

# Chitosan-based nanocarriers loaded with natural plant extracts for controlling fusarium wilt of banana: *in vitro* efficacy and greenhouse evaluation

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## INTRODUCTION & AIM

**The threat:** *Fusarium oxysporum* f. sp. *cubense* (Foc), particularly Tropical Race 4 (TR4), is a global threat to banana production. Current commercial varieties lack resistance.

**A possible solution:** nanoencapsulation of natural extracts using chitosan oligosaccharides (COS) to improve stability, bioavailability, and targeted delivery.

**Objective:** To evaluate *Rubia tinctorum* and *Uncaria tomentosa* nanocarriers (NCs) against Foc TR4/STR4 *in vitro* and in greenhouse trials with cv. Dwarf Cavendish.

## METHODOLOGY

### Nanocarriers:

- NC-A:** COS- $g\text{-C}_3\text{N}_4$  loaded with *Rubia tinctorum* (14.3% extract).  $g\text{-C}_3\text{N}_4$  = graphitic carbon nitride.<sup>[1,2]</sup>
- NC-B:** COS-HAp- $g\text{-C}_3\text{N}_4$  loaded with *Uncaria tomentosa* (22.2% extract). HAp = hydroxyapatite.<sup>[3,4]</sup>

**In vitro assays:** EC<sub>50</sub> and EC<sub>90</sub> determination + time-kill kinetics against Foc STR4 and TR4.

**Greenhouse trial:** Dwarf Cavendish plantlets inoculated with Foc STR4; root dip application. Assessed for 79 days for disease severity (SAUDPC) and physiology.

## RESULTS & DISCUSSION

### A. In vitro potency & kinetics

Both NCs showed concentration-dependent inhibition (Table 1), but *R. tinctorum*-loaded NCs demonstrated superior kinetics.

**Table 1.** Effective concentrations (EC<sub>50</sub> and EC<sub>90</sub>) in µg/mL of NC treatments against Foc STR4 and TR4. Values in parentheses indicate equivalent concentration of encapsulated plant extract.

Nanocarrier treatment	Effective concentrations	<i>F. oxysporum</i> f. sp. <i>cubense</i>	
		STR4	TR4
NC-A ( <i>R. tinctorum</i> )	EC <sub>50</sub>	620.5 (88.7)	328.2 (46.9)
	EC <sub>90</sub>	953.8 (136.4)	960.4 (137.3)
NC-B ( <i>U. tomentosa</i> )	EC <sub>50</sub>	610.3 (135.5)	291.7 (64.8)
	EC <sub>90</sub>	956.3 (212.3)	938.2 (208.3)

### Mode of action:

- NC-A (*R. tinctorum*): Fungicidal.** Achieved complete kill (0 CFU) within 24 hours (Table 2).
- NC-B (*U. tomentosa*): Fungistatic.** Significant suppression, but residual colonies persisted at 72h.

**Table 2.** Time-kill kinetics of nanocarrier treatments (3000 µg/mL) against Foc STR4 and TR4.

Treatment	T0 (h)	T24 (h)	T48 (h)	T72 (h)
Control (Foc)	2.00 ± 0.04 <sup>a</sup>	3.84 ± 0.03 <sup>a</sup>	6.66 ± 0.05 <sup>a</sup>	7.38 ± 0.03 <sup>a</sup>
NC-A ( <i>R. tinctorum</i> )	1.96 ± 0.03 <sup>a</sup>	0.17 ± 0.11 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>
NC-B ( <i>U. tomentosa</i> )	2.01 ± 0.02 <sup>a</sup>	1.15 ± 0.11 <sup>b</sup>	0.67 ± 0.28 <sup>b</sup>	0.38 ± 0.17 <sup>b</sup>

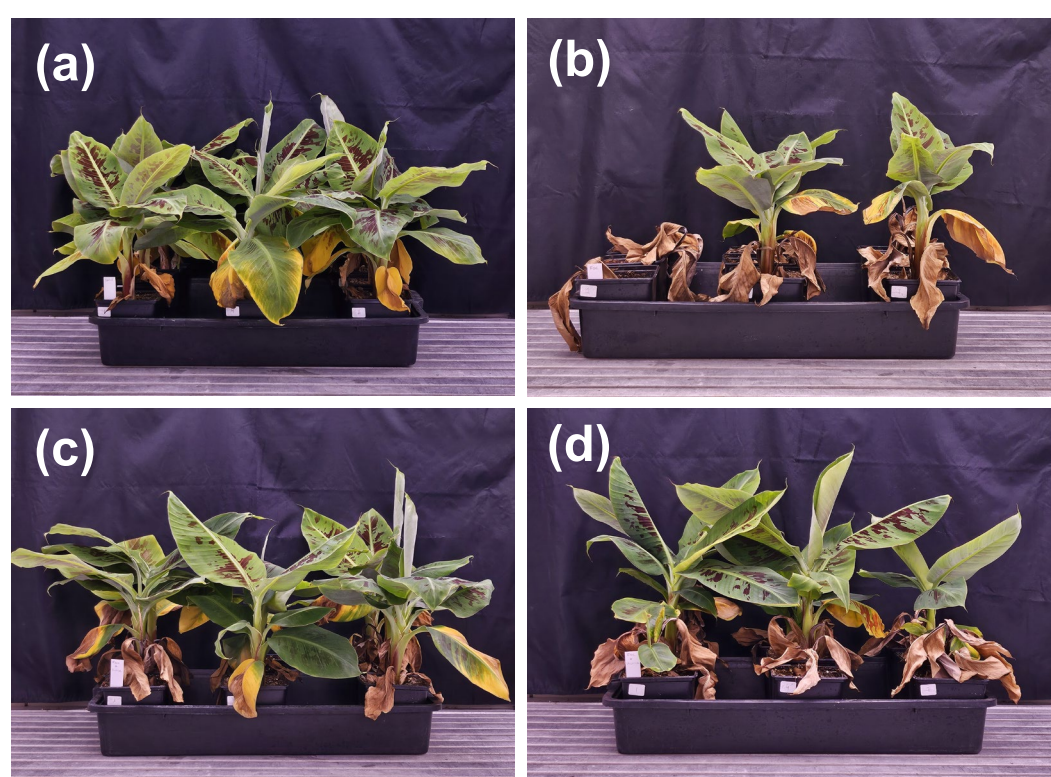
## RESULTS & DISCUSSION (cont.)

### B. Greenhouse efficacy (disease suppression)

In planta, the rapid fungicidal action of *R. tinctorum*-loaded NCs translated to significant disease control, whereas *U. tomentosa*-loaded NCs did not (Table 3, Figure 1).

**Table 3.** Standardized AUDPC (SAUDPC) at 79 days post-inoculation for banana cv. Dwarf Cavendish plants treated with NCs and challenged with Foc.

Treatment	SAUDPC	Efficacy (%)	Statistical grouping
Healthy control	0.00	—	—
Foc control	2.61	—	a
NC-A ( <i>R. tinctorum</i> )	1.70	34.9	b
NC-B ( <i>U. tomentosa</i> )	2.41	7.8	ab



**Figure 1.** Visual comparison at 79 DPI. (a) Healthy control, (b) Foc control, (c) NC-A (*R. tinctorum*), (d) NC-B (*U. tomentosa*). The NC treatments show reduced wilting and a healthier canopy compared to the inoculated control

### C. Physiological impact & defense elicitation

Treatment elicited strong defense responses and promoted growth despite pathogen pressure.

- Defense markers:** flavonoid content increased by 95% in NC-A-treated plants.
- Biomass:** NC-A treatment significantly increased fresh weight by 16.4%.
- Stress adaptation:** NC-A-treated plants showed reduced stomatal conductance (g<sub>sw</sub>), indicating a water-conservation strategy during infection

## CONCLUSIONS

- Efficacy:** *Rubia tinctorum*-loaded nanocarriers provided 34.9% disease reduction in greenhouse conditions, significantly superior to the control.
- Mechanism:** *Rubia tinctorum*-loaded NCs act as a fungicide (rapid kill <24h) and a defense elicitor (doubling flavonoid content).
- Application:** This nano-formulation represents a promising component for integrated pest management (IPM) strategies against Fusarium Wilt.

## REFERENCES

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