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Biorational-loaded Nanocarriers: Development and Field Validation for Sustainable *Botrytis* Control in Viticulture

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INTRODUCTION & AIM

Viticulture faces significant challenges from *Botrytis cinerea* Pers., the causal agent of gray mold, which can cause 30-40% fruit losses under favorable conditions. This pathogen not only reduces yield but also affects wine quality through:

- Alteration of grape chemical composition
- Production of toxic compounds that inhibit fermentation
- Detectable sensory quality loss even with just 5% infected grapes

Environmental concerns and increasing fungicide resistance necessitate sustainable alternatives. Plant extracts offer promising antimicrobial properties but face challenges in field application due to rapid degradation and limited bioavailability.

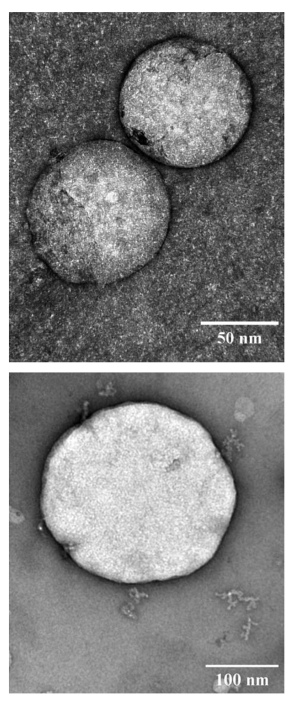
This study aimed to:

- Evaluate chitosan-based nanocarrier (NC) systems loaded with extracts from *Rubia tinctorum* L. and *Uncaria tomentosa* (Willd. ex Schult.) DC. against *B. cinerea*
- Assess protective effects under laboratory and *ex-situ* conditions
- Validate performance in vineyard field trials in D.O.P. Ribera de Duero

METHOD

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

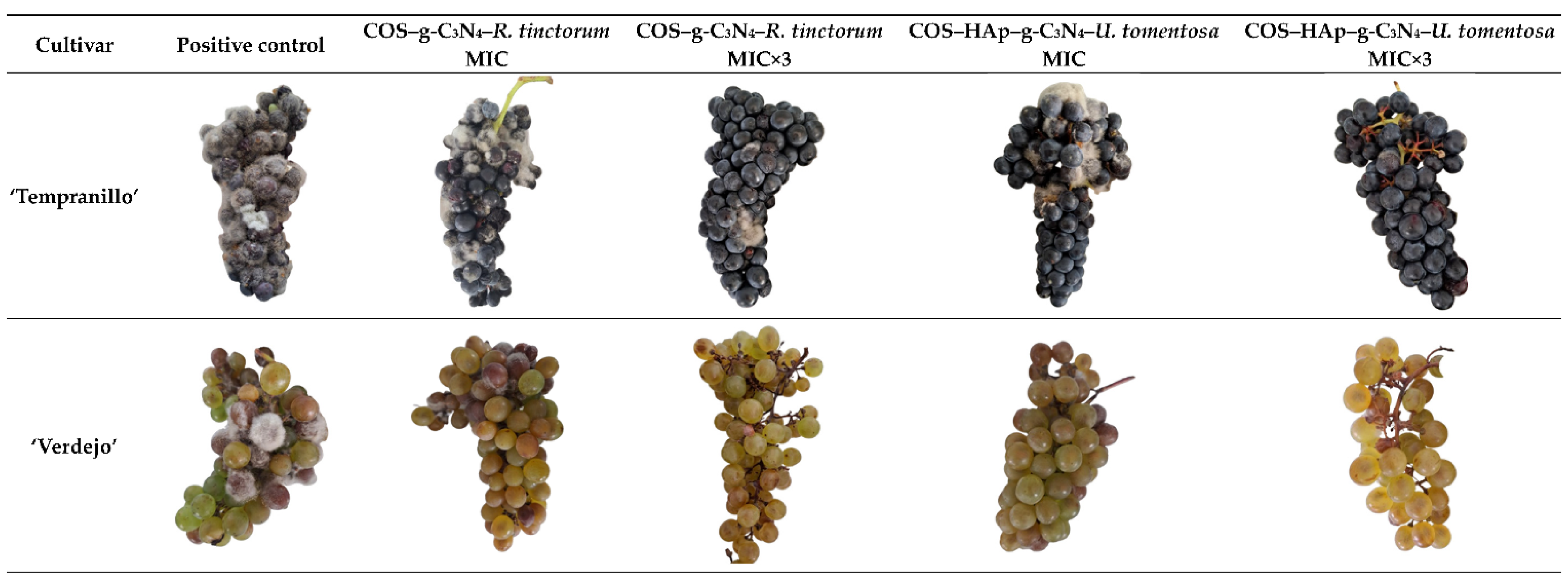
- *In vitro*: Agar dilution method following EUCAST procedures
- *Ex-situ*: Protection assays on artificially inoculated 'Tempranillo' and 'Verdejo' grape varieties
- *Field trials*: Randomized block design at Dominio Fournier winery (D.O.P. Ribera de Duero)
  - Treatments applied at MIC×3 concentration
  - Disease severity assessed using Hill *et al.*'s [5] standardized visual assessment
  - Effectiveness calculated using Abbott's formula

RIBERA DEL DUERO

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

Treatment	*R. tinctorum* extract	*U. tomentosa* extract	COS-g-C_3N_4-*R. tinctorum*	COS-HAp-g-C_3N_4-*U. tomentosa*
MIC	93.75	375	375 (53.6)	250 (55.6)

Figure 1. Representative images of 'Tempranillo' (*top row*) and 'Verdejo' (*bottom row*) grape bunches 7 days after inoculation with *B. cinerea* and treatment with different formulations.



RESULTS & DISCUSSION

In vitro antifungal activity (Table 1)

- NC-delivered extracts showed enhanced efficacy compared to unencapsulated forms
- COS-HAp-g-C_3N_4-*U. tomentosa*: More effective against *B. cinerea* (MIC = 250 µg/mL)
- COS-g-C_3N_4-*R. tinctorum*: Required higher concentration (MIC = 375 µg/mL)
- Equivalent extract concentrations in NCs showed significant improvement in efficacy

Ex-situ grape protection (Figure 1)

- Complete protection achieved at higher concentrations (MIC×3)
- 'Tempranillo': Both NCs provided significant protection through day 7
- 'Verdejo': COS-g-C_3N_4-*R. tinctorum* at MIC×3 showed best protection at day 7
- Protection diminished over time under highly favorable conditions for fungal growth

Field trial performance (Table 2, Figure 2)

- COS-HAp-g-C_3N_4-*U. tomentosa* showed the highest effectiveness (77.5%)
- NC treatments (68.8-77.5% effectiveness) significantly outperformed non-encapsulated extracts (50.0-56.3%) and commercial chitosan (53.8%)
- No phytotoxicity observed with NC treatments
- No negative effects on wine organoleptic properties

Table 2. Severity of *B. cinerea* infection (% affected area) in 'Tempranillo' grape clusters after field application of different treatments and their effectiveness.

Treatment	Incidence (%)	Effectiveness (%)
Untreated control	80	-
R. tinctorum extract	40	50
U. tomentosa extract	35	56.3
Commercial chitosan	37	53.8
COS-g-C_3N_4-*R. tinctorum*	25	68.8
COS-HAp-g-C_3N_4-*U. tomentosa*	18	77.5

Figure 2. Effective protection against *B. cinerea* achieved with *R. tinctorum* NCs (*upper left*) and *U. tomentosa* NCs (*upper right*) *vs.* untreated control (*bottom*).

CONCLUSION

- Chitosan-based nanocarriers loaded with botanical extracts demonstrated significant antifungal activity against *B. cinerea* in laboratory, *ex-situ*, and field conditions
- Field trials validated promising effectiveness (68.8-77.5%) against gray mold, substantially outperforming non-encapsulated extracts and commercial chitosan The NC approach offers several advantages:
  - Protection of bioactive compounds from degradation
  - Improved bioavailability
  - Controlled release properties
  - Synergistic effects with chitosan antimicrobial properties
- Nanoencapsulated plant extracts represent a viable sustainable approach to reducing synthetic chemical inputs while maintaining acceptable crop protection efficiency
- These formulations integrate compatibly with current vineyard practices without affecting wine quality

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