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Novel copper nanoparticles for the control of tomato foliar and fruit diseases

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Introduction

Grey mould and Late Blight caused by *Botrytis cinerea* and *Phytophthora infestans,* respectively are the most important foliar and fruit diseases of tomato. Applications of fungicides are the main control measures of these diseases. However, development of resistance to single-site inhibitors by both pathogens necessitates research for the development of alternative products.

Aim

This study was performed to investigate the efficacy of 4 novel Nano-copper (CuNPs) formulations against these 2 major tomato diseases.

Materials and Methods

✓ Formulations: Four types of Cu-based NPs were synthesized by Plin Nanotechnology (Thessaloniki, Greece). As reference compounds, two commercial copper products were included in the study, copper oxide (Nordox 75 WG, K&N Efthimiadis, Thessaloniki, Greece) and copper hydroxide (Kocide 2000, 35 WG, K&N Efthimiadis, Thessaloniki, Greece).

✓ *In vitro* assays: The assessment of isolates` sensitivity to four CuNPs was based on the inhibition of mycelial growth at different concentrations. CuNPs stock solutions were added into autoclaved nutrient media to achieve concentrations of 10, 50, 100, and 200 μg·mL^{-1} for CuNPs CN_S2_X1 and CN_S4_X1, of 10, 50, 100, 200, and 400 μg·mL^{-1} for CC_S4_X2, and of 10, 50, 100, 200, 400, 600, and 800 μg·mL^{-1} for CN_S1_X1, copper oxide and copper hydroxide. For the assessment of mycelial growth inhibition, mycelial plugs from the margins of actively growing 7-day-old *B. cinerea* and *P. infestans*, colonies on PDA and Oatmeal substrate, respectively were removed, with a 5 mm diameter cork borer and placed upside down on the center of 9 cm dishes containing the CuNPs-amended or -unamended media. Cultures of *B. cinerea* and *P. infestans* were incubated at 23°C and 19°C in the dark for 7 days, respectively. The EC_{50} value for each isolate was calculated by plotting the relative inhibition of mycelial growth against the Log_{10} CuNPs concentrations using SAS (JMP, SAS Institute, Cary, NC).

✓ *In planta* assays: The efficacy of the two most efficient formulations (CN_S4_X1 and CC_S4_X2) from the *in vitro* assay against *B. cinerea* and *P. infestans* were evaluated on tomato seedling plants (cv. Belladona) at the 4th true leaf growth stage under controlled environmental conditions in a plant growth chamber. The concentration of CuNPs applications were 240 μg ml^{-1}. To evaluate the protective and curative activity of the CuNPs tested, spray treatments were conducted 24, 48 and 96h before (protective treatments) or after (curative treatments) the inoculation of the plants.

- *B. cinerea* inoculation: Artificial inoculation was conducted by pipetting 10 μL of conidial suspension of the adaxial surface of each leaf. After inoculation, inoculated and control plants were incubated in a growth chamber at 23 ± 2°C for 6 days. For each plant the disease severity value was measured as follows: 0= no disease symptom; 1= disease symptoms covered 20% of the leaf; 2= disease symptoms covered 50% of the leaf; 3= disease symptoms covered 70% of the leaf; 4= dead leaf (**Figure 1A**).
- *P. infestans* inoculation: Plants were sprayed to run-off with a sporangial suspension. After inoculation, inoculated and control plants were incubated in a growth chamber at 19°C for 10 days. For each plant the disease severity was measured as mentioned above (**Figure 1B**).

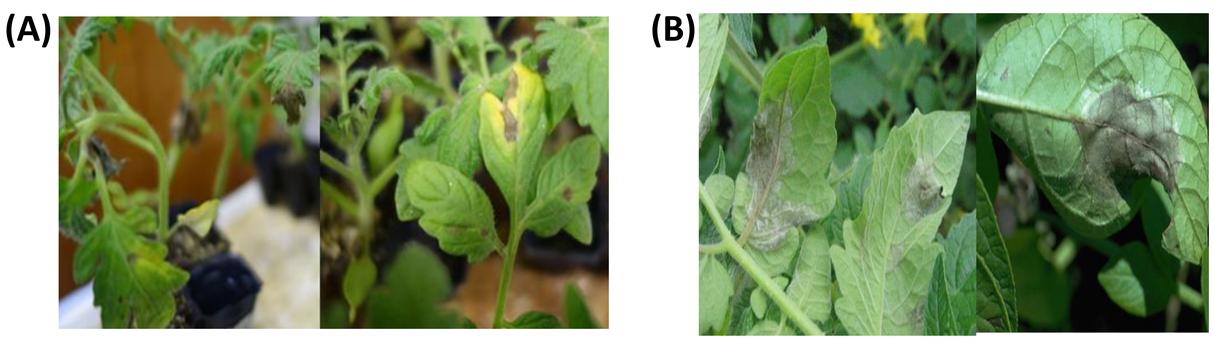

Figure 1. Symptoms of **(A)** Gray mold caused by *B. cinerea* and **(B)** Late Blight caused by *P. infestans* on tomato plants.

Results

- Measurements of *B. cinerea* and *P. infestans* isolates sensitivity to Cu-NPs revealed that the 2 fungal species had a similar sensitivity to the Cu-NPs tested.
- The 2 most efficient formulations *in vitro* against both pathogens were CN_S4_X1 and CC_S4_X2 with EC_{50} values ranging from 150 to 289 and from 23 to 45 μg ml^{-1} for *B. cinerea* and *P. infestans*, respectively (**Table 1**).

Table 1. Sensitivity in terms of EC_{50} values of *B. cinerea* and *P. infestans* isolates to different copper formulation products.

	EC_{50}					
	Copper formulation products					
	CN_S2_X1	**CN_S4_X1**	**CC_S4_X2**	**CN_S1_X1**	**Nordox**	**Kocide**
B. cinerea	185	150	289	325	200	745
P. infestans	35	23	45	32,7	12	15

- For both pathogens, disease severity on plants treated curatively was higher than in plants treated preventively (**Figure 2 and 3**).
- Applications of the two CuNPs against *B. cinerea* 24h prior to the inoculation led to lower disease severity with means of 1.5 and 1.3, respectively, compared to 48h and 96h before or after inoculation.
- Similarly, the 2 CuNPs tested were found to be highly effective against *P. infestans* as provided lower disease severity at 24h and 48h pre-/post-inoculation (**Figure 3**) .
- For both pathogens, disease severity was significantly higher on plants treated with the commercial copper products both at protective and curative treatments (**Figure 2 and 3**).

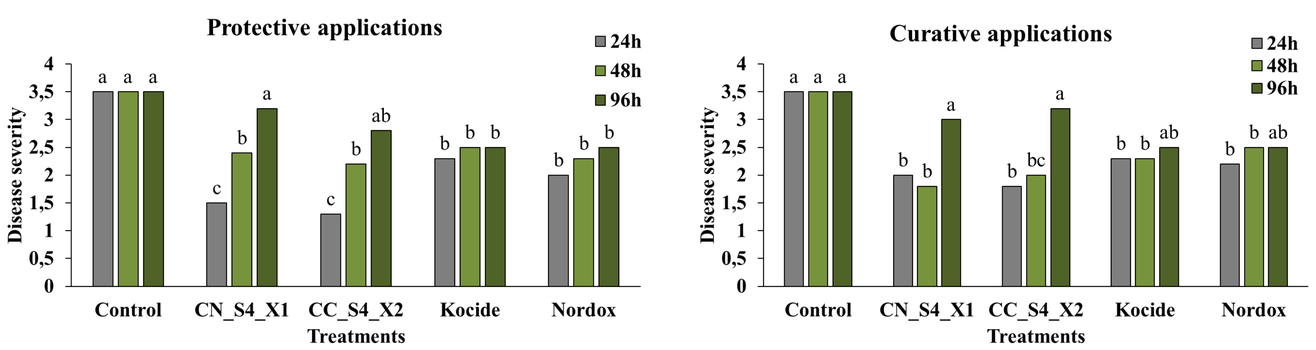

Figure 2. Grey mould severity of protective and curative applications of copper-based formulation treatments against *Botrytis cinerea* on tomato plants at three different timepoints.

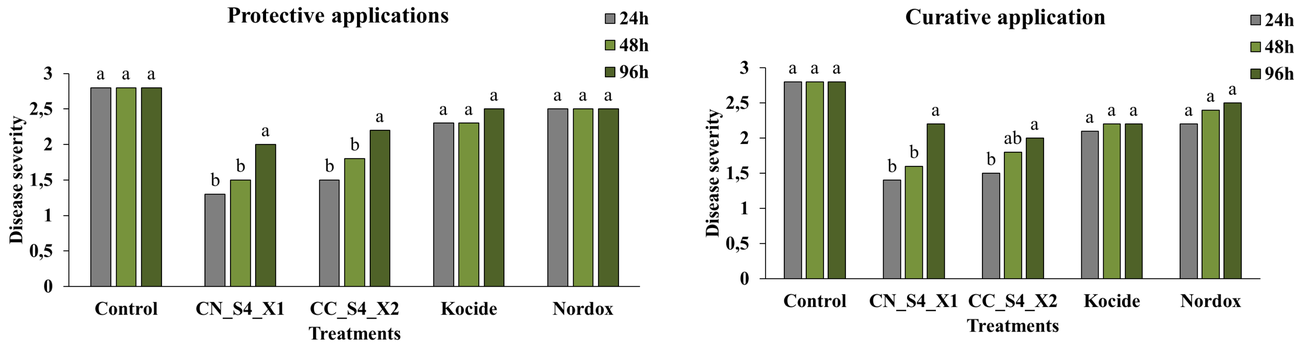

Figure 3. Late Blight severity of protective and curative applications of copper-based formulation treatments against *P. infestans* on tomato plants at three different timepoints..

Conclusion

The results of this study are expected to contribute to the optimization of tomato plant diseases control and reduce the yield losses caused, using a new generation of biocides.

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