. tinctorum*: Higher activity against *C. beticola* (125 µg/mL) and *S. sclerotiorum* (187.5 µg/mL)
- COS-HAp-g-C_3N_4-*U. tomentosa*: More effective against *B. cinerea* (250 µg/mL)
- Both NC formulations exhibited comparable or superior activity to copper-based fungicides (Clarus®, Yukon®)

Protection under controlled conditions (Figure 1)

- Complete protection achieved at MIC×2 concentrations (250-1000 µg/mL)
- Carrot: Full protection against *B. cinerea* with *U. tomentosa*-loaded NCs at 500 µg/mL
- Sugar beet: Complete protection against *C. beticola* with *R. tinctorum*-loaded NCs at 250 µg/mL
- Both NC formulations effectively controlled *R. solani* and *S. sclerotiorum*

Field trial performance (Table 2)

- NC treatments (40-41% effectiveness) significantly outperformed organic alternatives (12%)
- By season's end, disease severity difference between conventional and NC treatments was modest (105.59% *vs.* 108.51-109.24% affected leaf area)
- No phytotoxicity observed with NC treatments

Table 2. Cercospora leaf spot disease severity in terms of affected leaf area (%), area under disease progress curve (AUDPC), standardized area under disease progress curve (SAUDPC), and treatment effectiveness (%) for different treatment strategies in sugar beet field trials at San Román de Hornija, Spain (2024 growing season).

Treatment	Affected leaf area (%)				∑AUDPC	SAUDPC	Effectiveness (%)
	29/08/24	13/09/24	14/10/24	18/10/24			
Untreated control	28.57	92.66	135.02	152.70	5416.13	53.10	0%
Conventional treatment	7.59	35.46	91.76	105.59	2808.96	27.54	48%
Intermediate treatment	15.92	59.22	97.89	112.34	3651.25	35.80	33%
Organic agriculture treatment	26.82	83.21	118.14	134.37	4786.07	46.92	12%
COS-g-C_3N_4-*R. tinctorum*	9.64	48.34	95.06	109.24	3183.51	31.21	41%
COS-HAp-g-C_3N_4-*U. tomentosa*	10.40	51.86	94.40	108.51	3263.12	31.99	40%

CONCLUSIONS

- Chitosan-based nanocarriers loaded with botanical extracts demonstrated significant antifungal activity in laboratory and field conditions
- Field trials validated promising effectiveness (40-41%) against Cercospora leaf spot, approaching conventional fungicides (48%) and outperforming organic alternatives (12%)
- The NC approach offers several advantages over conventional application methods for plant extracts, including protection of bioactive compounds from degradation, improved bioavailability, and controlled release properties.
- Nanoencapsulated plant extracts represent a viable approach to reducing synthetic chemical inputs while maintaining acceptable crop protection efficiency

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Acknowledgments: This research has been funded by the Junta de Castilla y León under project VA148P23, with ERDF co-funding.

Junta de Castilla y León

European Union European Regional Development Fund

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